NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS

MEDICAL SCHOOL

LABORATORY OF PHARMACOLOGY

Director Prof. N. Sitaras

EFFECTS OF ANTIOXIDANT U-74389G ON A PORCINE MODEL OF INTRACEREBRAL HAEMORRHAGE: DETERMINATION OF INFLAMMATORY MARKERS AND CRUCIAL ENZYMES

DOCTORATE THESIS

ALEXIOS G. BIMPIS

NEUROSURGEON

ATHENS, 2013

ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΕΡΓΑΣΤΗΡΙΟ ΦΑΡΜΑΚΟΛΟΓΙΑΣ

Διευθυντής Καθηγητής : Νικόλαος Σιταράς

ΔΡΑΣΗ ΤΟΥ ΑΝΤΙΟΞΕΙΔΩΤΙΚΟΥ ΠΑΡΑΓΟΝΤΑ U-74389G ΣΕ ΠΕΙΡΑΜΑΤΙΚΟ ΠΡΟΤΥΠΟ ΕΝΔΟΕΓΚΕΦΑΛΙΚΟΥ ΑΙΜΑΤΩΜΑΤΟΣ ΣΕ ΧΟΙΡΟ:ΠΡΟΣΔΙΟΡΙΣΜΟΣ ΠΑΡΑΜΕΤΡΩΝ ΦΛΕΓΜΟΝΗΣ ΚΑΙ ΣΗΜΑΝΤΙΚΩΝ ΕΝΖΥΜΩΝ

ΔΙΑΤΡΙΒΗ ΕΠΙ ΔΙΔΑΚΤΟΡΙΑ

ΑΛΕΞΙΟΣ Γ. ΜΠΙΜΠΗΣ

ΝΕΥΡΟΧΕΙΡΟΥΡΓΟΣ

ΑΘΗΝΑ, 2013

Dedicated

To my parents and especially my father Georgios A. Bimpis

ΕΘΝΙΚΟΝ ϟ ΚΑΠΟΔΙΣΤΡΙΑΚΟΝ

ΠΑΝΕΠΙΣΤΗΜΙΟΝ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ϟΧΟΛΗ

ΟΡΚΟΣ

ΟΜΝΥΜΙ ΑΠΟΛΛΩΝΑ ΙΗΤΡΟΝ ΚΑΙ ΑΣΚΛΗΠΙΟΝ ΚΑΙ ΥΓΕΙΑΝ ΚΑΙ ΠΑΝΑΚΕΙΑΝ ΚΑΙ ΘΕΟΥΣ ΠΑΝΤΑΣ ΤΕ ΚΑΙ ΠΑΣΑΣ, ΙΣΤΟΡΑΣ ΠΟΙΕΥΜΕΝΟΣ, ΕΠΙΤΕΛΕΑ ΠΟΙΗΣΕΙΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ ΟΡΚΟΝ ΤΟΝΔΕ ΚΑΙ ΣΥΓΓΡΑΦΗΝ ΤΗΝΔΕ· ΗΓΗΣΕΣΘΑΙ ΜΕΝ ΤΟΝ ΔΙΔΑΞΑΝΤΑ ΜΕ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ ΙΣΑ ΓΕΝΕΤΗΣΙΝ ΕΜΟΙΣΙ ΚΑΙ ΒΙΟΥ ΚΟΙΝΩΣΕΣΘΑΙ ΚΑΙ ΧΡΕΩΝ ΧΡΗΙΖΟΝΤΙ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ, ΚΑΙ ΓΕΝΟΣ ΤΟ ΕΞ ΕΩΥΤΟΥ ΑΔΕΛΦΟΙΣ ΙΣΟΝ ΕΠΙΚΡΙΝΕΕΙΝ ΑΡΡΕΣΙ ΚΑΙ ΔΙΔΑΞΕΙΝ ΤΗΝ ΤΕΧΝΗΝ ΤΑΥΤΗΝ, ΗΝ ΧΡΗΙΖΩΣΙ ΜΑΝΘΑΝΕΙΝ, ΑΝΕΥ ΜΙΣΘΟΥ ΚΑΙ ΣΥΓΓΡΑΦΗΣ, ΠΑΡΑΓΓΕΛΙΗΣ ΤΕ ΚΑΙ ΑΚΡΟΗΣΙΟΣ ΚΑΙ ΤΗΣ ΛΟΙΠΗΣ ΑΠΑΣΗΣ ΜΑΘΗΣΙΟΣ ΜΕΤΑΔΟΣΙΝ ΠΟΙΗΣΕΣΘΑΙ ΥΙΟΙΣΙ ΤΕ ΕΜΟΙΣ ΚΑΙ ΤΟΙΣ ΤΟΥ ΕΜΕ ΔΙΔΑΞΑΝΤΟΣ ΚΑΙ ΜΑΘΗΤΗΣΙ ΣΥΓΓΕΓΡΑΜΜΕΝΟΙΣΙ ΤΕ ΚΑΙ ΩΡΚΙΣΜΕΝΟΙΣ ΝΟΜΩ ΙΗΤΡΙΚΩ, ΑΛΛΩ ΔΕ ΟΥΔΕΝΙ. ΔΙΑΙΤΗΜΑΣΙ ΤΕ ΧΡΗΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ ΚΑΜΝΟΝΤΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΡΙΣΙΝ ΕΜΗΝ, ΕΠΙ ΔΗΛΗΣΕΙ ΔΕ ΚΑΙ ΑΔΙΚΙΗ, ΕΙΡΞΕΙΝ. ΟΥ ΔΩΣΩ ΔΕ ΟΥΔΕ ΦΑΡΜΑΚΟΝ ΟΥΔΕΝΙ ΑΙΤΗΘΕΙΣ ΘΑΝΑΣΙΜΟΝ ΟΥΔΕ ΥΦΗΓΗΣΟΜΑΙ ΣΥΜΒΟΥΛΙΗΝ ΤΟΙΗΝΔΕ· ΟΜΟΙΩΣ ΔΕ ΟΥΔΕ ΓΥΝΑΙΚΙ ΠΕΣΣΟΝ ΦΘΟΡΙΟΝ ΔΩΣΩ. ΑΓΝΩΣ ΔΕ ΚΑΙ ΟΣΙΩΣ ΔΙΑΤΗΡΗΣΩ ΒΙΟΝ ΤΟΝ ΕΜΟΝ ΚΑΙ ΤΕΧΝΗΝ ΤΗΝ ΕΜΗΝ. ΟΥ ΤΕΜΕΩ ΔΕ ΟΥΔΕ ΜΗΝ ΛΙΘΙΩΝΤΑΣ, ΕΚΧΩΡΗΣΩ ΔΕ ΕΡΓΑΤΗΣΙΝ ΑΝΔΡΑΣΙ ΠΡΗΞΙΟΣ ΤΗΣΔΕ. ΕΣ ΟΙΚΙΑΣ ΔΕ ΟΚΟΣΑΣ ΑΝ ΕΣΙΩ, ΕΣΕΛΕΥΣΟΜΑΙ ΕΠ' ΩΦΕΛΕΙΗ, ΚΑΜΝΟΝΤΩΝ, ΕΚΤΟΣ ΕΩΝ ΠΑΣΗΣ ΑΔΙΚΙΗΣ ΕΚΟΥΣΙΗΣ ΚΑΙ ΦΘΟΡΙΗΣ ΤΗΣ ΤΕ ΑΛΛΗΣ ΚΑΙ ΑΦΡΟΔΙΣΙΩΝ ΕΡΓΩΝ ΕΠΙ ΤΕ ΓΥΝΑΙΚΕΙΩΝ ΣΩΜΑΤΩΝ ΚΑΙ ΑΝΔΡΩΩΝ ΕΛΕΥΘΕΡΩΝ ΤΕ ΚΑΙ ΔΟΥΛΩΝ. Α ΔΕ ΑΝ ΕΝ ΘΕΡΑΠΕΙΗ, Η ΙΔΩ Η ΑΚΟΥΣΩ, Η ΚΑΙ ΑΝΕΥ ΘΕΡΑΠΕΙΗΣ ΚΑΤΑ ΒΙΟΝ ΑΝΘΡΩΠΩΝ, Α ΜΗ ΧΡΗ ΠΟΤΕ ΕΚΛΑΛΕΙΣΘΑΙ ΕΞΩ, ΣΙΓΗΣΟΜΑΙ, ΑΡΡΗΤΑ ΗΓΕΥΜΕΝΟΣ ΕΙΝΑΙ ΤΑ ΤΟΙΑΥΤΑ. ΟΡΚΟΝ ΜΕΝ ΟΥΝ ΜΟΙ ΤΟΝΔΕ ΕΠΙΤΕΛΕΑ ΠΟΙΕΟΝΤΙ, ΚΑΙ ΜΗ ΣΥΓΧΕΟΝΤΙ, ΕΙΗ ΕΠΑΥΡΑΣΘΑΙ ΚΑΙ ΒΙΟΥ ΚΑΙ ΤΕΧΝΗΣ ΔΟΞΑΖΟΜΕΝΩ, ΠΑΡΑ ΠΑΣΙΝ ΑΝΘΡΩΠΟΙΣ ΕΙΣ ΤΟΝ ΑΙΕΙ ΧΡΟΝΟΝ· ΠΑΡΑΒΑΙΝΟΝΤΙ ΔΕ ΚΑΙ ΕΠΙΟΡΚΟΥΝΤΙ, ΤΑΝΑΝΤΙΑ ΤΟΥΤΕΩΝ.

ΙΠΠΟΚΡΑΤΗΣ

ΟΡΚΟΣ

ΟΡΚΙΖΟΜΑΙ ΕΙΣ ΤΟΝ ΑΠΟΛΛΩΝΑ ΤΟΝ ΙΑΤΡΟΝ ΚΑΙ ΕΙΣ ΤΟΝ ΑΣΚΛΗΠΙΟΝ ΚΑΙ ΕΙΣ ΤΗΝ ΥΓΕΙΑΝ ΚΑΙ ΕΙΣ ΤΗΝ ΠΑΝΑΚΕΙΑΝ ΚΑΙ ΕΙΣ ΟΛΟΥΣ ΤΟΥΣ ΘΕΟΥΣ ΚΑΙ ΕΙΣ ΟΛΑΣ ΤΑΣ ΘΕΑΣ, ΕΠΙΚΑΛΟΥΜΕΝΟΣ ΑΥΤΟΥΣ ΩΣ ΜΑΡΤΥΡΑΣ, ΟΤΙ ΘΑ ΤΗΡΗΣΩ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΑΤΑ ΤΗΝ ΚΡΙΣΙΝ ΜΟΥ ΑΥΤΟΝ ΕΔΩ ΤΟΝ ΟΡΚΟΝ ΚΑΙ ΑΥΤΗΝ ΕΔΩ ΤΗΝ ΕΓΓΡΑΦΟΝ ΣΥΜΦΩΝΙΑΝ. ΘΑ ΘΕΩΡΩ ΜΕΝ ΕΚΕΙΝΟΝ, Ο ΟΠΟΙΟΣ ΜΕ ΕΔΙΔΑΞΕ ΤΗΝ ΤΕΧΝΗΝ ΑΥΤΗΝ, ΙΣΟΝ ΠΡΟΣ ΤΟΥΣ ΓΟΝΕΙΣ ΜΟΥ ΚΑΙ ΘΑ ΤΟΝ ΚΑΜΩ ΚΟΙΝΩΝΟΝ ΤΟΥ ΒΙΟΥ ΜΟΥ ΚΑΙ ΘΑ ΤΟΝ ΒΟΗΘΩ ΟΤΑΝ ΕΥΡΙΣΚΕΤΑΙ ΕΙΣ ΟΙΚΟΝΟΜΙΚΗΝ ΑΝΑΓΚΗΝ, ΚΑΙ ΘΑ ΘΕΩΡΩ ΤΟΥΣ ΑΠΟΓΟΝΟΥΣ ΤΟΥ ΩΣ ΑΔΕΛΦΟΥΣ ΜΟΥ ΚΑΙ ΘΑ ΤΟΥΣ ΔΙΔΑΣΚΩ ΤΗΝ ΤΕΧΝΗΝ ΑΥΤΗΝ ΕΑΝ ΘΕΛΟΥΝ ΝΑ ΤΗΝ ΜΑΘΟΥΝ, ΧΩΡΙΣ ΑΜΟΙΒΗΝ ΚΑΙ ΕΓΓΡΑΦΟΝ ΣΥΜΦΩΝΙΑΝ. ΘΑ ΠΑΡΕΧΩ ΔΕ ΣΥΜΒΟΥΛΑΣ, ΕΙΔΙΚΑ ΠΡΟΦΟΡΙΚΑ ΜΑΘΗΜΑΤΑ ΚΑΙ ΟΛΑΣ ΤΑΣ ΥΠΟΛΟΙΠΟΥΣ ΓΝΩΣΕΙΣ ΤΗΣ ΙΑΤΡΙΚΗΣ ΚΑΙ ΕΙΣ ΤΑ ΠΑΙΔΙΑ ΜΟΥ ΚΑΙ ΕΙΣ ΤΑ ΠΑΙΔΙΑ ΤΟΥ ΔΙΔΑΣΚΑΛΟΥ ΜΟΥ ΚΑΙ ΕΙΣ ΤΟΥΣ ΜΑΘΗΤΑΣ, ΟΙ ΟΠΟΙΟΙ ΚΑΙ ΕΓΓΕΓΡΑΜΜΕΝΟΙ ΕΙΝΑΙ ΚΑΙ ΕΧΟΥΝ ΔΟΣΕΙ ΟΡΚΟΝ ΣΥΜΦΩΝΟΣ ΠΡΟΣ ΤΟΝ ΙΑΤΡΙΚΟΝ ΝΟΜΟΝ, ΕΙΣ ΚΑΝΕΝΑ ΔΕ ΑΛΛΟΝ.

ΘΑ ΧΡΗΣΙΜΟΠΟΙΩ ΔΕ ΤΗΝ ΘΕΡΑΠΕΥΤΙΚΗΝ ΑΓΩΓΗΝ ΠΡΟΣ ΩΦΕΛΕΙΑΝ ΤΩΝ ΑΣΘΕΝΩΝ ΚΑΤΑ ΔΥΝΑΜΙΝ ΚΑΙ ΚΑΤΑ ΤΗΝ ΚΡΙΣΙΝ ΜΟΥ. ΘΑ ΠΡΟΛΑΜΒΑΝΩ ΔΕ ΒΛΑΒΗΝ ΥΓΕΙΑΣ ΚΑΙ ΑΔΙΚΙΑΝ, ΔΕΝ ΘΑ ΔΩΣΩ ΔΕ ΦΑΡΜΑΚΟΝ ΘΑΝΑΤΗΦΟΡΟΝ ΕΙΣ ΚΑΝΕΝΑ, ΟΥΤΕ ΚΑΙ ΕΑΝ ΜΟΥ ΖΗΤΗΘΗ, ΚΑΙ ΟΥΤΕ ΘΑ ΔΩΣΩ ΤΟΙΑΥΤΗΝ ΣΥΜΒΟΥΛΗΝ. ΩΣΑΥΤΩΣ ΟΥΤΕ ΕΙΣ ΓΥΝΑΙΚΑ ΘΑ ΔΩΣΩ ΕΚΤΡΩΤΙΚΟΝ ΦΑΡΜΑΚΟΝ. ΘΑ ΔΙΑΤΗΡΩ ΔΕ ΤΟΝ ΒΙΟΝ ΜΟΥ ΚΑΙ ΤΗΝ ΤΕΧΝΗΝ ΜΟΥ ΚΑΤΑ ΤΡΟΠΟΝ ΑΓΝΟΝ ΚΑΙ ΣΥΜΦΩΝΟΝ ΠΡΟΣ ΤΟΝ ΘΕΙΟΝ ΝΟΜΟΝ. ΑΚΟΜΗ ΔΕ ΔΕΝ ΘΑ ΧΕΙΡΟΥΡΓΩ ΟΥΤΕ ΚΑΙ ΤΟΥΣ ΠΑΣΧΟΝΤΑΣ ΑΠΟ ΛΙΘΙΑΣΙΝ, ΑΛΛΑ ΘΑ ΤΟΥΣ ΠΑΡΑΠΕΜΠΩ ΕΙΣ ΑΝΔΡΑΣ ΧΕΙΡΟΥΡΓΟΥΣ, ΕΙΔΙΚΟΥΣ ΔΙΑ ΤΟ ΕΡΓΟΝ ΤΟΥΤΟ. ΕΙΣ ΟΣΑΣ ΔΕ ΟΙΚΙΑΣ ΜΕΤΑΒΑΙΝΩ, ΘΑ ΕΙΣΕΡΧΩΜΑΙ ΠΡΟΣ ΒΟΗΘΕΙΑΝ ΤΩΝ ΑΣΘΕΝΩΝ, ΑΠΕΧΩΝ ΠΑΣΗΣ ΕΚΟΥΣΙΑΣ ΑΔΙΚΙΑΣ ΚΑΙ ΑΛΛΗΣ ΔΙΑΦΘΟΡΑΣ ΚΑΙ ΣΑΡΚΙΚΩΝ ΣΧΕΣΕΩΝ ΜΕΤΑ ΓΥΝΑΙΚΩΝ Η ΑΝΔΡΩΝ ΕΛΕΥΘΕΡΩΝ ΚΑΙ ΔΟΥΛΩΝ.

ΟΣΑ ΔΕ ΙΔΩ Η ΑΚΟΥΣΩ ΚΑΤΑ ΤΗΝ ΘΕΡΑΠΕΙΑΝ Η ΚΑΤΑ ΤΗΝ ΕΠΙΚΟΙΝΩΝΙΑΝ ΜΟΥ ΜΕΤΑ ΤΩΝ ΑΛΛΩΝ ΑΝΘΡΩΠΩΝ ΕΙΣ ΤΗΝ ΚΑΘΗΜΕΡΙΝΗΝ ΤΟΥΣ ΖΩΗΝ, ΤΑ ΟΠΟΙΑ ΔΕΝ ΠΡΕΠΕΙ ΠΟΤΕ ΝΑ ΑΝΑΚΟΙΝΩΘΟΥΝ ΕΙΣ ΤΟΥΣ ΑΛΛΟΥΣ, ΘΑ ΚΡΑΤΩ ΜΥΣΤΙΚΑ ΠΙΣΤΕΥΩΝ ΟΤΙ ΤΑ ΤΟΙΑΥΤΑ ΕΙΝΑΙ ΑΠΟΡΡΗΤΑ. ΕΦ' ΟΣΟΝ ΜΕΝ ΛΟΙΠΟΝ ΤΗΡΩ ΑΥΤΟΝ ΕΔΩ ΤΟΝ ΟΡΚΟΝ ΚΑΙ ΔΕΝ ΤΟΝ ΠΑΡΑΒΑΙΝΩ, ΕΙΘΕ ΝΑ ΑΠΟΛΑΥΩ ΚΑΙ ΤΗΣ ΖΩΗΣ ΚΑΙ ΤΗΣ ΤΕΧΝΗΣ, ΕΚΤΙΜΩΜΕΝΟΣ ΕΣΑΕΙ ΑΠΟ ΟΛΟΥΣ ΤΟΥΣ ΑΝΘΡΩΠΟΥΣ· ΕΑΝ ΟΜΩΣ ΠΑΡΑΒΩ ΑΥΤΟΝ ΚΑΙ ΓΙΝΩ ΕΠΙΟΡΚΟΣ, ΝΑ ΠΑΘΩ ΤΑ ΑΝΤΙΘΕΤΑ ΤΟΥΤΩΝ.

ΙΠΠΟΚΡΑΤΗΣ

Oath of Hippocrates

I swear by Apollo the physician, and Aesculapius, and Health, and All-heal, and all the gods and goddesses, that, according to my ability and judgment, I will keep this Oath and this stipulation– to reckon him who taught me this Art equally dear to me as my parents, to share my substance with him, and relieve his necessities if required; to look upon his offspring in the same footing as my own brothers, and to teach them this art, if they shall wish to learn it, without fee or stipulation; and that by precept, lecture, and every other mode of instruction, I will impart a knowledge of the Art to my own sons, and those of my teachers, and to disciples bound by a stipulation and oath according to the law of medicine, but to none others. I will follow that system of regimen which, according to my ability and judgment, I consider for the benefit of my patients, and abstain from whatever is deleterious and mischievous.

I will give no deadly medicine to any one if asked, nor suggest any such counsel; and in like manner I will not give to a woman a pessary to produce abortion.With purity and with holiness I will pass my life and practice my Art. I will not cut persons laboring under the stone, but will leave this to be done by men who are practitioners of this work. Into whatever houses I enter, I will go into them for the benefit of the sick, and will abstain from every voluntary act of mischief and corruption; and, further from the seduction of females or males, of freemen and slaves. Whatever, in connection with my professional practice, or not in connection with it, I see or hear, in the life of men, which ought not to be spoken of abroad, I will not divulge, as reckoning that all such should be kept secret.While I continue to keep this Oath unviolated, may it be granted to me to enjoy life and the practice of the art, respected by all men, in all times! But should I trespass and violate this Oath, may the reverse be my lot!

- Submission date for PhD study at Medical school of Athens 1/10/2008, protocol number 1021 .

- The advisor comitee for the PhD, was nominated on 16/10/2008, protocol number 1732-24/10/2008.

- Submission of PhD protocol, signed by the three member commitee 3/02/2009, protocol number 4937-03/02/2009.

- Nomination of the seven members advisory commitee: 22/04/2013.

- The PhD approval from the Medical School of Athens, does not mean aggrement of the Medical School, with the content of the PhD.

law.5343/1932 article 202

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I offer my regards to all those who supported me in any respect during the completion of this project.

For all the above and the years that we spent together in order to finish this study, I would like to share a known to the Greeks from the ancient era phrase:

"Εάν μη έλπηται ανέλπιστον, ουκ εξευρήσει. Ηράκλειτος" (544-484 π.Χ.)

"If someone does not hope the unexpected, he will not discover it. Heraclitus" (544-484 B.C.)

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01/10/1992 – 06/12/1999	Degree (ptychio) in Medicine, National and Kapodistrian University of Athens, year 162nd, Greece. Grade: ‘’Very Good’’.
1999.	Licence to practice Medicine, Registration Number: 22561 Hellenic Republic, Prefectural Authority of Athens – Piraeus, Prefecture of Athens, Central Division, Directorate of Health and Public Hygiene, Greece.
18/11/2009	Licence to practice the qualification of Medical Specialty: ‘’Neurosurgery’’, Unified Prefectural Authority of Athens – Piraeus, Division of Western Athens, Department of Health Services, Greece.
11/2010 - present	General Medical Council, Full and Specialist Registration as a neurosurgeon,GMC Ref. No: 7089162.

Professional experience

Current post:	Spr equivalent position in Neurosurgery, Major Trauma Centre, St Marys Hospital, Imperial College NHS Trust, London, UK.
Previous posts:	
27/02/2012-03/08/2012.	Spr equivalent position in Neurosurgery, Walton Centre For Neurology and Neurosurgery NHS Foundation Trust, Liverpool, UK.
4/01/2011- 27/02/2012.	Middle Grade Trust Doctor, Queen’s Hospital, Department of Neurosurgery, Romford, Essex, UK. Spr equivalent position.
23/12/2009-23/12/2010.	Consultant in the Department of Neurosurgery at General‘’Panarkadian’’ Hospital of Tripoli, Greece.

15/10/2004 – 17/06/2009.	Specialty Training post in the Department of Neurosurgery at General Hospital of Elefsina ‘’Triassio’’, Magoula, Greece.
05/05/2003 – 05/10/2004.	Compulsory post as a Military Doctor in Hellenic Air Forces, Greece.
12/07/2001 – 16/01/2003.	Specialty Training rotation in the Department of Surgery (one and a half years in Surgery as part of my training in Neurosurgery), at General Panarkadian Hospital of Tripoli, Greece.
31/01/2000 – 11/07/2001.	Compulsory post as a Rural General Practitioner in National Ministry of Health, General Hospital of Kiparissia, Mesinia, Greece.

Languages:

Greek (native).
English (fluently).
French (basic).

Editorials:

During the last 3 years, I have participate in the editorial of two medical books, which were translated the first one from German and the second one from English.

The first one’s title is: Health up to 100, Siegfried Meryn publisher ‘’kritiki’’.

The second one’s title is: Shield your brain against time. Zaldy S. Tan. Publisher ‘’Kritiki’’.

Awards:

Best oral presentation-

Stilidis P., A. Bimpis, K Papatheofani ,I. Ziagkos Theofillogiannakos K, Varotsis F. ’Chronic subdural Haematomas in the elderly and Neurosurgical treatment’’.

Arcadia Medical Association 2002: presented at Panpeloponisian medical congress, 2002.

Current Memberships

Arcadia Medical Association.
Hellenic Pain Society.
Hellenic Neurosurgical Society.
EANS individual membership.

Medical education

Courses:

2006: (BLS), Basic Life Support, Greece. (Thriassio General Hospital, ICU unit) .

2006: (ATLS), Advanced Trauma Life Support, Greece. (University of Athens, ACS State/Provincial Committee on Trauma).

Attendance of seminars/workshops (with diploma):

2003 International symposium of neuromodulation.
2005 European Association of Neurological Societies. Eans Research Course (Methodology of Research and Publication).
2007 23rd Seminar in Microsurgery. Department of Orthopaedics Ioannina, Greece.
2008 European Spine Review and cadaver hands on course. Cleveland Clinic.
2009 32nd week in Microsurgery. Department of Orthopaedics Ionian.
2010 5th international Hydrocephalus Workshop. Crete, Greece.
2011 Cambridge Lectures in Neurosurgical anatomy, PGM Centre, Cambridge.
2012 Neuroanatomy of Operative Approaches (Part II), The Leeds Teaching Hospitals, Leeds.

Teaching experience

2012, I carried out lectures for medical students at the Walton centre of Neurology and Neurosurgery NHS foundation trust at Liverpool.
2001-2009, I have participated in lectures and lessons in several topics such as surgery topics, neurology topics, neurosurgery topics, anatomy topics, during my residency as part of the educational programme.
2007, employed as teacher for nurses specializing in ITU. This employment was a 6 month job provided by the Hellenic ministry of Health.
2001, I carried out lessons to the Hellenic Red Cross during my residency in Surgery.

Publications
International (peer review) journals:

1. Pantazis G, Tsitsopoulos P, Alexios Bimpis, Mihas C, Chatzistamou I, Kouzelis C. ''Symptomatic ossification of the ligamentum flavum at the lumbar spine: a retrospective study. Spine. 2008; 33(3):306-11.

2. P.Tsitsopoulos, O.Kiskira, A. Kolotoura, Alexios Bimpis, E. Anastasiou. ''Effects of Gabapentin on postoperative pain and morphine consumption after lumbar fusion surgery''.
Acta Neurochirg 2007; CCIII,(Suppl 1).

3. G. Pantazis, P. Tsitsopoulos, A. Bibis, P. Papageorgopoulos, C. Kouzelis. ''Ossification of Ligamentum flavum and spondylolisthesis''. A case report.
Acta Neurochirg 2007; CCXXX,(Suppl 1).

4. Alexios Bimpis, G. Dimogerontas, K.Kouzelis, A.Zarros, C.Liapi. Lazaroids: ''A new category of neuroprotective agents''.
Review of clinical Pharmacology and Pharmacokinetics 2008,p.115.

5. Alexios Bimpis, Apostolos Papalois, Stylianos Tsakiris, Apostolos Zarros, Konstantinos Kalafatakis, John Botis, Vasileios Stolakis, Konstantinos M. Zissis, Charis Liapi. Activation of acetylcholinesterase after U-74389G administration in a porcine model of

intracerebral hemorrhage.
Metab Brain Dis. 2012 Jun;27(2): 221-5.

6. Alexios Bimpis, Apostolos Papalois, Stylianos Tsakiris, KonstantinosKalafatakis, Apostolos Zarros, Vasiliki Gkanti, Nikolina Skandali, Hussam Al-Humadi, Constantinos Kouzelis, Charis Liapi.
Modulation of Crucial Adenosinetriphosphatase Activities due to U-74389G Administration in a Porcine Model of intracerebral haemorrhage.
Metabolic Brain Disease 2013.

7. Vasileios Stolakis, Charis Liapi, Apostolos Zarros, Hussam Al-Humadi, Konstantinos Kalafatakis, Alexios Bimpis, Ismene Dontas, Stylianos Tsakiris .
''Gestational Thiamine - Deprivation Alters Crucial Offspring rat Brain Enzyme Activities: The role of Equilibrated - Diet Restoration during lactation''.
(Manucript in preparation).

Hellenic Journals. (abstract in English)

1. Tsitsopoulos P., C. Pantazis G., Bimpis A., Kiskira O., Pankos A., Isaakidis D., Zymaris S.
Spontaneous cerebellar bleeding. Review of 20 cases.
Brain 2006; 43:71- 78, 2006.
2. Tsitsopoulos P., C. Rizos, A. Bimpis, A. Pankos, S. Zymaris.
Diagnosis and conservative treatment of lumbar spondylodiscitis.
Hellenic Neurosurgery 2006; 13 (3): 116-123.
3. Evaluation of the results of anterior cervical discectomy and fusion in the treatment of radiculopathy and myelopathy.
Vamvas Neuroscience news 2006; 8:14-20.

Abstracts in Congress book

1. Stilidis P , Bibis A., Avramopoulos D., Varotsis F.
Non-traumatic aetiologies of brain haemorrhage
World Congress Morocco, 2005.
2. . Stilidis P *., Bibis A. Varotsis F. Neurosurgical approach to post-traumatic rhinorrea. A case report.
World Congress Morocco, 2005.

Abstracts in Hellenic congresses

(Not all included)

1. Non traumatic aetiologies of cerebral hemorrhage.
P. Stylidis, A. Bimpis, D. Avramopoulos, F. Varotsis.
18th Panhellenic Neurosurgical Congress, 2004.
2. Outcome and follow up of myelopathic patients, treated with ACDF.
A. Bimpis, A. Pankos, P. Tsitsopoulos, Isaakidis D., C. Pantazis, C. Rizos, S. Zymaris
19th Panhellenic Neurosurgical Congress, 2005.
3. Spontaneous cerebellar bleeding. Results of 18 cases.
P. Tsitsopoulos, Pankos A., A. Bimpis, D. Isaakidis, P. Papageorgopoulos, M. Christopoulos, S. Zymaris.
19th Panhellenic Neurosurgical Congress, 2005.
4. The new era in treatment of supratentorial ICH after STICH. Literature review.
G. Pantazis, P. Tsitsopoulos, A. Bimpis, V.Katsiva, M.Sartzi, S.

19th Panhellenic Neurosurgical Congress, 2005.

5. Treatment of ethmoid tumours with acute symptomatology of the C.N.S.
   G.Pantazis, A.Bimpis, P. Tsitsopoulos, A.Pankos, D.Isaakidis, St.Zymaris, N. Marangos.
   19th Panhellenic conference of maxillo-facial surgery, 2005.
6. Postoperative lumbar spondylodiscitis. Diagnosis, Treatment, Results.
   A. Bimpis, C. Rizos, A. Pankos, M. Christopoulos, S. Zymaris.
   20th Panhellenic Neurosurgical Congress, 2006.
7. Cervical myelopathy and radiculopathy, results of surgical treatment.
   A. Bimpis, C.Rizos, A. Pankos, D. Isaakidis, S. Zymaris.
   20th PANHELLENIC Neurosurgical Congress, 2006.
8. Diabetus insupidus as aresult of traumatic brain injury of pituitary. Case report.
   A. Bimpis, O. Athanasiou, D. Isaakidis, C. Pantazis, I. Gerasimou, P. Tsitsopoulos, K. Rizos, C. Kouzelis.
   21st Panhellenic Neurosurgical Congress, 2007.
9. Surgical treatment of two rare cases of ossification of posterior longitudinal ligament in lumbar spine in colleration with spinal trauma.
   G. Pantazis, A. Bimpis, P. Tsitsopoulos, N.Stefanoglou, K. Kouzelis.
   21st Panhellenic Neurosurgical Congress, 2007
10. Brain abscesses rationale and treatment.
    A. Bimpis, T. Gerasimou, K. Ilias, B.Economides, C. Rizos, M. Christopoulos, C. Pantazis, P. Papageorgopoulos, F. Pasta, C. Kouzelis.
    22nd Panhellenic Neurosurgical Congress 2008
11. Stereotaxy and HIV. Case report and review of the literature.
    A. Bimpis, Gerasimou Th, K. Ilias, V. Economidis, P. Papageorgopoulos, F. Pasta, K. Kouzelis.
    22nd Panhellenic Neurosurgical Congress ,2008.
12. Pilocytic astrocytoma and intracerebral haemorrhage. Case report and literature review.
    A. Bimpis, T. Gerasimou, K. Ilias, V. Economidis, P. Papageorgopoulos, M. Christopoulos, C. Kouzelis.
    22nd Panhellenic Neurosurgical Congress, 2008.
13. Forestier of the cervical spine.
    G. Pantazis, A.Bimpis, T. Gerasimou, K. Ilias, N. Economides, K. Kouzelis.
    22nd Panhellenic Neurosurgical Congress, 2008.

CONTENTS

PART A: INTRODUCTION

CHAPTER I. Historical aspects of intracerebral haemorrhage, from early definition to first diagnosis and treatments.

CHAPTER II. Definition and epidemiology of intracerebral haemorrhage.

CHAPTER III. Risk factors of intracerebral haemorrhage.

CHAPTER IV. Clinical presentation and diagnosis.

CHAPTER V.Pathophysiology of intracerebral haemorrhage.

CHAPTER VI. Surgery as a treatment of intracerebral haemorrhage.

CHAPTER VII. Current management of intracerebral haemorrhage.

CHAPTER VIII. Prognosis of intracerebral haemorrhage.

CHAPTER IX. Preclinical models of intracerebral haemorrhage.

CHAPTER X. Neuroprotection in intracerebral haemorrhage.

The importance of antioxidants.

CHAPTER XI. Neuroprotective antioxidant efficacy of lazaroids.

PART B: MAIN PART OF THE THESIS

Protocol, results.

LIST OF TABLES

LIST OF PICTURES
Picture 1: One of the most important books in the history of cerebrovascular disease.
Picture 2: Cerebellar Haemorrhage
Picture 3: Putaminal haemorrhage with intraventricular expansion.
Picture 4: Putaminal haemorrhage.
Picture 5: Thalamic haemorrhage.
Picture 6: Putaminal haemorrhage with ischaemic surrounding oedema (delayed Ct head scan).
Picture 7: Parieto-occipital haemorrhage.
Picture 8: Parietal haemorrhage.
Picture 9: Massive lobar haemorrhage, in patient on Warfarin treatment and uncontrolled INR.
Picture 10: Intracerebral haematoma with obvious the distraction of the blood between planes of white matter as shown on plain CT of the head.
Picture 11: Pathological changes in vessels in ICH cases.
Picture 12: CT scan revealing cerebellar haemorrhage
Picture 13: Computed tomography revealing putaminal haemorrhage
Picture 14: MRI showing cavernoma.
Picture 15: MRI showing haemorrhagic transformation of previous stroke.
Picture 16: Ct head with unusual site of bleed.
Picture 17: DSA of the same patient, showing AVM.
Picture 18: Shows the basic set-up in order to do the incision and the induction of haematoma.
Picture 19: Shows the approximately 10cm sagittal incision above bregma, in the midline.
Picture 20: Shows the craniectomy in order to remove the whole brain after the haematoma induction.
Picture 21: Shows the dura after the removal of the bone flap, with the brain underneath.
Picture 22: Shows the cut of the dura edges bilaterally.
Picture 23: Shows the ligation of the sagittal sinus.
Picture 24: Shows coronal section of the brain that was preserved in formalin after removal.
Picture 25: Shows, another deep seated basal ganglia haematoma in coronal section.
Picture 26: Shows the final result of the technique.

LIST OF FIGURES

Abbreviations

AchE: acetylocholinesterase
Apo (A): Apolipoprotein A
Apo (E): Apolipoprotein E
Apo (H): Apolipoprotein H
ATP: adenosine triphosphate
ATPase: enzyme of adenosine triphosphate
AVM: Arteriovenous Malformation
BB-1101: MMP inhibitor
BB-94: MMP inhibitor
BBB: blood Brain Barrier
BGT: Basal Ganglia Territory
Ca^{++} ATPase: Calcium ATPase
CBF: Cerebral Blood Flow
CCL 5: Chemokine (C-C motif) ligand 5
CCT: Cerebral Cortex Territory
C-Fn: glycoprotein of extracellular matrices implicated in the adhesion of platelets to fibrin
CINCH trial: Cooling in INtraCerebral Haemorrhage
CNS: Central Nervous System
CPB 2: carboxypeptidase B2
CPP: Cerebral Perfusion Pressure
CR2: complement receptor 2
CSF: CerebroSpinal Fluid
CT: Computed Tomography
CTA: Computed Tomography, Angiography
CVA: Cerebro Vascular Accident
C3: complement
C1080T: Apolipoprotein H polymorphism
CD 14: Cluster of differentiation 14
C-857T: nucleotide polymorphism of TNF-α
C-863A: nucleotide polymorphism of TNF-α
DD Genotype: Angiotensin Converting Enzyme gene genotype.
DSA: Diffuse Substractial Angiography
DVT:Deep Venous Thrombosis
E2: Apolipoprotein E allele(genotype)
E4: Apolipoprotein E allele(genotype)
ECG: ElectroCardioGraphy
EEG:ElectroEngephaloGraphy
ERLIN 1: ER lipid raft associated 1
FACTOR V Leiden: is the name of a specific gene mutation that results in thrombophilia
FBN 1: Fibrillin 1
F2 isoprostane: marker of oxidative stress
GCS: Glasgow Coma Scale
G-CSF: granulocytecolony stimulating factor
GFAP: glial fibrillary acidic protein

GI691A: Factor V Leiden mutation
GM 6001: MMP inhibitor
GPX-1: Glutathione peroxidase 1
G1025C : Apolipoprotein H polymorphism
G-308A: nucleotide polymorphism of TNF-α
G341A: Apolipoprotein H polymorphism
G817T: Apolipoprotein H polymorphism
HO-1: Haeme oxygenase 1
HO-2: Haeme oxygenase 2
ICH : intracerebral haemorrhage
ICP: Intracranial Pressure
Il-1β: Interleukin 1β
Il-6: Interleukin 6
INR: International Normalized Ratio (for Prothrombin Time)
LIPC: lipase hepatic
LLC-PK1 cell layers: . Lewis Lung Carcinoma–Porcine Kidney
MAST: Multicentre Acute Stroke Trial
Mg^{++} ATPase: Magnesium ATPase
MIP-2: macrophage inflammatory protein
MMP: Matrix Metalloproteinase
MRA: Magnetic Resonance Angiography
MRI: Magnetic Resional Imaging
MTHFR gene: responsible for the enzyme methyltetrahydrofolate
NADPH: Nicotinamide adenine dinucleotide phosphate
Na^{+},K^{+} -ATPase: Sodium-Potassium ATPase
NF-κB: Nuclear Factor
NINDS: National Institute of Neurological Disorders and Stroke
NMDA: N-Methyl-D-aspartic acid
Nrf-2: nuclear factor erythroid-2
NXY-059: agent with free radical scavenger
OAC: Oral anticogulation
PAR (1,2,3)
PPAR-γ: Peroxisome proliferator-activated receptor -γ
PAF: platelet activating factors
PET: Positron emission tomography
PECAM-1:Platelet endothelial cell adhesion molecule
Q43P: polymorphism of beta tubulin
ROS: reactive oxygen species
RANNTAS I, II: A randomized trial of tirilazad mesylate in patients with acute stroke
RARs: retinoid acid receptors
Rt-PA: recombinant tissue plasminogen activator
SPECT: Single-photon emission computed tomography
STICH : Surgical Trial In Intracerebral Haemorrhage
SICHPA: Stereotactic treatment of intracerebral hematoma by means of a plasminogen activator
SOD: superoxide dismutase

TACE: TNF-alpha converting enzyme
THP1 cells: (Human acute monocytic leukemia cell line
TIMP: Tissue Inhibitor of Metalloproteinases
TBI: traumatic Brain Injury
TIMI study Group: Thrombolysis In Myocardial Infarction
TIMP-2:Tissue metalloproteinase
TNF-α: tumor necrosis factor-α
tPA: tissue plasminogen activator
TRAPPC9: trafficking protein particle complex 9
Tris-HCL: Tris (hydroxymethyl) aminomethane-HCL
TUDCA: Tauroursodeoxycholic acid
T-1031C: nucleotide polymorphism of TNF-α
UCP 1: Uncoupling protein 1
V279F: Lipoprotein-associated phospholipase A2 gene polymorphism.
Mutation for a phospholipid contained to platelets inactivated by PAF acetylhydrolase
Val 34Leu, Tyr 204phe, Pro 564 Leu: Polymorphisms of factor XIII
VCAM: Vascular cell adhesion protein
VEGF: (vascular Endothelial Growth Factor)
323Ins: polymorphism of Factor VII.

Preface

Intracerebral haemorrhage is a devastating disease which despite the overall improvement of medical care, remains of high mortality and morbidity. Many papers and many researchers currently investigate any aspect of intracerebral haemorrhage. The reasons is that the entity was in the past underestimated, because of the disappointing final outcome, the differences in pathophysiology in comparison to the better and wider explored entities of ischaemic stroke, subarachnoid haemorrhage and traumatic brain injury and the overall lack of clinical and experimental data regarding intracerebral haemorrhage.

Recently only, and especially the last decade, ICH has been started to be investigated as a completely different entity. We decided to propose and carry out an experimental study of ICH because I believe that this pathological entity is of high incidence, high mortality and morbidity, has lacked attention in the past and there are many unanswered questions regarding time course, pathways involved, surgical and non surgical treatment options. Our effort aims to pose questions and answer at the same time aspects of the pathophysiology of ICH and the role of antioxidants if any and in the present study the role of U 74389G. I believe that there is no better way to give answers than to try to pose them. In an entity that even the experimental protocols, models and agents tested are minimal, there is no better answer than to study and search, especially when it is about a devastating disease with high mortality, morbidity and poor treatment options.

Taking into account the different experimental models of ICH, we performed small changes in order to solve some of the methodological limitations, without changing the established models by previous researchers. Technical and funding limitations were the reason that we did not use stereotactic devices but the use of hard in construction swan-ganz catheter finally produced valid results of deep seated around basal ganglia territory haematomas. I, finally chose the porcine model in order to better study the pathophysiology and better simulate the physiology to the humans.

The aim of the study was to reveal aspects of the pathophysiology of ICH that have not described in the past such as the role of Na^+,K^+- Atpase, Mg^{2+}Atpase and acetylcholinesterase opening the discussion of the cholinergic system changes in the pathophysiology of ICH, given also the prolonged experience of the laboratory with these enzymes. We also aimed to observe the changes of inflammatory mediators such as TNF-α and IL-1, which are known to be involved in ICH, in order the results regarding the role of the antioxidant agent to be comparable. The use of the specific agent U-74389G, was based on the belief that the research regarding 21-aminosteroids should not stop after the disappointing results of the clinical trials of tirilazad mesylate especially taking into account the limitations of these trials. Unfortunately, limitations

regarding pathology evaluation was the reason for limiting the pathology results only to the inflammatory mediators described above in the time limit of this study.

The experimental model that was used, follows the established models widely used in the past as extensively being explained in the relevant chapter. We also believe that the use of the lazaroid U-74389G in a model of ICH, opens the discussion of its potential therapeutic role in ICH and maybe other pathologies of the brain that could benefit by an antioxidant, taking on the other hand into account the limitations of the role of antioxidant treatment in ICH as they are also described in the relevant chapter.

I believe that the results as described in part B, despite of being significant, are also important, revealing new aspects of the ICH pathophysiology especially regarding the unexplored field of the changes in cholinergic system and also the potential neuroprotective and antioxidant role of U-74398G in ICH. I hope that this effort might open the discussion and give new ideas regarding ICH basic research to other researchers also.

At last but not at least, I apologise for any limitations in the use of English language, which is obvious that is not my native one, throughout the text.

PART A
INTRODUCTION

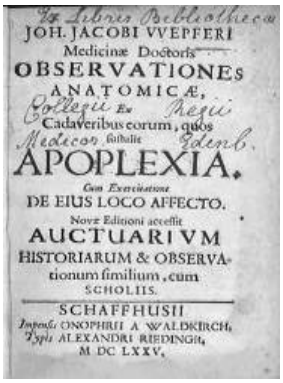
Chapter I. Historical aspects of ICH. From early definition to first diagnosis and treatments.

I. Early descriptions-definitions.

The earliest reports of stroke and familiar stroke date back to the 2nd millennium BC, in ancient Mesopotamia and Persia, (Ashrafian, 2010). Hippocrates (460-370 BC) was the first who described the phenomenon of sudden paralysis, often associated with ischemia. He was the first to describe 'apoplexy' (Thompson, 1996). Later Avikenas (980-1037AC), was the second in the history to describe the entity in his writings. Many centuries later the word 'stroke' was for the first time used to describe the apoplectic seizure (1599) and was included in the Barnhart concise dictionary of etymology and terminology (Barnhart,1995). From that period of time, the word 'stroke', used to describe the apoplexy, started to have more precise meaning and the pathophysiology started to be explored, defined and specifically determined.

II. Defining the anatomical and physiological base of cerebrovascular disease for the first time.

It was in 1658, when Johann Jakob Wepfer (1620 – 1695), a Swiss pathologist and pharmacologist, described for first time that the effects of stroke could be the final result of bleeding in the brain or the occlusion of one of the supplying arteries of the brain. He described the vascular anatomy of the brain and also cerebrovascular disease as a result of the vascular anatomy and physiology changes, for the first time. He conducted many postmortem studies and collected significant data regarding the carotid and vertebral artery pathology, as a cause of ischaemic and haemorrhagic stroke. His findings were finally documented in the 'Historiae apoplecticorum', published in 1658.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Typis ALEXANDRI RIEDINGII,
M. DC. LXXV.

Picture 1. One of the most important books in the history of cerebrovascular disease. From Paciaroni et al.,(2008).

It is without doubt that he was the physician who for the first time described the pathology of cerebrovascular disease and also gave data for the role of the vascular system in the brain vascular anatomy and physiology of cerebral blood supply in health and disease. Following his effort, the previous entities of apoplexy, stroke and ischemia, started to have for the first time what we call 'pathophysiological determination' (Barnhart, 1995).

Decades later, Friedrich Hoffmann (1660-1742) a German physician and chemist, tried to explain the functions of the body, relying on the Cartesian hydrodynamic schemes. His hypothesis helped to expand the way of thinking about the brain supply, circulation and function, giving new and very important data regarding the brain physiology. At the same period of time, Giovanni Battista Morgagni (1682-1771), was the first to describe the difference between intraventricular haemorrhage (IVH) and ICH.

III. First operations and techniques.

It took more than a century for the first operation as treatment of intracerebral haemorrhage. In 1888, Sir William Macewen, CB, FRS, (1848–1924), a Scottish surgeon, pioneer of the modern brain surgery,did the first operation for the removal of an intracerebral clot.

Many years later, Harvey Williams Cushing, (1869-1939), (pioneer neurosurgeon; considered the father of modern neurosurgery), was the one who did the first successful operation of removal of intracerebral haematoma in 1903.

At the same approximately period, Percival Sylvester Bailey (1892-1973), American neuropathologist, neurosurgeon and psychiatrist ,described treatment options related to the position of haematoma. The famous pioneer neurosurgeon Wilder Penfield (1891–1976), in his study: "The operative treatment of spontaneous intracerebral haemorrhage" in 1933, described the available data regarding the surgical treatment of ICH and suggested haematoma drainage with craniotomy and cortical incision.

Three decades later in 1932, G. W. Robinson**,** in his study "Encapsulated brain haemorrhages", described the first series of ICH and their overall outcome. This was the first publication of a series of intracerebral haematoma cases focusing on the outcome. At the same approximately period, (1936),W.M. Craig and A. W. Adson, described cases of ICH related to Charcot-Bouchard aneurysms for the first time.

IV. The angiography era.

After 1950, the introduction of classical angiography, played a major role in the diagnosis and treatment of ICH. Most important papers date back in 1953 and 1958. In 1953, Lofgren Fo, publishes the role of carotid angiography in the diagnosis of intracerebral haemorrhage, while in 1958**,** S. Briani and Ore G. Dall , publishes the angiographic aspects of intracerebral haematomas for the first time.

Not quite later, in 1961, W. Mc Kissock et al, were the first who referred to the similar outcome in conservative and surgical treatment of ICH; a study that entered the treatment options of ICH in a new era, opening a discussion regarding the best treatment that currently continues. Many decades after this study, the uncertainty continuous regarding the treatment options, especially the role of surgery in ICH.

V. The computed tomography (CT) and magnetic resonance imaging era (MRI).

The new era (until present) after the discovery of the CT scan and later on of MRI was evolutionary, with better understanding of the physical course of ICH. There is now, better understanding regarding the onset, the expansion, the clot formation, the ischaemic oedema and the clot lyses. CT and MRI findings triggered the discussion of new therapeutic options for the treatment of ICH, conservative or surgical, and gave birth to the idea that we could schedule and establish more precise criteria for more appropriate and effective treatments. On the other hand, there is still no universal agreement regarding the best treatment options and not much have been done regarding the overall mortality and morbidity.

CHAPTER II. Definition and epidemiology of intracerebral haemmorhage.

I. Definition.

With the term ICH, we describe many different conditions with known underlying causes or not. We can categorize the underlying causes into small and large arterial vessel disease, venous disease, vascular malformation, haemostatic disorders, and ICH in the presence of other conditions and diseases. Under the term ICH, we also include the entity of 'spontaneous intracerebral haemorrhage'. 'Spontaneous' means that no cause has been isolated with the available diagnostic tests, although we assume that there is an underlying cause for the bleed. With the term 'spontaneous', we also include the cases that the diagnostic tests didn't reveal the cause but also, there is no suspicion about a concept for a cause (idiopathic). A proposal for a detailed ICH classification by causes is currently prepared in the frame of the new ICH guidelines from the European Stroke Organization (Steiner et al., 2011).

II. Epidemiology.

ICH, accounts for approximately 10% to 15% of all cases of stroke and is associated with high rates of morbidity and mortality worldwide, higher mortality and morbidity rates in comparison to ischaemic stroke. Recent data, estimates that the incidence of ICH is twice the incidence of subarachnoid haemmorhage (Broderick et al., 1993a).

According to the World Health Organization (2010), approximately 15.3 million stroke incidents occur every year worldwide, 2-3 million of them being haemorrhagic. Recent studies have focused on the cost of stroke which for the European Union has been estimated at 27 billion euros/annum, of which 8.5 billion euros are indirect costs (Weimar et al., 2003; Di Carlo, 2009).

The additional cost of ICH is estimated to be 30,000–45,000 euros/survivor every year (Weimar et al., 2003). According to these studies (Weimar et al., 2003; Di Carlo 2009) , during the last decade, spontaneous ICH accounted for approximately 10% of all strokes in high-income countries and about 20% of all strokes in low/middle-income countries. One-month case fatalities were found to be 25–35 and 30–48%, respectively (Feigin et al., 2009).

It is also mentionable that not only the incidence rates are different between low to medium and medium to high income countries, but that the trends between those countries are different (increased rates in the medium to low and decreased rates in the medium to high income countries) (Feigin et al., 2009).

Similar publications regarding the epidemiology of cerebrovascular disease showed that mortality also varies widely in Europe,with central Europe having lower rates than Southeastern and Mediterranean countries. The one-month case fatality after ICH does not appear to have changed over the last few decades (Broderick et al., 1993a; Van Asch et al., 2010; Manno, 2012), with the volume of haematoma and the Glasgow Coma Scale, being predictors of the thirty day mortality (Broderick et al., 1993b).

Regarding the differences between race in the occurrence and fatality of ICH, published data suggests that the incidence of ICH is higher in asians (Van Asch et al., 2010) and blacks (Qureshi et al., 1995). Available data shows that the major risk factors for ICH include male gender, increasing age, arterial hypertension, excessive alcohol consumption, smoking, diabetes mellitus, poor diet and obesity calculated as waist-to-hip ratio, ' street drugs, liver dysfunction, previous cerebro vascular accident (CVA), (Ariesen et al., 2003; O'Donnell et al., 2010).

However, over the past decades, the incidence of ICH associated with prestroke hypertension appears to have declined, whereas ICH associated with use of antithrombotic drugs and presumed cerebral amyloid angiopathy in those aged between 6 to 75 years old, seems to have increased (Lovelock et al., 2007). According to the most recent data from the United States, ICH accounts for 15% of all types of stroke (Rincon et al., 2012). This study describes the epidemiological aspects of ICH in the period between 1978-2008. Interestingly enough, the admission rate in Unites States was 24.000 cases per annum, 12.9 per 100.000 per year in the first period between 1978 and 1983, while in the period between 1984 to 1988 the admission rate was 40,600 cases (17.0 per 100,000 persons per year). Since 1988, the annual admission rate after ICH remained stable with approximately 63.000 cases (21 per 100,000 persons per year) and remained unchanged until the end of their study (2008). From the same study, the in hospital mortality rate, for the first period (1979-1983) was 45%, decreased to 34% over the second period (1984-1988) and remained unchanged since then. (Rincon and Mayer, 2012).

The finding that the mortality rate remains the same over the last years, is generally accepted by many authors and studies (Steiner et al., 2011; Rincon and Mayer, 2012; Manno et al., 2012).

In the study of Rincon and Mayer (2012), in terms of ethnic data, non- whites experienced higher incidence in comparison to whites, data that is relevant to other works revealing that black and asians, have higher incidence rate of ICH in comparison to whites (Qureshi et al., 1995a**;** Klatsky et al., 2005; Smeeton et al., 2007; Pathak et al., 2009; Van Asch et al., 2010).

The incidence of ICH, was found to be higher in women than men in all age subgroups (Rincon and Mayer, 2012). Groups with higher in-hospital mortality were whites, women and persons older than 65 years. Higher incidence was also observed to black women younger than 45 years, and middle-aged black men. Other authors have demonstrated that the difference in

incidence between black and other ethnic groups is mainly because of hypertension which is higher especially to young blacks (Smeeton et al., 2007).

Other authors have demonstrated that the incidence of ICH increases by age. A recent study by Stein et al., (2012), estimates that by 2050, patients suffering of ICH with age more than 80 years of age, will be 2.5-fold higher in comparison to the data available in 2009. The total number of ICH cases is estimated to increase approximately 35.2% with the assumption that the ICH probability remains the same. In the same study, the estimation of in hospital mortality, reaches an increase of approximately 60.2%. The total number of patients with severe disability due to ICH will increase approximately 36.8%. The widespread use of anticoagulation and antithrombolytics raises the incidence of ICH (Stein et al., 2012).

Most of the cases of ICH are considered to be due to hypertensive atherosclerosis and the recently explored entity of amyloid angiopathy (Mayer et al., 2005; Sutherland and Auer, 2006).

Secondary ICH accounts for 15–20% of patients and usually results from vascular malformation, arteriopathies (other than amyloid angiopathy, lipohyalinosis, cerebral arteritis), neoplasia, coagulation and clotting disorders (iatrogenic, leukemia, thrombotic thrombocytopenic purpura, aplastic anaemia) ,central neurvous system infections (more frequently fungal, granulomas and herpes simplex), drugs (alcohol, cocaine, amphetamine), sympathomemetics, ephedra alkaloids. Other causes are eclampsia and preeclampsia with risk of 1per 9500 births and those of acutely increased Cerebral Blood Flow, such as carotid endarterectomy, after previous CerebroVascular Accident, or after exposure to cold. (Mayer et al., 2005; Sutherland and Auer, 2006).

Epidemiology of locations of intracerebral haemorrhage.

50% approximately to basal ganglia, with most common being the putamen, lenticular nucleus, internal capsule, globus palidus

15% thalamus

10-15% to the pons

10% cerebellum

10-20% cerebral white matter

1-6% brain stem

Source: Schmidek and Sweet, (1982).

Overall, deep hemispheric ICH (deep ICH) is a result of rupture of small arterioles most commonly in the putamen or thalamus, observation that was already reported as early as 1978 (Mohr et al., 1978; Wiggins et al., 1978; Furlan et al., 1979; Kase et al., 1982; Kunitz et al.,

1984), while, lobar ICH, accounts for 33–42% of all ICH according to Massaro et al. (2002) and Woo et al. (2002).

The cases of cerebral amyloid angiopathy are considered to result from the rupture of small and medium-sized arteries in the subcortical white matter; an observation that was recently done by O'Donnell et al.(2000).

CHAPTER III. Risk factors of intracerebral haemorrhage.

The major risk factors for ICH include male gender, increasing age, arterial hypertension, excessive alcohol consumption, smoking, diabetes mellitus, poor diet and obesity (O'Donnell et al., 2010; Ariesen et al., 2003) street drugs, liver dysfunction, previous CVA. Table 1, summarizes the most important risk factors and aetiologies of intracerebral haemorrhage.

Table 1. Risk factors of intracerebral haemorrhage.

Age, increases significantly by the age of 55, relative risk 7 after 70 years old
Gender, more common in men
Race affects blacks and asians more than whites
Previous Cerebrovascular Accident increases the risk by 23:1
Alcohol consumption
Cigarette smoking, Does Not increase the risk of ICH
Drugs such as cocaine, amphetamine, phencyclidine, phenylpropanolamine, pseudoephedrine, ephedra alkaloids, warfarin, aspirin
Liver dysfunction
Hypertension, acute and chronic
Associated with increased Cerebral Blood Flow, in areas previously ischaemic. Following carotid endarterectomy, haemorrhagic transformation, migraine, after Arterio Venous Malformation (AVM) surgery, physical factors.
Vascular anomalies. AVM, aneurysm rupture, Venous angioma rupture.
Arteriopathies, amyloid angiopathy, fibrinoid necrosis, lipohyalinosis, cerebral arteritis
Brain tumors
Coagulation or clotting disorders. Anticoangulation therapy, thrombolytic therapy, aspirin, leukemia, thrombocytopenia
Central Nervous System (CNS) infection, fungal infections, granulomas, herpes simple engephalitis
Venus or dural sinus thrombosis
Post traumatic
Pregnancy related. Preeclampsia, eclampsia
Post-operative. Following carotid endarterectomy, following craniotomy
Amyloid angiopathy
Microaneurysms of Charcot-Bouchard
Genetic Factors

I. Hypertension.

The role of hypertension in ICH, was recognized very early. First publication in PubMed, dates back in 1946 (Dunn et al., 1946), describing spontaneous ICH in a patient with essential hypertension. Many publications followed the following years and the first population based study regarding the role of hypertension was that of Takahashi et al. (1957). Aurell and Hoob. (1964), in their population based study in Sweden, reported that the decrease of incidence in ICH between 1964 and 1976 was related to the better control of hypertension in the general population. Similar findings were reported in Rochester by Furlan et al.(1979) and Garraway et al.(1979; 1983). Following these studies, many authors from different countries reported similar findings, in different populations, such as Ueda et al. (1981), regarding Japan.

These figures correlated with a decrease in the prevalence and severity of hypertension in this population. Many years later, the role of antihypertensive treatment in reduction of ICH incidence was recorded in the PROGRESS trial (Hasegawa et al., 2004). According to Woo et al. (2002), hypertension is the most important factor for deep hemispheric ICH. Other authors have observed higher blood pressure on admission (Ojemann and Heros, 1983).

II. Genetic factors.

Through the literature, many genes are investigated or currently being under investigation regarding their role to the ICH. In the past, of main interest were especially those genes responsible for the renin-angiotensin-aldosterone system and the coagulation system, also genes related to apolipoproteins inflammation pathways and those of homocysteine metabolism.

More precisely, amongst the most widely investigated genes are those involved in the renin-angiotensin-aldosterone system (such as angiotensin-converting enzyme), the coagulation pathway (such as factor XIII, factor VII, platelet-activating factor acetylhydrolase, factor V Leiden, and beta 1-tubulin), lipid metabolism (such as apolipoproteins, ApoE, Apoa, ApoH), homocysteine metabolism (such as methylenetetrahydrofolate reductase), inflammation pathways such as interleukin-6 (IL-6) and tumour necrosis-alpha (TNF-α) and other candidate pathways.

Analytically,

i. Regarding the renin-angiotensin-aldosterone system, the most studied is the angiotensin converting gene (Slowik,2004). The results have been inconclusive because although there are studies that support the relation with DD genotype in Indian and Polish population (Peck et al., 2008; Kalita et al., 2011) other studies suggested that there is no definite relation, in

Chinese (Wei et al., 2000), Greek populations (Dardiotis et al., 2011), or patients from Leeds (Catto et al., 1996).

Blood coagulation genes, coagulation involves both a cellular (platelet) and protein (coagulation factor) component. Deficiency or dysfunction of any coagulation factor can lead to bleeding disease. Until now many genes are investigated regarding their role in ICH, such as factor XIII, VII, platelet activating factor acetylydrolase and other platelet-activating factors.

Studies regarding factor XIII, focus especially on the deficiency of the proenzyme factor XIII, condition associated with high rate of intracranial haemorrhage. Many studies have focused on the Val34Leu polymorphism of factor XIII gene, and its importance but the results were overall conflicting (Catto et al., 1998; Corral et al., 2000; Gemmati et al., 2001; Cho et al., 2002; Endler et al., 2003; Slowik et al., 2005). Other polymorphisms have also been studied that showed higher incidence of ICH especially in white women. These are the Tyr204Phe and Pro564Leu, polymorphisms of Factor XIII (Reiner et al., 2001).

Factor VII, it's role in various bleeding disorders.

Congenital deficiency of this factor is associated with increased rate of spontaneous bleeding in these candidates. A study regarding patients carrying the 323Ins allele of factor VII showed 1.54-fold risk for ICH (Corral et al., 2001). Other studies did not confirm this finding (Greisenegger et al., 2007).

Platelet-activating factor acetylhydrolase

Is a phospholipid contained to the platelets granues that is being inactivated by PAF acetylhydrolase. The responsible mutation is V279F. Deficiency of this enzyme, found to be a generic risk factor for stroke (Yoshida, 1998). Further studies were carried out regarding haemostatic variants, such as the factor V GI691A Leiden mutation, but showed no significant difference, while the beta-tubulin Q43P polymorphism increased the ICH risk in men and was associated with an earlier ICH occurrence (Reiner et al., 2000; Iniesta et al., 2003).

ii. The lipid metabolism-related genes as a risk factor .

Many studies have demonstrated the role of apoE as a genetic factor of ICH. As known apoE, plays major role in lipid transport and metabolism. Data supports that ApoE e2 and e4 alleles, are risk factors for cerebral amyloid angiopathy (Premkumar et al., 1996; Greenberg et al., 1996). On the other hand, conflicting results have been produced regarding the predisposition to ICH. Other studies have focused in the past to the association between the e4 allele and ICH rate, but the metaanalysis of all these studies finally confirmed that the e2 and not the e4 genotype was associated with ICH. Interesting there was a stronger association with deep than lobar

haemorrhages and the risk was higher in Asians than in Europeans (Sudlow et al., 2006; Seifert et al., 2006; Tzourio et al., 2008).

Recently apoH, has been implicated in lipid metabolism, haemostasis and anitphospholipid antibody production. There are four polymorphisms of ApoH currently recognised, G341A, G817T, G1025C, and C1080T. Amongst them, only frequencies of the A allele of G341A found significantly higher in ICH patients than in controls in a Chinese population based study (Xia et al., 2004). Apo (a), a glycoprotein that comprises lipoprotein (a), has also been associated with haemorrhagic stroke, in a Chinese study (Sun et al., 2003).

iii. Homocysteine metabolism-related genes.

Elevated homocysteine levels have been associated with ischemic and haemorrhagic stroke. The results of the relevant studies are conflicting. So, in a Turkish, Caucasian population, elevated plasma levels of homocysteine were associated with increased risk of ICH and collated with two polymorphisms in the MTHFR gene which found to reduce plasma activity of the enzyme methyltetrahydrofolate, responsible for remethylation of homocysteine to methionine and finally elevate the plasma levels of homocysteine (Sazci et al., 2006). On the other hand this coloration is not proven to be parent in Chinese populations or in India (Li et al., 2003; Somarajan et al., 2011). Overall the results could be considered conflicting regarding the role of homocysteine metabolism related genes as a genetic factor of ICH stroke.

Inflammation-related genes.

Interleukin-6 is an inflammatory cytokine that may play a role in ICH. Until now there is a large clinical study available with the participation of 3151 Japanese individuals that showed that there is a significant relation between the G572>C polymorphism of IL-6 and ICH (Yamada et al., 2006). However, the G 174>C polymorphism showed no association with respect to ICH (Strand et al., 2007).

Tumour necrosis factor-alpha (TNF-α), is a proinflammatory cytokine that plays an important role in regulating inflammation. Until now the only available clinical study comes from Taiwanese population and shows that there is an association between spontaneous deep ICH and the four single nucleotide polymorphisms (T1031C, C863A, C857T, and G308A) that are gender dependent (Yamada et al., 2006; Strand et al., 2007) .

iv. Other pathways being currently under investigation.

Many pathways and genes are found or being found to be relative with ICH. Amongst them, more important are, those of extracellular matrix degradation (Reuter et al., 2009), oestrogen

receptor signalling (Schuit et al., 2005), and antioxidant systems (Travis and Salvesen, 1983). Most important data currently is that, regarding the tissue metalloproteinase (TIMP-2), found to be associated with increased risk of ICH in Caucasians (Reuter et al., 2009). Glutathione peroxidase 1 (GPX1), is also found to be associated with entire ICH but not non-lobar ICH (Travis and Salvesen, 1983). The important role of GPX1 in the antioxidant system is well established.

Other pathways involved and investigated, are those of oestrogen receptor alpha, participating in vasodilatation and atherogenesis, The osteoprotegerin 1181C/C, that of the C > T polymorphism (rs1324694) of ERLIN1, C>T polymorphism (rs12679196) of TRAPPC9, and G>T polymorphism (rs16936752) of WNK2 found to be associated with ICH prevalence, in IL-6, TNF, CD14, FBN1, PECAM1, UCP1, CPB2, LIPC, and CCL5, were also related to ICH (Strand et al., 2009; Pera et al., 2008; Lim et al., 2011; Alberts et al., 1997).

v. Genetic polymorphisms and recurrence of intracerebral haemorrhage.

Genetic variants found also to play a significant role in ICH recurrence. From many studies regarding the pattern of ICH recurrence in Asia populations, the most common pattern found to be that of ganglionic-ganglionic (Bae et al., 1999; Inagawa , 2005; Misra et al., 2011) whereas most recurrences in the European population, are those of lobar-lobar type (Hanger et al., 2007; Bailey et al., 2001). Until now there is association established between recurrence of lobar ICH and ApoE ε2/ε4 genotype (O'Donnell et al., 2000). The available data, regarding the genetics of ICH, was recently reviewed by Liu et al. (2012).

III. Cerebral amyloid angiopathy, other angiopathies, dementia related intracerebral haemorrhage.

Recently recognized cause, difficult to establish the diagnosis. Pathologic deposition of beta amyloid protein in the wall of small meningeal and cortical vessels, especially found in the white matter without evidence of systemic amyloidosis. Fibrinoid necrosis of the vessels wall may be present (Gilles et al., 1984; Mandybur, 1986; Vonsattel et al., 1991).

Other angiopathies related to ICH are those of fibrinoid necrosis (Rosenblum, 1977; Ojemann and Heros, 1983), lipohyalinosis (Fisher, 1971) and cerebral arteritis.

IV. Dementia.

Dementia has generally been considered a major risk factor for Cerebral Amyloid Angiopathy (CAA)-related lobar ICH because of the close molecular relationship between CAA and Alzheimer's disease. The most important study that is carried out so far, is that of Ellis et al. (1996), that investigated 117 brains with Alzheimer's disease, and revealed common CAA disease in 25.6% of specimens and CAA-related hemorrhages in 5.1% of the cases. Although there is a definite high percentage of CAA in patients with dementia, on the other hand several studies have demonstrated that 60-80% of the patients diagnosed with CAA-related ICH don't have dementia symptoms before the ICH recurrence. (Mandybur, 1986; Vinters, 1987; Greenberg et al., 1996).

The increased incidence of ICH in Alzheimer disease seems to be related to apoE, e4, despite the differences regarding the role of apoE in the two disorders.

V. Other risk factors related to intracerebral haemorrhage occurrence.

Many other risk factors regarding ICH, extensively investigated in the past, such as those of cigarette smoking, alcohol consumption and serum cholesterol levels. In a systematic review of the risk factors of ICH in the general population (Ariesen et al., 2003), four risks were identified as independent regarding the occurrence of ICH, male sex, age, hypertension and alcohol intake, while smoking and diabetes mellitus found to be weak risk factors.

i. Smoking as a risk factor.

On the other hand opposite results were recorded in other population based studies. For example, in Japanese ancestry Hawaiian men, a 2.5-fold increased risk of subarachnoid and ICH, was found in smokers (Abbott et al., 1986). Data from the Physicians health study and the Women's health study, showed significant association between cigarette smoking and ICH risk (Kurth et al., 2003a, b). In this study, smoking of more than 20 cigarettes per day was found to be an independent risk factor for ICH, with relative risk found to be 2.06 for men and 2.67 for women smoking more than 15 cigarettes per day. The overall data suggests that cigarette smoking has not been proved to be definite risk factor for ICH.

ii. Alcohol as a risk factor.

Regarding alcohol consumption, (Ariesen et al., 2003) found an increased risk of ICH in patients consuming alcohol. Overall a 2.05 OR was found (95% CL, 1.35 to 3.11) for moderate

intake and a risk of 4.11 (95% CL, 2.54 to 6.65) for high intake. Other studies demonstrated the dose dependent alcohol consumption. Juvela et al.(1995), for example, showed the dose dependent effect of alcohol 24 hrs and 1 week prior to ICH, while Woo et al.(2002), found that frequent alcohol consumption was independently associated with lobar rather than deep ICH. The overall data suggests that alcohol consumption is an independent risk factor for ICH.

iii. Serum cholesterol levels as a risk factor.

Regarding the association between serum cholesterol levels and ICH, low serum cholesterol levels has been found to be associated with ICH in several population based studies in the past, by many authors, such as Tanaka et al. (1982), Iso et al. (1989), Yano et al. (1989), Gatchev et al. (1993), Lindenstrom et al. (1994), Giroud et al. (1991); Iribarren et al. (1996).

In a recent study of Ariesen et al. (2003), the overall OR for high cholesterol, was found to be 1.22 (95% CL, 0.56 to 2.67), while in the study of Tanaka et al. (1982), cholesterol levels less than 160 mg/dl, were associated with higher risk of haemorrhage in Japanese and Hawaiian population. On the other hand, other studies such as these of Giroud et al. (1991), Kubota et al. (1997), Thrift et al. (1998) and Zodpey et al. (2000), showed conflicting results. Except the relevance of serum cholesterol levels and ICH incidence, studies have been carried out also regarding the coloration between ICH, serum cholesterol levels and anatomical site of haemorrhage. In the study of Giroud et al. (1991), low levels of serum cholesterol were associated with lobar and deep ICH in a similar manner, while on the other hand, low serum cholesterol levels, in the study of Segal et al. (1999), were associated mainly with deep ICH rather than lobar. The overall results are conflicting.

Further to the above, many other causes are recognized as risks factors for ICH. These are: haematologic abnormalities, congenital and acquired factors deficiency disorders, thrombocytopathic disorders and lymphoproliferative disorders (Hart et al., 1995).

iv. Haematologic abnormalities and intracerebral haemorrhage.

According to several studies, the haematologic abnormalities account for approximately 8% of all ICH cases (Bamford et al., 1988; Gebel et al., 2002). Non- iatrogenic causes are those of leukemia, thrombotic thrombocytopenic purpura and aplastic anaemia. It is proved that most common coangulopathies are iatrogenic and there is an increased prevalence by the years because of the widespread use of anticoagulants.

v. Warfarin- related intracerebral haemorrhage.

Very early data revealed the increased risk of ICH in individuals using warfarin. As early as 1959, reports suggested that the incidence of ICH in warfarin users is even higher to those that are hypertensive; this data was further confirmed by later studies (Barron and Fergusson, 1959; Wintzen et al., 1984; Kase et al., 1985; Dawson et al., 1993; Hylek and Singer, 1994; Smith et al., 2002).

The annual risk to those using warfarin, is estimated to be 0.3-1.7% according to Hart et al. (1995). The available data,not only reveals that anticoagulation therapy and especially warfarin, is associated with 6 to 11 fold increase in the relative risk of ICH (Furlan et al., 1979; Wintzen et al., 1984; Albers et al., 1991; Atrial Fibrillation Investigators, 1994; Hart et al., 1995), but also that patients on warfarin, have double risk of ICH (Radberg et al., 1991; Hart et al., 1995; Rosand et al., 2004; Cervera et al., 2012). We need to mention here that recent studies shows that the annual risk seems to be lower that 1.3%, the reason is that the initial data was based on older studies as mentioned above. This data when analyzed regarding the subgroup above 80 years old, showed that the annual risk was elevated only in this subgroup of patients and found to be as high as 1.8% (Fihn et al., 1993; Kawamata et al., 1995). So according to Blackshear et al. (1996), the annual risk is calculated from 0% to 0.3% per year.

Other studies suggest that age plays an important role in intracerebral haemorrhage in patients on anticoagulation. Age more than 70 y.old, and patients that combine warfarin and aspirin, found to have increased risk of ICH (Landefeld and Goldman, 1989; Hart and Pearce, 1993; Hylek and Singer, 1994; Hart et al., 1995). On the other hand, Hart et al. (1999), observed that aspirin when added to oral vitamin K antagonists may increase the risk of ICH with differences between different populations, suggesting that the result needs further confirmation. Regarding the characters of ICH in patients on anticoagulation and the differences in comparison to other individuals, it seems that the main differences are those of slow progression of haemorrhage that prolongs up to 48-72 hours, and the mortality in comparison, seems to be higher (Radberg et al., 1991; Hart et al., 1995; Rosand et al., 2004).

vi. Heparin-related intracerebral haemorrhage.

The risk of ICH on heparin is well examined. It seems that heparin is associated with high risk of ICH in cases of acute treatment of ischaemic stroke, while on the other hand, in all other uses of heparin, there is evidence that there is an increased risk of ICH; data on this issue are available as early as 1972 (Drapkin and Merskey, 1972; Handley et al., 1972).

vii. Fibrinolytic agents and risk of intracerebral haemorrhage.

Regarding fibrinolytic agents, there is enough data available to support that they are associated with increased risk of ICH. Rt-PA, commonly used in acute coronary syndromes, found to have 0.4-1.3% risk of ICH (TIMI Study Group, 1989). ICH in the use of rt-PA, occurs early, within the administration of the drug, or within the first 24 hrs after the induction to therapy, according to Gore et al. (1991). Usually these haemorrhages are multifocal, lobar, and are characterized with high mortality rate (Kase et al., 1990), the exact mechanism is not known, and probably underlying small vessels pathology is present. The National Institute of Neurological Diseases and Stroke (NINDS), rt-PA Stroke Study (NINDS and Stroke rt-PA Stroke Study Group, 1995; 2005), determined that there is a 6.4% risk of haemorhage the first 36 hours after rt-pa administration.

The results of several studies and trials regarding streptokinase returned even higher incidence of ICH than those of rt-pa. (Multicentre Acute Stroke Trial Italy [MAST-I] Group, 1995; Multicenter Acute Stroke Trial Europe Study Group, 1996).

viii. Antiplatelet drugs and intracerebral haemorrhage.

Antiplatelet drugs and especially aspirin have shown also conflicting results in several trials and studies, regarding their role in ICH. The risk of ICH, is as high as 1 per 10000 per year, according to He et al. (1998) and the Antithrombotic Trialists' Collaboration. (2002).

On the other hand, other studies have demonstrated that there is no increased risk in patients after ICH that are on aspirin (Viswanathan et al., 2006), and that there is not higher risk to patients on aspirin after ischaemic stroke. This data comes from both the Canadian Cooperative Study Group. (1978), the American Canadian Co-Operative Study Group. (1985) the UK-TIA Study Group. (1988) and the European Stroke Prevention Study (ESPS Group, 1987; Diener et al., 1996).

Other medicines that are found to be responsible for ICH occurrence are amphetamines, cocaine and phenylpropanolamine. Cocaine seems to be responsible because of the picks of high blood pressure and not the vasculitis or vasculopathy which is a rare phaenomenon (Levine et al., 1990; Peterson et al., 1991; Aggarwal et al., 1996; Nolte et al., 1996).

ix. Rare causes of intracerebral haemorrhage.

Other causes of ICH, are those of structural lesions such as arteriovenous malformations, aneurysms and cavernomas, rarely tumors, all of these causes together, represent a small amount of ICH cases amongst the whole. The estimated percentage regarding the vascular malformations are approximately 4-5% of the whole cases of ICH, according to Broderick et al.

(1992) and Furlan et al. (1979). In cases with lobar haemorrhage, structural lesions are responsible for the bleed in as much as 65% of cases with lobar haemorrhage according to Zhu et al. (1997). There is also increased risk of ICH after ischaemic stroke, as reported in several studies with relative risks between 5 and 22-fold (Okada et al., 1976; Brott et al., 1986; Woo et al., 2002). Woo et al. (2002), in their multivariable analysis, showed that there is increased risk of bleeding after ischaemic stroke, which is 13-fold increased for deep ICH and 4.1-fold risk of lobar ICH.

Associated with elevated CBF.

Following carotid endarterectomy, ICH cases have been reported (Russell and Gough, 2004; Bernstein et al., 1984). Rare cases have been reported after migraine attacks (Cole and Aube, 1987; Raade and Krug, 1999). After extreme physical stretch and exposure to cold also has been described.

Haemorrhagic brain tumors.

Usually glioblastoma, lymphoma, metastatic (melanoma, choriocarcinoma, bronchogenic carcinoma), rarely other tumors such as medulloblastoma, gliomas and benign tumours (Greenberg, 7th edition, 2010).

CNS infections.

Very rare cause especially the fungal infections, granulomas and herpes simple encephalitis.

Pregnancy related.

The incidence is estimated 1 per 9,500 births (Wang et al., 1999). Usually associated with eclampsia and preeclampsia and the mortality is approximated to 6% (Salerni, 1988). Postpartum ICH has also been described (Witlin, 2000).

Other.

Venous sinus thrombosis, post traumatic, post operative, idiopathic. These are very rare cases of ICH.

CHAPTER IV. Clinical presentation and diagnosis.

I. Clinical presentation of intracerebral haemorrhage.

The clinical presentation of ICH differs that that of ischaemic strokes, although there may be similar presentation in some cases. The neurological deficit is gradual in onset and progressive over the time from minutes to hours. Severe headache, signs of raised intracranial pressure are usually present, while hypertension is also common especially at onset. TIA like symptoms can be prodrome signs in ICH, especially in the lobar pattern of ICH.

The neurological findings may be characteristic regarding the anatomical site involved and the extension of haematoma. It is not the aim of this study to extensively describe the neurological manifestation; thus we will only describe general aspects emphasizing common and usual neurological findings of ICH presentation and development of clinical picture.

i. Putaminal haemorrhage.

Is the most common site of ICH. Presents with contralateral hemiparesis that can progress to hemiplegia and coma or death. Gradual deterioration in 62% of patients.

ii. Thalamic haemorrhage.

Contralateral hemisensory loss is characteristic, hemiparesis if internal capsule is involved. If extension to the upper midbrain, further signs are those of nystagmus, vertical gauze palsy, skew deviation, ptosis, miosis, anisocoria. Hydrocephalus if CSF pathways are being obstructed. High mortality and morbidity rate.

iii. Cerebellar haemorrhage.

Various signs and symptoms, usually those of raised intracranial pressure, usually because of the underlying hydrocephalus. If the brain stem is involved, facial palsy can be observed and coma is usually the result.

iv. Lobar haemorrhage.

The signs and symptoms depend on the lobe involved. In frontal lobe haematoma, usually frontal headache, hemiparesis with predominance to the arm and mild leg and facial involvement, are the most dominant symptoms. In parietal lobe haematoma, mild contralateral hemiparesis and hemisensory deficit. In occipital lobe, contralateral homonymous hemianopia, eye pain ipsilateral and in temporal lobe ICH, fluent dysphasia maybe the presentation, poor auditory comprehension, good repetition (Ropper and Davis, 1980).

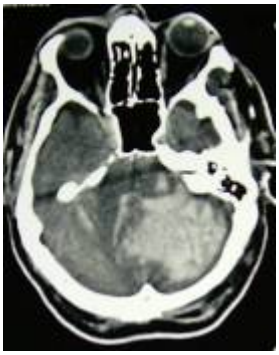
Delayed deterioration is usually observed in cases of ICH. It is usually a combination of rebleeding, oedema, hydrocephalus and seizures. Many studies focused on the early rebleeding. Data after the extensive use of CT, revealed that early rebleeding is more common in cases of basal ganglia haemorrhage, in comparison to lobar haemorrhage. Haematoma enlargement usually occurs the first 24 hrs after the onset and especially the first hours. The first 3 hrs from onset, the possibility of rebleeding is approximately 33-38% (Brott et al., 1997), dropping to 16% afterwards for the rest of the 24 hrs and 14% next day (Fuji et al., 1998). The overall outcome in cases with haematoma expansion was worst (Fuji et al., 1998).

Rebleeding can also occur later as delayed, with a rate of 1.8 up to 5.3% (Arakawa et al., 1998). Oedema and ischaemic necrosis around the haematoma site, causes deterioration; the present mass effect cannot usually explain the oedema formation. Many studies support that the cause of the excessive oedema could be due to thrombin or other toxins that is finally responsible for the delayed oedema formation and clinical deterioration (Ojemann et al., 1983). The haematoma after the final formation, follows the time course of oedema, ischaemia absorption and ,finally, resolution. This is a time course that could last for weeks.

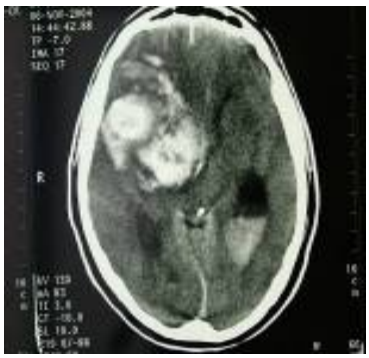
II. Diagnosis general terms.

In the CT era, diagnosis and follow-up is managed with plain CT head scan, where the presence of haematoma can be determined, the volume can be calculated and in cases of clinical deterioration the cause can be identified with a new CT head scan. In cases with suspicion of underlying pathology, an MRI and MRA brain is performed and if vascular abnormality is suspected, a Computed tomography-angiography (CTA), or classical angiography (DSA) is the appropriate examination.

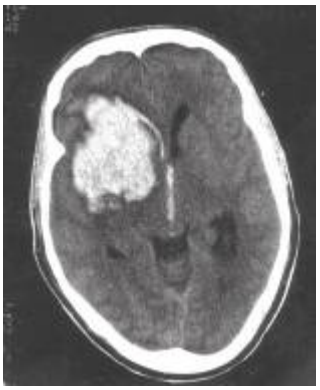
CT scan remains the golden standard for diagnosis and follow-up of the phaenomenon, is easy to use and gives very important data regarding the characters of the bleed, rebleeding in the case of clinical deterioration and simple follow-up of the resolution of haematoma. The pictures that follow are examples of ICH.



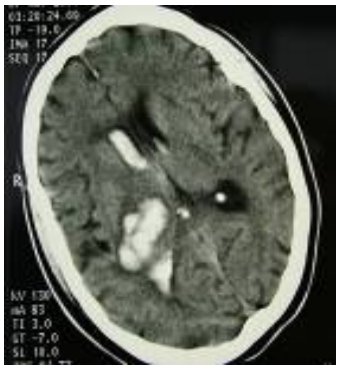
Picture 2. Cerebellar haemorrhage, personal collection.



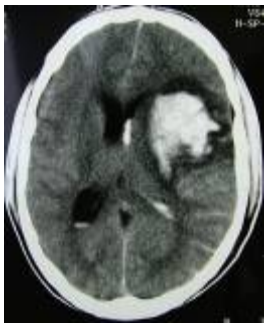
Picture 3. Putaminal haemorrhage with intraventricular expansion,personal collection.



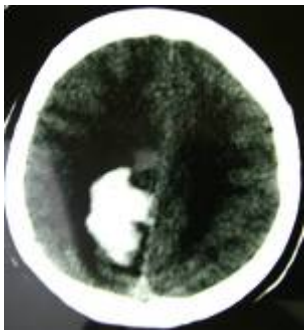
Picture 4. Putaminal haemorrhage, personal collection.



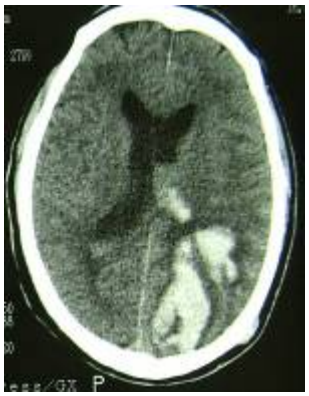
Picture 5. Thalamic haemorrhage, personal collection.



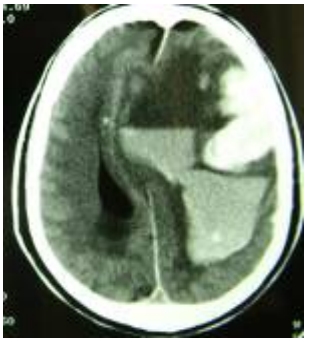
Picture 6. Putaminal haemorrhage with ischaemic surrounding oedema (delayed Ct head scan), personal collection.



Picture 7. Parieto-occipital haemorrhage, personal collection.



Picture 8. Parietal haemorrhage, personal collection.



Picture 9. Massive lobar haemorrhage, in patient on warfarin and uncontrolled INR, personal collection.

CHAPTER V. Pathophysiology of intracerebral haemorrhage.Time course of events, combined clinical and experimental data'.

I. The primary injury.

Intracerebral haematomas are usually the result of the rupture of small penetrating arteries that originate from the main intracranial vessels. Common arterial feeders of deep seated ICH are the lenticulostriates, thalamoperforators and paramedian branches of the basilar artery, while in lobar haemorrhages, more likely a structural abnormality is present. Usually the walls of these small vessels are degenerated of the media and smooth muscle and especially at their bifurcations (Cole and Yates, 1967).

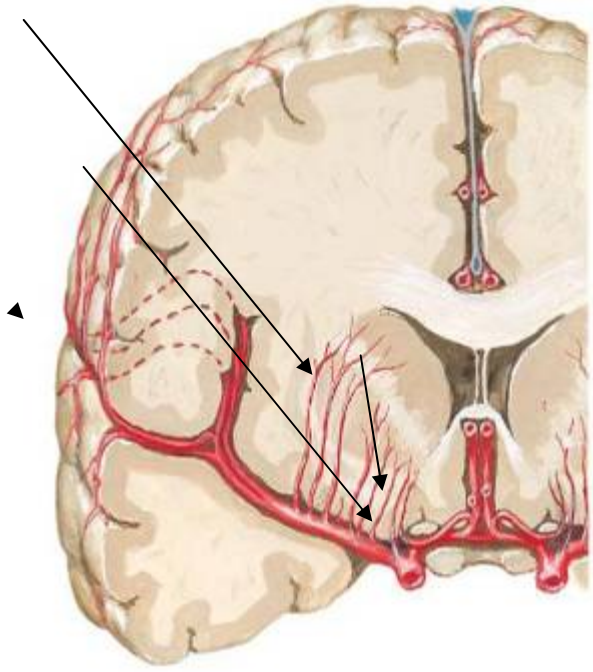


Figure 1: lenticulostriate arteries (arrows), that are usually the cause of ICH. Plate from Netter Neuroanatomy.

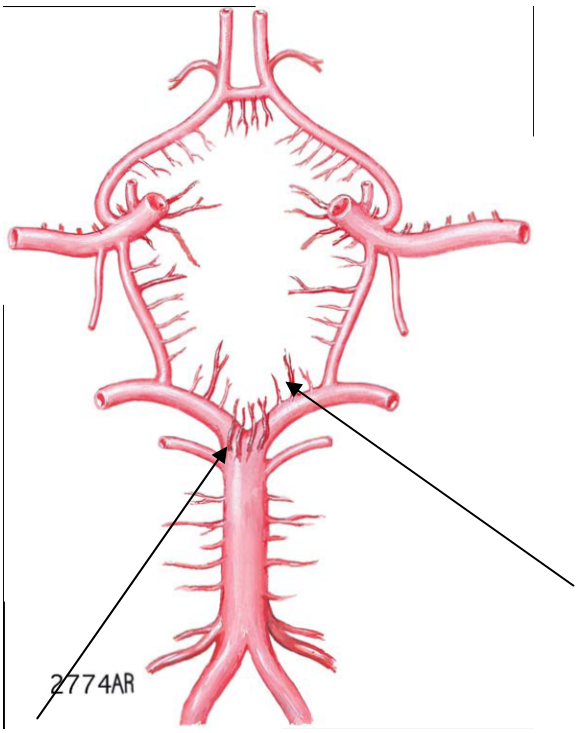
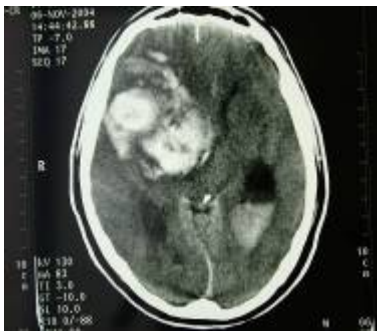
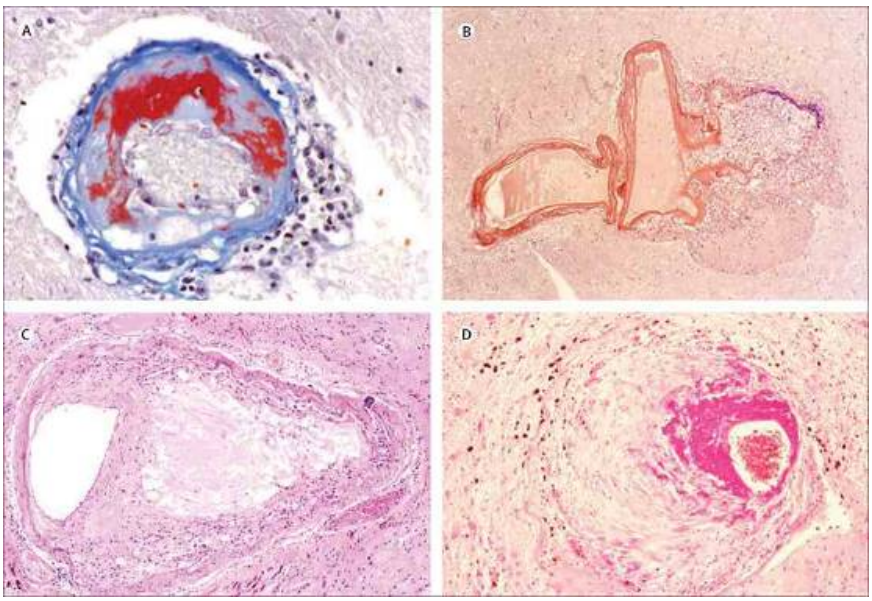


Figure 2: Thalamoperforators (right arrow) and branches of basilar artery (left arrow) that usually bleed in ICH. Plate from Netter Neuroanatomy.

After the primary event of bleeding, the blood spreads usually slowly between planes of white matter, causing at the beginning minor destruction. So the pattern of expansion at the early stages, more likely follows paths of less obstruction, leaving in between areas of unaffected brain tissue around the haematoma site (Mutlu et al., 1963). This is easily recognized in the CT head scan.



Picture 10. Intracerebral haematoma with obvious the distraction of the blood between planes of white matter as shown on plain CT of the head.



Picture 11. Pathological changes in vessels in ICH cases. Pathological features of small vessel disease (A) Lipoyalinosis (basal ganglia ×100). (B) Microaneurysm in the right thalamus in a 70-year-old hypertensive patient who died 42 days after developing a massive ICH in the left thalamus. Fibrinoid necrosis of the aneurysmal wall is almost ready to rupture. Phosphotungstic acid haematoxylin stain. Original magnification ×25. (C) Microatheroma (basal ganglia ×20). (D) Fibrinoid necrosis (pons ×20). Source: Pantoni L.; Lancet Neurology. (2010).

Nowadays, it is clear that the haematoma is not a phaenomenon that is the same from the beginning. The haematoma expands over time and develops over the first hours because of many possible causes such as progressive bleeding from the site of onset, disruption of the nearby vessels, hypertension and the presence of coagulation deficit (Olson 1993; Kazui et al., 1997; Kazui et al., 1996; Leira et al., 2004). This early phaenomenon is called primary injury in ICH. In the past mass effect because of haematoma itself was consider being the main mechanism of the pathophysiology of ICH while nowadays it is clear that of major importance is what we describe as ‘secondary injury’. That means the reactions and subsequent effects that follow the primary incident of haematoma accumulation and raise the intracranial pressure as well as the cellular response, most important part of which is the inflammation process.

II. The secondary injury.

i. Introduction.

Last decade, many researchers focused on the importance of inflammation and secondary injury after ICH revealing many new aspects for the pathophysiology of the phaenomenon.

Inflammatory markers such as Tumour Necrosis Factor-α, interleukin 6 (IL-6), were found to determine the haematoma expansion (Silva et al., 2005; Xi et al., 1998; Lee et al., 1996). There is evidence to support that Matrix Metallo Proteinases (MMPs) also play a significant role in the expansion of haematomas playing significant role in Blood Brain Barrier (BBB) disruption (Forsyth and Levinsky, 1990; Hamann et al., 1996; Lee et al., 1997; Aoki et al., 2002; Horstmann et al., 2003).

It is established that after the bleeding, significant amounts of thrombin appear at the haematoma site which is subsequently responsible for triggering several reactions, trigger chemotaxis of leukocytes, expression of adhesion molecules, cytokines release, BBB disruption, and local MMPs disruption (Silva et al., 2005).

The role of iron in this process is also very important, it is found to affect the BBB function, more likely through damage to the endothelial wall with the mediation of free scavengers. Most of the data suggests that all these mechanisms play a crucial role in the development of oedema formation after ICH but their excact role in early oedema formation remains undetermined. Results of previous studies, revealed that the above mentioned mediators are overexpressed in cases with early haematoma growth, but the mechanisms are still unclear. So in these cases there is evidence of higher number of leukocytes, fibrinogen, IL-6, TNF-α, in the serum of patients with ICH and especially to those that suffer early haematoma growth.

Both MMP-9 and c-Fn (glycoprotein of extracellular matrices implicated in the adhesion of platelets to fibrin, known as c-Fn) concentrations in serum, are found to be significant higher in patients with early haematoma growth, with c-Fn being the most powerful predictor of haematoma expansion. C-Fn is present mainly to the endothelium, so these findings suggest that high levels in these patients could be due to endothelial damage. This observation, permits to conclude that in cases with early haematoma growth, the lamina components such as c-Fn, laminin, and collagen IV, may be impaired. Similarly, MMPs' activation may cause loss of microvascular integrity in the tissue around the haematoma. Additionally to the above mechanisms, c-Fn is also important in mediating the adhesion of platelets to fibrin. Both the above observations could be an explanation of the phaenomenon.

However, the synthesis of c-Fn may be triggered during inflammatory processes by agents such as transforming growth factors and leukocytes, limiting the findings of these studies by general limitations. Overall, the above mechanisms are the more analytical description of the causes of haematoma expansion up to date, suggesting that c-Fn and MMPs are highly significant predictors of haematoma expansion (Forsyth et al., 1990; Hamann et al., 1995; Lee et al., 1997; Aoki et al., 2002; Horstmann et al., 2003).

The time course of haematoma expansion generally is over the first 24 hrs and usually the first 3 hrs (Fuji et al., 1994). This is the case for one third of ICH haematomas (Brott et al., 1997; Kazui et al., 1996).

The haematoma itself is responsible for early oedema formation in the surrounding parenchyma. Oedema forms from the release and accumulation of serum proteins that cause expansion in the brain tissue (Wagner et., al 1996; Wagner et al., 1998). This collection of fluid in the surrounding area, results in focal mass effect and intracranial pressure rise (Papo et al., 1979; Janny et al., 1982).

Subsequently, inflammation process starts to take place leading to neurotoxicity and more oedema. Of main importance in the process are the lysed red blood cells and blood products as well as leukocytes, macrophages, and plasma proteins. (Suzuki and Ebina,1980; Jenkins et al., 1990; Lee et al., 1995; Xi et al., 1998). Figure 3, summarizes part of the available data regarding early haematoma expansion.

After the primary injury, inflammatory mediator release, protease activation, microglia and astrocyte activation, brain tissue breakdown, and repairing mechanisms start the inflammatory process (Wang and Tsirka 2005a; Wang and Doré, 2007b). Inflammatory cells include blood-derived leukocytes and macrophages, resident microglia, astrocytes, and mast cells.

The role of microglia is very important. They are the first non neuronal cells that react to brain injury acting neuroprotectively to neuronal survival and overall function under various pathologies (Van Rossum and Hanisch, 2004).

Many researchers have showed that leukocytes/macrophages, activated microglia, astrocytes, and recently mast cells participate in the secondary damage via the release of inflammatory cytokines, chemokines, prostaglandins, proteases, ferrum and similar molecules (Aronowski and Hall, 2005; Zhang et al., 2006; Strbian et al., 2007; Wang and Doré, 2007b; Zhang et al., 2009, Zhang et al., 2010; Lindsberg et al., 2010). Figure 4 summarizes the available data regarding reactions between primary and secondary injury in ICH.

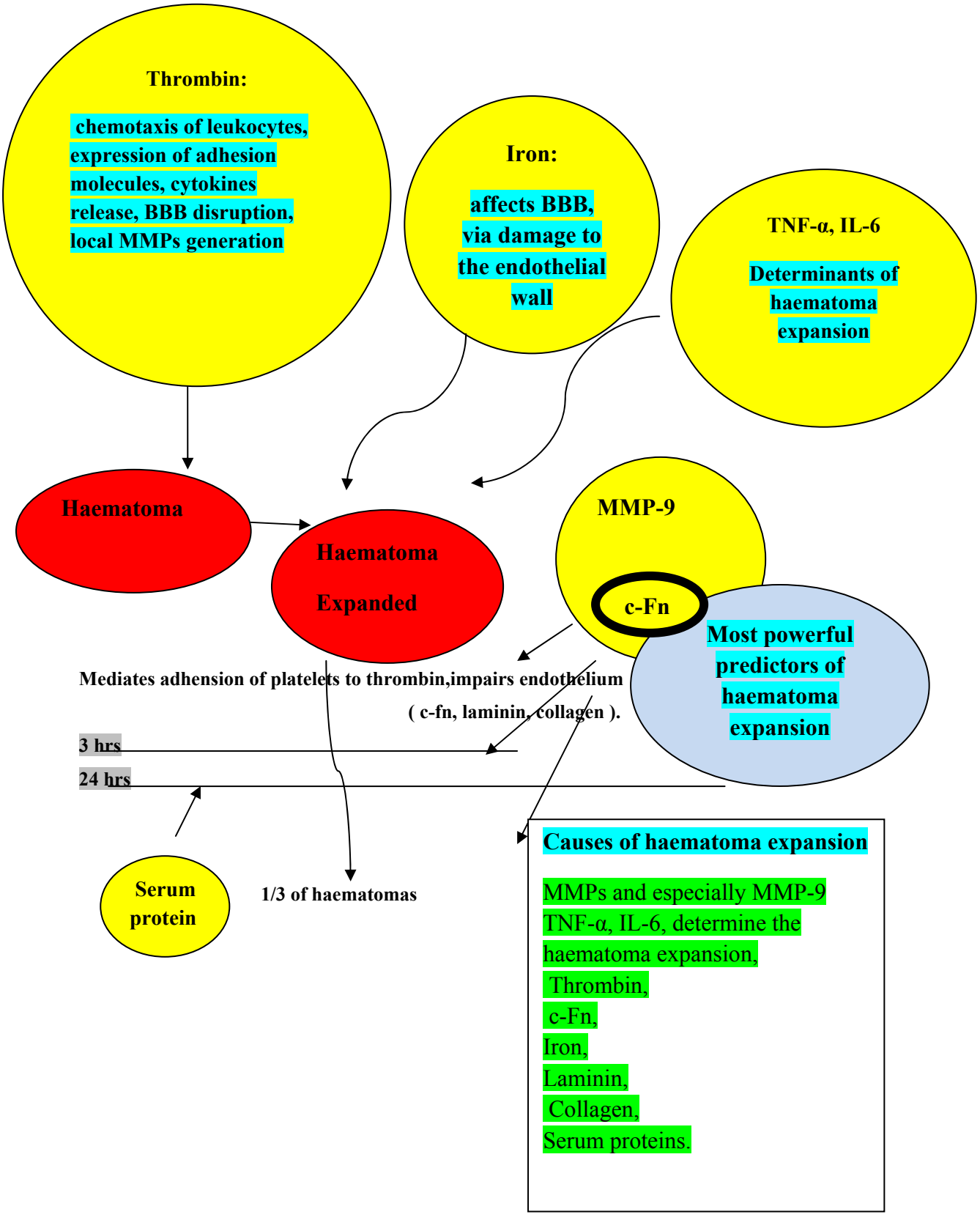


Figure 3. Early haematoma expansion: figure summarises part of the available data as described in the relevant text (the secondary injury in intracerebral haemorrhage).

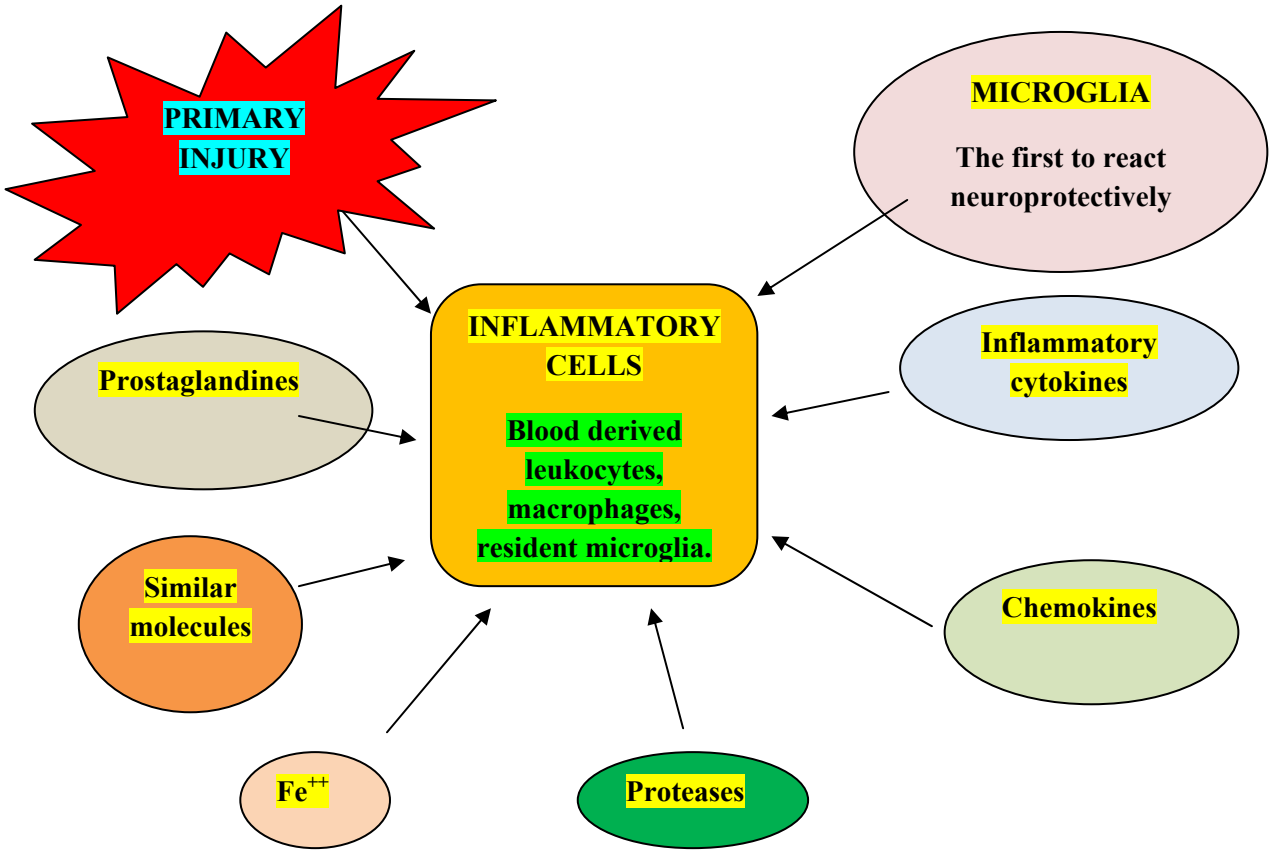


Figure 4. From primary to secondary injury in ICH. The relation between the primary incident and the inflammatory response, recently recognized as very important in the pathophysiology of ICH (simplified).

ii. The role of leukocytes in the secondary brain injury in ICH.

There is histopathological evidence that there is leukocytes infiltration around the haematoma site in cases of ICH (Zhao et al., 2006c; Wang and Doré, 2007a,b). It is shown that neutrophils are the first leukocytes that infiltrate the haemorrhagic brain. The neutrophils act with releasing proinflammatory proteases, reactive oxygen species (ROS) (Nguyen et al., 2009) and changing BBB permeability (Joice et al., 2009). Other studies suggest that neutrophils may kill neuronal cells via excitotoxicity and/or oxygen-glucose deprivation (Dinkel et al., 2004). Their life cycle after entering the haematoma area lasts approximately two days and after that they die (Savill and Haslett, 2001). Their death, damages further the neuronal tissue by stimulating the microglia/macrophages to produce proinflammatory toxic factors. Experimental

data revealed that there is early appearance around the haematoma site as early as 4 hrs (Wang and Doré, 2007a). Other researchers observed that the number of neutrophils peaked at 3 days (Wang and Tsirka, 2005b). More recent data revealed that the β2 integrins (CD11/18) known to be expressed in leukocytes, when not expressed in a CD18-/- mice model, oedema formation in the perihaematomal area is less prominent (Titova et al., 2008). In this study, not only oedema was less but also so was myeloperoxidase and nitrotyrosine immunoreactivity and, finally, the mortality rate itself.

The data that was collected in the laboratory, further confirmed by clinical data collection regarding the role of leukocytes. Many studies revealed the presence of elevated leukocytes in the Cerebro Spinal Fluid (CSF) of patients suffering from ICH. Bestue-Cardiel et al. (1999) described that peripheral leucocyte count was collated with hematoma size. Until now, there are three studies that describe the presence and behavior of leucocytes to the haematoma site in intracerebral haemorrhage.

First description was by Wisniewski, as early as in 1961. He observed the presence of leukocytes in the perihaematomal area between 2^{nd} and 4^{th} day after the onset of ICH. Before this observation, there was evidence of elevated concentration of leukocytes in surrounding blood vessels as early as 6-12 hrs after the onset. In the second study, by MacKenzie and Clayton (1999), there was evidence of leukocyte infiltration in the brain tissue after 5-8 hrs of onset, and disappeared 72 hrs after; that was a postmortem study. A third study examined the inflammation and cellular responses in the area around the haematoma, during craniotomy and revealed that presence of leukocytes appeared at 6-12 hrs from onset, increasing by 12-24 hrs (Guo et al., 2006).

Giaume et al. (2010) argued that the role of leukocytes is important in the pathophysiology of ICH but there is little data about the underlying mechanisms. Based on the clinical data, there is enough evidence to support that peripheral leukocyte count, is an independent factor of early deterioration in ICH. Figure 5, summarizes the available data regarding the role of lymphocytes in ICH.

iii. The role of microglia and macrophages in the secondary brain injury in ICH.

Microglial cells are constantly scavenging the CNS for plaques, damaged neurons, and infectious agents (Gehrmann et al., 1995). Their actions are similar to those of macrophages. They interact with the nearby neurons, astrocytes and blood vessels (Nimmerjahn et al., 2005).

Microglia becomes activated in several pathologies affecting the brain and spinal cord, that means that changes in morphology and function take place in order to play its role as a defender of the nervous system. The changes are mainly body enlargement, proinflammatory proteins

production, cell migration, proliferation and phagocytoxicity (reviewed by Wang and Tsirka, 2005a).

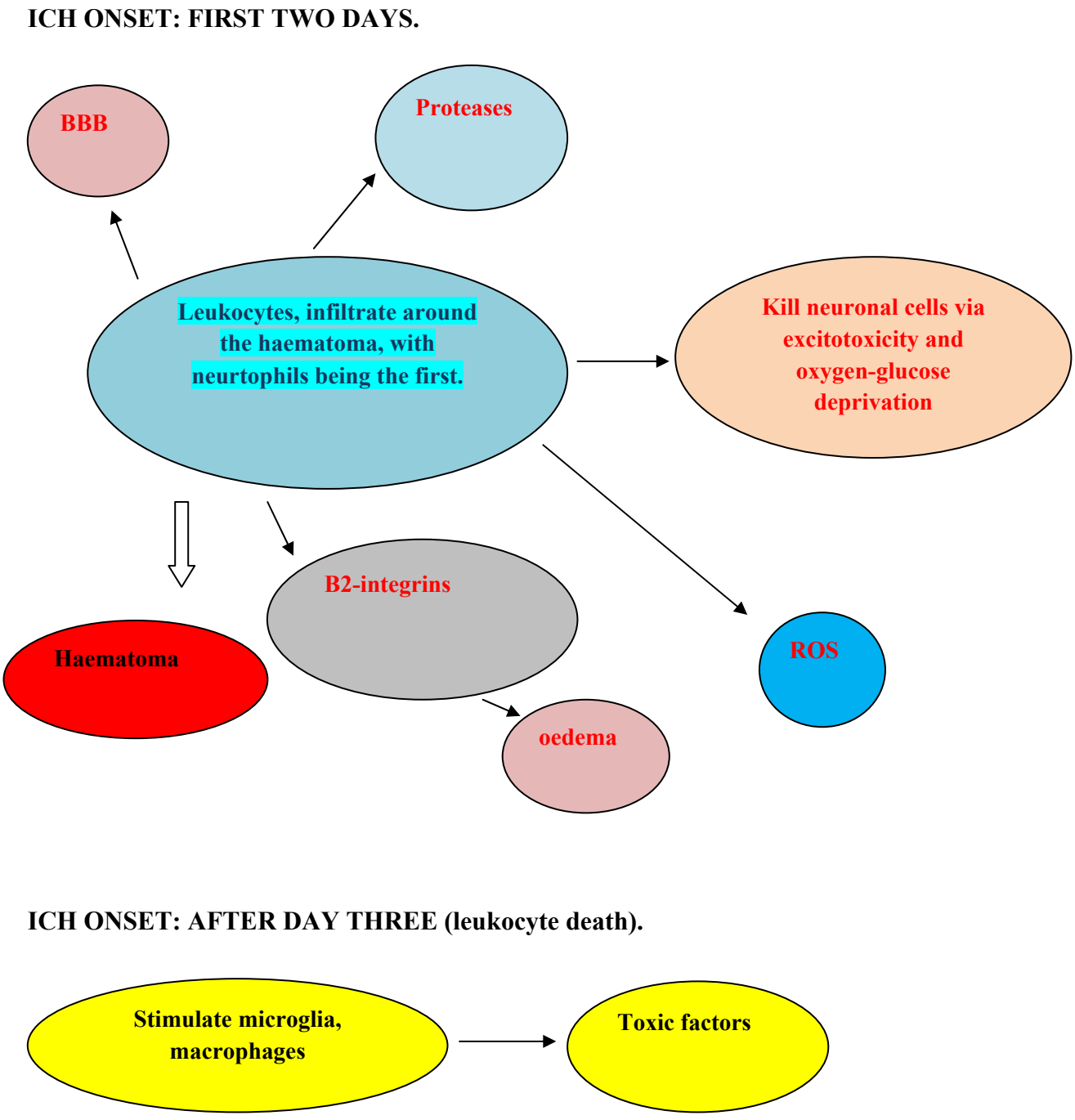


Figure 5. The role of leukocytes in ICH: figure summarizes the available data regarding their role, the first two days and after their death at day three. Leukocytes, appear as early as 4 hrs after the haematoma onset while peripheral leukocyte count is an independent factor of early haematoma expansion.

The activated microglia, depending on the condition, may play a neuroprotective or neurotoxic role in different pathologies of the nervous system (Van Rossum and Hanisch, 2004). Between the brain pathologies that microglia is already studied, it's role in ICH is in some extension explored.

Several authors, have studied and reviewed the role of microglia, either activated or inactivated in ICH. Techniques for the identification of activated microglia are precisely described in many studies in the past (Miao et al., 2005; Wang and Tsirka, 2005c; Fan et al., 2007). Current studies report that microglia and macrophages are being activated early after ICH, triggering secondary brain injury (Wang and Tsirka, 2005a; Wang and Doré, 2007b; Gao et al., 2008). Precise description of the differences between activated and non (resting) microglia have recently been published (Wang and Doré, 2007a; Wang et al., 2008).

So there is enough data regarding the role of microglia, well defined, at least regarding the main aspects. Amongst the many roles of most importance is the defense against the haematoma and neuronal tissue debris. There is evidence regarding the many reactions that take place in microglia, such as the expression and release of several factors or mediators, most well studied of which are cytokines, chemokines, ROS, proteases, cyclooxygenase-2, prostaglandins, haeme oxygenase -1 and its metabolites (Wang and Tsirka, 2005a; Wang and Doré, 2007a,b). The release by activated microglia of all the above mentioned factors, is highly suggestive of a possible toxic role in the surrounding the haematoma tissue.

Other studies, suggest that activated microglia may be responsible, or at least play important role in early brain injury after ICH (Aronowski and Hall, 2005; Keep et al., 2005; Wang and Doré, 2007b).

Wang and Doré (2007b) showed that the microglial activation starts within the first hour of the ICH onset, while leukocytes are observed later at least 4-6 hrs after onset (Wang and Doré, 2007a). Nimmerjahn et al. (2005) showed that focal microglia is immediately activated after BBB disruption. Wang et al. (2003) and Wang and Tsirka. (2005a) have reported that reactive microglia are prominent the first day after ICH, reached maximum on day seven after the onset and are subsequently normalized in three weeks time.

Similar data has also been published by Gong et al. (2000), Xue and Del Bigio. (2000) and Zhao et al. (2007b), after infusion of autologus blood into the rat striatum. The timeline in the last study was elevation 1-4 hrs after ICH, peak between day 3 to 7 and normalizes after three weeks. Recently, Wu et al. (2009a) described a connection between the activation of microglia and Nuclear Factor-kB activation in rats. In this study an increase in MIP-2 (macrophage

inflammatory protein), appears as early as 2 hrs, and peaks at day two. Figure 6, summarizes the role of microglia in ICH.

Conclusion.

The available data suggests that microglia plays a very important role in the process of inflammation after intracerebral haemorrhage. The available data, suggests that microglia, is responsible for expression and release of several inflammatory factors such as cytokines, chemokines, ROS, proteases, cyclooxygenase-2, prostaglandins, HO-1 and its metabolites. Most researchers point out that the main role of microglia is neurotoxic. Many studies have determined also possible time course of microglia activation and the pattern that follows.

Table 2, Summary of the available data regarding the time course of the microglial activation in ICH.

Author(s), Year	**Initial activation of microglia**	**Time of maximal activation and final normalisation**
Wang and Dore. (2007b)	**Activation within the first hour of ICH onset**	
Nimmerjahn et al. (2005)	**After the BBB disruption**	
Wang et al. (2003)	**The first 24hrs after the onset**	**Maximum in a week, normalizes in three weeks**
Wang and Tsirka. (2005a)		**Maximum in a week, normalizes in three weeks**
Gong et al. (2000)	**Similar to the above data**	
Xue and Del Bigio. (2000)	**Similar to the above data**	
Zhao et al. (2007b)	**Elevation 1-4hrs after ICH**	**Peak between day three to seven and normalizes after three weeks**
Wu et al. (2009a)	**Connection between activation of microglia and NF-kB activation in rats**	**An increase in MIP-2, appears as early as 2 hrs, and peaks at day two.**

iv. Available data regarding the role of astrocytes in intracerebral haemorrhage.

As known, astroglia, are characteristic star-shaped glial cells in the nervous system. They are the most common cells of the human brain. They outnumber neurons by tenfold. They perform many functions; most important and well known are the biochemical support of endothelial cells , the participation in the blood brain barrier formation, the nutrition of the nervous tissue, homeostasis of extracellular ion balance, and their role in the repair of the nervous system after damage. They remodel functionally and morphologically depending on the time and type of nervous system injury (Giaume et al., 2010).

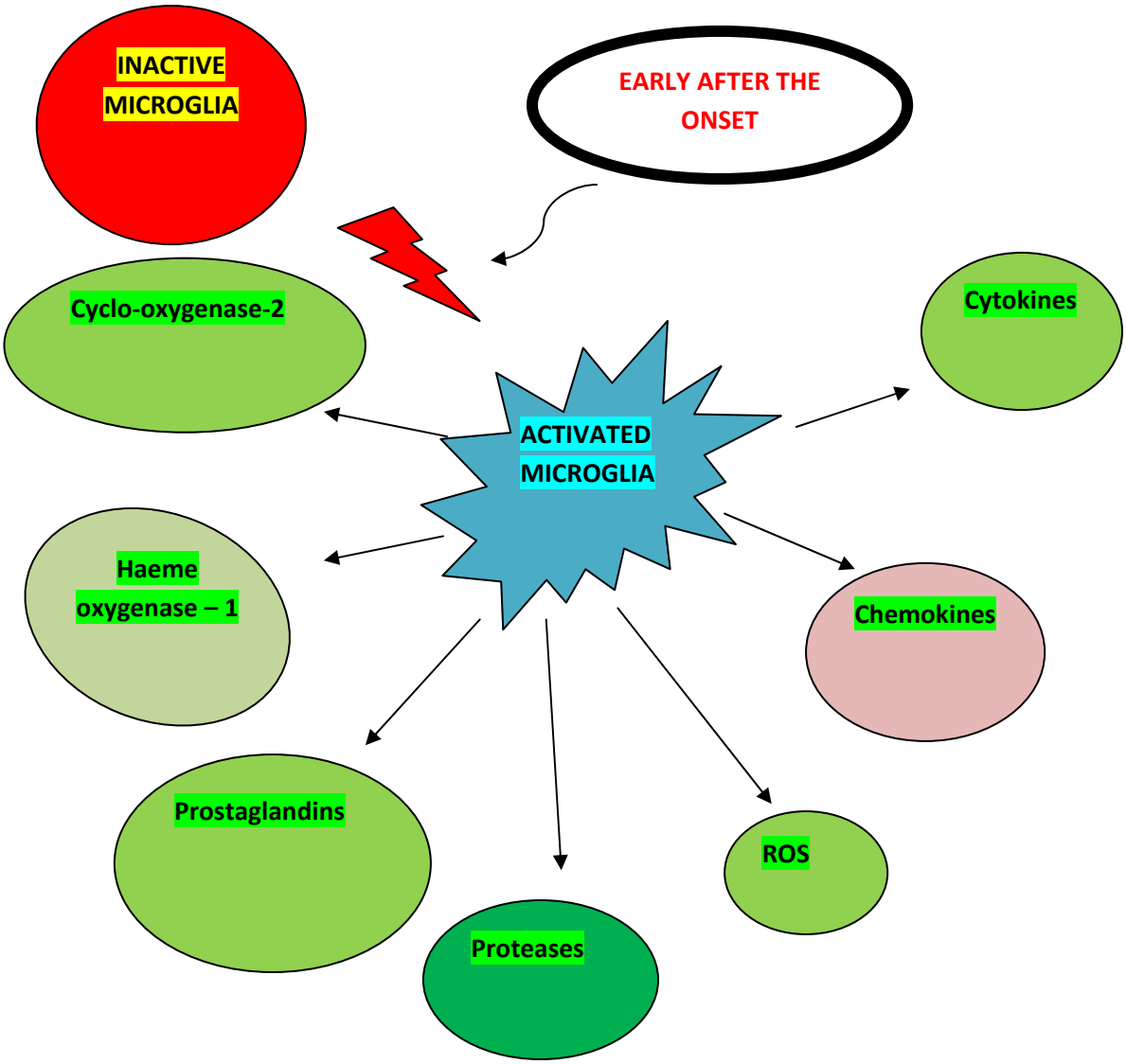


Figure 6. Summary of the role of microglia in ICH, as described in the text, simplified.

It is well established since last century that astrocytes propagate intercellular Ca^{2+} waves over long distances in response to stimulation, and, similar to neurons, release gliotransmitters such as glutamate, ATP, TNF-α, and D-serine, which play role in the modulation of neuronal excitability, synaptic activity, and plasticity even at long distances from the injury site. Any change in their function, in many pathologies of brain injury, affects neuronal functionality and finally viability. Their presence is quite important in many pathological conditions in the central nervous system (Anderson et al., 2003; Miller, 2005; Zhang, 2010).

Despite their well recognized importance in many pathological processes in the central nervous system, and their well defined role in many pathologies, especially to secondary ischaemic brain injury, their role in ICH is not well defined, not well established and the available overall data regarding this pathology is overall limited.

Wang and Dore. (2008), Wang et al., (2008), observed that astrocytes are activated in the perihematomal region early after ICH in a manner of greatest expression around the hematoma area, gradually decreasing when the distance alters. It is established from previous studies that after ICH, brain astrocytes are being activated and secrete glial fibrillary acidic protein (GFAP) in a process called gliosis. Astrogliosis can inhibit axonal regeneration, so this could be a potentional role of astroglia. Previous data (Wang and Tsirka, 2005b; Tejima et al., 2007), demonstrated that after ICH, MMPs are released by astrocytes, participating in that way in the inflammatory process. Plasma proteins are playing very important role in their activation.

Very important in their overall role, is that they have strong antioxidative potential, stronger than other cells in the central nervous system, such as neurons, meaning that they are highly resistant to ROS and recently observed that they are more resistant in ICH in comparison to neuronal cells (Wang and Doré, 2007a). The above findings suggest the very important role of astrocytes in ICH although limited and prelimiminary data of their excact role is available. Their specific role is not completely understood. Only some assumptions can be made regarding their more precise role.

According to the available data, activated astrocytes, found to produce neurotrophic factors, observation that suggests a neuroprotective role to neural cells (Brahmachari et al., 2006). Other researchers have observed that activated astrocytes, modulate the expression of microglial inflammatory mediators, observation which also suggests neuroprotective role (Pyo et al., 2003).

Min et al. (2006), observed ROS production of astrocytes in ICH, observation that is also suggestive for their neuroprotective role. Overall, it seems that astrocytes may play a neuroprotective role in ICH according to the above observation; however, early after ICH when

the activation is rapid and excessive, more likely they play a neurotoxic role by participating in inflammatory pathways.

(Rosenberg et al., 2001), showed that active astrocytes, interact with microglia, causing MMP activation. It seems that the interactions between microglia and astrocytes after ICH play very important role in the regulation of the following phaenomena and could be a target for therapeutic intervention (Wang and Doré, 2007b).

Further data from clinical studies; have revealed that astocytes may play an independent role in generating amyloid peptides in cases of cerebral amyloid ICH. This was a postmortem study of 6 patients that were diagnosed with amyloid angiopathy related ICH. Astrocytes were labeled in this study with Alzheimer A4 and gamma-trace peptides (Yong et al., 1992).

Tejima et al. (2007), in their study, demonstrated the astrocytic induction of MMP-9 in areas around the haematoma, supporting the important role of astrocytic overactivation early after ICH. Finally, in the recent study of Wu et al., (2008); Thrombin, its endogenous inhibitor protease nexin-1, and water channel protein aquaporin-4, have also been identified by immunohistochemistry in astrocytes, neurons, and cerebral vessels of the ipsilateral hippocampus at 5–96 hrs post-ICH in postmortem specimens.

Conclusion.

The exact role of astrocytes in ICH needs to be further examined and determined. There is data available suggestive of their important role neuroprotective or neurotoxic (in the early stages of ICH). The available (mainly experimental data), suggests that they play an important role in triggering inflammatory pathways, producing neurotrophic factors, ROS and MMPs. Very interesting is the interaction between microglia and astrocytes which could play a regulatory role in the triggering of the following phaenomena. It is without doubt that the available data is limited and further experimental and clinical research is crucial for determining the role of astrocytes in ICH and the identification finally of possible therapeutic targets. Figure 7,8, summarizes the role of astroglia in ICH.

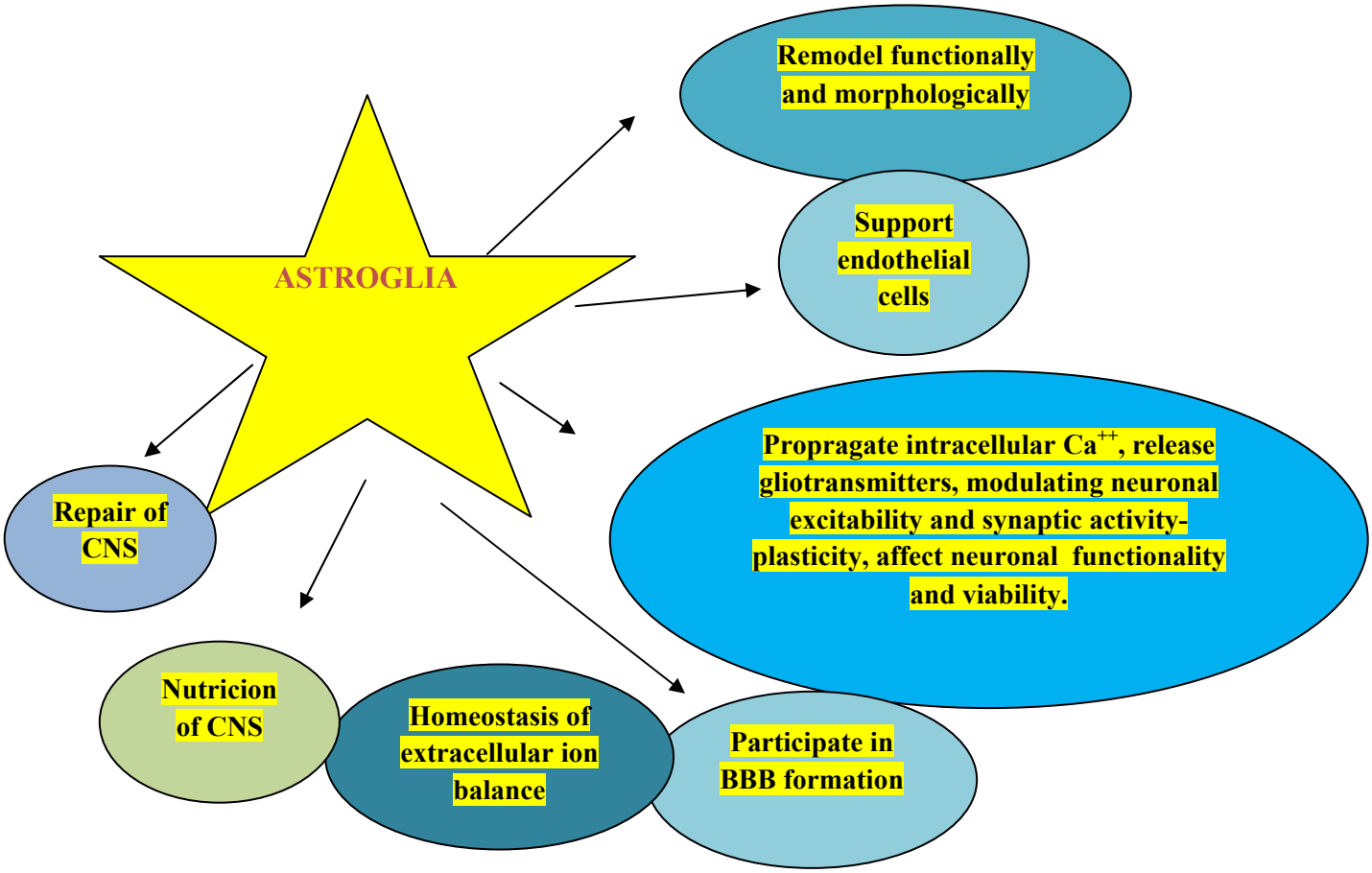


Figure 7: The role of astroglia. Schematic representation of the available data regarding the role of astroglia in intracerebral haemorrhage, simplified. For precise descrpiption see the relevant text.

III. Inflammatory mediators.

i. Matrix metalloproteinases.

It has been 40 years since the identification of the first matrix metaloproteinase (1962) by Gross and Lapiere. They identified the collagenase-1, the prototype of MMPs. Since the first identification of collagenase, many have been identified, so as far as now, 23 have been recognised and isolated (Gueders et al., 2006). Their role is very important in tissue remodeling.

They are zinc-dependent endopeptidases, belonging to a larger family of proteases the superfamily of metzincin. Although they were early recognized as possible therapeutic targets as early as late 1990s, especially in the cancer and arthritis research, failure of the clinical trials,

revealed that their role and physiological aspects, are more complex than was thought in the beginning. Their role in the CNS physiology and disease is currently being under investigation.

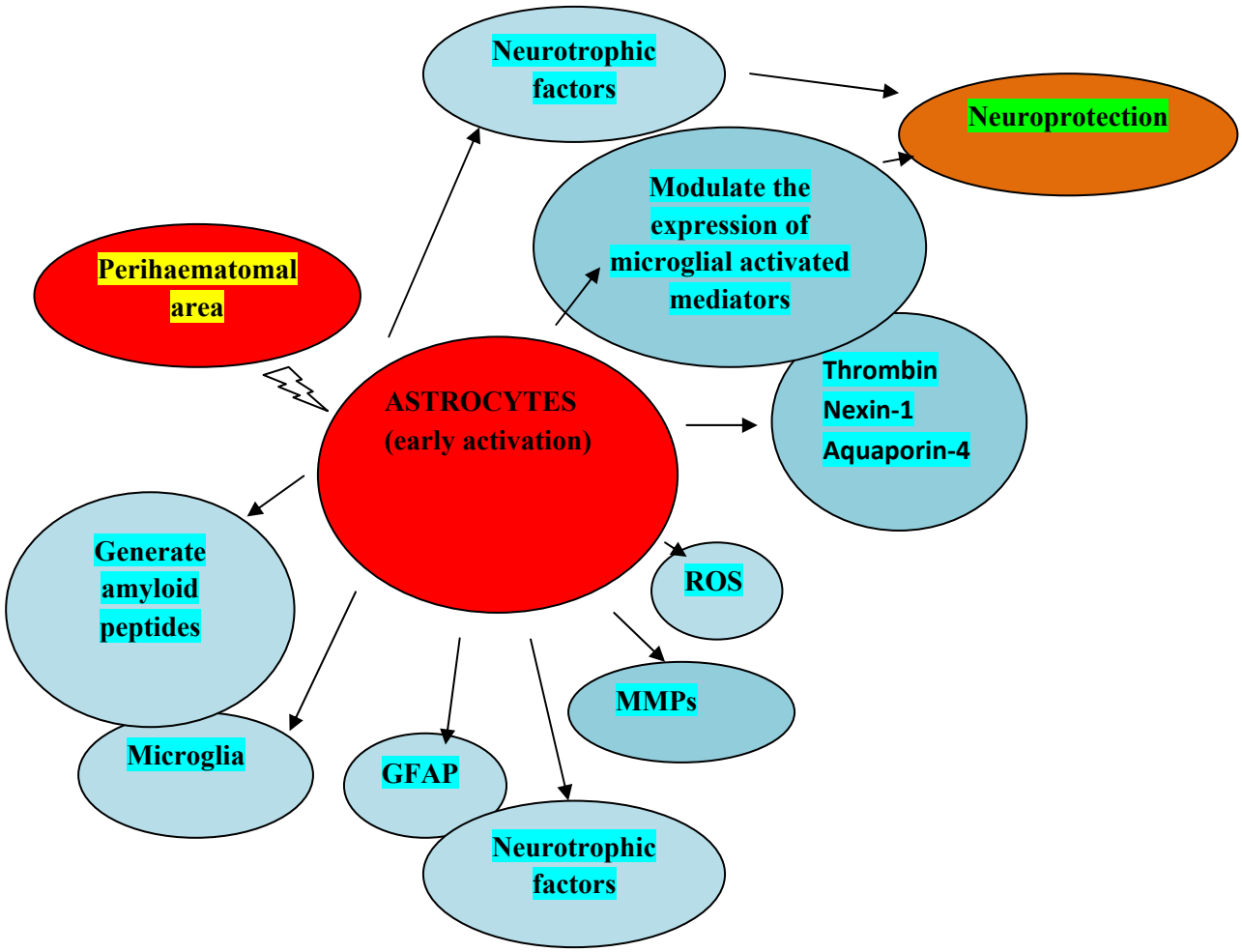


Figure 8, astrocytes in ICH. At early stages of intracerebral haemorrhage astrocytes are likely to play neurotoxic role by participating in inflammatory pathways. For more extensive description, see relevant text.

Breakdown of the BBB has been related to the presence of various MMPs. MMPs are endogenous family of zinc-dependent enzymes that are responsible for matrix remodeling. The extracellular matrix molecules, including type IV collagen, laminin, and fibronectin, constitute the basement membrane and help maintain the integrity of the BBB. Several types of MMPs have been shown to participate in the degradation of basal lamina and disruption of the BBB in animal models, and their inhibition has been shown to be helpful in reducing the vasogenic edema in ICH (Rosenberg and Navratil, 1997; Mun-Bryce and Rosenberg, 1998). Tissue inhibitor of metalloproteinases (TIMP), especially (TIMP-2), which is found in the brain

parenchyma, can be administered in experimental ICH to decrease perihematoma oedema by protecting the BBB (Rosenberg et al., 1992). In humans, a high blood concentration of MMP-9 detected within the first 24 hrs of ICH (Abilleira et al., 2003), was associated with early edema formation and subsequent progression in the following days, whereas high MMP-3 concentration correlated with mortality and residual scar volume (Alvarez-Sabín et al., 2004). MMP-9 concentration was also found to be a biomarker for predicting ICH complications after thrombolytic therapy in human ischemic stroke (Montaner et al., 2003) and haematoma expansion (Silva et al., 2005), which would suggest that MMPs are predisposing factors for haemorrhage. Interestingly, activation of MMPs was observed in heart transplant recipients when donors died following spontaneous ICH. These heart transplant recipients demonstrated upregulation of MMP-2 and MMP-9, which was associated with cardiac remodeling and subsequent development of coronary vasculopathy (Yamani et al., 2003).

In general, they are found degrading extracellular matrix proteins, but also they are involved in triggering numerous bioactive molecules, react with many surface receptors, trigger apoptosis, proliferation, migration, differentiation and angiogenesis. We could say that overall, they are involved in extracellular remodeling and neuroinflammatory response. There is enough data to support that MMPs are located in the cytosol when there are inactive and in their active state, they are clived by proteases especially plasmin and plasminogen activator (tPA), and other MMPs (Wang and Doré, 2007b; Xue et al., 2009a). Under normal conditions their presence in the nervous system is low. In pathological conditions they are activated and upregulated, in response to various brain pathologies. For an overview of the general role of MMPs, see figure 9.

Data regarding their role in ICH.

First study regarding their role in ICH was carried out in 1997 by Rosenberg and Navratil. In this study, MMP-2 and 9, were found to be active 24 hrs after the induction of ICH, in a collagenase model of ICH in rats.

In 2003, Wang et al., using the same model, observed similar response in gelatinase activity the first 24 hrs and subsequently in 2005, dramatic increase in MMP-9 was observed (Wang and Tsirka, 2005b). Similar results were observed by other researchers in the same period in other models with equal conclusions. Power et al in 2003, Wells and all in 2005, observed increased levels of MM3 and 12 (Power et al., 2003; Wells et al., 2005) after ICH. These findings were also supported by similar studies in different animal models (Lee et al., 2003; Mun-Bryce et al., 2004a; Lu et al., 2006; Xue et al., 2006; Wu et al., 2010b).

Xue et al. (2009a, 2006), observed that MMP 3 and 9, can directly kill human fetal neurons. This finding was further supported by the observation that mice models of MMP 3, - 9, - 12 genetic deletions, were found to have less haemorrhagic brain injury than wild type mice (Wells et al., 2005; Xue et al., 2006, 2009a,). In a similar study on the other hand, opposite results were found regarding MMP- 9 (Tang et al., 2004). Recently, (Xue et al., 2009a), described the synergistically role of MMP-3, -9 with thrombin.

The observations of MMPs role after ICH, triggered several investigations regarding the importance of MMP inhibitors, in order to therapeutically block MMP activity.

As early as 1997, Rosenberg and Navratil reported that rats treated with BB-1101 (a broad-spectrum hydroxamic acid-based MMP inhibitor), had reduced oedema formation after administration of BB-1101. Later (2005), GM6001 inhibitor, when administered to mice with ICH, attenuated gelatinase activity, neutrophil infiltration and ROS production. The result was both decreased early brain injury and finally better overall outcome (Wang and Tsirka, 2005b). These findings were further confirmed by Xue et al. (2009a, b).

BB-94 another MMP inhibitor was also investigated for a possible neuroprotective role and found to reduce recombinant tissue Plasminogen Activator - induced haemorrhage and similar neuroprotective results in ischaemic stroke (Lapchak et al., 2000; Pfefferkorn and Rosenberg, 2003). On the other hand, Grossetete and Rosenberg (2008) found that another inhibitor, BB-94, increased cell death and hemorrhage volume when tested in a collagenase model of ICH.

Minocycline was also extensively studied regarding a potent neuroprotective role. First study was that of Power et al.(2003). In this study, minocycline found to be neuroprotectant by inhibition of MMP-12, which resulted in protecting the morphology of neurons and improving functional recovery. Yong et al., in 2004, reported the promising role of minocycline as a neuroprotectant. Later in 2007, Wasserman and Schlichter, studied more precisely the role of minocycline as neuroprotectant, observing the effect after administering minocycline 6 hrs after ICH induction. The results showed that minocycline, decrease the TNF-α upregulation and the MMP-12 expression, which result in protection of BBB and reduction of brain oedema, but failed to reduce final neuronal death (Wasserman and Schlichter, 2007a, b). In contrast, Szymanska et al. (2006) failed to reveal any neuroprotective role of minocycline, when administered 3 hrs after the onset of ICH.

In the era of the preclinical findings regarding the role of MMPs in the ICH, many studies were recently carried out, most of them after 2000. Most of the studies, suggested the central role of MMP-9. Abilleira et al. (2003), Alvarez-Sabin et al. (2004), Silva et al. (2005), Castellazzi et al. (2010), observed high levels of MMP-9 after ICH. Most important findings,

are the time course (12-24 hrs after the onset), the coloration with perihaematomal oedema formation their role in enlargement of haematoma and final worsening of the neurological function.

Alvarez-Sabin et al. (2004), found that elevated MMP-9 levels, correlated with increase perihaematomal oedema and deterioration, especially during the acute stage of the pathology. In their study MMP-3 was found to collate with mortality.

Montaner et al. (2001a,b, 2003); Castellanos et al. (2003); Ning et al. (2006); Castellanos et al. (2007), reported the association between high levels of MMP-9 and haemorrhagic transformation of ischaemic stroke treated with thrombolysis. Recently, Barr et al. (2010), reported association between MMP-9 plasma levels and BBB disruption.

Up to date only three Histopathological studies are available regarding the role of MMP- 9 in ICH. Rosell et al. (2006) and Tejima et al. (2007), showed that MMP-9 was increased in neurons and astrocytes around the haematoma area, while (Wu et al., 2010a), recently reported that high levels of MMP- 9, nuclear factor-kappa B/p65 subunit, and macrophage inflammatory protein-2 were each upregulated on the hippocampus at the side of the haematoma.

Although there is clinical data available regarding the MMP- 9, there is no clinical data available regarding MMP- 3, MMP - 12. Table 3 (next page) summarises the available data regarding the role of MMPs in ICH.

Conclusion.

The role of MMP in ICH, only recently is being under investigation. The data experimental or clinical dates only since 1997, although the MMPs were first described as early as 1962. Available data supports that the role of MMP 9, MMP 3 and MMP 12 at least is important in the inflammatory response. Up to date there is only available data regarding mainly MMP 9 and experimental data regarding MMP 3 and MMP 12.

Data suggests that there is up-regulation of MMP 9 around the haematoma area, coloration with oedema formation especially to the early stages of onset, enlargement of the haematoma and worse neurological outcome. Interesting is the finding that high levels of MMP 9 are present in the hippocampus of the brain of ICH patients. Further studies experimental and clinical are needed in order to determine the role, importance and time course of MMPs in ICH. Especially their clinical role, given the lack of clinical data needs further investigation.

Table 3. Summary of the available data regarding the role of MMPs in ICH.

Author(s), year	MMPs Tested	MMPs Result
Rosenberg and Navratil. (1997)	MMP 2, -9	Increased, 24hrs after the induction of haematoma.
Wang and Tsirka. (2005b)	MMP 9	Increased levels.
Power et al. (2003) ; Wells et al. (2005)	MMP 3, -12	Increased levels.
Lee et al.. (2003); Lu et al.. (2006); Mun-Bryce et al.. (2004); Wu et al.. (2010b); Xue et al.. (2006)	MMP 3, -12	Increased levels.
Xue et al. (2009a, 2006)	MMP 3, -9	Can directly kill human fetal neurons.
Xue et al. (2009a)	MMP 3, -9, synergistic role with thrombin complex.	
Abilleira et al.(2003); Alvarez-Sabinet al.(2004); Silva et al. (2005); Castellazzi et al. (2010)	High levels of MMP 9 after ICH	Focused on the central role of MMP-9 in intracerebral haemorrhage.
Alvarez-Sabin et al.(2004)	High levels of MMP 3, - 9.	Increased oedema, neurological deterioration. MMP-3 was collated with high mortality.
Castellanos et al.(2003, 2007); Montaner et al. (2001a,b, 2003); Ning et al. (2006)		Coloration of high levels of MMP 9 with haemorrhagic transformation of ischaemic stroke treated with thrombolysis.
Barr et al. (2010)		Coloration between high levels of MMP 9 and BBB disruption.
Rosell et al. (2006) Tejima et al. (2007)		MMP 9 was increased in neurons and astrocytes around the haematoma area.
Wu et al. (2010a)		reported that high levels of MMP 9, nuclear factor-kappa β/p65 subunit, and macrophage inflammatory protein-2 were each upregulated on the hippocampus at the side of the haematoma.

Oxidative stress starts very early in perihaematomal white matter after ICH, fact that is evident with the upregulation of haeme oxygenase-1 in the area around the haematoma and also because of the carbonylation of proteins around the area (Wagner et al., 2002). Figure 10, shows the most important pathways of oxidative stress in ICH.

Several studies with infusion of plasma into the white matter showed that induction of protein carbonyl formation seems to induce oxidative stress plasma infusions into the hemispheric white matter also induce independentently protein carbonyl formation, observation that suggests that plasma alone can induce oxidative stress (Wagner et al., 2005).

Many mechanisms can explain this finding. It could be explained by glutamate-induced receptor stimulation leading to increasing of intracellular Calcium levels (Lipton et al., 2006) or as an interaction between holotransferrin and thrombin leading to intracellular iron deposition (Nakamura et al., 2005). Oxidative stress and HO-1 induction are also present in gray matter after ICH (Wu et al., 2003).

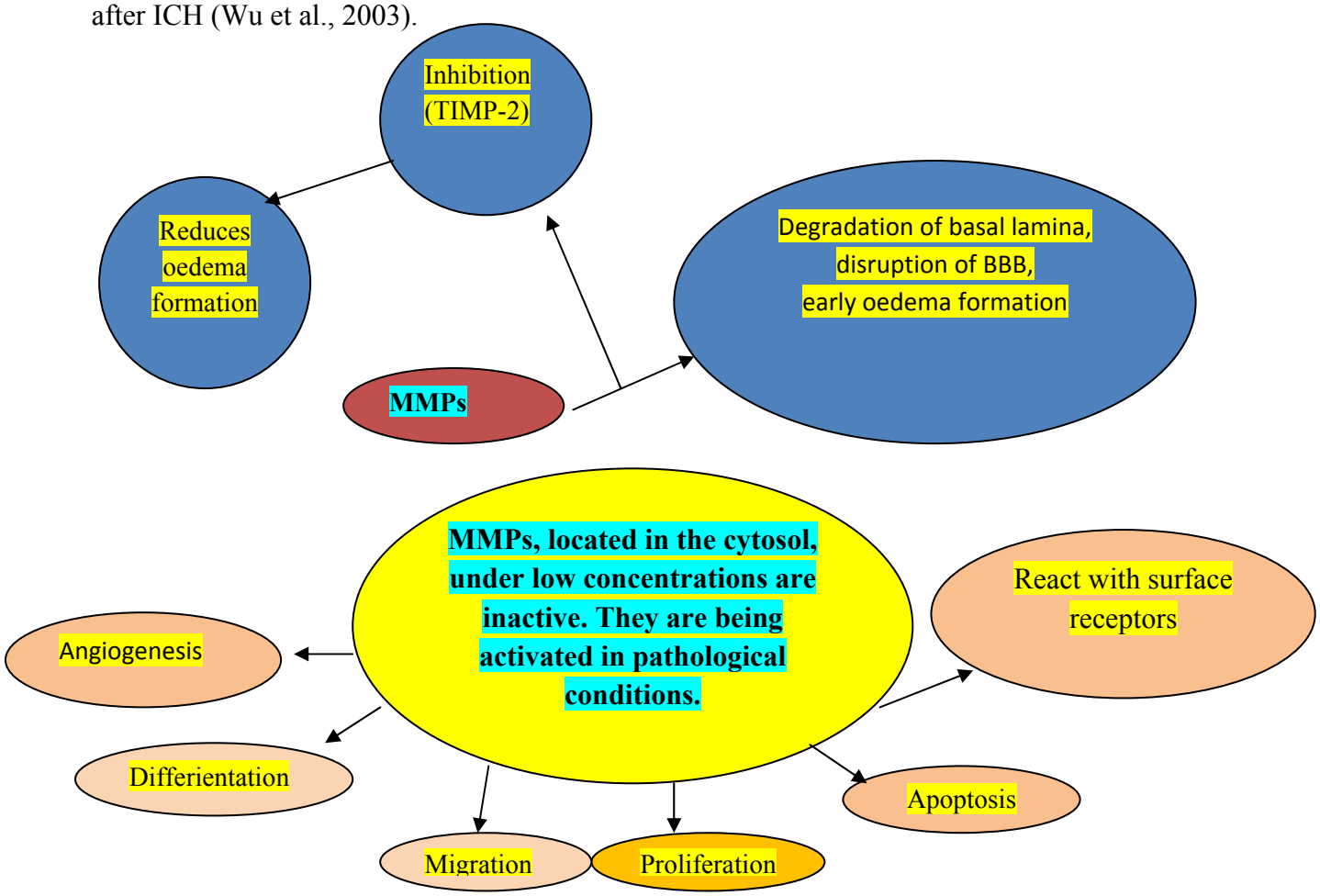


Figure 9. Simplified diagram of the role of MMPs in ICH. For precise description of their role, please see the relevant chapter.

Oxidative stress leads to DNA damage after ICH. (Matsushita et al., 2000; Wagner et al., 2003; Nakamura et al., 2005; Wagner et al., 2005, Tang et al., 2005), studied oxidative stress in NADPH oxidase KO mice after ICH. They observed that the total oxidative product was lower in NADPH oxidase KO mice after ICH, further to that, brain edema, neurological deficit and mortality, were higher in the wild-type but not in KO mice. This finding can only be explained by the fact that oxidative stress resulting from NADPH oxidase activation contributes to ICH and promotes brain injury. Hall early in 2000, and recently Aronowski and Zhao. (2011), have reviewed extensively the role of oxidative stress in ICH.

ii. The role of nuclear factor erythroid-2 (Nrf2), haeme-oxygenase, and iron, oxidative stress in ICH.

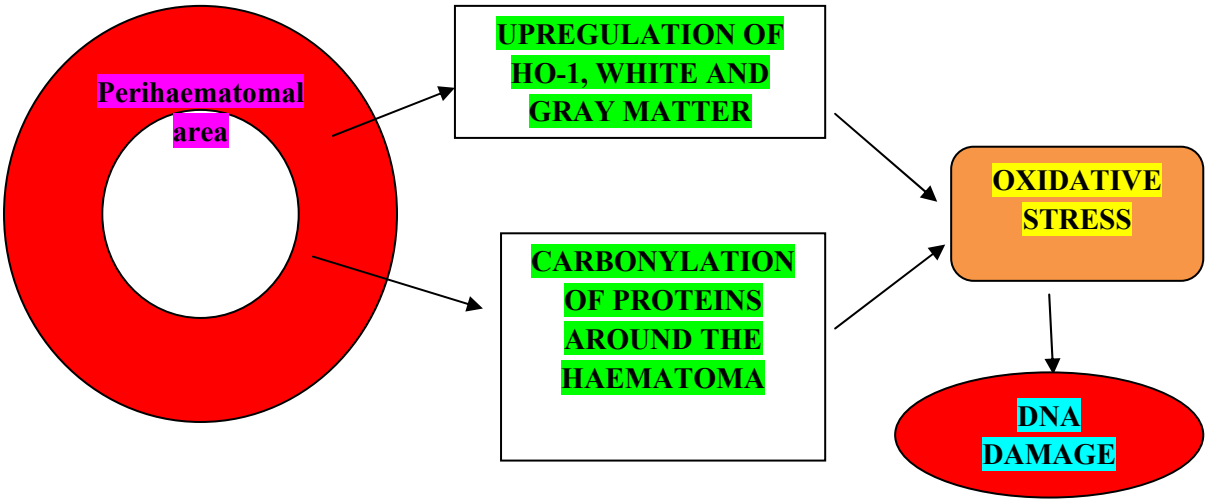


Figure 10. Summary of oxidative stress pathways in ICH.

iii. The role of nuclear factor erythroid-2 (Nrf2) in ICH.

Nrf2, is present in many systems and recently reviewed regarding its anti-oxidative role. In the brain, we can find Nrf2, in astrocytes, neurons and microglia**.** First findings regarding the antioxidant role of Nrf2 (nuclear factor erythroid-2) were published by Lee et al. (2003) and Kraft et al. (2004).

Recently, nrf-2, was recognized to play very important role in the oxidative stress in intracerebral haemorrhage. (Zhao et al., 2007a). According to the authors, Nrf2 is a regulator of antioxidative defense with neuroprotectant role for the central nervous system. In their study, Nrf 2-deficient mice found to be more vulnerable to oxidative stress, having finally severe neurological dysfunction in comparison to control mice, finding that was also observed in previous similar studies (Lee et al., 2003; Kraft et al., 2004).

Chen et al. (2006), and Thimmulappa. (2006), described also anti-inflammatory effects of Nrf 2 and showed that this effect more likely is mediated through NF-Kβ, observing that lipopolysaccharide and TNF-α are higher in Nrf 2-deficient mice. Further to that observation, they found that in inhibition of NF-kB, reduced numbers of neutrophils were observed, probably because of the expression of monocyte chemo attractant protein-1 and vascular cell adhesion molecule-1 (VCAM), which are NF-kB targets involved in the regulation of neutrophil infiltration. Overexpression of Nrf2, blocks them and reduces finally NF-kB production, Chen et al. (2006).

Increased expression of Nrf-2, found to stimulate the expression of several antιoxidative enzymes such as catalase, SOD, NAD (P) H dehydrogenase, quinone-1, and glutathione *S*-transferase, which are very important in the oxidative stress. Many authors have demonstrated that up regulation of catalase and SOD, may play neuroprotective role in ICH. Gu et al. (2004), Zhao. (2006), Wang and Dore. (2007a).

Chen and Kunsch. (2004), Nguyen et al. (2009), showed that Nrf2 is a regulator of genes that act in order to remove ROS, via NAD (P) Hquinine oxidoreductase 1, glutathione S transferases, glutamate– cysteine ligase, glutathione peroxidase, and Haeme Oxygenase-1 (HO-1). So it seems that plays important role in the production and release of HO-1, HO-2, responsible proteins for degrading haeme (from the haematoma) into biliverbin, carbon monoxide and iron (Ryter and Tyrrell, 2000), we know that iron induced oxidative stress seems to cause neurodegeneration (Zecca et al., 2004). Figure 11 summarizes the role of Nrf2 in ICH.

iv. The role of haeme oxygenase-1, 2 (HO-1, HO-2).

According to the available studies, HO-1 is mainly present in glial cells, while, HO-2 is expressed throughout the brain (Koeppen et al., 2004; Matz et al., 1997; Nakaso et al., 2000; Koeppen et al., 2004; Wang and Doré, 2007a). These findings suggest that they may have different roles in ICH.

Furthermore, regarding the role of haeme oxygenase 1, 2 in ICH, the experimental data is controversial. Some studies, provide evidence that HO-1 and HO-2 have cytoprotective functions (Parfenova and Leffler, 2008; Takahashi et al., 2004). On the other hand, several preclinical studies have demonstrated that non-selective HO inhibitors are neuroprotective in blood ICH models (Huang et al., 2002; Koeppen et al., 2004; Wagner et al., 2000). Seems that, HO-1 does not exert a direct neuroprotective effect early after ICH because it selectively localizes to microglia/macrophages.

Although activation of microglia/macrophages participate to the resolution of haematoma, (Wang et al., 2003), it is involved in early brain injury after ICH (Wang et al., 2003; Wang and

Tsirka, 2005c). So we could assume that HO-1 plays a neuroprotective role early after ICH. On the other hand previously data suggests that excessive levels of OH-1 could result in cytotoxicity (Suttner and Dennery, 1999).

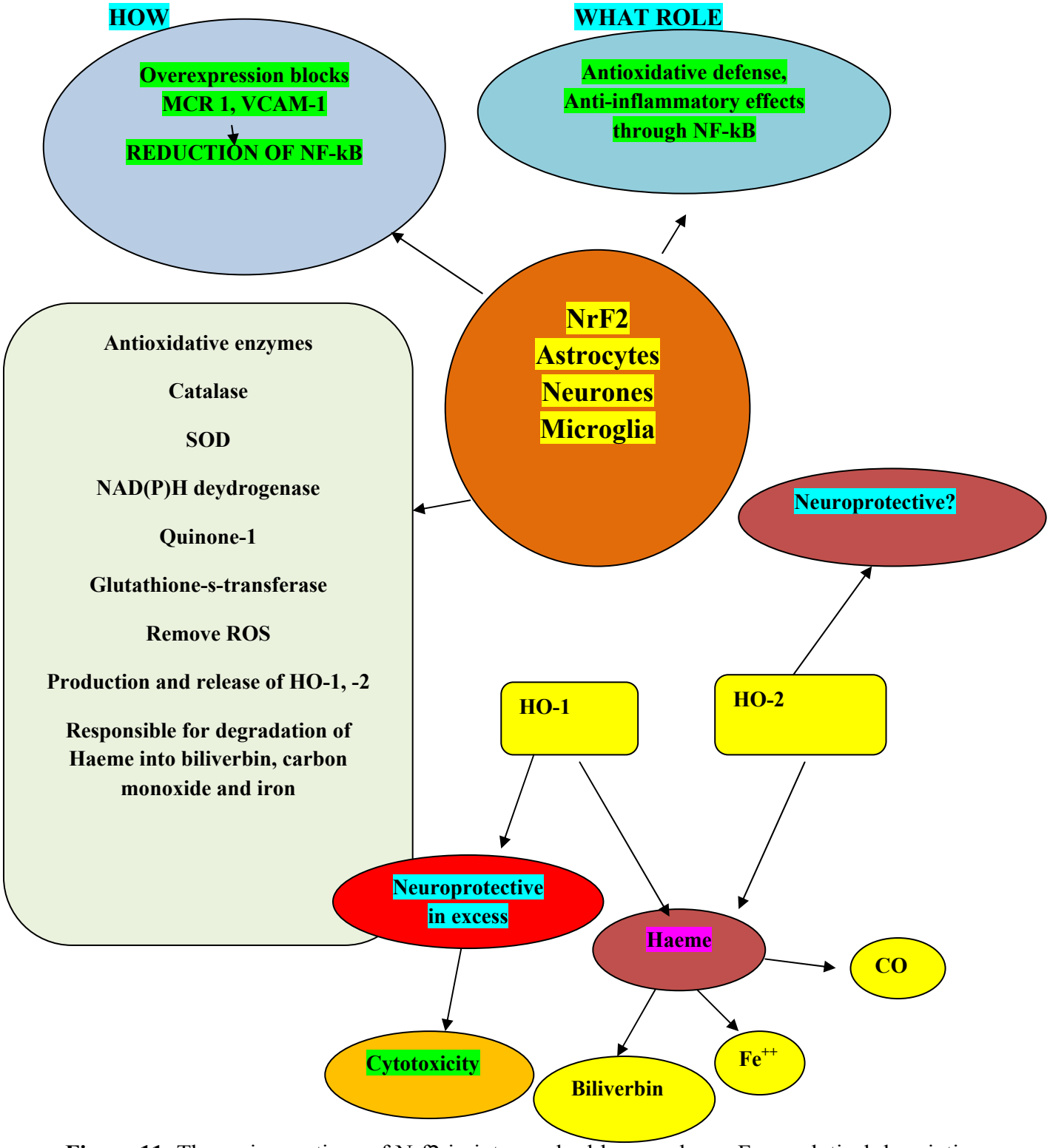


Figure 11. The main reactions of Nrf2 in intracerebral haemorrhage. For analytical description, see the relevant text.

In ICH, the haeme-induced upregulation of HO-1, might exceed the protection threshold and result in brain injury. In OH deficient mice, it is observed that the injury volume was smaller in comparison to the control group and also decreased inflammation and free radical levels according to Wang and Doré. (2007a,b). Many authors have reported the coloration between HO-1 and oxidative stress in models of whole blood ICH (Wagner et al., 2002; Wu et al., 2003; Chen and Regan, 2007).

Wang et al, showed that HO-2 deletion renders primary cultured neurons more vulnerable to haemin (oxidized haeme)-induced toxicity (Wang et al., 2006) and that HO-2 deficient mice are more vulnerable than wild type mice to collagenase-induced ICH (Wang et al., 2006b; Wang and Dore, 2008). Furthermore, the exacerbation of brain injury in HO-2 deficient mice is associated with increased neuroinflammation and ROS production.

In a rat model of ICH, brain hemorrhage led to iron deposition and a threefold increase in non-haeme iron (Wu et al., 2003). Excess iron in the brain can result in lipid peroxidation and the formation of free radicals (Halliwell and Gutteridge, 1992, Gutteridge, 1994), which finally results in neuronal damage (Thompson et al., 2001; Zecca et al., 2004). Haemolysis and haeme/iron-mediated toxicity occur 2–3 days after ICH (Wagner et al., 2003). Considerable evidence suggests that haemoglobin breakdown and subsequent iron accumulation within the brain mediates secondary brain injury after ICH (Wagner et al., 2003; Xi et al., 2006). Brain atrophy and neurologic deficits observed up to 2 months after ICH have been attributed to iron deposition within the striatum (Hua et al., 2006a). Interestingly, and in support of the iron mediated toxicity after ICH, treatment with an iron chelator such as deferoxamine has shown to provide neuroprotection in a whole-blood ICH model in rats (Hua et al., 2006a; Nakamura et al., 2004a; Okauchi et al., 2009b; Song et al., 2007) and in piglets (Gu et al., 2009), but not in a collagenase-induced ICH model in rats (Warkentin et al., 2010).

Currently, no histopathological data are available on Nrf2 in human ICH brain. Two studies have shown induction of HO-1 in microglia/macrophages and increased iron content associated with the haemorrhagic lesion (Beschorner et al., 2000; Lou et al., 2009). Interestingly, increased HO-1 concentration was reported to be associated with worse neurological outcome after traumatic brain injury in infants and children (Cousar et al., 2006). In a small clinical study, it was reported that the levels of nonprotein-bound iron were elevated in the cerebrospinal fluid from preterm infants with intraventricular haemorrhage compared to the control ones (Savman et al., 2001).

In patients with spontaneous ICH, an association between serum ferritin level and perihaematomal oedema volume on days 3–4 has been reported (Mehdiratta et al., 2008). High

serum ferritin levels did not correlate with acute phase reactions and were found to be associated with poor outcomes (De la Ossa et al., 2010).

Recently, magnetic resonance imaging revealed a relationship between iron within the haematoma and perihaematomal edema in the human brain after ICH (Lou et al., 2009), which links to iron-mediated toxicity to the surrounding brain, edema formation and delayed neuronal death after ICH.

v. The role of NF-kB in intracerebral haemorrhage.

Available data shows that ICH induces gene expressions that can be either neurotoxic or neuroprotective. (Tang et al., 2002; Wagner et al., 2003; Lu et al., 2006). Figure 12, represents the known roles of NF-kB, in ICH.

NF-kB, seems to have different and multiple roles in trauma, ischaemia, Alzheimer's disease, oncogenesis, Parkinson (Cohen, 1988) and other degenerative diseases (Pizzi et al., 2006). Recent data, finds that there are several subunits of NF-kB (most important are P50, P65), with different roles regarding different responses in brain damage. NF-kB, and its subunits, is found to be modulated by several pathways such as neurorotransmitters, electrical activity, cytokines, neurotrophic factors and oxidative stress.

Generally, NF-kB seems to have different roles under different stimuli and doesn't play only one and isolated role of promoting cell death, but more likely plays a role of mediator and regulator of the inflammatory response in many brain pathologies including ICH. This regulatory role, results to the different final outcome with NF-kB being a mediator. Specific to ICH data, suggests that NF-kB, sensitive to oxidative stress (Grilli and Memo, 1999; Hickenbottom et al., 1999) is early and rapidly activated in perihaematomal brain after ICH (Hickenbottom et al., 1999; Wagner and Broderick, 2001; Wagner et al., 2003; Wagner et al., 2004; Aronowski and Hall, 2005). Recently, its role has been extensively reviewed by Mattson and Meffert. (2006) ,Wagner. (2007).

According to the data available, NF-kB is found to play a crucial role in upregulation of central nervous system genes and especially proinflammatory cytokines and HO-1 (Wang and Shuaib, 2002; Allan and Rothwell, 2003).

Aronowski demonstrated that after ICH, peroxisome proliferator activated receptor 'y (PPAR-y) is being activated, affecting the NF-kB activation and precisely suppress NF-kB (Zhao et al., 2006). Further to that, they have also observed that 15-deoxy-D12, 14-prostaglandin J2 (an antagonist of PPAR-y) suppresses NF-kB expression. Treatment with 15d-PGJ2 was

found to reduce inflammation, behavioral dysfunction and neuronal damage of ICH (Zhao et al., 2006c).

Overall, NF-kB, regulates the expression of several genes, plays the role of mediator between the extracellular environment and nucleus, resulting either in cell survival or cell death. So we could say that NF-kB, is a bridge between the primary pathology and the final result of the phaenomenon in the decision of cell death or survival. So NF-kB could be considered a bridge between early phaenomena and apoptosis. In several studies, has been found to have either apoptotic or antiapoptotic role. (Lin et al., 1995, Kitajima et al., 1996).

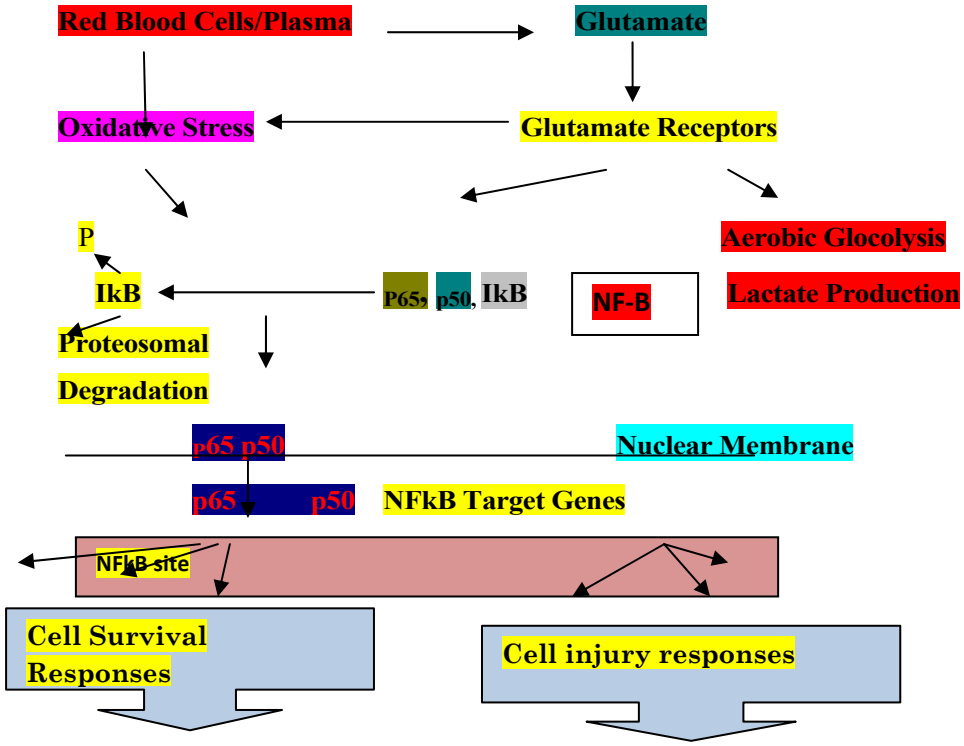


Figure 12. The central role of NF-kB in intracerebral haemorrhage. Wagner, 2007

vi. The role of thrombin and blood degradation products.

Very important role in ICH, are playing the haemostatic mechanisms, blood and blood degradation products. This role was first described by Suzuki et al. (1980), while later on Jenkins, et al. (1990), described the role of blood constituents in intracerebral haemorrhage. The available data shows evidence of brain oedema formation 3 days after the injection of blood into the brain when red cells were injected and 24 hrs after when lysed blood was injected. Lysed red blood cells are increasing the BBB permeability and finally trigger oedema formation.

The role of thrombin is very important for the phaenomena that follow the blood release in the brain parenchyma. There is evidence that thrombin, when administered in BBB disrupted environment, is responsible for inflammation and oedema. (Lee et al., 1995; Lee et al., 1996; Lee et al., 1997; Xi et al., 1998). Thrombin is known to been associated with apoptosis. (Gong et al., 2001).

More recent studies by Hua, et al. (2007), showed that the role of thrombin is more complex than thought in the beginning. In their study, authors concluded that thrombin causes brain damage in high concendrations, while in low concentrations seems to play a neuroprotective role. So the modulation of thrombin in several phases during the ICH pathophysiology is of major importance to understand and determine.

The coloration between thrombin and apoptosis is very important given that apoptosis results in programmed cell death and final phagocytosis by normal cells. (Beilharz, 1995; Leist, 1998). Apoptosis involves single cells and results in programmed cell death, with subsequent phagocytosis by adjacent normal cells (Beilharz et al., 1995; Leist and Nicotera, 1998).

There is evidence that apoptosis starts as early as the haematoma onset and lasts up to 72 hrs after the haematoma induction. (Matsushita et al., 2000; Nakashima et al., 1999). The available data suggests that apoptosis as a form of cell death in intracerebral haemorrhage follows a delayed manner with many mediators such as tumor necrosis, and caspaces being involved.

vii. The role of glutamate, lactate and hypermetabolism.

Glutamate is produced by the activation of post-synaptic N-methyl-D-aspartate (NMDA) receptors. NMDA receptors, when activated trigger intracellular calcium ions influx which finally owe to increase in sodium concentration in the cells, cause oedema formation and neuronal death (Lees et al., 1997).

Glutamate and other excitatory amino acids, are found increased early in the perihaematomal area because either from the regional compression or by the release of the blood components in the area.

It seems that there is a delay in the development of oedema and BBB disruption after ICH, which suggest that probably, other mediators, play their role in the secondary injury process. Most studies until now, conclude that this mediator is glutamate.

Glutamate is produced by the activation of post-synaptic N-methyl-D-aspartate (NMDA receptors). Wagner et al. (2007), in their porcine model of intracerebral haemorrhage, showed that oedematous perihematomal white matter is characterized by increased lactate levels quite early after the occurrence of ICH (even from the first hour). They also showed that lactate is

elevated not by anaerobic glycolysis but by aerobic glycolysis (Wagner, 2007). The main cause seems to be the penetration of brain white matter by blood glutamate, which stimulates glutamate receptors and triggers aerobic glycolysis, leading finally to lactate production. Similar findings were observed by microdialysis study (Qureshi et al., 2003a). In this study, glutamate levels were elevated by 4-fold at the haematoma region as early as 30 minutes after the haematoma and remained elevated for the first 5 hrs.

Regarding the metabolism status, Ardizzone et al. (2004) observed an increased [14C]-2-deoxyglucose consumption in the perihaematomal area as early as 3 hrs after the haematoma induction, which was inhibited by glutamate receptor antagonists. These data can only be explained by the fact that glutamate receptor activation increases the metabolism of glucose in the perihaematomal white matter, at early times after ICH, observation that can explain the increased lactate levels at the perihaematomal area.

Although expression of cytokines and their contribution to neural injury and inflammatory responses are well characterized in the pathology of cerebral ischaemia and head injury, ICH was not associated with expression of TNF-α, IL-1β, or IL-6, either in the perihaematoma region or in other regions of the brain, when blood and cerebrospinal fluid (CSF) were tested 1 hr after the onset of haemorrhage (Qureshi et al., 2001b). The increase of inflammatory markers only becomes evident in situations in which the BBB is permeable (Castillo et al., 2002).

The perihaematomal area, plays significant role regarding the inflammatory response. The migration of blood components and especially leukocytes and platelets into the area around the haematoma, plays main important role in the secondary injury that follows ICH. Experimental data suggests that when platelets and lymphocytes are reduced in the area around the haematoma, formed oedema is finally less prominent (Castillo, 2002).

viii. Regarding the role of TNF-α in intracerebral haemorrhage.

TNF-α is known to be a very important cytokine. It seems that results in brain damage from the studies available in traumatic brain injury and stroke (Barone and Feuerstein 1999; Hallenbeck 2002). The mechanisms of action seems to be those of BBB disruption (Yang et al., 1994; Wagner et al., 1999; Xi et al., 2001a) neutrophils and monocytes activation (Gong et al., 2000; Xi et al., 2001a) and apoptosis (Matsushita et al., 2000).

In the past there was controversy regarding expression of TNF-α and especially the time course of event, in ICH. Data that is available regarding especially the role of this cytokine, suggests that in in vitro models of rats and pigs, TNF-α increases after ICH, triggering complement activation (Hua et al., 1998; Xi et al., 2001a, b).

In other studies, inhibition of TNF-α, finally reduced brain injury (Mayne et al., 2001) but in a similar model in dogs, TNF-α levels were normal one hour after ICH (Qureshi et al., 2001). Hua, et al.(2006b), in their study in rats, showed opposite results. They observed that there is a timeline pattern in TNF-α expression that follows an increase as early as 2 hrs and return to baseline after 24 hrs. In my study, similar to the last findings were observed. In the porcine model of ICH that we studied, TNF-α , found to be increased as early as 4 hrs after the haematoma induction and returned approximately to baseline 24 hrs after the haematoma induction. The results of this study, are analytically being presented in part B.

Other investigators emphasized in the TACE enzyme, which is known to be a very important enzyme in activation of TNF-α and early increase in his levels was reported (Hua, 2006b). They observed that TACE was increased within one hour in their study, suggesting relevance to TNF-α activation. In the TNF-α knock-out mouse, TNF-α was absent and the neurological deficit was reduced in comparison, oedema also. In other similar studies, blocking TNF-α results in less brain damage (Barone FC et al., 1997). Clinically, Castillo et al. (2002), reported relevance in the plasma TNF-α levels and oedema formation, which was evident early, 5 hrs after the onset..

ix. The role of thrombin and TNF-α.

Hua et al.(2006b), in their study, showed that there is increase in TNF-α when thrombin is infused as early as 2 hrs. Further to that they also observed that very high doses of thrombin limit ICH because those doses, result in cell death. There have been recognized three thrombin receptors in neurons and astrocytes (PAR1, 2, 3) (Niclou et al., 1994; Weinstein et al., 1995; Xue et al., 2009b).

x. TNF-α and neuroprotection.

Ya Hua. (2006b), tested the relation between thrombin concentrations and TNF-α. It is known also from previous studies that there is a concentration effect in the role of thrombin. In low concentrations is neuroprotective (Vaughan et al., 1995; Xi et al., 1999; Striggow et al., 2000; Jiang et al., 2002), while in high concentrations is neurotoxic. In their study they found that TNF-α blockage didn't affect thrombin preconditioning, on the other hand, they found that pretreatment with TNF-α is neuroprotective. An early increase in TNF-α, results in brain oedema and neurological impairment. On the other hand, thrombin triggers TNF-α increase.

xi. Apoptosis in intracerebral haemorrhage.

Qureshi et al.(2003b), in their study, described apoptosis in humans taking into account all the previous data regarding apoptosis in ICH in preclinical studies, which had earlier been described and reviewed by Leist and Nicotera.(1998). Before this study, experimental data found that in areas near the haematoma, there were necrotic and damaged cells as well as normal cells (Qureshi et al., 2001a). Other authors had shown that in a collagenase model in rats, 24 hrs after the induction of haematoma, there were apoptotic cells around the haematoma area, most of which were neurons and astrocytes (Matsushita et al., 2000).

Matsushita et al. (2000) and Qureshi et al. (2001a), analyzing the apoptosis pattern around the haematoma, found that apoptosis, was orientated in the area of the haematoma, while in the same study (Qureshi et al., 2001a) as well as in the study of (Nakashima et al., 1999), authors found that apoptosis is evident as early as 24 hrs and persists up to 72 hrs.

Qureshi et al. (2003a), in a retrospective clinical trial, they studied a series of patients that were operated for removal of intracerebral haematoma clot, between 1992 and 2000 at Buffalo General Hospital and found that in the perihaematomal region, there was evidence of apoptosis. Apoptosis was present in 10 out of 12 specimens that were surgically obtained and the onset was present as early as 24 hrs after the haematoma induction. It is interesting to mention that in the same study, apoptosis was very difficult to identify in the cerebellar according to the authors because of the different cell populations.

Later on, Wagner. (2007) in the very important article "Modeling Intracerebral Hemorrhage Glutamate, Nuclear Factor-kB Signaling and Cytokines", reviewing the available data regarding intracerebral haemorrhage pathophysiology, describes the important role of NF-kB as mediator to cell death and survival, giving an overview of the final connection of the important pathways in intracerebral haemorrhage. Recently, Hwang et al.(2011), also reviewed the role of apoptosis in ICH emphasizing possible therapeutical treatments and strategies.

In conclusion there is enough evidence to support that the result of secondary injury in ICH, finally induces apoptosis at the haematoma site. This could be a therapeutic target.

xii. The role of complement activation in intracerebral haemorrhage.

Recently Ducruet et al. (2009), reviewed the role of complement in ICH. There is enough evidence to support that complement activation is very important regarding the secondary brain injury in ICH. Data available is still limited in order to understand and definitely describe the role of complement in ICH. It is reported that C3 is the more important in ICH secondary injury amongst the many complement parameters. C3 seems to act directly toxically, playing on the other hand a role in recovery in ICH. Analytically, Bellander et al. (1996), were the first to

determine that C3 implies the activation of complement in ICH. C3d was found to be a product of C3 after its activation, while CR2 was found to be its receptor. At the same period, Dempsey et al. (1996), observed that CR2, belongs to a family of proteins that play a regulatory role with main importance in defense and immunity. Complement depletion reduced oedema formation after ICH, late after the onset of ICH, 24 and 72 hrs as described by Hua et al., (2000), Xi et al., (2001b, 2002).

Zhang et al. (2006), showed that after ICH in the rat there is upregulation of C3 and CR2, which play significant role in secondary injury in terms of inflammation, oedema and finally brain injury in ICH. In conclusion complement seems to be activated in ICH, playing important role regarding activation of pathways of defense and immunity, with enough evidence to support that one of the roles in ICH is participation in oedema formation, inflammation and brain injury.

IV. Pathology of perihaematomal region from physiological point of view.

The presence of hematoma triggers oedema formation and causes neuronal damage in the surrounding brain parenchyma. It is known today that oedema is present as early as 1 hr after ICH, as first was observed by Wagner et al. (1996). In his model of porcine ICH, higher concentration of water was calculated in the haematoma site in comparison to the contralateral hemisphere. This early oedema around the haematoma, is the result of osmotic active serum proteins from the clot and water, while the BBB proved to be intact in his study. Between the first day and the second day after the onset of ICH, main role plays, the activation of coagulation and proteolytic enzymes that trigger the inflammatory response, resulting in direct cellular toxicity, BBB disruption and depression of the metabolism around the haematoma. All the above mechanisms together result in decrease of Cerebral Blood Flow (Nath , 1987; Zazulia , 1999; Lee, 1996a).

Cytotoxic oedema, which we could schematically call late oedema, is the result of cell death and BBB disruption, observed between day 5 and 6 after the intracerebral haemorrhage onset (Sansing , 2003).

The parenchyma around the clot, is oedematous, characterized by blood cell's lysis products while histopathologically is characterized by oedema, neuronal damage, and the presence of cells such as macrophages and neutrophils. These findings have been described as early as 1963 (Mutlu, 1963; Morris, 1999).

Recently, Staykov et al. (2011), studied the time course of oedema formation after ICH in 219 patients. In conclusion, the authors found that oedema develops early after ICH and

becomes double in size by day 7 while significant increase of oedema was present already at day 1 after ICH. The study observed that increase of oedema was present and after the day 7 up to day 11. Other observations, reported prolonged increase of oedema even two to three weeks after the onset, Zazulia.(1999). Figure 13, shows the most important pathophysiological changes that take place in ICH pathology.

V. The role of cerebral blood flow.

In the beginning, ischaemic insults in the perihaematomal area were believed to play an important role in the pathophysiology of ICH and the overall mechanisms underlying the phaenomenon. In the first studies, many authors suggested that ischaemia was a part of the pathophysiology in the surrounding area, playing significant role in oedema formation. Most of this data was based in experimental studies (Kobari et al., 1988; Bullock et al., 1998; Nehls et al., 1998; Yang, 1999). All these studies described the relation between haematoma formation, intracranial pressure (ICP), cerebral blood volume (CBV) and cerebral blood flow (CBF), concluding that there is ischaemia around the haematoma. With the use of new diagnostic and experimental technology and especially with the use of MRI, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) studies, these findings have began controversial. Recent data especially after the use of the recent technological innovations, suggests that ischaemia, does not play a role in edema formation after ICH.

Although there is not currently available universally accepted method to directly measure the metabolic changes in cells around the haematoma, MRI and more specifically, SPECT and PET, helped a lot last decade to determine the pathophysiology of the surrounding to haematoma neuronal tissue, especially with the determination of CBF changes.

On MRI studies, with the use of diffusion sequences, it was observed that in both hemispheres 6 hrs after the haematoma onset, there is increase in diffusion which is present at both hemispheres the one with the haematoma and the controlateral ‘unaffected’ hemisphere. These increase, can be explained only with the rise of water in the perihaematomal area as a reaction of the brain to maintain normal CBF. Vasogenic oedema also occurs secondary to inflammation this global reaction may represent an adaptive process to a new steady state (Kamal et al., 2003).

At the same period, Butcher et al. (2004), studied the characters of oedema around the haematoma with MRI sequences that are sensitive for ischaemia in order to determine if there is evidence of ischaemia in the perihaematomal area. Their study, which included 95 patients, showed that there was a reduction of CBF in the perihaematoma area but this was normalized 3

to 5 days after the onset. There was no MRI evidence that there is ischaemia or ischaemic oedema around the haematoma area. Their conclusion was that the early oedema formation is plasma derived, although they were limitations of the study.

Recently, Fainardi et al. (2012), in similar study, examined 35 cases regarding the characters of oedema in ICH, concluding that there is vasogenic oedema that progressively resolves while on the other hand, there is increase of cytotoxic oedema by time. They observed that perihaematomal vasogenic values were more prominent at day 2 in comparison to day 7, while on the other hand values associated with increased cytotoxic oedema were more prominent in day seven that in day 2 in spontaneous ICH.

Further to the MRI studies, SPECT studies were also performed. Mayer. (1998), reported decreased CBF during the first day that normalized on day three after ICH. In the studies of Sills et al. (1996), Kidwell et al. (2001) the authors concluded that hypoperfusion was present and highest in the early hours following ICH. Similar results were also found in PET studies. Perihaematomal CBF was reduced in the hematoma site, without any evidence of ischaemia. The first study was as early as 1986 by Uemura et al. This study involved 21 patients and observed that there is CBF and CMO_2 reduction around the haematoma site which was localized. In their study, Sills et al. (1996), described hypoperfusion also around the heamatoma site. There is enough evidence to support that there is hypoperfusion around the clot but without ischaemia (Rosand et al., 2002, Carhuapoma et al., 2000).

So the perihaematomal area is hypoperfused more likely because of a hydrostatic mechanism that takes place the first hours after ICH, as an effort to normalize the perfusion. Another explanation could be a reduction in metabolic rate of oxygen, which could result in hypoactive tissue and not ischaemia (Zazulia et al., 2001). When this early hypoperfusion resolves, vasodilatation occurs and more likely implicates the secondary damage in ICH.

Overall, three phases of CBF can be identified. The first phase is an acute phase that is characterized by hypometabolism, involving the region around the haematoma, present the first 2 days of onset. The reduction in CBF and oxygen consumption is present at both hemispheres.

Second phase, is between 2 days and 14 days, characterized by the phase of reperfusion. In that phase there are mixed areas of normal flow, hypoperfusion and hyperperfusion.
The last phase is after 14 days with resolution of CBF in all areas except the dead tissue areas.

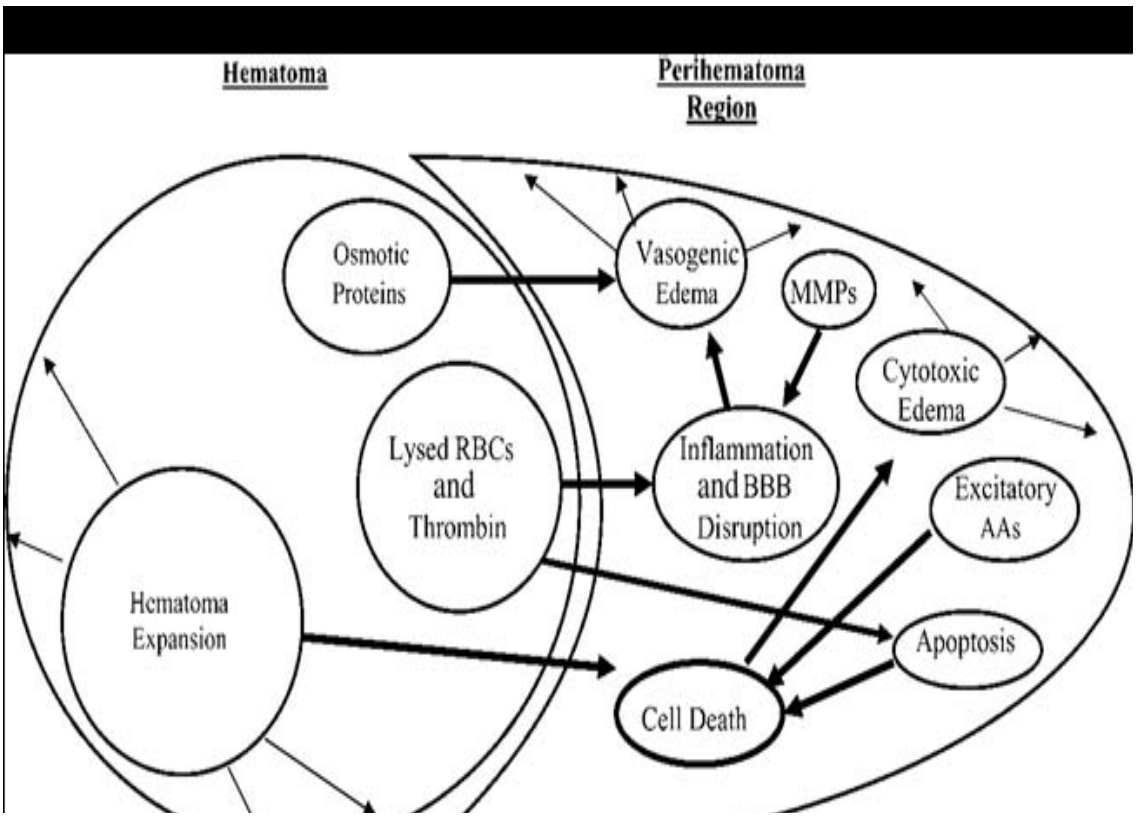


Figure 13. Summarizes the pathophysiology in the perihaematomal area of ICH. Gustavo et al. (2007).

CHAPTER VI. Surgery as a treatment of intracerebral haemorrhage.

I. Surgical treatment of intracerebral haemorrhage.

One small phrase can currently describe the surgical management in ICH: the uncertainty continuous. Currently, there are no guidelines regarding the surgical treatment of ICH. In the last edition of one of the most worldwide and common used neurosurgical textbooks, (Greenberg, 2010), the surgical input is described as follows: *'amazingly, after repeated attempts to penetrate this dilemma, considerable controversy persists regarding indications for surgery. Surgery may lower morbidity, from rebleeding, oedema or necrosis from mass effect of haematoma (unproven), but rarely causes neurologic improvement. Meta-analyses yield inconclusive or conflicting results'.*

Since the first attempt to surgically evacuate ICH clots, back in 1888 (Sir William Macewen) and 1903 (Harvey Williams Cushing), surgeons believed that for many and different reasons, surgical removal of ICH clot is of benefit for the patient, especially for those that suffer from medium to large haematomas. Until now there is no evidence that surgical treatment is better for patients despite the many different attempts, techniques, subgroups e.t.c.

It was McKissock the first that published as early as in 1961 that there is no difference in the outcome of patients suffering of ICH regarding the conservative or surgical treatment. Since this study, no much have change in the rationale of ICH treatment. So, fourty years later the overall rationale regarding surgical treatment of ICH, is well described by Fernandes et al. (2000), confirming the same overall aspects in their paper: **"**Surgery in Intracerebral Hemorrhage: The uncertainty continues".

Last decade, Surgical Treatment of Intra Cerebral Haemorrhage international trial (STICH) was undertaken in order to give an answer regarding the main question of the role of surgery in ICH. In this study, 1083 patients were enrolled. The conclusion was that for supratentorial ICH, there was no benefit of early surgery. On the other hand much criticism and many limitations of the study followed the conclusions, mainly because of selection bias, prolonged median time to surgery, high percentage of crossover (26%) and late crossovers (Mendelow et al., 2005)**.**

A recent metaanalysis of smaller studies regarding the role of early surgery, revealed opposite results (Prasad et al., 2009). In this metaanalysis, ten trials and 2059 participants were included. Surgery was associated with statistically significant reduction in death or dependency at final follow up and the heterogeneity was not significant, especially after excluding the STICH trial. These studies had acceptable quality.

Another metaanalysis of ICH treatment, revealed that surgical treatment has better outcome in comparison to medical treatment when the haematoma volume is more than 40ml and the Glasgow Coma Scale (GCS) more than 6 out of 15 (Anik et al., 2010).

Recently, Gregson et al. (2012), published another metaanalysis. This included 14 surgical trials up to 2010, concluding that there is enough evidence to support that surgery benefits if undertaken early before patient's deterioration. The ongoing STICH II trial and further studies regarding the subgroups of patients that could benefit of surgery are currently focusing on answering the precise role of surgery in ICH in more focused selected subgroups. So currently the overall practice regarding surgery in ICH, could be as detailed as described in Greenberg's textbook of Neurosurgery (2010), description which is only recommendation. At the same time, similar recommendations were also described by the Guidelines of American heart and stroke association for the treatment of ICH (2010). The 'guidelines' for considering surgery versus medical management could be described as follows:

II. Supratentorial haemorrhage.

i. NOT surgery: factors that favour non surgical management.

Small lesions with patients having Glasgow Coma Scale more than 10 out of 15 and no focal deficit, or focal deficit that is already established and surgical intervention cannot relief or improve it (Juvela and Kuurne, 1989). Severe neurological condition, large ICH and bad neurological condition on admission. High ICH score (see Table that follows), massive haemorrhage, and poor neurological condition, haemorrhage involving eloquent structures such as the dominant hemisphere, midbrain, brainstem, etc., age more than 75 years, severe coangulopathy, poor general medical condition of patient and basal ganglia haemorrhage (Batjer et al., 1990; Waga et al., 1986a,b).

Tables 4,5. ICH score and the clinical outcome. Hemphill et al., (2001).

Feature	Finding	Points
GCS score	3-4	2
	5-12	1
	13-15	0
Age	> 80	1
	< 80	0
Location	Infratentorial	1
	Supratentorial	0
ICH volume	>30	1
	<30	0
IVH	Yes	1
	no	0

ICH score	30 day mortality
0	0%
1	13%
2	26%
3	72%
4	97%
5	100%
6	? 100%

ii. Surgery, when should be considered, suggestions.

Supratentorial haemorrhage.

Consider surgery when there is marked mass effect, underlying oedema or evidence of eminent herniation. In these cases we could consider surgery as a heroic measure to a devastating condition. Usually in the current practice the decision is also conveyed to the family given the high mortality of the disease, especially when ICH score is high.

Consider surgery where there is impression and evidence on the imaging that the progression of the neurological deficit is because of the clot and the mass effect but not the direct neural damage in the brain parenchyma. That means in other words that the deficit itself is because of the raised ICP, or the focal raised pressure that could explain it. Consider surgery in medium volume clots on CT head. Usually, small haematomas (up to 10 cc), don't produce any significant deficit and the mass effect that they produce, is not of significance. In these cases surgery is not an option. In the cases of medium sized clot, sizes between 10 and 30ml of volume, we could consider surgical intervention. In cases with larger haematoma than 30cc volume, the outcome has been found to be poor (Broderick et al., 1993). In cases of haematoma volume more than 60ml, the 30-day mortality was up to 90% (Broderick et al., 1993), while in a series of patients with haematoma volume more than 85ml, mortality recorded as 100% in any kind of treatment (Volpin et al., 1984). STICH trial recorded that the outcome in patients presented with coma at onset, was devastating, confirming the previous observations of smaller studies (Mendelow et al., 2005).

The role of intracranial pressure monitoring.

In cases when patient has raised ICP or patients that they are monitored regarding their ICP, evacuation could be considered but there is no evidence if there is finally a favorable outcome. According to Monro-Kellie doctrine of course surgical intervention lowers intracranial pressure. The same consideration can apply in patients that are terminally ill with brain stem compression. A desperate measure to lower ICP and maintain life, despite the expected overall bad outcome. This could be considered especially in cases that the deterioration is rapid and the previous neurological status was at least fair.

Anatomical site of haematoma and surgical decision.

In terms of the anatomical site of haematoma, there is no enough data to support any definite decision. We could consider surgery, if the haematoma is located on the non dominant hemisphere because it seems reasonable but on the other hand not proven. The only data available that favor surgical treatment is regarding the cerebellar haemorrhage (Kobayashi et al., 1994). Better results have been observed in patients that are young, less than 50 years of age. Data regarding the timing of surgery suggests that the outcome is better in cases that the operation takes place the first 24 hrs after the onset.

Cerebellar haemorrhage.

More precise seems to be the recommendations for surgery in patients with cerebellar haematoma. Kobayashi et al. (1994), reported that patients who had GCS 14/15 or 15/15 and small cerebellar haematoma (less than 4 cm in volume) could be treated conservatively with good results. Patients with larger than 4ml haematoma volume and GCS lower than 13/15, needed surgery. In cases that deterioration is due to hydrocephalus of compression of the fourth ventricle, external ventricular drainage needs to be inserted while, when there is brain stem involvement, any intervention has poor outcome.

Location of the haematoma within 1cm from the cortical surface as mentioned in the STICH trial, seems to be favored by surgical intervention and currently is being under investigation in STICH I trial.

The above recommendations are the result of the uncertainty that continuous regarding the surgical input in the ICH. As described above, enthusiasm that followed the first operations for ICH clot removal by many surgeons at the first half part of the 20th century was overwhelmed by Mc Kissock et al. as early as 1961. This was the first prospective and randomized study regarding intracerebral haemorrhage treatment. In this study a comparison regarding the final outcome was done between surgical and conservative treatment. The result of the study was that

in patients surgically treated the outcome was overall worst in comparison to the patients that were treated conservatively.

Later on two more important studies regarding the comparison between surgical treatment and conservative management, were carried out. Both of them in 1989 with opposite conclusions-results. In the first study, Auer et al. (1989), reported the favourite outcome of clot removal in patients with endoscopically treated supratentorial haematoma. On the opposite, Juvela et al., (1989) in their prospective randomized study, showed that there is no benefit of surgery in patients with ICH.

So the century ended up with the same feeling, 'uncertainty'. This is very well described in the review of Fernandes et al. (2000), with the quite successful title: "Surgery in intracerebral haemorrhage . The uncertainty continues".

iii. The STICH trial and its importance. Changing the surgical input?

In order to reply to the question, STICH trial was commenced and carried out. As mentioned above this multicenter randomized controlled trial was one of the most important studies that were ever carried out for ICH. 1033 patients were enrolled finally, 83 centers around the world, and 27 countries. The numbers not only demonstrate the very good set-up of the study but more than that the interest for treatment solutions against this devastating with high morbidity and mortality disease.

STICH trial, divided patient in those that received surgical intervention and those that received conservative management. From the 1033 enrolled, 503 were offered surgery and 530 conservative treatment. From the follow up in 6 months, 51 were lost and 17 were alive but with unknown status. The result showed that comparing those that underwent surgery to those that were treated with conservative management, no overall benefit was evident with surgery. From the 468 patients that underwent operation, 122 had favorable outcome (26%), while from the 496 patients that were conservatively treated, 118 patients had favorable outcome (24%).

STICH trial showed that there is no benefit in surgical treatment in comparison to conservative management in patients with supratentorial ICH. But a lot of criticism and skepticism has been aroused regarding the validity and furthermore the translation of the result.

Selection bias, such as that the responsible for the decision neurosurgeon should be uncertain for the treatment (surgery or not), the fact that early surgery was determined by operation the first 24 hrs while other previous studies and the physical history of intracerebral haematoma showed that the deterioration (haematoma expansion, rebleed), usually happens the first 8 -12 hrs, so 24 hrs, could be considered late from one point of view. A lot of crossovers and especially late crossovers from the medical managed subgroup to surgery. Twenty six per

cent of the patients that had been randomized for medical treatment, crossed over to the surgical group and quite late (even 60 hours from onset).

As a conclusion, STICH trial could not answer the dilemma. Greenberg. (2010) states that : *'…This trial may be more accurately considered to be a comparison between early versus delayed surgery in patients subjectively judged to need surgery by the investigator…'*

iv. Surgical techniques for clot removal in intracerebral haemorrhage.

The idea of surgical intervention for ICH clot removal dates back in 1888, when the first operation was performed. The background was the belief that removing the clot, benefits patient because, results in lowering of the ICP and reduces the early and late oedema formation that follows ICH onset (Nehls, Mendelow , 1990; Teernstra et al., Chambers et al., 2001). Many authors have reported that except the above, perfusion is finally improved in the involved to the haemorrhage hemisphere (Nehls et al., 1990, Teernstra et al., SICHPA 2003; Mendelow et al., 2005).

The most widely, commonly and traditionally used technique, is that of clot removal via craniotomy-craniectomy, depending on the underlying oedema. The craniotomy is usually big enough in order to give access in the clot area and also to give place for oedematous brain to expand if needed. Following craniotomy, dura is opened and the site of cortex underlying the haematoma is recognized and a corticectomy is then performed in order to access the haematoma site. Subsequently, haematoma evacuation follows and the operation finishes with securing the cut bone, back in place or with the final removal of the craniotomy bone flap (craniectomy). With this operation, we finally achieve better access to the haematoma site, removal of the haematoma and more space for the oedema present intraoperatively or after the operation. Although surgical trauma is extensive especially to the deep seated haematomas affecting also the surrounding brain parenchyma, the result is good regarding the main aims of the operation (clot removal, oedema reduction, better lowering of the ICP and resolving mass effect phaenomena). Well studied procedure, commonly used with enormous follow-up because of the extension of use throughout the years.

Other more rare procedures are those of minimal invasive principles. Only recently introduced, these techniques are based as of principle in clot removal with minimizing the damage of the brain tissue and overall surgical trauma. They can also be performed without general anaesthesia, with the use of local anaesthesia, something that is of patient's benefit. Two techniques are widely used, one with stereotactic aspiration of the clot and the other with the use of endoscopy.

The stereotactic aspiration technique. Described, as early as in 1981, by Hayashi, et al. Four years later Niizuma et al. (1985), for the first time, described the use of urokinase for intracerebral clot lysis and finally removal. In (1988), Yamanaka et al. was the first to compare craniotomy, stereotactic aspiration and conservative management in putaminal haematomas. Stereotactic aspiration consists of creating a small opening in the skull and, using a small usually needle or catheter, accessing and aspirating the hematoma. First large series of patients that underwent stereotactic aspiration of haematoma, was published by Thomas et al. (1989), presenting 300 patients with CT guided stereotactic aspiration of haematoma or other lesions.

Since these first reports, attempts and series, many other authors published similar series or compare the classical craniotomy technique with stereotactic aspiration and conservative management. (Yamanaka and Satoh , 1988; Niizuma et al., 1990; Okimura et al., 1991; Horimoto et al., 1993; Kumon and Sakaki, 1993). The same period, first studies with similar techniques or slightly different technical methods where published, such as ultrasound imaging for stereotactic evacuation of ICH with aqua-stream and aspiration., by Iwamato et al.(1993).

In 1994 Lippitz et al. described the lysis of basal ganglia haematoma with recombinant tissue plasminogen activator (rtPA) after stereotactic aspiration, for the first time. This technique was used subsequently by other teams (such as Schaller et al., 1995). Similar technique with the use of streptokinase was also described the same period.

Combined use of stereotactic aspiration and intracerebral streptokinase infusion in the surgical treatment of hypertensive intracerebral hemorrhage.

Tzaan et al. (1997) was the first to introduce this technique. A multicenter randomized controlled trial (SICHPA), was published in 2003 (Teernstra et al., 2003), involving 70 patients divided in two groups. The overall conclusion was that stereotactic aspiration can be easily performed, achieves modest reduction to haematoma volume up to 18ml (in 7 days), while in the control group the haematoma reduction in the same period of time, was 7ml. This could mean that the use of rt-PA, could improve the outcome according to the authors.

In 2004 (Thiex et al., 2004), for the first introduced for the first time frameless stereotactic haematoma lysis with the use of fibrinolytic therapy. After that, many authors published similar techniques and series (Vespa et al., 2005; Barrett et al., 2005; Kim, et al., 2007). In most of the studies the use of catheter and fibrinolytic therapy of any kind, showed promising results regarding the haematoma volume reduction. The use of the technique is currently always combined with the use of fibrinolytic therapy and the results reported are overall promising. (Mohadjer et al., 1992; Niizuma et al., 1985).

Endoscopic technique for the aspiration of intracerebral haemorrhage. For the first time, endoscopic evacuation of ICH was described by Auer et al. as early as 1985**.** Following this first publication, many other authors and teams followed the technique, and compared it with stereotactic aspiration technique also (Kim et al., 1996; Kim et al., 1998; Nishihara et al., 2005; Hayashi et al., 2006). Recently, Miller et al. described image-guided endoscopic evacuation of ICH (2008).

The data regarding this technique is overall limited and is not as widely accepted as the stereotactic aspiration minimal invasive technique that was described above. Usually demands small craniotomy and via the craniotomy endoscope is being inserted for the clot removal. The results are similar to the stereotactic aspiration in terms of mortality (Kim et al., 1996 and show improved outcome in selected cases regarding medical treatment alone. (Zhu et al., 2011). While this year Zhu et al. (2012), publishes a series of K-hole endoscopic aspiration for the treatment of ICH.

Recently, STICH trial suggested that the improved use of endoscopic design and surgical technique, it could be possible to evacuate large deep seated haematomas with minimal brain tissue damage (Nakano, Ohkuma, 2005).

Conclusions.

For the time being the uncertainty continues. There is no definite answer for the role of surgery in the treatment of ICH. Despite improvements in techniques, anaesthesia, intensive care units, the overall result remains poor and as described in the epidemiology chapter unchanged the last years.

Regarding the role of the surgery, grossly for the moment only suggestions-recommendations can be done and no guidelines are available. The overall decision, should be individualized following the recommendations mentioned at the beginning of this chapter, until surgery itself find a more accurate role in the fight against this devastating condition. Newest surgical techniques need to be randomized further and data needs to be evaluated in order to determine their role in surgical treatment of ICH.

My opinion (if this permitted given my small experience) is that further studies with well defined groups of patients especially those that are borderline in terms of neurological condition and also those that are candidates for rebleeding, expansion of haematoma and early deterioration (regarding the deep seated haematomas especially), should be carried out. The same direction should also followed regarding the newly introduced and promising techniques of stereotactic aspiration and endoscopic removal of haematoma.

CHAPTER VII. Current management of intracerebral haemorrhage.

I. Diagnosis.

As previously discussed, ICH is diagnosed by the clinical picture and computed tomography (CT) or magnetic resonance imaging (MRI) of the brain.

i. Computerized Tomography and Magnetic Resonance Imaging.

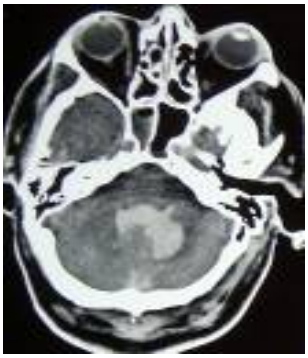
In general terms the clinical picture alone is usually non specific for the diagnosis of ICH. It can mimic ischaemic stroke like symptoms while on the other hand the presence of symptoms of raised intracranial pressure at onset, such as headache, nausea, vomiting, elevated blood pressure (as high as 90% of the cases), are usually characteristic in ICH. Another characteristic finding in intracerebral haemorrhage is the impaired level of consciousness. Comatose patients or hemicomatose at presentation, have high possibility for underlying bleeding (in comparison to ischaemic stroke).

The gold standard for diagnosis of ICH, remains the CT scan since introduction of the technique in 1974, even after the introduction of MRI (Kidwell et al., 2004).

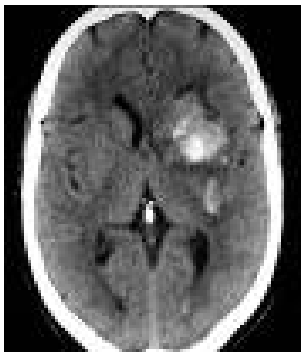
First publication dates back, as early as 1975, suggesting the useful role of CT head in diagnosis of ICH. CT scan gives us valuable data regarding the volume of haematoma, the site of haemorrhage and the relation to anatomical structures. It is very helpful for definition of surgical planning of possible intervention and is a very useful and easy to perform examination, for the follow up or in cases of deterioration.

With the use of CT head scan, we could recognize phaenomena such as rebleeding, haematoma expansion, late deterioration, lysis of haematoma and final resolution of the phaenomenon. Since the introduction of CT head, a clinical timeline of the pathophysiology could be revealed and key points in diagnosis and management could be identified. The volume of the blood can be calculated precisely or grossly estimated with the use of the ABC/2 rule, known as ellipsoid method (Kothari et al., 1995; Newman, 2007). Except of calculating the clot, the CT head scan gives as also details of an overall picture, and some useful but not diagnostic details, regarding the underlying pathology.

Magnetic resonance imaging, on the other hand, continues to be studied as a radiologic tool to diagnose ICH. For the moment, the role of MRI remains the estimation of the age and the possible aetiologies underlying the bleed such as aneurysm, AVM, tumors, or other more rare underlying pathologies such as cavernomas.



Picture 12: CT scan revealing cerebellar ICH, (personal collection).

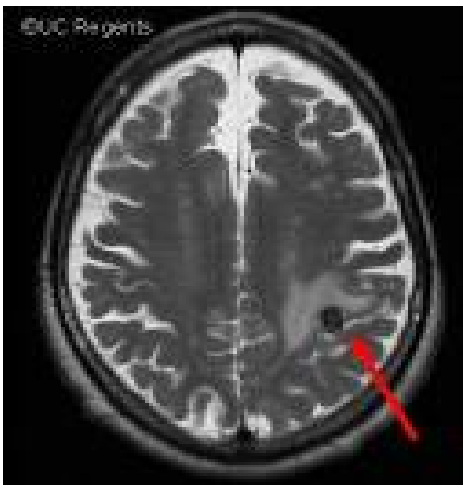


Picture 13: Computed tomograph revealing putaminal ICH.

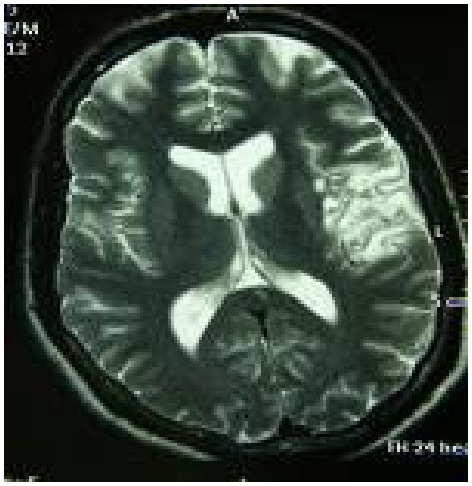
ii. Other techniques. CTangiography and Cerebral angiography (Diffuse Substraction Angiography).

The role of classical angiography, is quite diminished after the introduction of CT head scan. Although, in the past angiography played a very important role in diagnosis and treatment of intracerebral haemorrhage, and all the other brain pathologies (Raney,1950) without any doubt CT head scan replaced angiography as diagnostic tool after introduction. The earliest article specifically regarding the role of angiography in ICH, was published as early as 1953 by Lofgren .

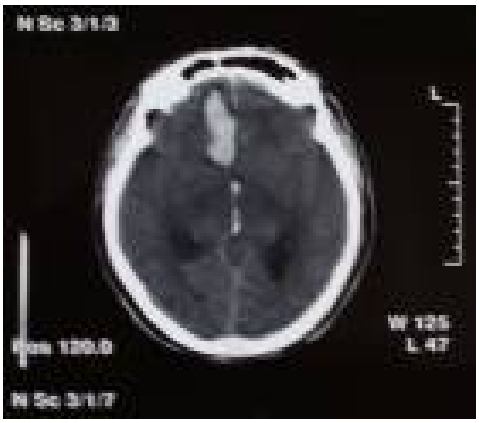
Currently cerebral angiography is only used rarely in patients with suspicion of underlying structural pathology. With the further evolution of CT, Computed Tomography Angiography (CTA), gives enough data especially for the presence of aneurysms and other vascular abnormalities, carries less risks in comparison to diffuse substraction angiography (DSA) and has been reported to be quite reliable (sensitivity 96%). In a recent comparative study and metaanalysis, the accuracy of CTA in detecting the underlying vascular abnormalities of ICH, showed that CTA, in comparison to DSA, has high sensitivity, specificity, positive (PPV) and negative (NPV) predictive value, and accuracy. The values were found to be 94.6%, 100%, 100%, 96.5%, and 97.8%, respectively. A total of 544 cases were included for meta-analysis. The pooled sensitivity, specificity, PPV, NPV, and accuracy of CTA for detecting the aetiology in ICH were 95.4%, 98.3%, 96.9%, 97.4%, and 97.2%, respectively. There was no substantial heterogeneity between the studies. Ma et al., 2012. The use of CTA (computed tomography angiography) and DSA (diffuse substraction angiography) in cases of ICH, is mandatory in cases where there is unusual anatomically haemorrhage, absence of hypertension, intraventricular haemorrhage, and in patients of young age. (Zhu et al., 1997).



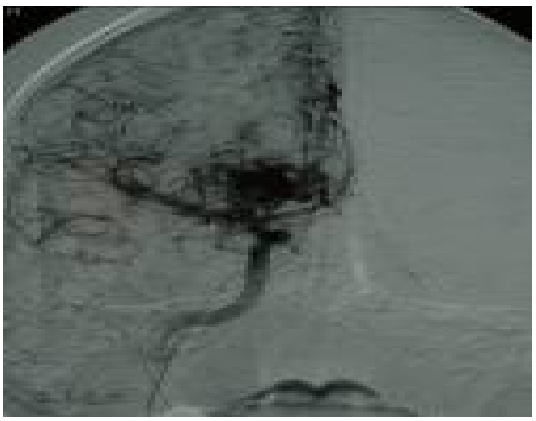
Picture 14*:* MRI showing Cavernoma.



Picture 15: MRI showing haemorrhagic transformation of previous stroke.



Picture 16: Ct head with unusual site of bleed, personal collection



Picture 17: DSA of the same patient,AVM, personal collection

II. Current recomendations and guidelines for the management of intracerebral haemorrhage. (Morgenstern, et al., 2010).

i. Integral components of the history, physical examination, and work up of the patient with ICH in the emergency department. (Table 6).

PARAMETER	COMMENT
History **Time of symptom onset (or time the patient was last normal) Initial symptoms and progression of symptoms**	
Vascular risk factors	Hypertension, diabetes,hypercholesterolemia, and smoking
Medications	Anticoagulants, antiplatelet agents,decongestants, antihypertensive medications, stimulants (including diet pills), sympathomimetics
Recent trauma or surgery	Carotid endarterectomy or carotid stenting in particular, as ICH may be related to hyperperfusion after such procedures
Dementia	Associated with amyloid angiopathy
Alcohol or illicit drug use	Cocaine and other sympathomimetic drugs are associated with ICH, stimulants
Seizures	
Liver disease	May be associated with coagulopathy
Cancer and hematologic disorders	May be associated with coagulopathy
Physical examination	
Vital signs	Fever is associated with early neurologic deterioration Higher initial blood pressure is associated with early neurologic deterioration and increased mortality
A general physical examination focusing on the head, heart, lungs, abdomen, and extremities **A thorough but time urgent neurologic examination**	A structured examination such as the NIH Stroke Scale can be completed in minutes and provides a quantification that allows easy communication of the severity of the event to other care givers. GCS score is similarly well known and easily computed, and the initial GCS score is a strong predictor of long term outcome. These can be supplemented as needed
Serum and urine tests	
Complete blood count, electrolytes, blood urea nitrogen and creatinine, and glucose	High creatinine is associated with haematoma expansion. Higher serum glucose is associated with hematoma expansion and worse outcome (although there is no data to suggest that normalization improves outcome
Prothrombin time or INR and an activated partial thromboplastin time	Warfarin related hemorrhages are associated with an increased hematoma volume, greater risk of expansion, and increased morbidity and mortality.
Toxicology screen in young or middle-aged patients to detect cocaine and other sympathomimetic drugs. Urinalysis and urine culture and a pregnancy test in a woman of childbearing age.	Cocaine and other sympathomimetic drugs are associated with ICH.
Other routine tests	
ECG	To assess for active coronary ischemia or prior cardiac injury that may indicate poor cardiac function and to obtain a baseline in the event of cardiopulmonary issues during hospitalization
Chest radiograph	
Neuroimaging	As described in the text

Table 7: The current medical management of intracerebral haemorrhage as defined by the guidelines can be described as follows:

RECOMMENDATIONS	INTERVENTION	COMMENT
Class 1 recommendations		
Emergency diagnosis and assessment of ICH and its causes	Rapid neuroimaging with CT or MRI is recommended to distinguish ischemic stroke from ICH.	(Unchanged from the previous guideline) Class I, Level A
Medical treatment for ICH patients with a severe coagulation factor deficiency or severe thrombocytopenia	Should receive appropriate factor replacement therapy or platelets, respectively.	(New recommendation) Class I Level C
Haemostasis/antiplatelets/DVT prophylaxis ,patients with ICH whose INR is elevated due to OAC	Should have their warfarin withheld, receive therapy to replace vitamin K–dependent factors and correct the INR, and receive intravenous vitamin K.	(Revised from the previous guideline) Class I, Level C
Patients with ICH should have intermittent pneumatic compression for prevention of venous thromboembolism in addition to elastic stockings.		(Unchanged from the previous guideline) Class I, Level B
Inpatient management and prevention of secondary brain injury		
General monitoring. **Initial monitoring and management of ICH patients should take place in an intensive care unit, preferably one with physician and nursing neuroscience intensive care expertise.**		(Unchanged from the previous guideline) Class I, Level B
Management of glucose. Normoglycemia is recommended		Class I, Level C
Seizures and antiepileptic drugs	Patients with clinical seizures should be treated with antiepileptic	(Revised from previous guideline) Class I, Level A

RECOMMENTATION	INTERVENTION	COMMENT
Patients with a change in mental status who are found to have electrographic seizures on EEG should be treated with antiepileptic drugs		Class I, Level C
Procedures. Surgery and clot removal	Patients with cerebellar hemorrhage who are deteriorating neurologically or who have brainstem compression and/or hydrocephalus from ventricular obstruction should undergo Surgical removal of the hemorrhage as soon as possible.	(Revised from the previous guideline) Class I, Level B
Prevention of recurrent ICH After the acute ICH, absent medical contraindications.	BP should be well controlled, particularly for patients with ICH location typical of hypertensive vasculopathy	(New recommendation) Class I, Level A
CT indicates computed tomography; MRI, magnetic resonance imaging; DVT, deep vein thrombosis; INR, international normalized ratio; oral anticoangulants		
Source: Guidelines for the Management of Spontaneous Intracerebral Hemorrhage A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association Lewis B. Morgenstern Et al., (2010).		

Table 8. Suggested recommended guidelines for treating elevated BP in spontaneous ICH (these are Class C recommendations).

SBP indicates Systolic Blood Pressure; MAP, Mean Arterial Pressure.
1. If SBP is >200 mm Hg or MAP is >150 mm Hg, then consider aggressive reduction of BP with continuous intravenous infusion, with frequent BP monitoring every 5 min.
2. If SBP is >180mm Hg or MAP is >130 mm Hg and there is the possibility of elevated ICP, then consider monitoring ICP and reducing BPby using intermittent or continuous intravenous medications while aiming a cerebral perfusion pressure above ≥60 mm Hg.
3. If SBP is >180mm Hg or MAP is >130 mm Hg and there is no evidence of elevated ICP, then consider a modest reduction of BP (eg, MAP of 10mm Hg or target BP of 160/90 mmHg) using intermittent or continuous intravenous medications to control BP and clinically reexamine the patient every 15 min.

ii. Management of the raised intracranial pressure (ICP).

Intracranial hypertension higher than 20mmhg and persisting is defined as surgically treated as described to the trauma algorithm by the trauma foundation (as early as 2000). Patient suffering of elevated intracranial pressure according to Diringer. (1993), should be treated with intracranial icp monitor. Preferable with the insertion of an intraventricular catheter which allows cerebrospinal fluid draining. But this is not widely accepted and currently not in common use. The ICP bolt insertion allows as taking measures in order to lower ICP values. For example the head position can be adjusted in order to lower the ICP.

Mannitol or hypernatraemia. There is insufficient data to support the use of either mannitol or hypertonic saline as a prophylaxis. Bhardwaj and Ulatowski. (1999), suggested the use either of mannitol or hypertonic saline for the treatment of raised ICP in patients with increased oedema and eminent herniation. The limitations are osmolarity 310-315 mOsm/Lt when mannitol is used or serum sodium 145-155 mEq/lt.

Hyperventilation, as known from the Traumatic Brain Injury management is beneficial and fast. It is well known that causes decrease in cerebral blood flow with immediate effect. This measure has many limitations and especially the high possibility of rebound intracranial hypertension. The aim is for $PaCO_2$ goal of 27 to 30 mmHg and usually in a gradual manner over 24-48 hrs.

Corticosteroids are proven to be ineffective in ICH management (Poungvarin 2004; Poungvarin et al., 1987). A recent clinical trial, for the use of steroids in ICH and aneurysmal subarachnoid haemorrhage, concluded that there is no evidence of a beneficial or adverse effect of corticosteroids in patients with either subarachnoid haemorrhage or primary ICH (Feigin et al., 2005). Further studies are of need in this area because the data is old before determining more precise predictors of deterioration and outcome in ICH.

Neuromuscular paralysis. Sedation can be used for lowering intracranial pressure .On the other hand, barbiturate coma, is one the last weapons in cases of uncontrolled intracranial pressure. All these data, comes from the experience of traumatic brain injury and only limited data comes from patients suffering of ICH (Dereeper et al., 2002).

The role of hypothermia in ICH, is currently under investigation with the CINCH trial (Kollmar et al., 2012), in order to determine the efficacy of hypothermia or not in ICH patients, based on previous experimental data available regarding the neuroprotective role of hypothermia, Ginsberg. (2003).

Decompressive craniectomy in ICH is also a measure for lowering ICP in these patients. There is not enough data to support the wide use of decompressive craniectomy although cohorts suggest that it could reduce mortality (McDonald , Carter, 2002; D'Ambrosio et al., 2005; Fung et al., 2012). Further clinical trials are of need in order to determine the role of decompressive craniectomy in ICH.

III. Recommendations for surgery (see also relevant Chapter).

For most patients with ICH, the usefulness of surgery is uncertain *(Class IIb; Level of Evidence: C).* (New recommendation).

Specific exceptions to this recommendation follow.

Patients with cerebellar haemorrhage who are deteriorating neurologically or who have brainstem compression and/or hydrocephalus from ventricular obstruction should undergo surgical removal of the haemorrhage as soon as possible *(Class I; Level of Evidence: B)*. (Revised from the previous guideline). Initial treatment of these patients with ventricular drainage alone rather than surgical evacuation is not recommended *(Class III; Level of Evidence: C).* (New recommendation).

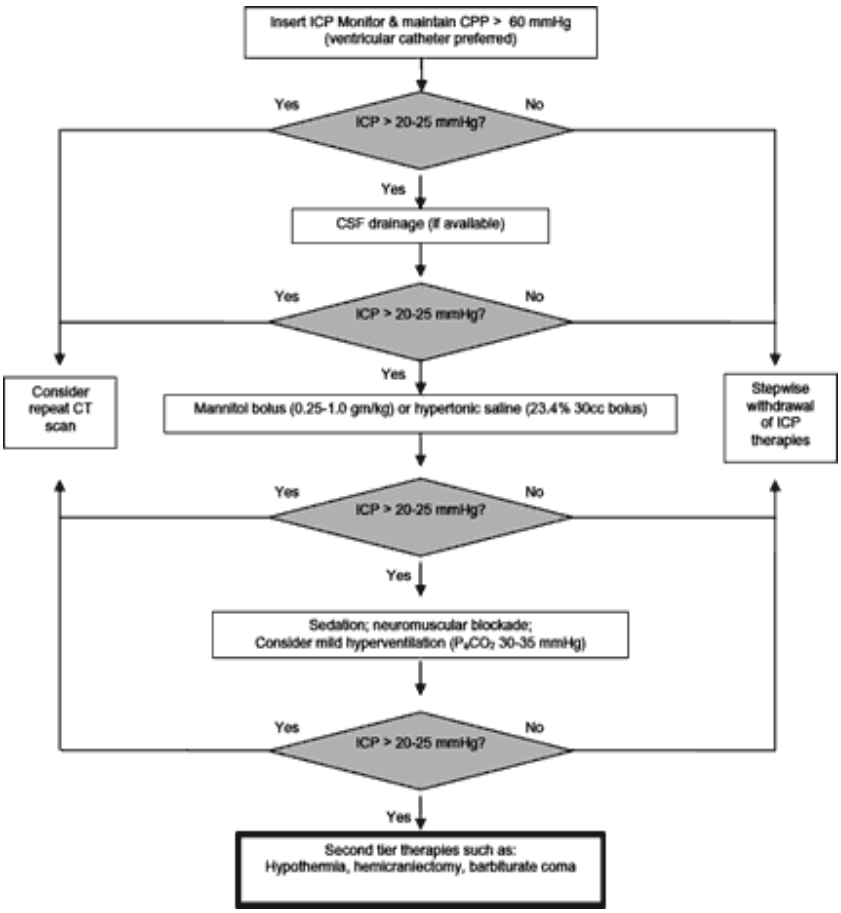


Figure 14. Intracranial pressure treatment algorithm. CPP, indicates cerebral perfusion pressure; CSF, cerebrospinal fluid. Adapted from Brain Trauma Foundation Head Injury Guidelines. Copyright 2000, Brain Trauma.

For patients presenting with lobar clots >30 mL and within 1 cm of the surface, evacuation of supratentorial ICH by standard craniotomy might be considered *(Class IIb; Level of Evidence: B).*(Revised from the previous guideline)

The effectiveness of minimally invasive clot evacuation utilizing either stereotactic or endoscopic aspiration with or without thrombolytic usage is uncertain and is considered investigational *(Class IIb; Level of Evidence: B).* (New recommendation)

Although theoretically attractive, no clear evidence at present indicates that ultra-early removal of supratentorial ICH improves functional outcome or mortality rate. Very early craniotomy may be harmful due to increased risk of recurrent bleeding *(Class III; Level of Evidence: B).* (Revised from the previous guideline).

Summary.

The overall current data suggests that there is much to determine in ICH treatment. In contrast to the previous decade, we have more answers regarding the treatment of ICH. There are more precise Class I recommendations regarding coagulation issues, epilepsy, glucose management, thromboprophylaxis, as described above. Currently the appropriate management of BP in ICH is under investigation. The management of elevated intracranial pressure in ICH follows the general measures that are used for the management of traumatic brain injury, while the role of surgery is not overall defined given the uncertainty regarding the precise role of surgery in the undoubtedly multiparammetric ICH management, as described extensively in the separate chapter of this study (surgical treatment of ICH). There are many questions to answer in developing a finally effective overall and individual strategy for a better overall outcome in these patients.

CHAPTER VIII. Prognosis of intracerebral haemorrhage.

I. General prognostic features.

There is marked heterogeneity in the available data regarding ICH. A lot of studies from different authors, different methods of collection, analysis, cohorts and subgroups have been carried out in the past. There are a lot of studies regarding large groups of patients but with general classifications in the inclusion criteria and less studies with smaller number of patients, regarding more specific haemorrhagic locations and more precisely selected data.

Early studies, focused on determining general terms in association with the final prognosis. Age, anatomical site of haemorrhage, previous history of hypertension and other prognostic factors that have been extensively described in the chapter of risk factors, were studied regarding their effect in prognosis. Amongst them the level of consciousness on presentation, seems to be the most consistently associated clinical sign with the outcome in ICH (Furlan et al., 1979; Garde et al., 1983; Mayr et al., 1983; Nath et al., 1983; Drury et al., 1984; Helweg et al., 1984; Steiner et al., 1984). Several authors have described the independent predictors of outcome in intracerebral haemorrhage (Dixon et al.,1985; Portenoy et al., 1987; Senant et al., 1988; Tuhrim et al., 1991; Daverat et al., 1991; Tuhrim et al., 1999 ; Hemphill et al., 2001; Garibi et al., 2002; Cheung , 2003).

Summarizing, the haemorrhage volume, intraventricular expansion, the level of consciousness are found to be independent factors determining survival. Other factors such as midline shift, oxygen saturation, electrocardiographic abnormalities, age, or glucose levels, were not consistent to allow a definite strong association as a result.

II. Long term prognosis.

Most of the data collected, is regarding the first month outcome and especially the mortality rate. Fewer studies have been carried out regarding the long term survival and disability after 6 months.

Garde et al., as early as in 1983, reported the outcome in 100 cases of ICH, mentioning that, 63 patients amongst them, survived over the first month. They found GCS and haematoma size to be predictor of the final outcome and the overall final status was 34 paretic (with only 5 of them not being ambulatory), 35 able to return to work and 6 remained in hospital or in rehabilitation units. Fieschi et al., 5 years later (1988), reported that the long term outcome of 104 patients was 69 survivors, and in the first year follow up, 51 had made a good or excellent

recovery. From the total, 18 patients had severe neurological deficit that remained unchanged. These patients were older, with significant larger haematoma at onset than the rest patients.

Daverat et al. (1991), followed up 166 patients with ICH and the results were 95 survivors more that 6 months while 78% where found to be independent. The authors didn't find relation between age and outcome, but age was found to be related with the final functional recovery.

In another study, Tuhrim et al. (1991), studied the 30-day survival in relation to GCS, volume of haematoma, pulse, pressure and intraventricular extension. They used Barthel's index in terms of outcome and they observed that the model they used correctly determined the outcome in 95% of the patients in one year's follow-up.

STICH trial secondary outcomes in terms of 6 months survival and disability, gives valuable and up to date data regarding the overall outcome in ICH.

Table 9. Secondary outcomes of the STICH trial.

STICH TRIAL OUTCOME	**Early surgery N=468**	**Conservative management N=496**	**Absolute benefit**
Primary outcome			
Favorable	122 (26%)	118 (24%)	2.3 (-3.7 to 7.7)
Unfavorable	346 (74%)	378 (76%)	2.3 (-3.7 to 7.7)
Not recorded			
Secondary outcomes			
mortality			
Alive	304 (64%)	316 (63%)	1.2 (- 4.9 to 7.2)
Dead	173 (36%)	189 (37%)	1.2 (- 4.9 to 7.2)
Prognosis-based Rankin Index (modified)			
Favorable	152 (33%)	137 (28%)	4.7 (-1.2 to 10.5)
Unfavorable	312 (67%)	351(72%)	4.7 (-1.2 to 10.5)
Not recorded	4	9	4.7 (-1.2 to 10.5)
Prognosis based Barthel Index			
Favorable	124 (27%)	110 (23%)	4·1 (–1·4 to 9·5)
Unfavorable	341(73%)	377 (77%)	4·1 (–1·4 to 9·5)
Not recorded	3	10	4·1 (–1·4 to 9·5)

In this study although the primary goal wasn't the long term follow up of patients with intracerebral bleed, we can collect very useful data regarding the outcome in both surgical and conservative management. We can see that the mortality rate is 36% for the surgical and 37% for the conservative management, while the prognosis based Barthel index is favorable in 27% and unfavorable in 73% for the surgical group and 23% and 77% subsequently for the conservatively treated group. That means that the overall outcome despite all methods of treatment remains poor in patients suffering of ICH.

III. Specific prognostic Features.

i. Hydrocephalus.

The impact of hydrocephalus in the final outcome, is not quite clear. It seems that hydrocephalus (when present) is a poor sign for the final outcome. It is important also to deferientate hydrocephalus caused by expansion of large intracerebral bleed with hydrocephalus resulting from a small thalamic haemorrhage. It seems that hydrocephalus is present in as much as 50% of the cases of intracerebral bleed.

Kumral et al. (1995), observed that hydrocephalus was an important predictor in mortality in a study of 100 patients with thalamic haemorrhage. Diringer et al. (1998), in their multivariate analysis of 81 patients suffering of ICH, observed that 40 of them, had a degree of hydrocephalus and worse outcome in comparison to those without hydrocephalus. In the hydrocephalus group, mortality rate was as high as 50% while in the group without hydrocephalus mortality rate was 2%.

Phan et al. (2000), studied 100 ICH cases, divided them in two groups, those with medial (thalamic and caudate), or lateral (putaminal) haemorrhage. They found that hydrocephalus was present in 86% of the patients who finally died and especially in the lateral group which was associated with much larger intracerebral bleed that could explain the high mortality rate in that group.

ii. Anticoagulant related haemorrhage.

First reports regarding the role of anticoagulation in ICH, underlined the signigicance of anticoangulation. Forsting et al. (1991), reported that 20 amongst 40 patients suffering of ICH (on anticoagulation), did not survive and 5 of them developed subdural haematomas. Another study, by Radberg et al. (1991), reported that amongst 200 patients that suffered ICH and was on warfarin, 57% died in the group that ICH was anticoagulant related, while, the mortality rate for the whole group was 30%.

Rosand et al. (2004), studied 435 ICH patients above the age of 55. Amongst them 102 were on warfarin at the time of haemorrhage. The use of warfarin, more than doubled the 3-month mortality. The higher the INR on admission, the higher the mortality rate was. Fang et al., (2005), observed that in those that were not on anticoagulation, elevated prothrombin time was recorded to those that finally died. This report although it comes from a population based study (Taiwan), suggests that non iatrogenic coagulopathies play an independent role in ICH. Haematoma in patients on warfarin, seems to expand more than the expected and later than the usual expansionwe observe in common ICH cases (Flibotte et al. 2004).

iii. Haematoma expansion.

Excluding warfarin as a cause of haematoma expansion, other causes associated with haematoma expansion are those of high systolic blood pressure. One study estimated that patients with high systolic blood pressure had twice the rate of haematoma expansion (Willmot et al., 2004). The expansion over the first 24 hrs found to be as high as 32% (Mayer et al.,2005).

iv. Blood pressure.

There are many studies suggesting that elevated blood pressure is associated with haematoma expansion and worse outcome. Chen et al. (1989) was the first to note that persistent hypertension is present in 6 out of 8 ICH before haematoma enlargement, 4 of them died. Clinical deterioration was associated with haematoma enlargement. Leira et al. (2004), showed that early neurologic deterioration was associated with systolic blood pressure, IVH and enlargement of haemorrhage.

v. Intraventricular haemorrhage.

Independently studied as a factor associated with bad outcome, showed that there is independent association between intraventricular haemorrhage and outcome (Tuhrim et al., 1991; Young et al., 1990; Willmot et al., 2004).

IV. Anatomical features regarding prognosis.

i. Putaminal haemorrhage.

Kanaya et al. (1992), proposed a classification scheme. He indicated that lesions that involve only the anterior limp of the internal capsule (Grades I and II) have better outcome in comparison to those involving the posterior limb (Grades III and IV) or thalamus (Grade V). The overall prognosis of putaminal haemorrhage is poor.

ii. Caudate haemorrhage.

Patients with caudate haemorrhage, are divided into two groups. In both groups the final outcome is overall good. The first group has the symptoms of raised intracranial pressure with headache, vomiting and usually intraventricular haemorrhage. These patients usually recover completely. The second group consists of patients that have involvement of internal capsule in some degree, so they usually have hemiparesis and conjugate gaze paresis. These patients have low mortality rate. (Stein et al., 1984).

iii. Thalamic haemorrhage.

Thalamic haemorrhage account for 10-15% of ICH cases. Overall survival in thalamic haemorrhage is poor and worse in comparison to putaminal haemorrhage. Several studies showed that haematoma size is a prognostic factor for the outcome in these cases. There is no definite size of haematoma associated with mortality rate. As early as in 1977, Walshe et al. showed that cases with haematoma diameter more than 3.3 cm, were finally fatal, while the overall mortality was 50%. Similar studies showed similar results also.In conclusion, thalamic lesions more than 3cm in diameter have very high mortality rate. Intraventricular extension of the haematoma when present, isusually is associated with worse outcome (Weisberg, 1986a).

Other useful data is that in thalamic haemorrhage, the level of consciousness at presentation is associated with the final outcome. It is also observed that posterior thalamic lesions are associated with mild final neurological deficit (Piepgras et al., 1981; Hirose et al.,1985)

iv. Lobar haemorrhage.

Lobar haemorrhage may carry a lower mortality rate than ICH in other locations. Reported mortality rates range from 9% to 32%. In approximately one third of cases there is history of hypertension, those with hypertension, had worse outcome (Ropper and Davis ,1980; Weisberg,1985; Tanaka et al., 1986). In the same studies, it was observed that haematoma size, initial presentation in terms of consciousness level, intraventricular expansion and midline shift, were prognostic factors of the final outcome.

v. Pontine haemorrhage.

Rare entity of approximately 5% of all cases. (Silverstein, 1967).Prognosis depends on the extension of the pontine haemorrhage. In cases where haemorrhage is extensive, with extension in the ventricles, the result is usually fatal. Smaller haemorrhages are not usually fatal (Tuhrim et al., 1982). Prognosis in these small haemorrhages is excellent. (Payne et al., 1978; Burns et al., 1980; Weisberg et al., 1986b; Yamanaka, 1988).

vi. Cerebellar haemorrhage.

Cerebellar haemorrhage usually has a good outcome if the presentation is good. If the presenting neurology is that of coma, final prognosis is poor despite the surgical intervention for evacuation of haematoma. The overall mortality rate in these cases is as high as 72% (Ott et al., 1974; Little et al., 1978). The overall survival rate for patients with cerebellar bleed is 80% in thirty years follow-up.(National Institute of Neurological Disorder and Stroke Data Bank series).

Conclusion.

It seems that there is a slight improvement in prognosis of ICH that last decades, and this could be attributed to the wide use of neurointensive care units. (Diringer and Edwards, 2001). Not only the treatment offered is better but the intensive observations, give valuable data for improving the overall treatment and finally prognosis. Other authors suggested that there is no overall change in the prognosis of ICH despite the technological improvement in diagnosis, treatment and overall evolution of medicine.

CHAPTER IX. Preclinical models of intracerebral haemmorhage.

I. Introduction.

Preclinical models of ICH, are without any doubt a very important tool in research of the pathophysiology and treatment options. Although there are several limitations most of which we will extensively describe in this chapter, these models have provide us with valuable data in order to describe the possible mechanisms involved in ICH and finally to explore solutions in a old pathology with poor results of any followed treatment.

More than that given, that the post-mortem studies of ICH and the clinical data is limited, especially regarding the pathophysiology of the entity, experiments gave as the data to schedule techniques and also to direct clinical research in several areas. Without any doubt, data regarding pathophysiology, and treatment options, has mostly been based on experimental observations.

The importance of research, clinical or laboratory, has recently been described and prioritized by the National Institute of Health (Stroke workshop, 2005). In this report the need of developing experimental models that will be clinically relevant to the actual pathophysiology of ICH, was prioritized, aiming to better understand the pathophysiology and establish new therapeutic approaches ,determining finally effective treatment options to reduce the mortality and morbidity rates.

At present, there are two models available and widely used in experimental ICH. These are the collagenase model and the autologus blood injection model. In the past other models have been used in order to produce intracerebral haematoma such as the hypertensive model and the amyloid angiopathy model.

Preclinical models of ICH, date back in 1963 introduced to caninnes, next effort was in 1987 by Kingman and Mendelow, introducing the microballoon model, following years Rosenberg et al., introduced the collagenase model in 1990 and Yang et al. (1994), presented the double injection model. All the above models have been used in several animal species, such as canines, rats, rabbits, pigs and mice (Ma et al., 2011).

In general terms, hypertensive models are those that more precisely represent the pathophysiology of ICH, given that they are relevant to the actual phaenomenon but their disadvantage is that they have high variability. The extend and site of haematoma; as well as the overall parameters of the haematoma occurrence, are difficult to be compared and analyzed. Similar to that model, another one has been proposed, with the use of the diversion of the femoral artery to the brain, in order to produce the haematoma. This has the advantage of well

and accurately producing clot but on the other hand is quite difficult to reproduce. The history and most important models of experimental ICH have recently been reviewed by Lucas et al. (2008) and Ma et al. (2011). Currently, the most usually and widely used preclinical models of ICH, are those of collagenase and the double injection model.

II. The collagenase model.

Introduced for the first time by Rosenberg et al. (1990). The main idea, was based on the collagenase actions. Collagenases are intracellular enzymes that catalyze the degradation of collagen which is structural element of basal lamina and part of BBB. The injection of collagenase, causes haemorrhage at the injection site as a result of its main action. This model is near the real human pathophysiology of ICH and also has high reproductivity of the haemorrhage. It is simple and the haemorrhage is usually dose dependent. It has been successfully used in many species such as pig mouse and rat (Rosenberg et al., 1990).

The problem with this model is that collagenase, has an inflammatory result in the brain parenchyma. There is no definite answer regarding the influence of the independent inflammatory action of collagenase in the brain parenchyma, although in vivo experiments with the use of collagenase does' not produce inflammatory response (Matsushita et al., 2000). This is the main disadvantage of the collagenase injection model in comparison to the whole blood injection model. Advantages of the collagenase model, are the relativeness of the real pathophysiology, easiness of data collection and accumulation, easy reproduction and the usefullness in studying transgenic effects. Lastly, this model by the effect of collagenase, mimics conditions of rebleeding. The main disadvantage remains the direct inflammatory action of collagenase itself to the brain parenchyma.

III. The autologus blood injection model.

Widely used, first described in the mid – 80s. The model consists of the injection of autologus blood in the brain parenchyma, via cannula over a specific period of time and while the animal is anaesthetized. This model has also disadvantages. Main disadvantage is that the haematoma being developed, is not from the rupture of small vessels as it happens with collagenase for example and that there is high possibility of back flow of blood at the injection site.

This was an early and often observation when this model was introduced. Other disadvantages are those of intraventricular rupture which changes the overall pathophysiology

of the experiment, and that the model does not mimics the rebleed conditions that the collagenase model mimics (Yang et al., 1994).

Later on, in order to resolve this techniqual issue, (Deinsberger et al., 1998), introduced the double injection technique to the already used whole blood injection model. By this technique, blood injection into the brain parenchyma,follows two phases. Firstly small amount of autologus blood is being injected in the brain and is being left to clot around the needle. Secondly a further (the rest) amount of blood is being subsequently injected over a specific period of time. The result of the technique is a well formed clot without any back flow incidents. The model also allows for transgenic observations.

Whichever is the model used, there are advantages and disadvantages, given that currently there is no definite model to precisely mimic human intracerebral haematoma. The use of one or the other model and especially the animal species for research, should take into account what is the main question of the experiment scheduled, the cost and current rationale. Generally it seems that large animal models are better for physiology studies of ICH, whereas, rodent models are better for observing molecular mechanisms.

IV. Regarding animal species (which and for what experiment).

i. Primate.

Excellent for translational studies, but very complex to organize a study with them. They are very good for physiology studies, and measurement of very important physiological parameters in the past such as CBF changes, oedema, penumbra around the haematoma site, study of vessels changes and ischaemia (Laurent et al., 1976; Bullock et al., 1988). The cost, housing, and ethics, difficult to afford. These are the reasons that they are rarely used in experiments in our days.

ii. Feline.

More often used in the past, rarely used at present because of the cost, replaced by other models. In the past they were used mainly to study physiological parameters such as intracranial pressure and cerebral blood flow (Kobari et al., 1988; Tomita et al., 1994).

iii. Canine.

Used in the past for physiological and surgical studies. Two main models have been applied to canines in the past. the hypertension model and the autologus blood injection models. Many studies have been done, regarding regarding parameters, such as oedema, lethal volumes of clot,

ischaemia in the perihaematomal region, CSF, ICP, CBF and even microdialysis (Lillehei et al., 1974; Sussman et al.,1974; Steiner et al., 1975; Sugi et al., 1975; Takasugi et al., 1985).

Currently, they are in use for standardizing methods such as surgical techniques, or diagnostic studies (Mukai et al., 1991; Tamura et al., 2006; Wessmann et al., 2009; Zhou et al., 2010; Fulkerson et al., 2012; Wu, 2012).

Many studies have been carried out regarding possible treatments of ICH, with the option of clinical appliance of the findings (Qureshi et al., 1999; Powers et al., 2001; Qureshi et al., 2002; Ware et al., 2005). The use of these models is widely spread; with many studies related to these models..

iv. Porcine models.

There are a lot of similarities in the anatomy and physiology of porcine in comparison to human brain. They are commonly used when there is need for large model of ICH.

Their use is valuable in determining neurophysiological aspects in ICH, and especially neuromonitoring (changes in ICP, CPP,CBF,microdialysis), oedema formation and changes in the perihaematomal area, evoked potential dynamics, changes in the cortex excitability, new surgical techniques, pathophysiological changes in terms of primary and secondary injury and CT and MRI studies. There is wide use of this promising model in neurophysiological studies, and especially surgical techniques.

v. Lapine.

Introduced for the first time in 1980 by Kaufman et al. the autologus blood lapine model of ICH, is used in studying surgical techniques (such as the clot lysis with the use of urokinase), pathophysiology (such as the role of iron and haeme oxygenase-1), even MRI techniques regarding ICH. (Narayan et al.,1985; Koeppen et al., 1995; Gustafsson et al., 1999; Qureshi et al., 2001b; Alemany, et al., 2002; Qureshi et al., 2003; Koeppen et al., 2004; Thai et al., 2006; Aydin et al., 2006). The model has good appliance, is cost effective, and is widely used because can be considered a useful model between large and small animals. So it is commonly used when larger animals are difficult to be used.

vi. Murine.

Widely used because they have many advantages regarding other models and especially the most expensive ones. Both models have been used in the past in murine (autologous blood and collagenase). The most important difference regarding other models is the percentage of white

matter which is more that the other above mentioned models. They were at first used for ICP studies (Bullock et al., 1984; Nath et al., 1986; Sinar et al., 1987; Kingman et al., 1988).

Table 10. Selection of the most important studies regarding porcine models in intracerebral haemorrhage experiments.

Electrophysiology	Mun-Bryce et al. (2001), Mun-Bryce et al. (2004a,b), Manley et al.(2006), Mun-Bryce et al. (2006), Orakcioglu et al.(2012)
Neuromonitoring	Wagner et al. (1998), Shimoda et al. (1998), Rise et al. (1998), Rieger et al. (1999), Malhotra et al. (2004)
Oedema and perihaematomal regional changes	Wagner et al. (1996), Mayfrank et al. (2000), Wagner et al. (2005)
Surgical techniques	Wagner et al. (1999), Rohde et al. (2001), Rohde et al. (2002), Thiex et al. (2005).
Pathophysiology, antioxidant treatment	Clark et al.(2008), Rohde et al. (2008), Gu et al., (2009), Gu et al., (2011)
Diagnostic exams (CT , MRI)	Miot-Noirault, (1997), Küker et al., (2000), Rohde et al., (2001),

v. The importance of preclinical models in intracerebral haemorrhage research.

Without any doubt the extensive use of preclinical models thelast decades, was very effective. Without the use of preclinical models, ICH mechanisms and pathophysiological pathways wouldn't have been understood and the data would have been quite limited. With the use of preclinical models, it was achieved to study the secondary injury mechanisms underlying the pathology, introducing new options for therapeutical interventions. It was important to determine the role of inflammatory processes that follow the primary insult. Inflammatory processes were revealed and in some details explored. In summary, oxidative stress, cytokine release, microglia activation, astrocytic activation, apoptosis, the role of NF-kB, MMPs, iron, blood degradation products, etc, are few of the most well explored pathways in ICH, which without the use of preclinical models it would be very difficult to explore. On the other hand, the models that are currently in use have their limitation. They do not precisely represent the pathophysiology of ICH in humans. They don't mimic the phaenomenon because of techniqual limitations at present.

In one of the next chapters of this study, regarding the antioxidants treatment in ICH, we will see that most of the antioxidants agents that was studied in in vivo models of ICH, and finally was clinically tested in patients, didn't have the expected from the preclinical models results. This could be translated by the fact that the models are not representing the actual pathophysiology of humans or that there are differences in the physiology between the species.

CHAPTER X. Neuroprotection in intracerebral haemorrhage. The importance of antioxidants .

The basic mechanisms that are involved in the pathophysiology of ICH have extensively been discussed in the first chapter. Most important of them are in summary, the role of: thrombin, matrix metalloproteinases, haeme and iron, glutamate, complement, proinflammatory cytokines and other inflammatory mediators. The idea of antioxidant therapy in ICH is old and aims in finding solutions regarding a possible treatment in a pathology that few effective strategies have been established. The overall idea, dates back when antioxidants were started to be used for many other pathologies such as ischaemic brain injury due to stroke, or traumatic brain injury. Last decade, new data regarding the pathways of ICH, gave more specific and detailed targets for possible intervention. Many studies have already been carried out especially regarding possible neuroprotective agents. Most of the agents come from experimental data from ischaemic stroke and traumatic brain injury because these are the pathologies that were investigated firstly and most extensively, before the first data regarding ICH started being collected. In the second part of this chapter we will analytically describe the available data regarding lazaroids (21-aminosteroids), while on this chapter we will focus in general aspects of oxidative stress and antioxidant treatments.

I. Underlying mechanisms of central nervous system pathology from the perspective of oxidative stress.

i. Acute Central Nervous System Injury.

Since the early 80ties, lipid peroxidation of cell membranes, ferum ions and physical antioxidants, were found to play an important role in CNS pathology (Braughler et al., 1986; Youdim et al., 1988; Bromont et al., 1989; Wang et al., 2004; Back et al., 2004). Nowadays we know more about pathophysiological mechanisms that follow the first insult in CNS injury, while new underlying mechanisms, reactions and procedures are continuously being described. Ischaemic injury, presents to be a common insult in most CNS pathological situations, such as cardiac arrest, subarachnoid hemorrhage, CNS trauma and stroke of any kind. We can recognize that three steps do take place up to the final ischaemic result:

1. The first ischaemic event, the primary cause in other words.
2. The first physiological reaction against the ischaemic incident. That means a variety of pathophysiological procedures that take place in order to respond to the ischaemic insult. The physiological defense. The period that follows the first event, overwhelms the acute

physiological endogenous reaction and results to the final (secondary ischaemic) injury (Taylor et al., 1996; Wang et al., 2004; Back et al., 2004; Dietz et al., 2004; Lucas et al., 2006).

3. Two main pathophysiological procedures can be described. The first one is the whole CNS ischaemia (global) and the second one is the limited in an area ischemia (local). In the first condition ischaemia is referred to the whole brain (such as in cardiac arrest), and in the second condition, ischaemia is limited in a small or greater area of the brain, depended on the artery that has been occluded, or where the traumatic area is located. This two pathological situations are very different in comparison to each other, due to the difference of the cause and the events that follows the acute injury.Changes that follow the first event, are extremely complex and not fully understood for the time being. The most important changes that follow the ischaemic incident in general terms are:

Blood Brain Barrier (BBB) integrity disruption (Zucarello et al., 1993; Brown, 2003), which results in membrane permeability changes, changes in ions homeostasis (Halliwell and Gutteridge, 1989, Brown et al., 2003), consequently in ion's concentration changes inside neuronal cells, most important of which is the increase of calcium ions concentration (Brown et al., 2004). Other events include changes in protein expression (Oliver et al., 1990; Pulsinelli, 1992), DNA damage (Back et al., 2004), free radicals formation, release and action (Braughler et al.,1986). The underlying mechanisms that follow the ischaemic event, result in ischaemic injury of the nervous system tissue with neuronal cells being the most vulnerable (Braughler et al., 1986, Gennarelli, 1997).

The main reason for this vulnerability is that neuronal cells, depend their metabolism to glucose. The final result is apoptosis and neuronal cell death (Back et al., 2004; Gennarelli, 1997; Pulsinelli, 1992). Recently, free radicals and their action in the pathophysiology of neuronal damage, are considered to be of great importance (Braughler et al., 1986; Halliwell and Gutteridge, 1989). Many experiments and clinical studies aim to fully reveal the role of free radicals in the ischaemic injury. So nowadays we know a lot about their role in most CNS injury pathologies.

ii. Oxidative stress and mechanisms of oxidative stress, the role of free radicals.

In CNS damage, several mechanisms that take part result in free radicals formation. It is well known that free radicals are being produced in mitochondria during ischaemic conditions, as a result of the lack of oxygen (Halliwell and Gutteridge, 1989; Simonian et al., 1996; Love, 1999, Lewen et al., 2000). Other procedures that form free radicals are neurotransmitter's oxidation, such as epinephrine, norepinefrine and dopamine (Simonian et al., 1996). Many enzymes such as mono-amino-oxydase, tyrosine-Hydroxylase, L-amino-oxydase, while

metabolized, produce H_2O_2, while common enzymes catalyze the production of active oxygen species, such as phospholipase-A_2. As a result, araxydonic acid is produced, which is further metabolized in eicosanoids, reactions that produce oxygen free radicals (Lewen et al., 2000). Ascorbic acid oxidation itself, also produce H_2O_2. So does catecholamine oxidation (Coyle et al., 1993). The most common free radical that is produced is $O_2^{\cdot -}$ This free radical react further with H_2O_2 producing OH·. Recently, another pathway reveals that $O_2^{\cdot -}$ reacts with endogenous NO·, producing $ONOO^-$, which further reacts with ONOOH, forming OH· (Ebadi et al., 1996; Yossi et al., 2002; David et al., 2004). Another pathway that results in free radicals production, is glutamate metabolism.

Free radicals are known as very reactive oxidants responsible for several changes that take place in neuronal tissue after central nervous system injury of any cause, such as trauma (Hall et al., 1989), stroke of any origin (Hall et al., 1989; Demirkaya, 2001), subarachnoid hemorrhage (Sandrzadeh et al., 1993; Gaetani, 1998). They affect molecular proteins, DNA and lipids. New findings, suggest that all the reactions described above and result in the production of free radicals, form together a new entity called oxidative stress. The exact description, is that oxidative stress is the situation that free radical's concentration cannot be overwhelmed by the endogenous mechanisms, imbalance which results in secondary neuronal tissue damage (Ebadi et al., 1996; Jenner et al.,1996; Simonian et al., 1996; Love, 1999; Lewen et al., 2000).

iii. Lipid peroxidation and neuronal damage.

As described above, during the ischaemic-reperfusion incident, many free radicals are being formed mainly in mitochondria, from monoamine neurotransmitters and leucocytes that invade central nervous system after trauma and hypoxia, especially during the reperfusion phase (Halliwell et al., 1984; Braughler et al., 1986; Minotti et al., 1989). Free radicals themselves are responsible for ferum ions emission from haemoglobin, transferritin, and ferritin. Free ferum ions that being released are responsible for severe damage in cell membranes, which finally leads to rapture of neuronal cell's membrane and death. Ferum ions, act catalyzing cell membrane lipid peroxidation forming free radical oxidants. The main result is damage in all lipid dependent enzymes that are present in the membrane lipid bilayer, Na^+,K^+-ATPase and Ca^{++}-ATPase (Garriedo et al., 1998). As a result the calcium ions concentration increases into the neuronal cell and phospholipase A_2 produces araxidonic acid (Lewen et al., 2000). Further metabolites production such as prostaglandin E_2, Leukotriene B_4 and platelet activating factor, enhances inflammation (Garriedo et al., 1998).

Oxygen free radicals and leukotriene B_4, activate neutrophils in the damaged tissues. The final result of all these mechanisms of oxidative damage, lead to cell death, leakage of

cytoplasm components and arachidonic acid into the extracellular environment. This action takes place in all cells neuronal, glial and vascular. The membrane lipid bilayer damage in vascular endothelium, leads in BBB damage in all brain tissue pathological situations, as described above (Coyle et al., 1993). Lipid peroxidation process as a radical-driven chain reaction, involving oxygen and follows a very complex series of reactions well described by Roberto Federico et al.(1992). Lipid peroxidation is a very important phenomenon in CNS pathological situations (Lebel et al., 1991; Roberto Federico et al., 1992), because cell's membranes of the brain, are very rich in polyunsaturated fatty acids, which have reactive hydrogens that can take part in several reactions of lipid peroxidation. Brain is poor in antioxidant capacity comparing with other organs, because is poor in catalase, superoxide dismutase and glutathione peroxidase. So CNS is more vulnerable in lipid peroxidation and free active scavengers formation. It is proven that some areas of the brain are rich in ferum ions, which are released during the injury process. CSF, has much less concentration in transferritin, compared to plasma, resulting in minor efficacy to react with free ferum ions. CNS, has a huge concentration of neurotransmitters that produce H_2O_2 when they get oxidized.

iv. Mechanisms that follow lipid peroxidation in central nervous system trauma and ischaemia.

One of the early incidents that take place, is glutamate release (Benveniste et al., 1984; Hagberg et al., 1985; Faden et al., 1989; Siesjo, 1992a; Siesjo 1992b; Bullock et al., 1995; Davalos et al., 1997). Glutamate releases into the extracellular space, which results in activation of glutamate's receptors and finally in neuronal depolarization. If depolararization insists, the result is cell death. The entire procedure, finally results in increase of intracellular calcium ions concentration, which further results in cell membrane ions permeability homeostasis disruption. In this environment, any change in CBF, can result in energy failure and cell death. Generally, it is believed that the two mechanisms that are involved in brain damage are free radicals and glutamate release, both mechanisms seems to affect calcium ions homeostasis (White et al., 1984; Bose et al., 1992; Siesjo, 1993).

There is evidence that there is a connection between the two pathways (Pellegrini et al., 1990). Further changes in calcium ions homeostasis, results in extracellular ions concentration increase, glutamate release and free radicals formation (phospholipase activation). Reperfusion in this environment, forms further free radical molecules. It is believed that NO acts in a similar way. The way that NO acts is not completely determined yet. It is well known that intracellular calcium ions increase, activates NO-synthetase, results in NO concentration increase, and

finally free active radicals are formed and released (Hayberry et al., 1992; Cazevielle et al., 1993; Fagni et al.,1994).

New data reveals many mechanisms that take part after the first incident of ischaemic injury in CNS. The formation of free radicals, results in genes expression, such as NF-kB (Schneider et al., 1999), AP-1, HIF-1, SP-1 (Sen, 1998; Chan, 2001), suspends cell death, leads to cytokines, free radicals, toxins formation, while intracellulary leads to cycloxygenase-2, NO, metalloproteinase, and cytokines formation. These gene expressions, results in free radical formation BBB integrity, leading finally in apoptosis and cell death (Chan, 2001).

The above description represents part of the complex reactions and mechanisms that follow the first incident in CNS pathology. In fact the mechanisms are more complex. New data suggests that many mechanisms and molecules get involved in the secondary brain damage. Recently a more detailed and accurate description, includes the role of xanthine oxidase, superoxide dismutase, catalase , glutathione oxidase, nitric oxide synthetase, metal chelators, polypolymerase, and many other molecules that take part in the complex reactions of oxidative stress.

II. The role of antioxidants in central nervous system pathology.

Very early, free radicals and their leading role in the pathophysiology of brain injury in several central nervous system pathologies, placed them in the center of most of most efforts to find new therapeutical solutions. Most research in neuroprotection last years aims to find a way to stop oxidative stress procedures by finding agents that scavenger free radical formation and consequently their oxidative capacity. The last two decades, many agents have been tested in order to find if they could be effective for therapies. (Hall, 1988a; Hall, 1988b; Hall et al., 1994; Hall et al., 1997). Antioxidants are molecules that react with free radicals, resulting in inactivating them. These molecules by scavenging free radicals, achieve to protect the brain tissue from oxidative stress and its harmful consequences. Most known and studied antioxidants are vitamins E, C (Braughler, 1989; Roberto Federico et al., 1992), carotinoids (Acheson et al., 1983; De Kumar et al., 1988; Padh, 1990; Bendich, 1993; Chang et al., 1998), synenzymeQ10 (Ogawa et al., 1986; Noack et al., 1994; Forsmark et al., 1997; Grieb et al., 1997), melatonin (Beyer et al., 1998; Kilic et al., 1999) alpha-lipoic acid (Cao et al., 1995; Woltz et al., 1996; Packer, 1998) , hyperoxidismutase analogs (Tagaya et al., 1992; Young et al., 1996), n-acetylcysteine (Moldeus et al., 1986) glutathione (Larsson et al., 1983; Muller et al., 1984; Shivakumar et al., 1995; Iwata et al., 1999), metal ions (Palmer et al., 1994; Zhang et al., 1998; Sarco et al., 2000), uric acid (Ames et al., 1981; Benzie et al., 1996; Yu et al., 1998), creatinine

(Holtzman et al., 1998; Balestrino et al., 1999; Sullivan et al., 2000)., lazaroids (Hall et al., 1988b; Anderson et al., 1991; Singh,1991; Clark et al., 1995) , pyrimidines (Bundy et al., 1995; Hall et al., 1997) ,PBN, Ebselen (Muller et al., 1984; Johshita et al., 1990; Saito et al., 1998; Ogawa et al., 1999,) , MCI 186, NXY-059(Peeling et al., 2001), MDL74,180, Nicaraven, spin traps (Taylor et al., 1996), albumin, (Ginsberg, 2003) . Table 11, summarises the most well known categories of antioxidants in CNS pathology.

TABLE 11. Categories of antioxidants or free radical scavengers, with most important examples per category. Roberto Federico Villa and Antonella Gorin, (1997).

Antioxidant category	**Most important antioxidants compound**
Endogenous enzymes	superoxide dismutase, catalase, glutathione peroxidase
Endogenous antioxidants (most importants)	alpha-tocopherol, ascorbic acid
Other endogenous antioxidants substances	Uric acid,glutathione,melatonin,endogenous antioxidant cofactors, e.g., selenium, coenzyme Q10
Precursors and derivatives of endogenous antioxidants compounds and enzymes	acetylcysteine, polyethylene glycolsuperoxide dismutase,metal chelators, e.g.,deferoxamine
Metal chelators	deferoxamine
Naturally occurring plant substances	flavonoids (in Ginkgo biloba and black tea), lycopene, Guilingji (a Chinese herbal)
Synthetic free radical compounds	21-aminosteroids,pyrrolopyrimidines,ebselen
Compounds with other primary beneficial therapeutic effects	selegiline,probucol,carvedilol,aspirin,magnesium, statins.

TABLE 12: Efficacy of antioxidants in the treatment of cerebral ischemia and intracranial hemorrhage in clinical studies. Roberto Federico Villa and Antonella Gorini, (1997).

antioxidant	disease	efficacy	references
Vitamins E,C, Carotenoids, Flavonoids	Ischemic stroke	+ - 	Gey et al., 1993 Keli et al., 1996 Daviglus et al., 1997 Hennekens et al., 1996
Ebselen	SAH	+/- + -	Yochum et al., 2000 Ogawa et al., 1999 Saito et al., 1998
Tirilazad mesylate	SAH Head injury Spinal cord injury	-, + - -	Kassel et al., 1996 Haley et al .,1997 Marshall et al.,1998 Bracken et al ., 1997
Nicaraven	Head injury SAH	- +	Young et al., 1996 Asano et al., 1996

III. Recent summary of data regarding agents that are being under investigation, regarding their potential antioxidant role in intracerebral haemorrhage.

i. Free radical scavengers.

In the collagenase injection model, the use of dimethylthiourea (a scavenger of hydroxyl radical) or *α*-phenyl-*N*-*tert*-butyl nitrone (a spin trapping agent), showed that there was less severe neurological deficit but no difference in neuronal loss (Peeling et al., 1998). Edaravone, was clinically tested regarding its effectiveness in ischaemic stroke and ICH. Findings were oedema reaction and DNA damage when administered in rats (Nakamura et al., 2008). NXY-059, in the collagenase model in rats, showed that diminishes neurological impairment, and suspenses apoptosis in the perihaematomal region (Peeling et al., 2001). The results of the clinical trial that followed the experimental data were not encouraging (Lyden et al., 2007).

ii. Growth factors and cytokines.

A clinical study of patients with ICH, revealed that high levels of growth factors in serum, resulted in better recovery. These factors were the vascular endothelial growth factor, the G-CSF (granulocytecolony stimulating factor-cytokine) and angiopoietin-1. These observations

date as early as 1974 (Sobrino et al., 1974). Later on, (Park et al., 2005), extensively studied the effects of G-CSF and found that overall improves the final outcome in terms of neurological deficits, reduces oedema, preserves BBB idegrity, and suspenses apoptosis. Erythropoietin, found to reduce oedema, apoptosis, activated microglia, TNF-a expression, and being affected by activation of caspaces (Lee et al., 2006; Grasso et al., 2009).

iii. Minocycline.

Extensively studied regarding its neuroprotective properties, found to have anti-apoptotic, antioxidative and anti-inflammatory actions. Power et al. (2000), described the most important effect of minocycline in the brain, followed by many authors such as Yong et al. (2004). Generally minocycline was found to decrease IL-1β, expression, and the same findings were observed regarding MMP-12 (inhibition). Marked effects also were recorded in macrophages and microglia activation at the perihaematomal area with important impact in reducing apoptosis. Interesting is that the treatment with minocycline, was responsible for better overall behavioral outcome in the animals studied (Wu et al., 2009).

Szymanska et al.(2006), determined that there is a time window in order minocycline to be effective. They observed that if administered after 3 hrs, there is no beneficial overall effect in the brain infarct reduction and the final behavioral deficit. Other studies had different results, regarding the role of minocycline such as affecting the secondary injury accumulation in terms of decreasing activation of microglia, macrophages, expression of cytokines such as TNF-α, suppressing MMPs and especially MMP-12, but no effect in final neuronal loss outside the haematoma site. Recently, Xue et al. (2010), observed that when minocycline is locally administered in high doses, had better results in the overall neuroprotection in their mouse model.

iv. Statins.

Their main action is that of inhibition of hydroxymethylglutaryl-CoA reductase. Recently and currently under investigation, statins, found to significantly reduce the neurological deficits of ICH when administered in rats (Seyfried et al., 2004). Drugs that currently under investigation for their neuroprotective role from this category are atorvastatin and simvastatin, with differences in their action. The way they act is still not completely understood. It seems that they affect the synaptic plasticity. The final impact is that they reduce haematoma volume, tissue loss, oedema formation, brain atrophy, apoptosis, decreases neutrophils and microglia/macrophages population, improving finally the overall neurological outcome (Seyfried et al., 2004; Jung et al., 2004; Karki et al., 2009).

The experimental data seems highly suggestive for a positive neuroprotective role of statins while on the other hand the clinical data is not so encouraging. The first retrospective clinical study that was done, comes from Johns Hopkins Hospital. In their study, enrolling ICH patients treated between 1999 and 2006, pretreatment with statins showed decreased mortality, and perihaematomal oedema (Naval et al., 2008a; Naval et al., 2008b). While FitzMaurice et al. (2008), studying the effects of statins in ICH, found that there is no association between the prior use of statins and the final neurological outcome, in their prospective cohort study.

v. Nuclear receptor ligands.

In this recent category many agents have been studied. Amongst them PPARγ (Peroxisome proliferator-activated receptor-γ, responsible for lipids and glucose metabolism genes) stimulators, such as 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) (Drew et al., 2006; Zhao et al., 2006), rosiglitazone (Zhao et al., 2007) and dexamethasone (Lema et al., 2004; Savard et al., 2009), all of them with observations of antioxidant neuroprotective action. Van Neerven et al. (2008), observed that RARs (retinoic acid receptors), when stimulated, suppress micloglia activation, recently also observed that tamibarotene when administered protects dopaminergic neurons from inflammation (Katsuki et al., 2009; Kurauchi et al., 2010).

Matsushita et al. (2010), studied the effects of Am80 (tamibarotene), and found that there was no impact on the injury volume or effect on brain oedema formation but there was significant impact in reduction of microglia/macrophages population and oxidative stress in the area around the haematoma. They also observed that there was an overall improvement in the final neurological outcome.

vi. Other targets for pharmacological intervention in the intracerebral haemorrhage.

Rodrigues et al. (2003), studied the effect of tauroursodeoxycholic acid (TUDCA), known for his anti-apoptotic action. The effect was reduction of apoptosis and improvement of the overall neurological outcome. Sinn et al. (2007a), examined the impact of valproic acid, and histone deacetylase inhibitor, observing that they both upregulate neuroprotective genes. Other pathways such as cystamine, ubiquitin-proteasome system and AT1-angiotensin receptors have also been examined with limited data regarding their role (Jung et al., 2007; Sinn et al., 2007b; Okauchi et al., 2009). Hiroshi et al. extensively and recently (2010), the most important agents under investigation for their neuroprotective effects.

CHAPTER XI. Neuroprotective antioxidant efficacy of lazaroids.

I. Introduction.

In my experimental protocol, I decided to study the antioxidant role of U -74389G in ICH. The reason was that there are few overall studies regarding the antioxidant role of U-74389G and has never been tested in ICH. The category of lazaroids is a wide category of agents that are tested in the past regarding their antioxidant role and especially tirilazad mesylate (U-74006), showing promising results in several intracranial pathologies. They form a very important category in basic research and are considered quite potential neuroprotective agents. The clinical trials of tirilazad mesylate, were not of statistical significance but this doesn't mean that the same applies to the rest molecules of the category.

Twenty years have passed since the first experiments considering the possible neuroprotective – antioxidant role of 21-aminosteroids (Lazaroids), took place (Anderson et al., 1988; Hall et al., 1994; Hall et al.,1998b). Until now, the first very encouraging experimental results considering 21-aminosteroids to be of mayor neuroprotective importance in CNS pathology, have been overwhelmed by the results of the clinical trials that took place and finished last decade. So the last years, few only studies have been carried out (after the clinical studies results) considering the antioxidant role of 21-aminosteroids (lazaroids).

Many researchers consider that this incorrespondance between laboratory and clinical trials is a result of many potential reasons. Some of them believe that the main reason is the clinical trials schedule, and the way they were carried out. Other researchers believe that this is due to many parameters that are different between animals and humans, parameters that we don't exactly know for now, so it is difficult for the time being to combine laboratory tests with clinical trials. Many researchers believe that most of the causes of these incorrespondance played a separate role for the final result of tirilazad mesylate trials. It is important to schedule and perform new more detailed and accurate laboratory studies, so that to understand and bridge the difference in the results between laboratory and bedside in antioxidant therapy and finally establishing the possible antioxidant efficacy of 21-aminosteroids and other antioxidants in CNS pathology.

Similar results have been also observed for many other agents studied. Although for example statins found to play significant neuroprotective role in preclinical studies, clinical trials failed to show any positive result. On the other hand there is no doubt that many mechanisms that take place in several CNS pathological situations, have been more extensively examined and understood the last years (Back et al., 2004; Dietz et al., 2004; Wang et al., 2004; Lucas et al., 2006).

The available experimental data is more detailed now comparing to the last decade. It is also clear that many antioxidant agents haven't fully been examined in experimental studies and clinical trials, while many other are currently under investigation (Braughler et al., 1988a; Youdim et al., 1988; Minotti et al.,1989). From the "family" of 21-aminosteroids the only molecule that is extensively examined is tirilazad mesylate, while many other molecules from the same category have already proven to have major antioxidant efficacy in experimental data, but are not fully examined yet and especially in CNS pathology.

II. The antioxidant-neuroprotective role of lazaroids (21-aminosteroids).

Lazaroids are chemical compounds, which have similar to corticoids actions, without the known for the corticoids side effects. They haven't glucocorticoid or mineralcorticoid effects. The category includes many compounds with similar pharmacological characteristics, and some differences in chemical structure and possible in other pharmacological characteristics.

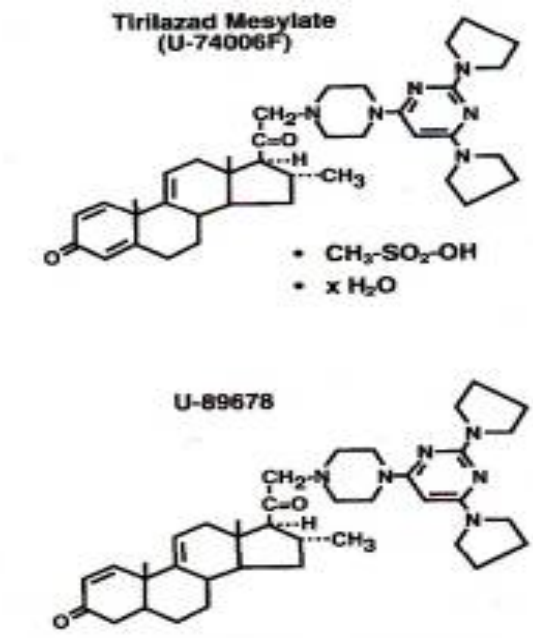


Figure 15: Tirilazad mesylate and its metabolite in humans. Roberto Federico Villa and Antonella Gorini, (1997).

Most known and well studied, is tirilazad mesylate (Hall 1988a; Anderson et al., 1991; Xue et al., 1992; Sanada et al., 1993; Park et al., 1994; Fleishaker et al., 1995; Kassel et al., 1996; Bracken et al., 1997; Haley et al., 1997, Marshall et al., 1998). Tirilazad mesylate, was the most

studied molecule last decade, and was studied not only in experimental studies, but also in clinical trials. Figure 15, shows the chemical structure of tirilazad mesylate and its metabolite in humans. Other molecules of 21- aminosteroids, less studied for their antioxidant efficacy are U 83836, U 74389, U 78517F, most of them under experimental studies the last years. Figure 16, shows the chemical structure of U 74389G that we used in our study.

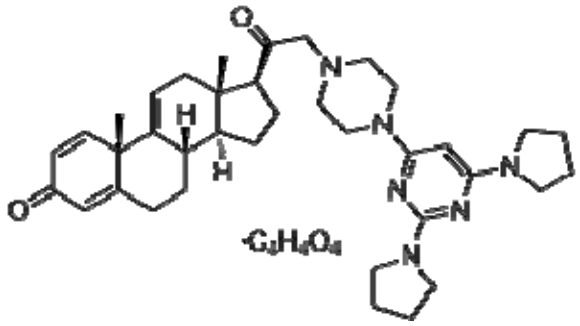


Figure 16: The U-74389G (21-aminosteroid) that we used in our study.

Synonym:

21-(4-(2, 6-Di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl)-pregna1, 4, 9(11)-triene-3, 20-Dione-(Z)-2-butenedionate

Description: A 21-aminosteroid (lazaroid) which is a highly lipid-soluble inhibitor of lipid peroxidation. Inhibits lipid peroxidation in a variety of systems, including cultured brain microvessel endothelial cells, Cu^{2+} treated monocytic THP1 cells, and LLC-PK1 cell layers, where it blocks F2 isoprostane production. It has also been shown to prevent organ damage in intestinal cold storage and preservation and in reperfusion injury (Katz et al., 1995; Salahudeen et al., 1995; Campo et al., 1996).

III. Pharmacokinetics- Pharmacodynamics- Effects and actions of lazaroids.

Lazaroids are synthetic antioxidants, and their main effect, is suspension of lipid peroxidation. They act with two main mechanisms:

1. They act as free radical scavengers (Hall et al., 1989; Hall et al., 1991; Sato et al., 1992). They stabilize the membrane lipid bilayer. The stabilization role is due to the efficacy lazaroids have, to react easily with the phospholipids of the lipid bilayer. (Hinzmann et al., 1992, Hall et al., 1994).

2. Their chemical attraction to the lipid bilayer, stabilizes the membrane and helps lipids to defend again lipid peroxidation. Figure 17, represents the way tirilazad mesylate reacts into membrane bilayer. New data reveals, that lazaroids have other effects, such as the defense against toxicity of glutamate, TNF-α, and cytokines, in CNS pathologies (Domenica Altavilla et al., 1998; Selda Kabadere et al., 2004). Some experimental data suggests that they act in glioma cells suspending glioma's cells multiplication. Their action is believed to be cytotoxic and not cytostatic (Durmaz et al., 1999).

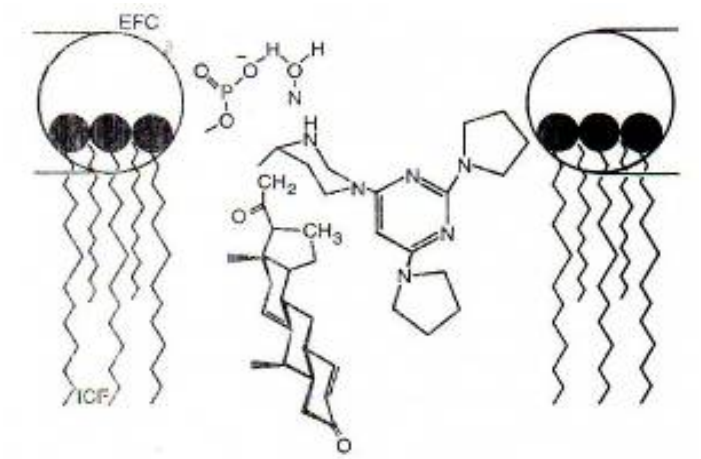


Figure 17 . Schematic presentation of the way tirilazad mesylate reacts into membrane lipid belayer. Roberto Federico Villa and Antonella Gorini, (1997).

IV. Tirilazad Mesylate.

Most studied molecule from the category of lazaroids is tilizarad mesylate. Basic characteristics of the molecule, is that react easily with lipids due to chemical attraction, enters poorly BBB and concentrates in the vessels endothelium. One of the most important actions is that protects BBB integrity, because protects the vessels endothelium (Hall et al., 1994; Fernandez et al., 1997).

i.Pharmacokinetics.

Tirilazad mesylate pharmacokinetics, are shown in the Table that follows, in the next page:

TABLE 13: Basic pharmacokinetics of tirilazad mesylate, the most well studied lazaroid

Author	Dose in mg/kg	administration	Plasma concentration	Half lifeh	Side effects	Steady state (days)	Excretion
Fleishaker et al., 1993a	0.25-0.50 1.00-2.00	IV 10or 30 min infusion at final concentration of 1.5mg/mL	20ng/ml> 99% bound to proteins in humans	Human 3.7 h Rat 50h	Local pain Increase in lymphocyte counts		In feces 12% in Urine
Fleishaker et al., 1993b	0.5-1.00 2.00-4.00 6.00-(day)	IV, every 6 hours	20ng/ml	35	Increase in liver enzyme activities	5	As above
Laizure et al .,1993	10	IV infusion after 0-10-20-40min and 1.5-2-3-4-6-8h from injection		Rat 2.4 Brain1.9 Heart 1.6 Liver 1.5			Clearance 6.1ml/min
Hulst et al.,1994	1.5 3.0	Single infusion over 10 min	10 ng/ml	14.7			25% lower in the elderly

In general terms tirilazad is well studied comparing to the other molecules, most experimental and clinical trials last decade referred to this compound. In brief, tirilazad, is metabolized in the liver, has half-life time of 35 hrs, (needs 5 days to reach stable concentration), and it's main metabolite is U-89678 (Weinkers et al., 1998). Tirilazad, reacts with other molecules such as phenyntoin, and becomes inactive. Deferences in secretion of the main metabolite, between males and females were also observed (Lew et al., 1993; Hulst et al., 1994). The possible reason is that there is a difference in liver blood supply between males and females or maybe tirilazad itself metabolizes differently in two sexes. (Wienkers et al., 1995).

ii. Side effects.

Both clinical trials and experimental studies, proved that tirilazad mesylate, is a safe molecule with no effects on cardiac rhythm, glucose plasma concentration, no glucocortcoid or mineralcortycoid effects. (Fleishaker et al., 1993).

V. Experimental and clinical data regarding tirilazad mesylate.

i. Experimental data.

The antioxidant efficacy of lazaroids, with the lack of considerable side effects on the other hand, in comparison to corticosteroids, put them under investigation from the early beginning. Many experimental and laboratory studies were carried out, especially with tirilazad mesylate which leaded to clinical trial and multicenter studies in humans. So lazaroids were examined in traumatic brain injury (McCall 1987; Hall et al., 1994a; Hall, 1995; Durmaz, 2003), spinal cord trauma (Anderson,1991; Hall,1992), subarachnoid haemorrhage (Hall et al., 1994), ischaemic stroke (McCall, 1987; Hall et al., 1994; Prange,1994; Hall, 1995). Recently enough, in other pathological situations such as Parkinson (Brundin et al., 2000) and gliomas (Durmaz et al., 1999). The experimental results were enthusiastic in most studies, so clinical trials were designed in order to prove the efficacy in humans. Clinical trials were scheduled and carried out using tirilazad mesylate (U-74006F), in traumatic brain injury (Straw et al., 1995; Marshall et al., 1998), ischemic strokes (RANTTAS,1996; RANTTAS II, 1998), subarachnoid haemorrhage (Kassel et al.,1996; Haley et al., 1997; Lanzino et al., 1999a,b), spinal cord injury (Bracken et al., 1997)

The results in contrast to the experimental data were disappointing. The result was the containment of research for the antioxidant role of lazaroids, although many researchers had several objections or posed questions regarding the schedule of the clinical trials and final results.

ii. Clinical trials regarding traumatic brain injury.

The pathophysiology of CNS trauma, involves free radical formation, and lipid peroxidation. Very important role in the pathophysiology are playing ferum ions that are being released from the blood cells. The final result is membrane lysis and cell death. Lazaroids act stabilizing the membrane's lipid bilayer and preserving in that way ions homeostasis. In TBI, two main polycentric clinical trials were performed. The two trials were finally contradictory. The first clinical trial, which took place in North America, stopped before ending, because an increase in death rates were observed in the tirilazad patient group, although finally, there was no statistical significant difference in death rates, when the results were further analyzed in six months follow up (Straw et al., 1995). This trial was stopped before ended. The second trial, carried out in centers in Europe and Australia, involving 957 patients with severe TBI, and 163 patients with mild TBI. In this clinical trial, no statistical significant difference was documented between the groups of patients. On the other hand a statistical significant difference was

observed in patients suffered of post traumatic subarachnoid haemorrhage (Marshall et al., 1998).

iii. Clinical trials regarding spinal cord injury.

In spinal cord trauma a double blind multicenter trial was carried out. In that trial a comparison between methylprednisolone and tilizarad mesylate was performed. In total, 499 patients were admitted and separated in three groups. All of them were admitted and treated the first 8 hrs from the onset of traumatic injury. All of them took a single dose of methylprednisolone. The first of the three groups, took methylprednisolone for 24 hrs, the second for 48 hrs and the third took tilizarad mesylate for 48 hrs. The results didn't establish any efficacy of tilirazad mesylate in comparison to methylprednisolone.The group that took tilizarad mesylate (2.5mg/kgr/6h for 48h), had in general less side effects in comparison to the other groups, but the difference wasn't statistically significant.

About the effectiveness and the clinical outcomes in patients, the tirilazad mesylate group, had equal recovery rates in comparison to the other two groups, but the better result was observed in the 48 hrs to the methyprednisolone group. Further more detailed analysis showed that tilizarad mesylate group had worse clinical kinetic results in comparison to the other two groups. Many investigators believe that the cause of this result is the low dose of tilizarad that was used, or the duration of the therapeutic scheme that was followed.

iv. Clinical trials regarding tirilazad mesylate in ischaemic stroke.

In ischaemic stroke, two clinical trials were carried out: RANTTAS I (The RANTTAS investigators, 1996; RANTTAS II, 1998). Both two trials, failed to reveal the efficacy of tirizarad mesylate in ischemic stroke. The first one, RANTTAS I. (1996), was a double-blind prospective trial, in which 660 patients were included. The patients took tirizarad mesylate in dose 6mg/kg/day for three days and the results were analyzed in three months follow up. In this clinical trial, no statistical significant positive effect of tirilarad mesylate was documented. The possible reason may be the dose or the therapeutic scheme that was followed. After these results, a second clinical trial was scheduled, based on the first one, the RANTTAS II.(1998), clinical trial. This trial included 252 patients that took higher doses of tirilazad mesylate compared to the RANTTAS I trial. The results were 14% reduction in death rates in both males and females, difference that probably due to the number of patients, didn't prove to be of statistical significance.

v. Clinical trials regarding tirilazad mesylate in subarachnoid haemorrhage.

Subarachnoid haemorrhage pathophysiology, presents with differences and similarities in comparison to other CNS pathologies. Vasospasm is of main importance, BBB disruption, edema formation and elevated intracranial pressure are also important mechanisms. Lipid peroxidation, especially in vessels plays the major role in vasospasp occurence. Clinical trials of tirilazad mesylate considering the possible positive effects in subarachnoid hemorrhage, started in 1995, with the first results (phase II), proving to be very encouraging in a three months follow up (Haley et al.,1995). Two multicentre clinical trials were scheduled and performed. One located in Europe and Australia-New Zealand and the other one in America. The two studies were finally contradictory. In details, in the first one, 1023 patients were included, separated into four groups (three groups with different tirilazad mesylate doses and one placebo group). From all these groups, the one that took tirilazad mesylate in higher dose (6mg/kg/day), had better results and less death rates compared to the other groups in a three months follow up (Kassell et al., 1996). In the second trial (Haley et al., 1997), a higher dose of tirilazad mesylate was administered (15mg/kg/day), for ten days. The three months follow up documented no statistical significance in neurological outcome and overall death rates. The possible reasons for the differences between the two studies probably are the therapeutic protocol (doses, duration), the group formation, and the use of other medicine that affect tirilazad mesylate such as phenyntoin.

New studies were scheduled and carried out recently. Two of them were of main importance. The first one was carried out in Europe, Australia, New Zealand and South Africa, including 819 women who took tirilazad mesylate in a dose of 15mg/kg/day. The study showed that there was a difference in vasospasm appearance in the tirilazad group (24.8%) in comparison to the placebo group (33.3%) (Lanzino et al., 1999). But a statistical significant difference between the two groups in a three months follow up failed to be established. A similar clinical trial, located in North America (Lanzino et al., 1999), included 830 patients and administration of high tirilazad mesylate doses, documented that there was a difference in death rates in patients that were stage IV, V of subarachnoid haemorrhage (poor prognosis) and took tirilazad (24.6%) in comparison to those that took only placebo (43.4%). In the majority of the studies that were carried out, a difference if efficacy of tirilazad mesylate was observed between males and females (Haley et al., 1995; Kassell et al.,1996).

Summary.

Last years, neuroprotection in CNS pathologies is considered of main importance. Many neuroprotective agents have been studied in the past and new ones are being under investigation

for the time being. It is generally accepted that neuroprotection must be considered as a strategy against the numerous mechanisms that take place in CNS injury. There is no doubt that all these mechanisms that being activated after the first event (primary injury), are complex enough, so the use of one and only agent separately may be not enough to overwhelm the pathophysiological changes that take place. On the other hand the pathophysiological changes are not yet fully understood (Kwan, 2002; Ginsberg, 2003). Under this era, it would be better to approach neuroprotection, as a multiparametric strategy. In comparison to the last two decades, new data has been collected, in several areas. We understand better the complexity of phaenomena that follow the first incident in CNS primary injury, we know more things about the homeostasis of neuronal tissue, and finally we have more experimental and clinical data about the role and the effectiveness of many antioxidant agents. The result is that we can approach the injured CNS, in a more detailed and more multiparametric manner. This seems to be the key point in establishing a more complete than in the past neuroprotective strategy (Delanty, 2000; Kwan, 2002; Ginsberg, 2003, Warner et al., 2004). Most researchers agree that neuroprotection needs a combined approach in which, antioxidant therapy plays an important role, not fully understood and explained yet (Danton et al., 2004, Warner et al., 2004,). Many neuroprotective-antioxidant agents have already been examined and many others are being under investigation for the time being (Kavanach et al., 2001; Danton et al., 2004; Warner et al., 2004). Although the experimental and laboratory data suggests the efficacy of some antioxidant agents that have already been examined, most clinical trials that were done, failed to prove the importance of these agents in humans. There are many reasons for such an outcome. Before concluding that antioxidants that were examined in clinical trials are not at all effective, there are some questions that must be answered. In table 14 (next page), synoptic presentation of the above is being made.

Firstly, one of the probable questions is if there are several not recognised parameters that affect the results of the trials. Is there any bias; a metanalyssis of tirilazad mesylate in stroke, that was published recently, concludes that bias played an important role (Emily Sena et al., 2007). Many questions arouse for the experimental models that are in use and the significance they have to the human's pathophysiology especially when most experiments that took place in the past were in small animal and a few only in bigger ones (Danton et al., 2004; Emily Sena et al., 2007). This argument could be a possible explanation of the insignificance between laboratory and bedside.

TABLE 14: Synoptic presentation of the main reasons that may have leaded in inability to establish the statistical significance (if present) of antioxidants in clinical trials in comparison to experimental data.

• Inappropriate theories about the action of the drug in the ischaemic territory.
• Inappropriate to human stroke animal models.
• Effectiveness in animal models doesn't means and in humans. • Differences between brain structure, function, vascular anatomy between humans and animals.
• Drugs may inhibit only one pathway and not the others that take place.
• The narrow time window may be missed when the drug is applied. And is not accurately known for the time being.
• Maybe the drug doesn't be delivered adequate in the injured territory.
• BBB integrity that prevents the drug to penetrate.
• Differences between the study trials, group formation, age environmental differences, highly heterogeneity of the brain damage.

Secondly. Were the clinical trials appropriate scheduled and carried out? Many authors believe that clinical trials themselves played a role in the failure to document the efficacy of antioxidants in clinical practice given the marked heterogeneity of stroke and clinical trials design (Keith, 2002). Maybe the way clinical trials were scheduled (groups, agent's doses, duration of therapeutic scheme) and carried out, was responsible in a way or another for the failure to document a statistical significance in tirilazad mesylate trials (Danton et al., 2004; Emily Sena et al., 2007). For example there is a difference in most studies between the time that the agent is injected in laboratory compared to the clinical trials. In most experiments, tirilazad mesylate, was administered shortly after the onset, while in clinical trials in humans, the administration was after several hours. This difference may be of major importance, in tirilazad mesylate therapeutical action. Similarly tirilazad mesylate has a fixed therapeutical width in animals. If an analogous therapeutic width exists in humans, maybe the doses that were used in humans are out of this therapeutical width (Danton et al., 2004; Emily Sena et al., 2007). In a similar way, the results of the tirilazad mesylate clinical trial considering the efficacy of the molecule in subarachnoid haemorrhage that performed in North America were unequivalent in comparison to the other clinical trial (that took place in Europe-Australia-New Zealand- South Africa) at the same time, due to the use of phenyntoin.

Finally most researchers believe that those differences between laboratory and bedside are due to the way the groups are formed, environmental, age related or other similar bias. Maybe all the above possible causes play an independent role, leading to this inequivalance. Most authors on the other hand believe that this inequivalance must be understood. The way that can be solved is to perform more detailed experiments in bigger animals so as to understand if the efficacy of tirilazad mesylate really exists (Grotta, 1999; Danton et al., 2004; Emily Sena et al., 2007).

Under this point of view, in our opinion any new antioxidant agent must be specifically examined. Antioxidant therapy is very important in ICH and not only. Maybe next years the main point would be to find a combined strategy in response to the complex mechanism that arouse in CNS injury. It is possible that antioxidant therapy itself could not be enough. Experiments that use many agents in comparison could give better results (Danton et al., 2004).

In our opinion lazaroids, are not fully investigated. The category has many chemical molecules with differences in actions, efficacy and even basic pharmacological characteristics. Although past decade failed to reveal the possible antioxidant role of tirilazad mesylate in humans, further detailed studies of any kind are needed in order to better understand the 21-aminosteroids and their action. Many other molecules are not examined yet, or have partly been examined. Similarly, some other effects and action of lazaroids have recently been documented. (Domenica Altavilla, 1998; Durmaz et al., 1999; Selda Kabadere, 2004). The few new experiments that have been carried out after tirilazad mesylate trials, suggest that other molecules of the 21-aminosteroids family are very effective and have also other effects not known from the previous studies. (Vignes et al., 2006).

PART B

MAIN PART OF THE THESIS

I. Introduction, basic idea and aims of the study.

Our study is an experimental preclinical model in large animals (porcine) of ICH. It is our belief that ICH, is a topic that still lacks the attention that deserves given the incidence, mortality and morbidity, despite the high number of studies, papers and overall research undertaken especially over the last decade. We believe that more research and clinical data is of need in order to find solutions regarding this devastating disease. It is mentionable that last decade, studies of any kind regarding ICH, multiply in all fields, giving us an idea regarding the importance of the entity and on the parallel the many unanswered questions which finally reflect to the poor outcome despite current technological evolution and inovation.Not many years have passed since the pathophysiology of ICH has been considered being a different entity than the better studied already pathophysiology of stroke, traumatic brain injury or subarachnoid haemorrhage.

Taking into account the limitations of the preclinical models regarding ICH (and not only), as they were described in the chapter of preclinical models, considering also the discussion for the antioxidants, regarding their role in brain pathology, we decided to study the effects of a lazaroid, the U74389G in a large (porcine) animal model of ICH.

As previously explained in the relevant chapter, large model and especially porcine, has been chosen, because provides valuable data for pathophysiological and neurophysiological studies, suitable for observation of biochemical and pathophysiological parameters and with significant relevance to human physiology and pathophysiology finally.

The chosen agent from the category of lazaroids,was based on the fact that lazaroids are very powerful antioxidants and although in the past tilirazad was found to be ineffective in clinical trials, they remain a category of potent antioxidants carrying the discussion for the differencies in the results between bench and bedside. Currently, no matter what antioxidant is used there is a barrier between laboratory and human application and many questions are still unanswered. There is strong belief that in basic research, the categories of agents studied in the past without establishing efficacy, regarding their antioxidant role in humans shouldn't discourage the ongoing research for antioxidant treatments in intracerebral haemorrhage. The antioxidant U-74389G, although belongs to the category of 21-aminosteroids (lazaroids) -with most important in the past being the tirilazad mesylate that failed the clinical trials-, is a different agent and has been scarcely tested in brain pathologies, resulting in quite limited basic research experimental data. On the other hand is a very powerful antioxidant.

I used U 74389G, because is a very powerful lazaroid that hasn't been tested in ICH and scarcely overall in the brain despite its well established antioxidant role in other systems and

diseases. I think that the overall discussion and research about antioxidants, is very important regarding the possible treatment options of brain secondary injury, in many different pathologies and regarding this study, in ICH. It is a duty for basic research to pose questions and find answers providing clinical research with tools for better treatment options and beneficial result for the patient.

We used a slightly modified experimental porcine model of intracerebral haemorrhage as being following described in the relevant chapter, that produced a deep seated intracerebral haematoma, around the basal ganglia territory.

Regarding the pathways that were examined, the aim of this study was both to check certain pathways and changes that were not studied before in intracerebral experimental haemorrhage and also to observe the effects of U 74389G as a potential antioxidant in ICH pathology. Given our availabilities and also our good experience in cholinergic system neurochemistry research, we studied the changes in Na^+,K^+-ATPase, acetylocholinesterase and Mg^+-ATPase in our model at 4 and 24 hrs after haematoma induction, with or without the administration of U74389G.

From the despription that follows regarding the cholinergic system and the Na^+,K^+-ATPase and the Mg^+-ATPase , it is obvious that the these neurophysiological mechanisms are very important in brain physiology, responsible for nerve signaling in health and disease. This importance is well recognized and also extensively studied in many brain pathologies. Mostly the role of acetylocholinesterase is being under investigation regarding Parkinson, Alzheimer, and myasthenia Gravis amongst other brain pathologies as described above. The cholinergic system, apart from its colossal importance as a neurotransmission system, seems to play an important role in brain injury and recovery also. It has been recently suggested that the brain possesses a cholinergic anti-inflammatory pathway that counteracts the inflammatory responses after ICH, thereby limiting damage to the brain itself (Lee et al., 2010). Moreover, Hijioka et al. (2011) have recently provided evidence of the significant neuroprotective effect of nicotine administration in the central region of hematoma, where ICH-associated inflammatory reactions (such as accumulation of activated microglia/macrophages and increased oxidative stress) are considered to be less prominent than in the peripheral region. Given the above data, we decided to study the changes of Mg^{2+}-ATPase, Na^+,K^+-ATPase and Ache in intracerebral haemorrhage, 4 and 24hrs after haematoma induction.

We also examined the changes in TNF-α, at the same timeline because of its importance as a mediator of inflammatory response, as well as the changes in IL-1 expression 4 and 24hrs after the haematoma induction. Both TNF-α and IL-1, were studied as a timeline as well as regarding the effect of U-74389G on their expression 4 and 24 hrs after the haematoma induction. The

importance of determining the changes in TNF-α and IL-1, in intracerebral haemorrhage is well described in the relevant chapter of the pathophysiology in intracerebral haemorrhage.

In the text that follows a brief overview of Na^+,K^+-ATPase, Mg^+-ATPase, and acetylcholinesterase is given .

As it is well known, Na^+,K^+-ATPase pump, was discovered in 1957 by Jens Christian Skou, a Danish scientist. Since the description of the pump, much experimental data has been collected regarding its role in CNS. Acetylocholinesterase on the other hand, is well known for its importance in neurotransmission. Mg^+-ATPase, also is a very important pump which is tested by the acetylcholine that is released into the synaptic cleft, reacting with the post-synaptic cleft and finally transferring the signal to the post-synaptic target. In the post-synaptic cleft, by hydrolisation of achetylocholine, the post synaptic signaling is being terminated. Analytically:

Na^+,K^+-ATPase is a membrane protein complex which uses the hydrolysis of ATP to transfer Na^+ and K^+ ions. Na^+,K^+-ATPase, is a member of the family of P-type proteins. Is a transmembrane protein which consists of two subunits A,B. A subunit (100-kDa) with ten segments is the catalytic subunit responsible for transport of ions and ATPase activity. The glycosylated beta subunit, approximately 50-60 kDa, seems to play an important role in regulation, by forming heterodimers a-b, the number of the sodium pump, and the operation of a subunit. At least four isoforms alpha 1 (a1), alpha 2 (a2), alpha 3 (a3) and alpha 4 (a4) have been recognized in a module which is composed of 1000 amino acids. Isoform (a2) is found mainly in skeletal muscles and brain and to a lesser extent in the heart and in the fat cells in the eye. Isoform (a3) is detected mainly in tissues and isoform (a1) in the kidney.

The beta subunit is a glycoprotein that has three isoforms b1, b2 and b3. The isoforms b1 and b2 are found in almost all tissues. The beta subunit acts as a receptor during the formation of the pump. Subunit a, is formed in the absence of subunit beta and undergoes rapid degradation in the endoplasm.

Beta subunit is important for the proper functioning of the enzyme, seems to be involved in contact with the K^+ and the regulation of the enzyme affinity for Na^+ and K^+. The module includes:

a) the slots for the connection of sodium ions in the protein portion which projects inside the cell .

b) Two slots for the connection of potassium ions inside the membrane.

c) slot for ATP and the inhibitor.

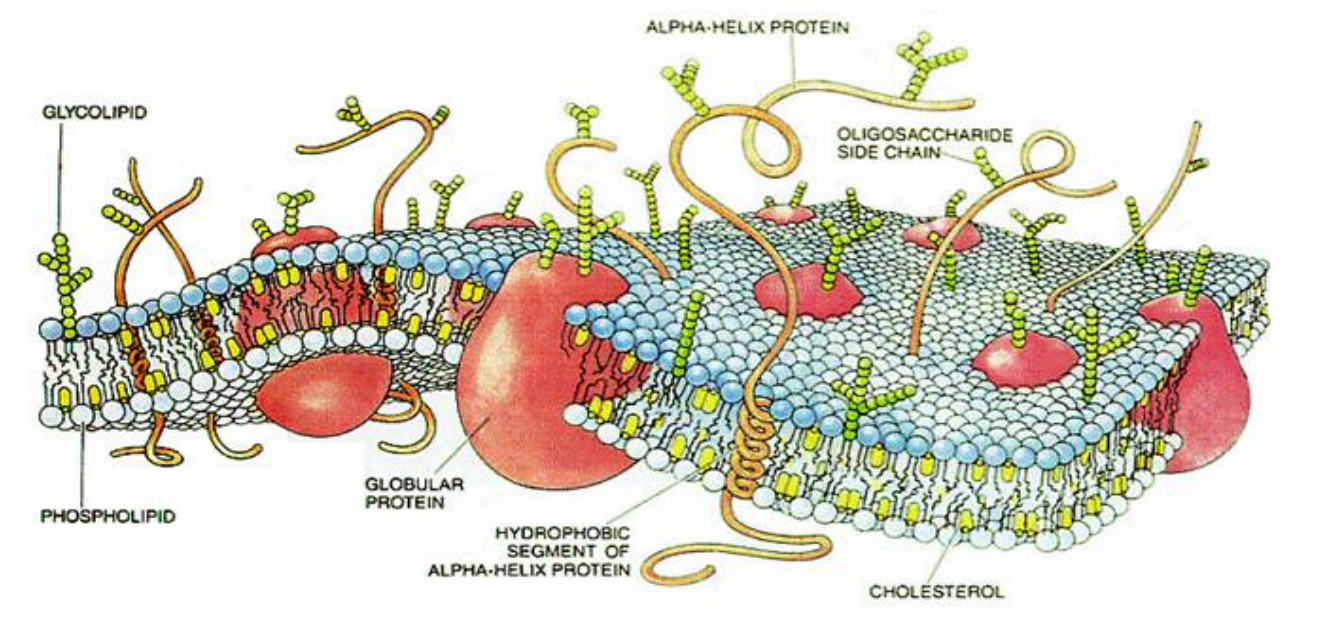


Figure 18: Represents the cell membrane.Source: en.wikibooks.org

It has been recently identified a gene family of at least seven small protein domain that appear to control the active ion transport of Na^+,K^+-ATPase. These proteins appear to regulate the activity of Na^+,K^+-ATPase.

Action of Na^+,K^+- ATPase pump

The pumping of Na+,K+ in opposite directions with hydrolysis of ATP is made from a complex protein that extends across the thickness of the cytoplasmic membrane. It was discovered by Jens Skoy in 1957 and named Na^+, K^+- ATPase. ATPase hydrolyzes ATP only if the presence of Na^+, K^+ and Mg^{2+} ions. Reaction is as follows:

$$3Na^+ (in) + 2\ K^+ (out) + ATP + H_2O \rightarrow 3Na^+ (out) + 2\ K^+ (in) + ADP + Pi + H^+$$

Uneven ions transport in both directions, makes the inside of the cell 50-90 millivolts more negative than the outside environment. This creates an electrical potential at the cell membrane.

Important is that the pump is phosphorylated by ATP in the presence of Na^+ and Mg^{2+}.

$$E + ATP \rightarrow E\text{-}P + ADP$$

Only the presence of K^+ is the dephosphorylation:

$$E\text{-}P \rightarrow E + Pi$$

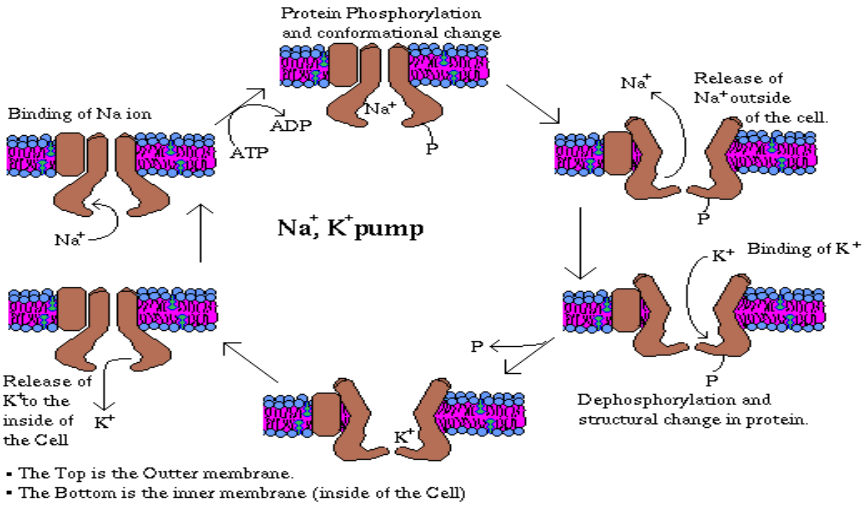


Figure 19: Represents the action of the pump. Source: medrounds.org

Isolation of Na^+, K^+- ATPase showed that it consists of two catalytic subunits associated with a glycoprotein that is found inside the membrane. The pump is able to take two forms, the E1 and E2. E1 communicates with the interior of the cell, while E2 communicates with the external environment. The pump in the formulation E1 indicates greater affinity for the Na^+ , while E2 formulation, indicates greater affinity for K^+. Na^+, K^+-ATPase has the following futures:

1) is located in the cell membrane

2) Cytosol compartment has greater affinity for the Na^+ ions in comparison to K^+ions.

3) The extracellular compartment has greater affinity for the ions K^+ in in comparison to Na^+ ions.

4) It has enzymatic activity and catalyzes the hydrolysis of ATP.

5) The hydrolysis rate depends on the cytoplasmic Na^+ concentration and from the extracellular K^+ concentration.

6) Is found in all cells where Na^+ and K^+ transport exists.

7) Participation of Na^+ and K^+ ions in the pump action and enzyme function, is related to the concentration of the ions.

Role of the Na^+, K^+- ATPase pump.

Na^+,K^+- ATPase pump, is present in all cells of the body and is responsible for maintaining the difference of the concentrations of sodium and potassium ions from both sides of the cell membrane and thus maintaining the intracellular electronegativity. Supplies the energy needed to transport components associated with sodium such as amino acids, glucose and vitamins. The

transport of ions such as Ca^{2+} and H^{+} from both sides of the membrane and the osmotic balance and the volume of cells, depends on the gradients of sodium and potassium ions. Na^{+}, K^{+}-ATPase, plays a special role in many tissues: maintains the food and ionic composition of cerebrospinal fluid and is responsible for the liquid movement during transport in the gastric system, in nasotracheal epithelium and kidney. The production of electrical potential is important for the function of tissues such as muscles, nervous system while reabsorption in the lungs at birth depends on the potential of the ions created by the pump Na^{+},K^{+}- ATPase. It is estimated that approximately 25% of ATP is used by the enzyme.

One of the most important functions of the pump is that controls cell volume. In particular cells are able to balance the osmotic pressure on both sides of the membrane with the excistense of Na^{+}, K^{+}- ATPase pump.

The production of electrical potential is also used as energy source for the depolarization and repolarization of the membrane potential. The pump is based on the ability of nerve cells to carry the nerve signals in each point of the nervous system. The transmission of nerve impulses in the form of the action potential due to instantaneous and transient depolarization of the membrane potential. The active transport of Na^{+} and K^{+} ions, plays a key role in the transmission of messages and control of substances between cells.

Na^{+}, K^{+}- ATPase, is important in many cellular functions closely connected and controlled by hormones (insulin and thyroxine), neurotransmitters, and growth factors. The catalytic subunit of Na^{+}, K^{+}- ATPase acts as a substrate for the protein kinase and is phosphorylated by both dependent cyclic AMP protein kinase and by the Ca^{2+} / phospholipid-dependent kinase. This action of kinases in vivo is conflicting issue and several observations need further study. Thyroid hormones and steroids increase the action of the pump. Other external factors that affect the action of the pump are neurotransmitters. Among the most studied is noradrenaline and serotonin. Na^{+}, K^{+}- ATPase, is also activated by serotonin. In vivo pharmaceutical or dietary changes in serotonin are followed by parallel changes in the action of the pump. Dopamine, inhibits the activity of the pump in isolated neurons.

Changes in the activity of Na^{+}, K^{+}-ATPase was found to be positively correlated with changes in the phospholipid and especially phosphatidylcholine, changes in the microenvironment generated by the phospholipids may reduce the activity of the Na^{+}, K^{+}-ATPase.

The role of cholinergic system, neurotransmission.

Acetylcholine (Ach) is a neurotransmitter of pre and post-ganglionic fibers of the autonomic nervous system (all parasympathetic and some sympathetic fibers) and in the the neuromuscular synapse.

Is being produced in mitochondria at the cholinergic system with the catalytic action of choline acetyltransferase, enzyme that catalyzes the reaction between choline and of acetyl coenzyme A in the presence of glucose and ATP. These substances are found in the mitochondria, except choline which can not penetrate cell membranes: it is possible that a specific transport system of choline from the extracellular space through membrane exists. Choline acetyltransferase is found in high concentrations in the cytoplasm of cholinergic endings.

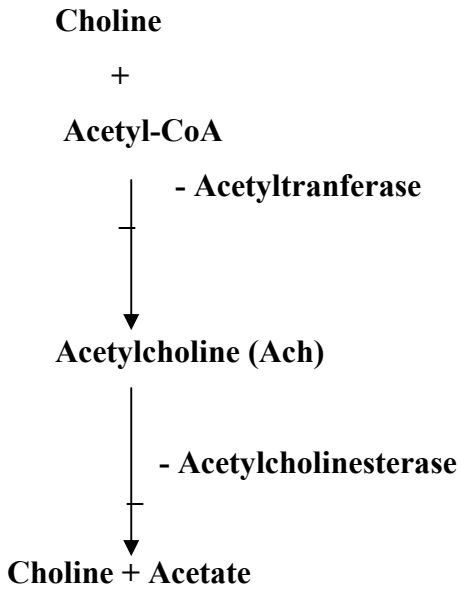


Figure 20: The circle of choline.

After synthesized, acetylcholine is transported and stored in the presynaptic neuron. Acetylcholine release: when potential reaches a nerve ending, the transmitter is released. The release of Ach is affected by the presence of ions Ca^{2+}, lack of Ca^{2+} inhibits the release. When the action potential reaches a nerve end, calcium channels in the presynaptic membrane open causing an increase in the concentration of intracellular calcium ions, resulting finally in the release of acetylcholine in the synapse.

Acetylcholine is released from synaptic vesicles in the synaptic gap and reacts with muscarinic and nicotinic receptors, or with receptors of the presynaptic membrane that released

acetylcholine. In this way the postsynaptic membrane depolarizes,or hyperpolarizes (excitatory or inhibitory activity).

With the arrival of the nervous stimulus to the nerve end, Ache is being released, reacts to the postsynaptic membrane changing ions permeability, resulting in action potential. Recycling of Choline: Choline is being reinstated back to the presynaptic neuron and stored until it is released by another action potential. Choline crosses the cell membranes by two processes referred to as high and low selectivity (affinity) transfer. The high selectivity of transport is saturated and depends from the sodium and the membrane potential. The low selectivity transport of choline is a process of passive diffusion and not dependent on the concentration of choline.

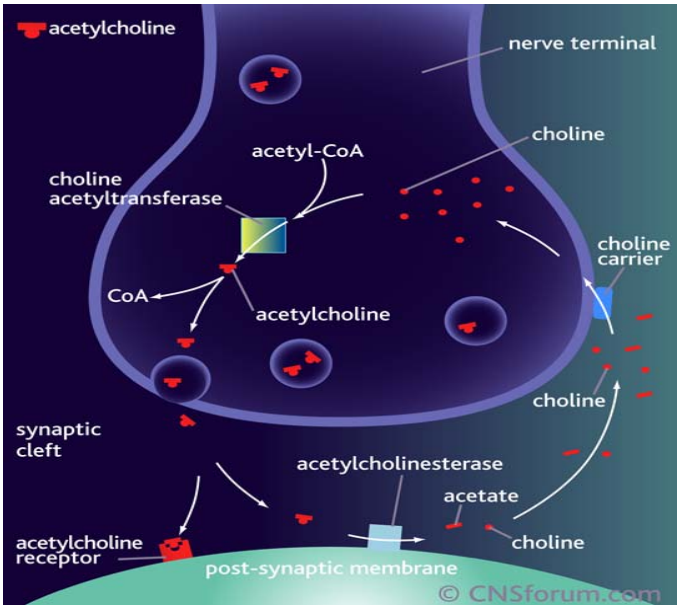


Figure 21: Synapse and the choline pathway. Source: CNSforum.com.

Localization of cholinergic synapses.

CNS is rich in Ach. Localising Ache synapses in the CNS cholinergic system is difficult.
Peripheral Nervous System

Acetylcholine is the neurotransmitter in the following cases:

1) Preganglionic axons of the autonomic nervous system.
2) Postganglionic parasympathetic nervous system.
3) Post-ganglionic sympathetic axons distributed to sweat and adrenal glands.
4) Motoneurons in skeletal muscles.
5) In the sympathetic fibers distributed to the blood vessels.

6) the reciprocating fiber from the motor Renshaw neurons are also cholinergic.

In the peripheral nervous system and possibly the perossoteres times and the CNS, acetylcholine acts as excitatory transmitter.

Main areas where acetylcholine plays a major role are the posterior horn of the gray matter of the spinal cord, cranial nerves nuclei, reticular formation, mesencephalic, thalamic neurons, hypothalamic nuclei, optic chiasm, cerebellum and the central layer IV of the cortex. High levels of acetylcholine appears to have a stimulatory and suppressive effect. The cholinergic system is one of the most important neurotransmition systems that controls the brain and activities that are essential part of conscious awareness. Degenerative diseases of the brain, and changes in consciousness are associated with lack of local cholinergic system.

In Alzheimer's disease for example,degeneration of cholinergic neurons and hypoactivity of cholinergic projections to the hippocampus and the cortex were found.

Also cholinergic fibers innervate the brain during the most dynamic period of neuronal differentiation and formation of synapses, suggesting the possible role in these events. From in vivo studies of the past decade shows that changes in cholinergic innervation during early postnatal development can alter characteristics of the cortex.

It has been shown that cholinergic muscarinic receptors facilitate learning and memory. Pontine neurons containing acetylcholine are important for the initiation and maintenance of sleep REM. Acetylcholine plays important role in the regulation of releasing growth hormone and somatostatin, and therefore play an important role in development.

Drugs inhibiting muscarinic receptors cause hallucinations and lowering of consciousness while nicotinic receptors associated drugs are associated with anaesthesia.

Hydrolysis of acetylocholine and the role in CNS pathology.

Cholinesterases are responsible for the fast degradation of acetylcholine within milliseconds after release from the synapse. Acetylcholinesterase (AchE) plays a key role in cholinergic synaptic transmission. This is a very well maintained enzyme in the animal kingdom, and distributed in many tissues of vertebrates where it is expressed in different molecular forms depending on the configuration of the structural and catalytic subunits. In the mammalian brain acetylcholinesterase is in a form of four 70-kDa units that merge together with a unit of 20-kDa. The role of Achetylcholine in the central nervous system disorders is not quite clear. Except myasthenia, may play an important role in familial dysautonomia, seizure disorders,Chorea Huntington, Alzheimer's disease, Parkinson's disease.

Figure 22: 3D representation of achetylcholinesterase. Source: wikibooks.org

Mg^{2+}-ATPase

The role of Mg^{2+}-ATPase activity in the nervous system is not quite determined yet. It seems that it plays a excitatoty-contraction cycle role in the nervous system. Component of Mg^{2+}-ATPase is M (r) 85.000 glycoprotein (85k-GP). Antibodies have been found in many tissues such as heart, spleen, smooth muscle, brain and lungs.

Although topographical localization of the enzyme both in the brain and skeletal muscles has not entirely defined, there is evidence that the part of the enzyme that cleaves ATP is located in synaptic side of the presynaptic membrane. Mg^{2+}-ATPases'actions:

1) can serve as a regulator of the concentrations of the extracellular ATP

2) may be able to regulate the availability of ATP

3) Specifically in the brain can alter the neurotransmitter role of ATP.

II. Animals.

IIa1. Groups and subgroups.

In our study, we used a slightly-modified porcine model of ICH based on the one presented by Wagner et al. (1996); taking into account all the discussion regarding back flow, the double injection model etc.

Forty, (40) male Landrace pigs (swines) aged 135–150 days old and weighting 30–35 kg were used. Preoperatively all pigs were allowed to food and water ad libitum. All surgical

procedures were performed under aseptic conditions. Pictures that follow, shows, the basic phases of the protocol.

The protocol of our study was approved by the Ethics Committee of Medical School of National and Kapodistrian University of Athens and by the Veterinary Authority of the Prefecture of Athens (permission #421/ 21.4.2009).

The experiment was done with the same principles in both groups and the first twenty animal brains, were then preserved at the time of the removal in -80^{o} C, while the rest 20 animal brains were preserved in formalyl for blocks and pathology evaluation.

So the groups and subgroups of the experiment are as follows:

Group I, consists of Subgroups A and B, E and F each of the subgroups consists of 5 animals.

Subgroup A consists of 5 animals. This is a control group that underwent haematoma induction at the right basal ganglia territory (deep seated). This subgroup hasn't been offered intravenous administration of U 74389G. All animal were intubated ventilated and the brain was removed via craniectomy (see below) and the animal euthanatized four hours (4 hrs) after the haematoma induction. The brain was then preserved in formalin.

Subgroup B consists also of 5 animals. This subgroup was offered intravenous administration of U 74389G, after the haematoma induction. The administration of the agent was done one hour after the haematoma induction. All animals of this subgroup were intubated, ventilated, underwent haematoma induction at the right basal ganglia territory (deep seated) and the brain was removed after four hours (4hrs) from the onset, via craniectomy (see below) and the animal was then euthanatized. The brain was then preserved in formalin.

Subgroup E consists of 5 animals. This is a control group that underwent haematoma induction at the right basal ganglia territory (deep seated). This subgroup hasn't been offered intravenous administration of U 74389G. All animals were intubated ventilated, for the haematoma inductions, then they were extubated and left free for twenty four hours (24 hrs). They were reintubated and ventilated and the brain was removed via craniectomy (see below) 24 hrs after the haematoma induction and the animals were then euthanatized. The brain was then preserved in formalin.

Subgroup F consists of 5 animals. They underwent haematoma induction at the right basal ganglia territory (deep seated). This subgroup was offered intravenous administration of U 74389G. All animals were intubated ventilated, for the haematoma inductions, then they were extubated and left free for twenty four hours (24hrs). They were reintubated and ventilated and the brain was removed via craniectomy (see below) 24 hrs after the haematoma induction and the animals were then euthanatized. The brain was preserved in formalin.

Group II, consists of subgroups C, D, G, H, each of the subgroups, consists of 5 animals.

Subgroup C, Consists of 5 animals. This is a control group that underwent haematoma induction at the right basal ganglia territory (deep seated). This subgroup hasn't been offered intravenous administration of U 74389G. All animals were intubated ventilated and the brain was removed via craniectomy (see below), the animal was euthanatized four hours (4hrs) after the haematoma induction. The brain was then preserved in -80^{o} C.

Subgroup D consists also of 5 animals. This subgroup was offered intravenous administration of U74389G, one hour after the haematoma induction. All animals of this subgroup were intubated, ventilated, underwent haematoma induction at the right basal ganglia territory (deep seated) and the brain was removed after four hours (4 hrs) of the haematoma induction via craniectomy (see pictures that follow) and the animal was then euthanatized. The brain was then preserved in -80^{o} C.

Subgroup G consists of 5 animals. This is a control group that underwent haematoma induction at the right basal ganglia territory (deep seated). This subgroup hasn't been offered intravenous administration of U 74389G. All animals were intubated ventilated, for the haematoma inductions, then they were extubated and left free for twenty four hours (24 hrs). They were reintubated and ventilated and the brain was removed via craniectomy (see below) 24 hrs after the haematoma induction and the animals were then euthanatized. The brain was then preserved in -80^{o} C.

Subgroup H consists of 5 animals. They underwent haematoma induction at the right basal ganglia territory (deep seated). This subgroup was offered intravenous administration of U 74389G, one hour after the haematoma induction. All animals were intubated ventilated, for the haematoma inductions, then they were extubated and left free for twenty four hours (24 hrs). They were reintubated and ventilated and the brain was removed via craniectomy (see pictures that follow) 24 hrs after the haematoma induction and the animals were then euthanatized. The brain was then preserved in -80^{o} C.

IIa2. Anaesthesiologic protocol.

All animals were anaesthetized with midazolam 0.5 mg/kg B.W and ketamine 15 mg/kg B.W. i.m. intratracheally. Atropine 0.045mg/kg B.W. intratracheally was administered 10 minutes before the intubation. Induction of anaesthesia was achieved with propofol 3mg/kg, fentanyl 0,012mg/kg, cisatracurium besylate 0,5mg/kg bolus i.v. during intubation.

Anaesthesia was then maintained with propofol 1% (6-8mg/kg/h), fentanyl (2mg=4amp σε 500ml N.S), cisatracurium besylate (200mg=10amp Nimbex σε 500ml N.S.), with the use of dial a flow 60-80 ml/h i.v.

Parameterers of ventilator were set to FIO2 40%, 20 breaths/min. Extubation was achieved with metoclopramide 10mg i.m./i.v., neostigmine 2,5mg/ml=1 amp i.m./i.v. After procedure analgesia was administered with carprofen 0, 5-4mg/kg/24h i.m., butorphanol 0,1mg/kg i.m. /4h and fentanyl 0,015mg/kg im/8h. Antibiotics were given, cephalosporin 20mg/kg/12h i.m.

II b. Surgical technique.

Antisepsis and general surgical preparation. All animals were intubated sedated and ventilated. During the experiment, they were on strict monitoring regarding the vital parameters and the ventilation. Care was taken in order to achieve pH and arterial blood gases within normal limits. All animals were then positioned for the main procedure.

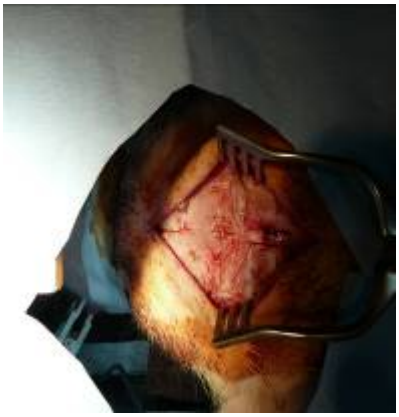
The preparation was same to all animals. Animals in prone position, head was secured in a frame and blocks for stability, prepped with betadine solution 10% and draped. Their ear was also prepped and prepared for the autologus blood sample. All instruments were also sterilized with the usual techniques and the whole procedure was aseptic. Skin incision of approximately 10cm was performed above the superior sagital sinus. Subcutaneous tissue, muscle and pericranium were then removed and the bone was recognized thought the surgical field (pictures 17-24).

Sagittal and coronal sutures were recognized and bregma was marked in every animal. Using the stereotactic atlas of Félix et al. (1999), a 3 mm burr hole was placed 1cm in front and 1cm lateral of the bregma. After the burr hole, dura was recognized and opened, in order to insert the catheter more easily and minimize the induced trauma.

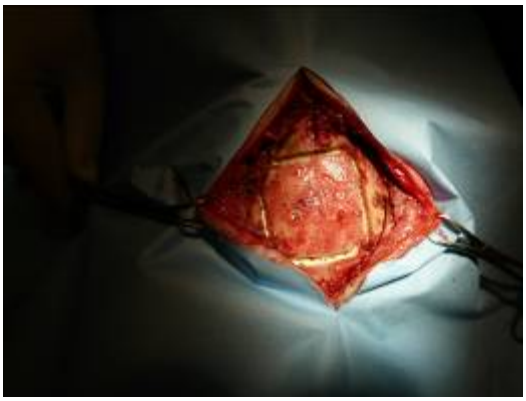
Bone thickness was measured in each animal individually for the accuracy of the depth insertion of the catheter afterwards. Following dura incision, a pediatric Swan-Ganz catheter was inserted via the burr hole at a depth of 2cm from the dura, in a vertical plane and remained in place during the whole procedure. The balloon was then inflated at a volume of 1 ml, 2–3 times, for several seconds each time, and consequently autologus blood was taken via femoral or ear vein. The blood was simultaneously slowly injected through the distal part of the Swan-Ganz catheter, during a ten minutes time period.

Blood injection was performed in such a manner, so while the balloon was decompressed, the blood was gradually being applied up to a total of 3ml volume. This method ensured that blood was gradually being embedded at the cavity, minimizing the possibility of back flow. A total of 3ml of autologus blood was injected in each animal's right basal ganglia territory, and finally, a deep seated hematoma was induced. After clot formation, the catheter was gradually removed with caution to minimize the mechanical trauma. The skin was then closed with sutures until the final brain removal. All procedures were performed under aseptic conditions.

Picture 18. Shows the basic set-up in order to do the incision and the induction of haematoma. The anaesthetized swine was placed in a frame in order to achieve stability of the head and the same haematoma site.



Picture 19. Shows the approximately 10cm sagittal incision above bregma, in the midline. Recognition of bregma and then induction of the haematoma at the right hemisphere through mini burr hole of 3mm. High speed sterilized drill was used for the burr hole.



Picture 20. Shows the craniectomy in order to remove the whole brain after the haematoma induction. High speed sterile drill was used for the craniectomy.

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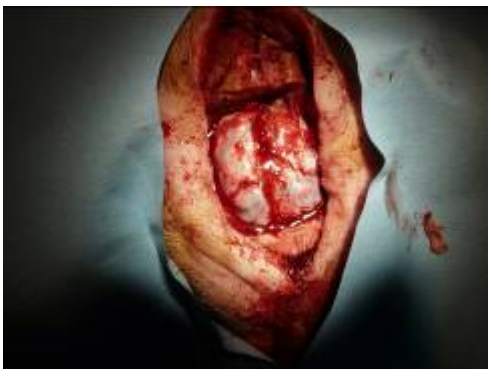
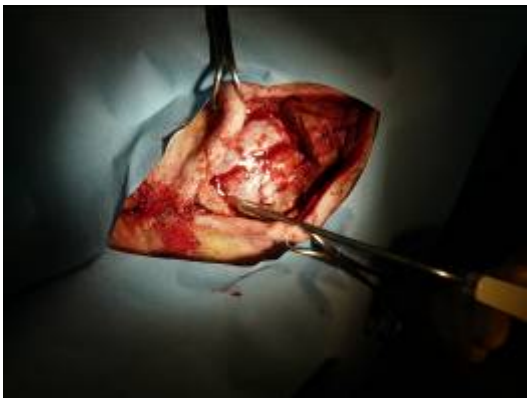
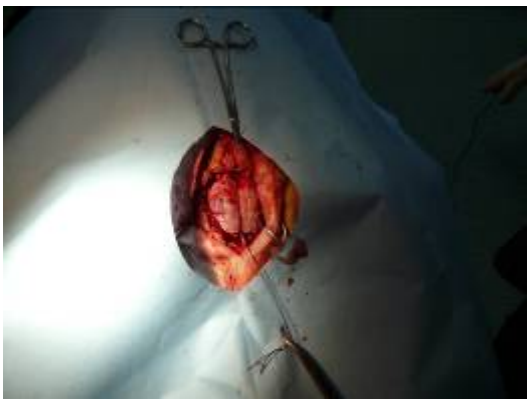


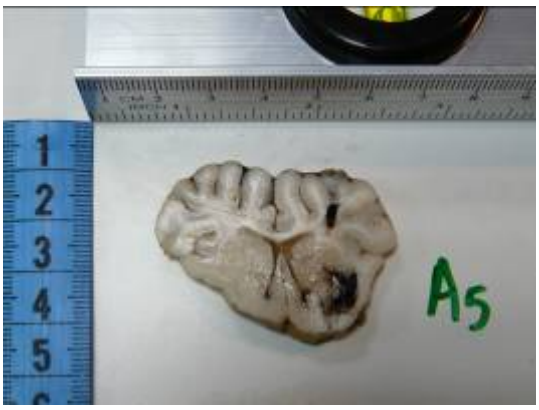
Picture 21. Shows the dura after the removal of the bone flap, with the brain underneath.



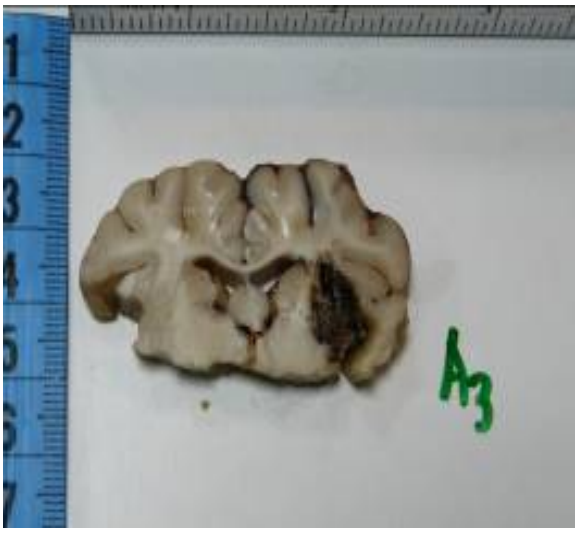
Picture 22. Shows the cut of the dura edges bilaterally in order to elevate it, see the brain and remove the brain underneath en block.



Picture 23. Shows the ligation of the sagittal sinus in its attachment frontally in order to cut it and remove the brain underneath en block.



Picture 24. Shows coronal section of the brain that was preserved in formalin after removal. It is obvious the basal ganglia haematoma, that is the result of our technique.



Picture 25. Shows, another deep seated basal ganglia haematoma in coronal section.

Table 15. Abbreviations used for the porcine brain samples studied.

ICH: intracerebral haematoma.

SALBGT4 − left (uninjured) basal ganglia territory, 4 hrs post ICH-induction, saline-treated
SALBGT4 + right (penumbra-related) basal ganglia territory, 4 hrs post ICH-induction, saline-treated
SALCCT4 − left cerebral cortex territory, 4 hrs post ICH-induction, saline-treated
SALCCT4 + right cerebral cortex territory, 4 hrs post ICH-induction, saline-treated
SALBGT24 − left (uninjured) basal ganglia territory, 24 hrs post ICH-induction, saline-treated
SALBGT24 + right (penumbra-related) basal ganglia territory, 24 hrs post ICH-induction, saline-treated
SALCCT24 − left cerebral cortex territory, 24 hrs post ICH-induction, saline-treated
SALCCT24 + right cerebral cortex territory, 24 hrs post ICH-induction, saline-treated
LAZBGT4 − left (uninjured) basal ganglia territory, 4 hrs post ICH-induction, lazaroid-treated
LAZBGT4 + right (penumbra-related) basal ganglia territory, 4 hrs post ICH-induction, lazaroid-treated
LAZCCT4 − left cerebral cortex territory, 4 hrs post ICH-induction, lazaroid-treated
LAZCCT4 + right cerebral cortex territory, 4 hrs post ICH-induction, lazaroid-treated
LAZBGT24 − left (uninjured) basal ganglia territory, 24 hrs post ICH-induction, lazaroid-treated
LAZBGT+ right (penumbra-related) basal ganglia territory, 24 hrs post ICH-induction,lazaroid-treated
LAZCCT24 − left cerebral cortex territory, 24 hrs post ICH-induction, lazaroid-treated
LAZCCT24 + right cerebral cortex territory, 24 hrs post ICH-induction, lazaroid-treated

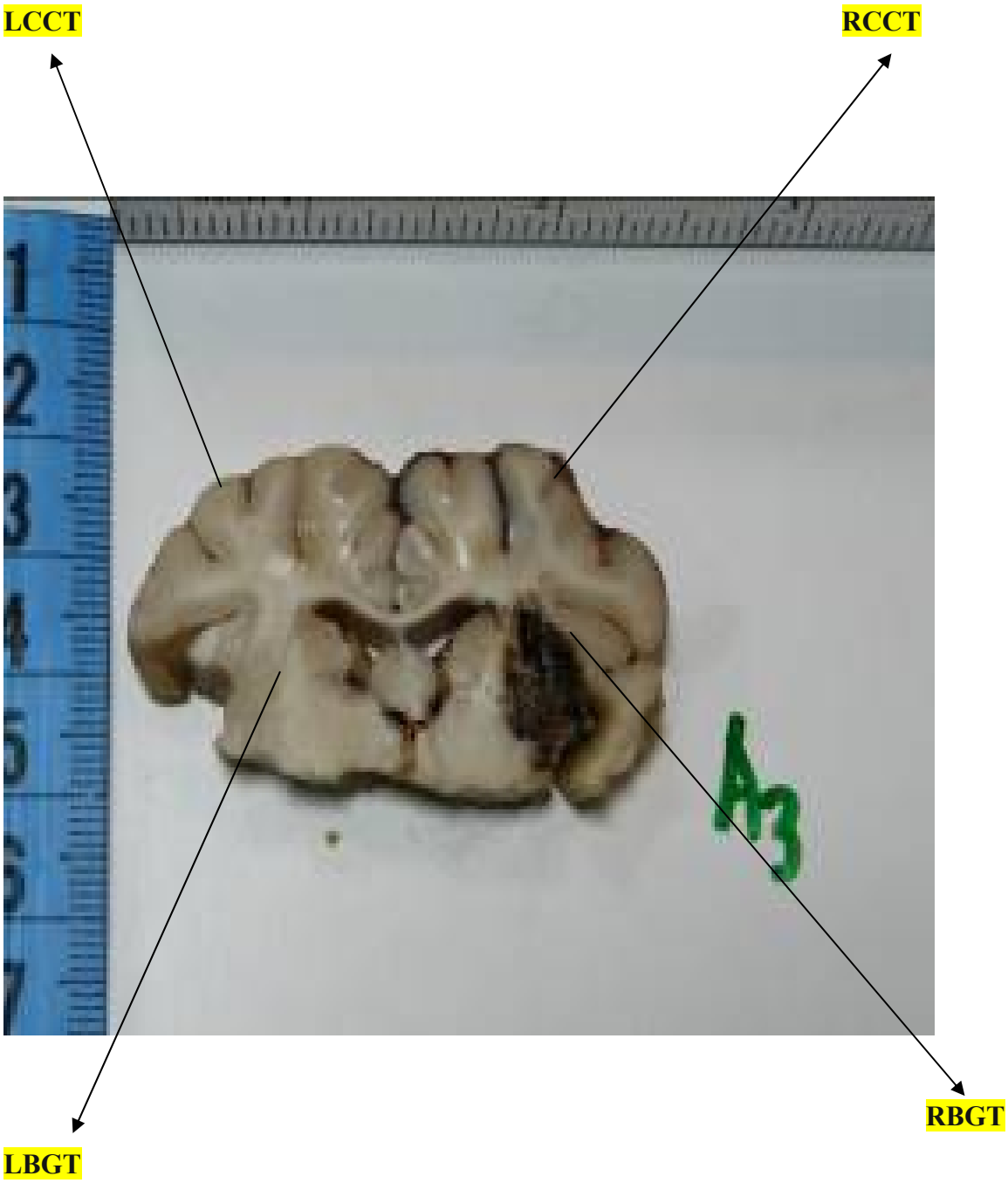


Figure 23: Overview of the sites that where examined.

LBGT: Left Basal Ganglia Territory, uninjured

LCCT: Left Cerebral Cortex Territory, uninjured

RBGT: Right Basal Ganglia Territory, haematoma

RCCT: Right Cerebral Cortex Territory,haematoma site

SAL: Saline treated

LAZ: Lazaroid treated

II c. Methods.

i. Tissue preparation for Na^+,K^+-ATPase and Mg^{2+}-ATPase activities and activation of acetylocholinesterase.

The obtained tissues were homogenized in 10 vol ice-cold (0-4^o) medium containing 50mM Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4 and 300 mM sucrose, using an ice-chilled glass homogenizing vessel at 900 rpm (4-5 strokes). Then, the homogenates were centrifuged at 1,000 x g for 10 min to remove nuclei and debris (Bowler, Tirri, 1974). In the resulting supernatant, the protein content was determined according to the method of Lowry et al. (1951) and then, the enzyme activities were measured.

ii. Determination of acetylocholinesterase enzymatic activities.

As described above, samples were obtained from the animals' brains at 4 hrs (5 saline- and 5 lazaroid-treated animals) and at 24 hrs (5 saline- and 5 lazaroid-treated animals) following the experimental-induction of ICH, from the right basal ganglia territory (BGT) around the haematoma (penumbra tissue), its respective (neuroanatomically-matched) left BGT, as well as from a standard cerebral cortex territory (CCT) from both hemispheres. Table 15, summarizes the abbreviations of the studied porcine brain samples.

The obtained tissues were homogenized in 10 vol ice-cold (0–4°) medium containing 50 mM Tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.4 and 300 mM sucrose, using an ice-chilled glass homogenizing vessel at 900 rpm (4–5strokes). Then, the homogenates were centrifuged at 1,000 × g for 10 min to remove nuclei and debris (Tsakiris, 2001). In the resulting supernatant, the protein content was determined according to the method of Lowry et al. (1951) and then the enzyme activity was measured. AChE activity was determined by following the hydrolysis of acetylthiocholine according to the method of Ellman et al. (1961), as described by Tsakiris. (2001).

The incubation mixture (1 ml) contained 50 mM Tris- HCl, pH 8, 240 mM sucrose and 120 mM NaCl. The protein concentration of the incubation mixture was 80–100 μg/ml. The reaction was initiated after addition of 0.03 ml of 5, 5′- dithionitrobenzoic acid (DTNB) and 0.05 ml of acetylthiocholine iodide, which was used as substrate.

The final concentration of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction followed spectrophotometrically by the increase of absorbance _ΔOD_ at 412 nm.

iii. Determination of Na^+,K^+-ATPase and Mg^{2+}-ATPase enzymatic activities.

Na^+, K^+-ATPase activity was calculated from the difference between total ATPase activity (Na^+, K^+, Mg^{2+}-dependent ATPase) and Mg^{2+}-dependent ATPase activity. Total ATPase activity was assayed in an incubation medium consisting of 50 mM Tris-HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 240 mM sucrose, 1 mMethylenediamine tetraacetic acid K_2-salt (K+-EDTA), 3 mM disodium ATP and 80-100 μg protein of the homogenate in a final volume of 1 ml. Ouabain (1 mM) was added in order to determine the activity of Mg^{2+}-ATPase. The reaction was started by adding ATP and stopped after an incubation period of 20 min by addition of 2 ml mixture of 81% lubrol and 1% ammonium molybdate in 0.9 M H_2SO4 (Bowler, Tirri,1974; Chen et al.,2011). The yellow color which developed was read at 390 nm.

iv. Determination of TNF-α and IL-1 by microarray.

All samples were embedded in standard paraffin blocks. On the respective H&E-stained sections, a representative brain area was selected from the ipsilateral site. The corresponding area was marked on the surface of the standard paraffin block. Tissue cores were punched from the designated area using a specialized puncher. The process was repeated on the contralateral side of injury by selecting topographically identical area. All tissue cores (0.6cm in diameter), were put into tissue microarray block, covering up to 20 cores.

From tissue microarray blocks, 4μm thick section were cut and placed on silanized slides, then rehydrated in increasing concentrations of alcohol. Heat induced antigen retrieval was performed in automated staining device (Leica Bond Max Autostainer). After non specific antigen and peroxidase blockage primary antibodies IL-1, and TNF-α, were applied in a dilution of 1:100 for 30 minutes in room temperature. For colorimetric reaction secondary antibody and polymer-HRP based visualization system (Bond Polymer Refine Detection; Leica) was used and finally slides were counter stained by hematoxilin for three minutes and coverslipped.

Picture 26: Shows the final results of the technique of microarrays.

IId. Statistical analysis.

i. Statistical analysis used for the determination of Na^{+},K^{+}-ATPase and Mg^{2+}-ATPase activities.

The data were analyzed by a two-tailed Student's *t*-test, through SPSS for Window Software. The *P* values of <0.05 were considered statistically significant.

ii. Statistical analysis used for the determination of TNF-α and IL-1.

Parametric data was expressed at mean+/-SEM. Data was analysed with two way ANOVA, using statistical software (SigmaPlot 11, Systat Software Inc., San Jose, CA, USA). For pairwise comparisons, the Student-Newman-Keuls post hoc test was used and P values<0.05 were considered statistically significant.

III. RESULTS - DISCUSSION.

IIIa1. Activation of acetylocholinesterase after U-74389G administration.

i.RESULTS.

Figure 24a presents the effects of U-74389G administration on left and right (injured) BGTs, 4 and 24 hrs following the induction of ICH. The experimental induction of ICH causes a marginally non-significant decrease in AChE activity (when compared to the uninjured hemisphere's respective BGT AChE activity at 4 hrs: SALBGT4+ vs. SALBGT4−, −29%, $p<0.10$). The porcine AChE activity remains unaltered at 24 hrs in the saline-treated BGTs.

The administration of U-74389G managed to increase AChE activity in the uninjured BGT at 4 hrs when compared to the uninjured respective saline-treated BGTAChE activity at 4 hrs (+182%, LAZBGT4−vs SALBGT4−, $p<0.05$) and increased AChE activity in the injured BGT at 4 hrs (although this did not achieve significance:+33%, LAZBGT4+ vs. SALBGT4+, $p>0.05$). However, the benefits of U-74389G administration on AChE activity were obvious at the crucial injured BGT at 24 hrs, where AChE was found statistically-significantly activated when compared to the injured respective saline-treated BGT at 24 hrs (+70%, LAZBGT24 + vs SALBGT24+, $p<0.05$). The ability of U-74389G to enhance AChE activity was also found in the studied CCTs (Fig. 24b).

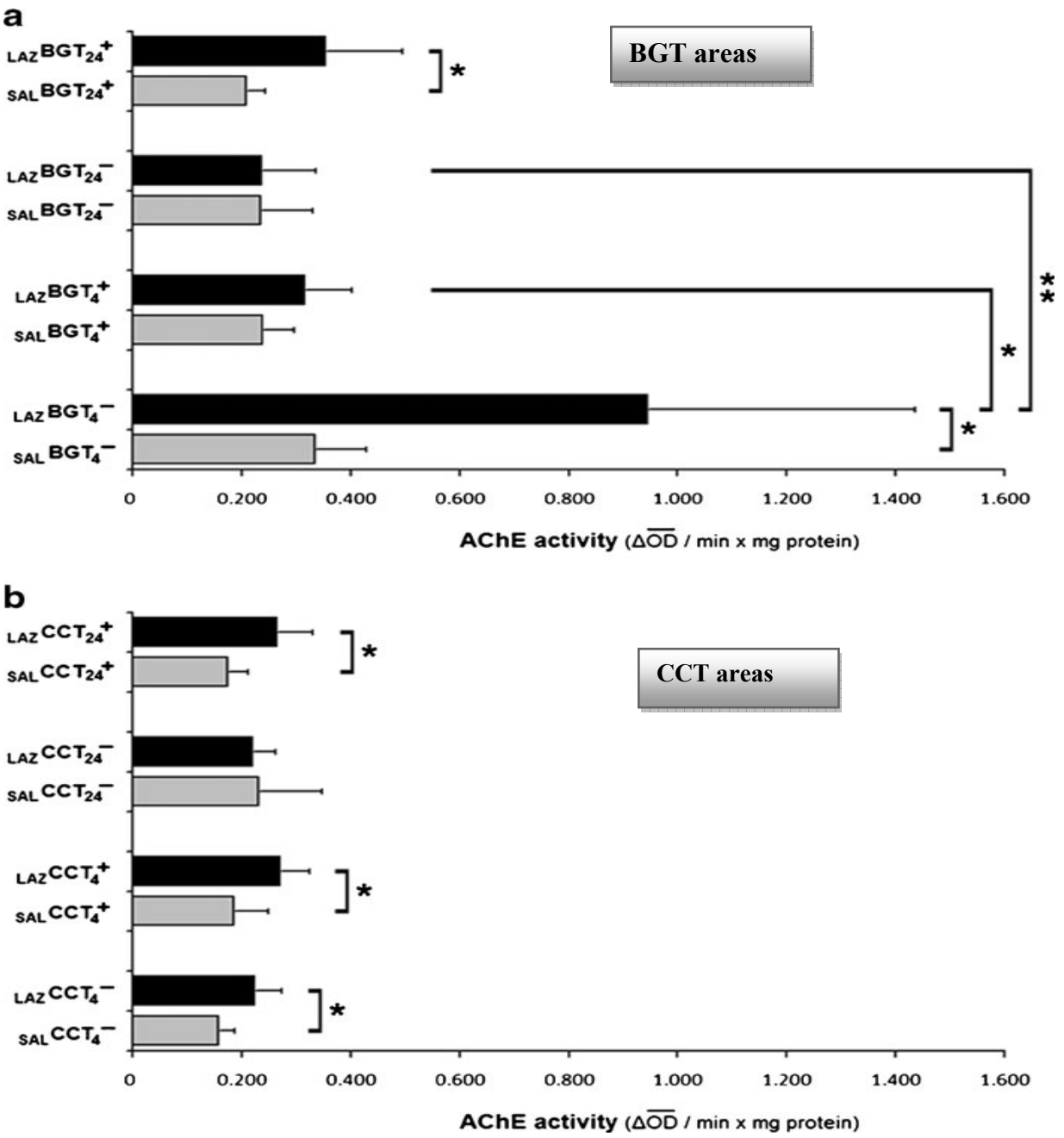


Fig 24 a,b : Modulation of acetylcholinesterase (AChE) activity in basal ganglia territory (BGT; a) and cerebral cortex territory (CCT; b) due to U-74389G (LAZ) or saline (SAL) administration in a porcine model of ICH. For more details concerning the experimental groups and the abbreviations used, see "Materials and Methods" and "Table 15". Each value indicates the mean±SD of five independent experiments (five samples for each value). The average of each experiment arose from three evaluations of the homogenized brain of each animal. Statistical analysis for Figure 24a: *$p<0.05$, **$p<0.01$. Statistical analysis for Figure 24b:*$p<0.05$.

ii DISCUSSION.

It is an established knowledge, that the model of ICH used in the present study is characterized by the induction of perihematomal oxidative stress, caused by plasma components through glutamate-stimulated increase in intracellular calcium levels and/or intracellular iron deposition (Wagner,2007).

A significant number of experimental studies have investigated the possible usage of the lazaroid U-74389G in cerebrovascular diseases, in particular through its antioxidant capacity. Durmaz et al. (2003) have shown that U-74389G presents a protective effect towards cerebral postischaemic reperfusion injury in terms of attenuating brain edema, reducing neuronal necrosis and enhancing bloodbrain barrier integrity.

Furthermore, the antioxidant potential of U-74389G has been already highlighted (as reflected by the reduction of extracellular superoxide anion concentrations and by restoring the activity of antioxidant enzymes) in cases of traumatic brain injury (Fabian et al., 1998) and ischemia / reperfusion-induced brain damage (Farbiszewski et al., 1994). The antioxidant capacity of U-74389G also seems to present neuroprotective effects in relation to the synaptic activity of neurons under conditions of hypoxia followed by reoxygenation (Vlkolinský et al., 1999) and under mild spinal cord compression (Harat and Kochanowski, 1999) (where the positive results were similar to those exerted by methylprednisolone for the same purpose).

Nevertheless, it should be noted that de Haan et al. (1998) failed to highlight any neurologic or histopathologic indications of the neuroprotective effects of U-74389G after spinal cord ischemia. The purpose of this study was to conduct a neurochemical evaluation (employing AChE activity as a biomarker) on the effects of U-74389G administration on a porcine model of ICH, based on the current knowledge concerning AChE activity behavior under conditions of edema, hypoxia and oxidative stress (such as those simulated in the present animal model); in fact, many experimental protocols have correlated neurotoxicity and acute oxidative stress with reduced AChE activity (Carageorgiou et al., 2004; El-Demerdash 2011; Gonçalves et al., 2010; Pari and Murugavel, 2007; Schallreuter et al., 2004; Shadnia et al., 2007; Weiner et al., 1994; Wyse et al., 2004; Zhou et al., 2003). Moreover, data indicating a down-regulation of cholinergic activity following brain injury already exist: patients with cerebral hemorrhage are known to present low AChE levels in their cerebrospinal fluid (CSF) compared to healthy control subjects (Egashira et al., 1999).

Our study demonstrates the activation of AChE activity following U-74389G administration in a porcine model of ICH. The lazaroid U-74389G seems to be an established neuroprotectant and this is the first report of its supporting role in the enhancement of cholinergic response to

the induction of ICH. Our study also demonstrates an initial (at 4 hrs post-induction of ICH) delayed increase in AChE activity within the injured BGT in comparison to the more rapid effect on AChE activity within the uninjured BGT, due to U-74389G administration. This finding, along with the fact that at a later time-point (at 24 hrs post-induction of ICH) AChE activity levels also present a delay in returning to basal levels in the lazaroid-treated injured BGT compared to that the presence of the ICH (injury) affects the U-74389G pharmacokinetics within the BGT injured areas, possibly due to disrupted microcirculation and ischemia.

It is assumed that 4 hrs after the experimental induction of ICH, the lazaroid-treated injured BGT (LAZBGT4+) demonstrates lower AChE activity when compared to the respective uninjuredBGT (LAZBGT4−) due to the fact that the induction of ICH does not allow for an equal distribution of the lazaroid among the two studied BGTs. On the other hand, 24 hrs after the induction of the ICH, the U-74389G demonstrates prolonged activating effects upon AChE within the lazaroid-treated injured BGT (LAZBGT24+) when compared to the respective time-matched uninjured (LAZBGT24−), possibly due to impaired venous drainage of the drug in the injured hemisphere that prolongs its actions. It would be very interesting to elucidate the U-74389G's potential direct role in cholinergic stimulation, as well as to conduct further studies concerning the pharmacokinetic properties of U-74389G within the central nervous system under the studied ICH-simulating conditions, in order to shed more light on the mechanisms involved in its neuroprotective properties.

Moreover, future experimental approaches would benefit from the inclusion of two shamoperated groups of pigs (intubated, anesthetized and having their dura drilled without any catheter insertion) that could reflect the state of the cholinergic system on the right hemisphere (consistently injured in our study) in terms of AChE activity, without the presence of any kind of traumatic injury or ICH, following saline- or lazaroid-administration.

IIIa2. Adenosinetriphosphatase activities due to U-74389G administration in a porcine model of intracerebral hemorrhage.

A parameter directly linked to neuronal viability following an ICH incident is, without doubt, the maintenance of crucial adenosine-triphosphate (ATP) levels. Oxidative stress is recognized as a key factor leading to mitochondrial dysfunction and ATP depletion in stroke (Moro et al., 2005; Zhan ,Yang, 2006), which in the case of ICH is triggered by both the primary and the secondary phases; apart from the oligemic and ischemic effect of the penumbra formation, the production of excitotoxicity and the generation of free radicals due to the release of haemoglobin (Hb) and other blood elements cause massive oxidative damage to nearly all cellular compartments (Qureshi et al., 2009; Aronowski , Zhao, 2011; Wagner et al., 2003; Nakamura et al., 2005). As a consequence of ATP depletion, the maintenance of crucial neuronal ATP-consuming functions, such as neuronal excitability, tend to fail (Santos et al., 1996; Chen et al., 2007).

We, herein, report the findings of our study concerning the role of two important adenosinetriphosphatases (ATPases) in a porcine model of spontaneous ICH that we have recently developed (Bimpis, Papalois et al., 2012). The one of these two ATPases is the sodium-potassium adenosine triphosphatase (Na^+, K^+-ATPase), which is a transmembrane enzyme implicated in neuronal excitability (Sastry, Phillis, 1977), metabolic energy production (Mata et al., 1980), as well as in the uptake and release of catecholamines (Bogdanski et al., 1968; Swann, 1984) and serotonin (Hernandez , 1987). It is responsible for the maintenance of the neuronal membrane potential, since it pumps three Na^+ ions out of the cell for every two K^+ ions pumped in, by consuming ATP; in fact, the functioning of Na^+,K^+-ATPase is responsible for a very large part of neuronal energy expenditure. The other studied ATPase is magnesium adenosine triphosphatase (Mg^{2+}-ATPase), which is an ATP-consuming enzyme that functions in order to maintain high brain intracellular Mg^{2+} ion levels, changes of which can control rates of protein synthesis and cell growth (Sanui, Rubin, 1982). The current study focuses on the effect of experimentally-induced ICH on the activities of these two ATPases within the first 4 and 24 hrs following the lesion's induction, in combination with a view of the effectiveness of the lazaroid antioxidant U-74389G administration; the latter is a 21-aminosteroid that is considered as a potent lipid peroxidation inhibitor (Hall et al., 1994), implemented as a potential neuroprotective approach to the used ICH model.

i. RESULTS.

Figure 25A, presents the effects of U-74389G administration on left and right (injured) BGTs' Na^+,K^+-ATPase activities, 4 and 24 hrs following the induction of ICH. The experimental induction of ICH caused no significant change in Na^+,K^+-ATPase activity (when compared to the uninjured hemisphere's respective BGT Na^+,K^+-ATPase activity at both 4 and 24 hrs time-points). However, saline-treated BGT Na^+,K^+-ATPase activities at 4 hrs post-ICH were found significantly higher than those at 24 hrs (+64%, SALBGT4- *vs* SALBGT24-, $P<0.01$; +62%, SALBGT4+ *vs* SALBGT24+, $P<0.05$) (Figure 25A). The administration of U-74389G caused a significant decrease in Na^+,K^+-ATPase activity in both the injured and the uninjured BGT at 4 hrs when compared with the injured and uninjured respective saline-treated BGT Na^+,K^+-ATPase activity at 4 hrs (-29%,LAZBGT4- *vs* SALBGT4-, $P<0.05$; -47%, LAZBGT4+ *vs* SALBGT4+, $P<0.01$) and maintained these Na^+,K^+-ATPase activity levels at 24 hrs (although these did not achieve significance when compared with the 24 hrs saline-treated ones) (Figure 25A).

However, the inhibitory effect of U-74389G administration on Na^+,K^+-ATPase activity at 4 hrs post-ICH was not obvious at the studied CCTs, where Na^+,K^+-ATPase was found statistically-significantly activated in the uninjured-side CCT when compared with the injured respective saline-treated CCT at 4 hrs (+31%, LAZCCT4- *vs* SALCCT4-, $P<0.05$) (Figure 25B). The ability of U-74389G to stimulate the CCT Na^+,K^+-ATPase activity was only evident in the uninjured CCT at 4 hrs and was not maintained till 24 hrs post-ICH (Figure 25B). However, in both saline-treated CCTs, Na^+, K^+-ATPase activity was found to be statistically-significantly increased at 4 hrs when compared with the 24 hrs respective regions (Figure 25B).

Figure 26A presents the effects of U-74389G administration on left and right (injured) BGTs' Mg^{2+}-ATPase activities, 4 and 24 hrs following the induction of ICH. The administration of U-74389G caused a significant decrease in the activity of Mg^{2+}-ATPase in the injured BGT at 4 hrs when compared with the respective saline treated one (-27%, LAZBGT4+ *vs* SALBGT4+, $P<0.05$); a phenomenon that was not maintained 24 hrs after (+55%, LAZBGT24+ *vs* LAZBGT4+, $P<0.01$). No other significant differences were recorder as concerns Mg^{2+}-ATPase activity among the studied CNS areas (Figures 26A and 26B).

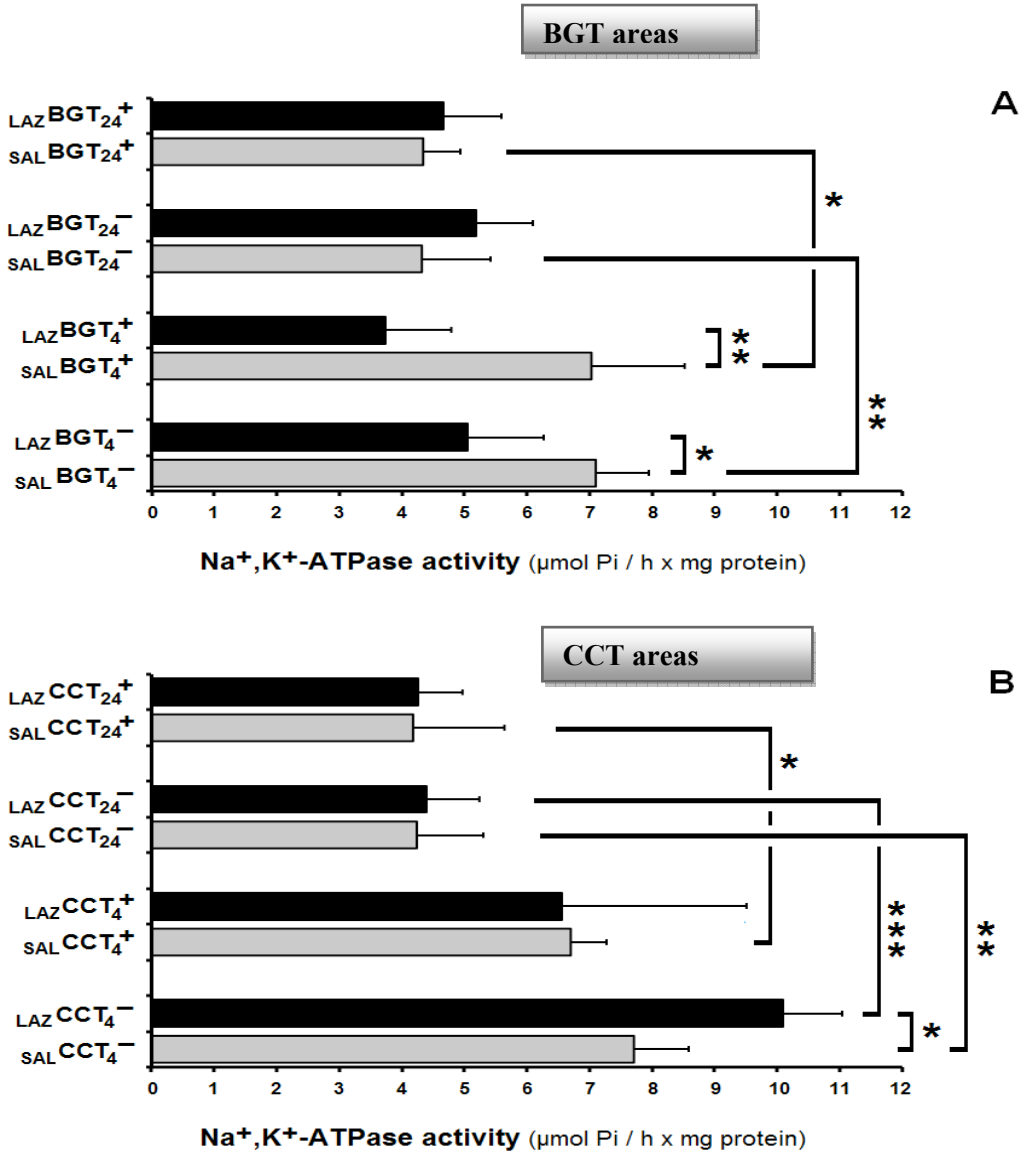


Figure 25 A, B. Modulation of Na^+,K^+-ATPase activity in basal ganglia territory (BGT; Figure 25A) and cerebral cortex territory (CCT; Figure 25B) due to U-74389G (LAZ) or saline (SAL) administration in a porcine model of ICH. For more details concerning the experimental groups and the abbreviations used see "Materials and Methods" and "Table 15". Each value indicates the mean } SD of five independent experiments (five samples for each value, each sample being measured in triplicate). Statistical analysis for Figure 25A: * $P < 0.05$, ** $P < 0.01$. Statistical analysis for Figure 25B: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

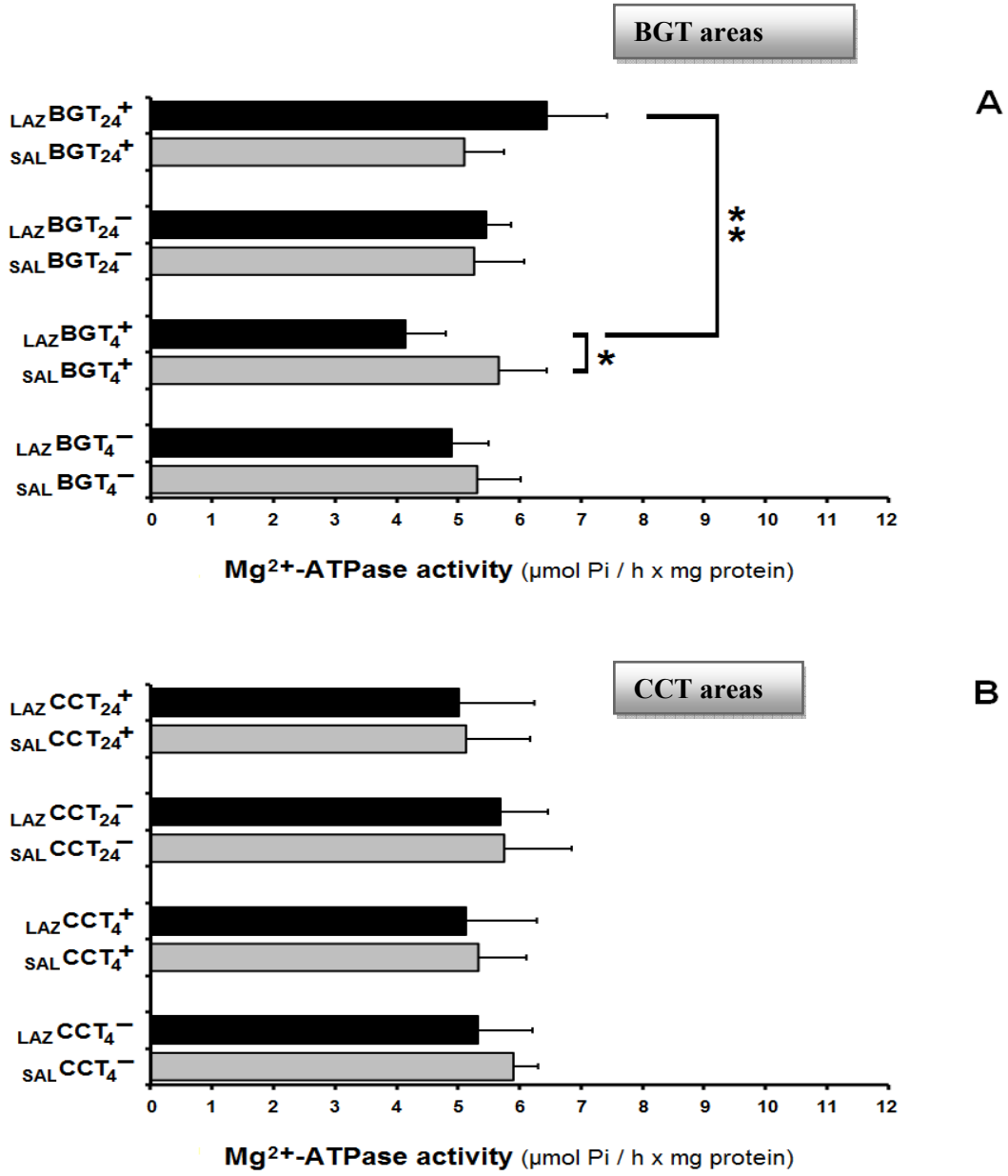


Figure 26 A,B. Modulation of Mg^{2+}-ATPase activity in basal ganglia territory (BGT; Figure 26A) and cerebral cortex territory (CCT; Figure 26B) due to U-74389G (LAZ) or saline (SAL) administration in a porcine model of ICH. For more detailsconcerning the experimental groups and the abbreviations used see "Materials and Methods" and "Table 15". Each value indicates the mean □} SD of five independent experiments (five samples for each value, each sample being measured in triplicate). Statistical analysis for Figure 26A: * $P < 0.05$, ** $P < 0.01$.under the studied ICH-simulating conditions.

ii. DISCUSSION.

The employed model of ICH is known to induce perihaematomal oxidative stress (Wagner 2007), and has already been studied by us in terms of its effect on AChE activity (an important neurochemical biomarker) (Bimpis et al. 2012). We, herein, report our findings concerning the role of two important ATPases in this same model of spontaneous ICH within the first 4 and 24 h following the lesion's induction. Of the two studied ATPases, Na^+,K^+-ATPase is the well studied one when it comes to the neurochemical evaluation of stroke, since: (a) Na^+,K^+-ATPase is considered to be particularly vulnerable to ATP depletion commonly observed in ischaemic stroke (Chen et al. 2011), (b) it is believed that the Na^+,K^+-ATPase failure (as an immediate consequence of stroke onset) evokes anoxic depolarization across the stroke-affected gray matter (White et al. 2012), (c) exposure to ICH-related erythrocyte-derived toxic elements such as ferrum ions is known to significantly decrease synaptosomal Na^+,K^+-ATPase activity (Keller et al. 1997), while (d) a decreased capillary Na^+,K^+-ATPase activity is known to occur both in vivo (after the onset of global cerebral ischemia) (Mrsulja et al. 1980) and in vitro (under hypoxic conditions) (Kawai et al. 1996).

It has been repeatedly shown that following either an in vivo or an in vitro experimental induction of ischemic stroke, both neurons and glial cells suffer from a failure of the Na^+,K^+-ATPase activity, a loss of membrane selectivity to ion permeation, as well as from a cytoskeletal disruption (leading to the development of swelling and cellular deformation) (Douglas et al. 2011). Zhu et al. (2008) have demonstrated that experimentally-induced hypobaric hypoxia on rat cerebral cortex causes a dramatic decrease in the activities of Na^+,K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase, while Jung et al. (2007) have reported a decrease in Na^+, K^+-ATPase protein expression in the brain slices of rats undergoing experimentally-induced cerebral ischaemia (produced by permanent middle cerebral artery occlusion).

Moreover, Yang et al. (2008) have recently suggested that the experimentally-induced cerebral infarction in rats could result in decreasing of the available ATP molecules, acidic metabolite's accumulation and epicyte injury, associated with a reduction of Na^+,K^+-ATPase activity. Haemorrhagic transformation (a common complication of cerebral infarction) was also studied by these authors (Yang et al. 2008), revealing a statistically-significant decrease of Na^+,K^+-ATPase activity at the 3 and 24 h timepoints but not at the 6 and 12 h timepoints after the operation. One should also notice that the neuropathological examination conducted within this study has revealed the existence of swollen mitochondria in the tissue of both cerebral infarction- and haemorrhagic transformation-undergoing groups, indicating an impairment in ATP supply

and a consequent damage of the Na^+,K^+-ATPase function (resulting in the edematic transformation of both brain mitochondria and cells) (Yang et al. 2008).

Wagner et al. (1998) have infused autologous blood (1.7 ml) into the porcine frontal lobe white matter and have studied the early brain metabolic alterations that occur after 1, 3, 5 and 8 h following the induction of ICH. Their impressive study has revealed that the rapid appearance of the oedema in the area surrounding the ICH and its high water content are not due to ATP deficiency, but probably a result of clot-derived plasma proteins' accumulation (Wagner et al. 1996; 1998). The authors revealed that the brain areas surrounding the ICH-induced haematoma appear with normal or significantly-higher ATP levels, and that several other metabolite levels (such as those of carbohydrate substrates) also increase (Wagner et al. 1998). Although the latter could be attributed to the perihaematomal plasma protein accumulation (Wagner et al. 1996) and are commonly described in oedematous regions (containing extravasated plasma proteins) adjacent to CNS tumors (Linn et al. 1989; Okada et al. 1992), the finding of elevated lactate concentrations in the oedematous white matter following the ICH-induction has led Wagner et al. (1998) to suggest the undergoing of increased aerobic glycolysis in these brain areas. Moreover, Wagner et al. (1998) also suggested that this enhanced aerobic glycolysis may reflect the occurrence of an intracellular compartmentalization of the glycolytic ATP production and utilization (as also seen in the case of muscle cells) (James et al. 1996; Paul et al. 1979), and could justify the carbohydrate metabolite accumulation through a reduction in metabolism.

Our findings seem to be in accordance with those of Wagner et al. (1998), as our experimental approach to ICH did not cause a reduction in Na^+,K^+-ATPase activity (Fig. 1a and b), as it might have been expected by the above-presented data acquired by other stroke models. The issue of whether a decrease or an increase in Na^+,K^+-ATPase activity has a definite effect upon the CNS cells coping with stroke simulating conditions is of a more complex nature than it initially seems. Oselkin et al. (2010) have recently reported that although increased Na^+,K^+-ATPase activity has been shown to protect hippocampal slice culture neurons against hypoxia-hypoglycemia, treatment with ouabain (the poisonous glycoside known to inhibit Na+,K+-ATPase at high concentrations) could stimulate Na^+,K^+-ATPase at low concentrations and, thus, could protect the neurons against ischaemia in a time-dependent way.

Moreover, although a decrease in Na^+,K^+-ATPase activity during ischaemia seems to be expected, the results of certain in vivo studies have varied (Djuričić et al. 1984; Shigeno et al. 1989). Our study has also revealed that the saline-treated experimentally-induced ICH causes a time-evolving reduction of Na^+,K^+-ATPase activity in all studied regions (BGT and CCT), when values are compared between the 4 and the 24 h timepoints (Fig. 25a and b). Although one could argue that this could be an effect related to the prolonged exposure to anaesthesia, the findings of

Wagner et al. (1998) might suggest for a gradual ATP depletion and/or expansion of oxidative epiphaenomena (directly related to ICH pathophysiology), leading to a significant inhibition of Na+,K+- ATPase. The latter could be partially justified by the finding that the lazaroid-treated group managed to maintain stable levels of Na^+,K^+-ATPase activity throughout the experiment's time-course in the BGT (Fig. 25a) but not in the CCT (Fig. 25b).

Since Na^+,K^+-ATPase is considered to be the major ATP consumer within the CNS (Astrup 1982; Clausen et al. 1991), the experimental application of Na^+,K^+-ATPase inhibitors have provided neuroprotective outcomes within the ischaemic stroke context (Cho et al. 2009; Wang et al. 2006). In our study, the administration of U-74389G statistically-significantly inhibited both the injured and the uninjured BGT Na^+,K^+-ATPase (Fig. 25a); a property not maintained until the 24 h timepoint, nor similarly established at the porcine CCT (Fig. 25b). The inhibited BGT Na^+,K^+-ATPase could suggest a very important role in the regional ATP economy (at the crucial first 4 h post-induction of the ICH) that needs to be further studied in order to be clarified. Moreover, the region-specific response of Na^+,K^+-ATPase to the lazaroid administration might point towards the involvement of other crucial parameters, such as the qualitative profile of the region's dominant systems of neurotransmission (Santos et al. 1996).

As concerns Mg^{2+}-ATPase, our study has only revealed a perihematomal BGT lazaroid-induced decrease in tis activity at 4 h after the ICH-induction, that was reversed to control levels by the 24 h timepoint (Fig. 26a). This finding also suggests a decrease in ATP consumption within thecrucial BGT+ due to U-74389G, that could indicate a very important (and potentially neuroprotective) contribution of the lazaroid into maintaining high ATP levels in the region.

It should, however, be noted that our herein reported findings concerning both the studied ATPases are not in accordance with those of Durmaz et al. (2003). Although conducted within a significantly different context, these authors have reported that the inhibition of Na^+,K^+-ATPase following traumatic brain injury in rats was accompanied by a parallel decrease in the activity of Mg^{2+}/Ca^{2+}-ATPase, and that both activities were found to be restored to control values by the prophylactic administration of U-83836E (a second generation lazaroid) (Durmaz et al. 2003).

In conclusion, our study demonstrates that the examined porcine model of ICH does not cause a decrease in Na^+,K^+-ATPase in the perihematomal BGT, nor a change in the activity of Mg^{2+}-ATPase. This is the first report focusing on these crucial ATPases in the experimental setting of ICH and is not in agreement with the majority of the findings concerning the behavior of these (crucial for CNS cell survival) enzymes under stroke-related ischaemic conditions. The administration of U-74389G in this ICH model revealed an injury specific type of behavior, that could be considered as neuroprotective provided that one considers that Na^+,K^+- and Mg^{2+}-ATPase inhibition might in this case diminish the local ATP consumption. Further experiments

are required in order to elucidate the U-74389G's potential direct role in ATPase inhibition, as well as to clarify the pharmacokinetic properties of U-74389G within the CNS under the studied ICH-simulating conditions. Moreover, future experimental approaches would benefit from the inclusion of two sham-operated groups of pigs (intubated, anesthetized and having their dura drilled without any catheter insertion) that could reflect the state of the studied enzyme activities (baseline levels) on the right hemisphere (consistently injured in our study), without the presence of any kind of traumatic injury or ICH, following saline- or lazaroid-administration.

IIIb. Time course of TNF-α and IL-1, effects of U-74389G on TNF-α and IL-1 4 and 24 hrs after the haematoma induction. Results of immunohistochemistry.

Two of the most important proinflammatory cytokines are Tumour Necrosis Factor-α and IL-1. These are very important regarding the secondary injury after ICH, proven to be responsible for oedema formation and triggering apoptotic pathways. According to Hua Y et al. (1998) and Xi et al. (2001), TNF-α increases after ICH, triggering complement activation. Mayne et al. (2001) showed that inhibition of TNF-α, finally reduces brain injury, while on the other hand, Qureshi et al. (2001), showed that TNF-α levels were normal one hour after ICH in a dog model of ICH. Hua et al. (2006), in their study in rats, observed that there is a timeline pattern in TNF-α expression, with the increase being evident as early as 2 hrs after ICH induction. In the same study, Hua et al. (2006), observed that TACE (known to play a key role in TNF-α activation), was increased within one hour, in the perihaematomal area, suggesting being involved in the early activation of TNF-α. In the TNF-α knock-out mouse, TNF-α was absent and oedema and neurological deficit found to be reduced in comparison to wild-type mice. Studies regarding the TNF-α, in traumatic brain injury and ischaemic stroke, revealed that TNF-α, may be responsible for brain injury by a number of mechanisms. There is enough data to support that after ICH, mechanisms that involve neutrophils and monocytes(Gong et al., 2000; Xi et al., 2001) , blood brain barrier disruption (Wagner et al., 1999, Xi et al., 2001, Yang et al., 1994) and apoptosis (Matsushita et al., 2000) are being involved in the pathophysiology of secondary brain injury. Barone et al. (1997) and Rosenberg et al. (1995), observed that exogenous TNF-α, results in oedema formation and also blood brain barrier disruption. On the other hand, blocking the synthesis of TNF-α, results in improved neuronal survival and recovery after traumatic brain injury, (Shohami et al., 1997). From the clinical studies, Castillo et al. (2002), reported relevance in the plasma TNF-α levels and oedema formation 5 hours after the onset of ICH.

Regarding the role of pro-inflammatory interleukin-1, several authors have described, increase of proinflammatory cytokines after ICH. Wagner et al. (2004, 2006), showed that upregulation of IL- 1, take place as early as 1-2 hrs after ICH induction, in the perihaematomal white matter. In these studies, Wagner et al. (2007) have also demonstrated the coleration of IL-1 upregulation, with oedema formation. In another study, Holmin, Mathiesen. (2000), described the administration of IL-1, in several brain pathologies and the coleration with inflammation, apoptosis and vasogenic oedema. Later on Allan, Rothwell. (2003), described the role of IL-1, in central nervous system injury. Lu et al. (2006), showed continuous upregulation of IL-1 gene expression the first 24 hrs after the haematoma induction in ICH. So overall, the role of IL-1, in various situations of brain injury, in neurodegeneration and situations of blood

brain barrier permeability, is well described. Masada et al. in their study, observed that IL-1 receptor antagonist overexpression, reduces oedema produced by thrombin. Wagner et al. (2004), showed that IL-1, in perihaematomal white matter in ICH, is mainly expressed to astroglia and microglia and chronologically after 24 hrs.

These two very important pro-inflammatory cytokines seems to play major role in the secondary brain injury that follows ICH.

Any inhibition of the upregulation of these cytokines, could be considered neuroprotective. In our study of experimental ICH in a large porcine model, we aimed to study the changes of TNF-α perihaematomal expression as well as the changes of IL-1, expression. We examined these changes at the first 4 hrs after the haematoma induction and also 24 hrs after the haematoma induction. At the same time, given that inhibition of the upregulation of these very important cytokines could be considered neuroprotective, we tested the effect of the administration of the lazaroid U-74398G on TNF-α and IL-1, expression at the perihematomal area in comparison to the opposite undamaged hemisphere. The lazaroid U-74389G was chosen because of his potent antioxidant effect (Hall ,1994). So the aims of our study was to determine the changes of these two proinflammatory cytokines and given that a timeline of their expression, could be a neuroprotective target, to see if a potent neuroprotectant such as U-74389G, could have a positive antioxidant and finally neuroprotectant role in ICH. The results are being presented below and further extensively discussed in the discussion that follows.

i. RESULTS.

Regarding TNF-α.

In the cerebral cortex and subcortical regions, TNF- α and Il-1 immunopositivity was observed in glial elements, microvessels and neurons. The number of neurons was approximately 10% of the total number of cells. There was no difference among the cortical and subcortical regions of the individual animals in the ipsilateral or contralateral site. Figure 27,28, summarizes the results regarding TNF-α, IL-1. TNF-α immunopositivity was significant higher on the ipsilateral side in the control group compared to the treated group (175+/-14 vs.101+/-12*cells/mm^{2}respectively) as well as there was significant difference between the ipsilateral and contralateral sites 4hrs following injury (Figure 27A). There was no difference 24 hrs following injury between groups and sites (Figure 27B).

Figure 27 A,B

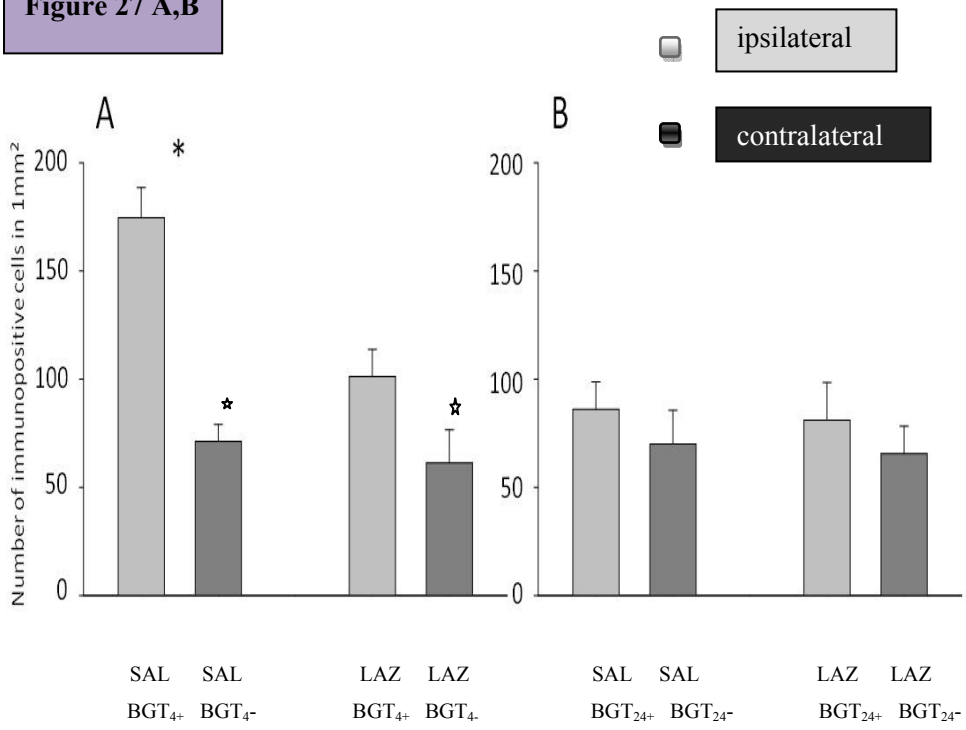


Figure 27.

Panel A shows the number of TNF-α immunopositive neurons 4 hours following ICH. The number of positive cells is significantly higher in the control group than in the group treated with antioxidant U-74389G (*p<0,05). There was significant difference in the number of TNF-α immunopositive cells between the ipsilateral and contralateral sites (*p<0,05). Panel B shows the number of TNF-α immunopositive neurons one day following ICH. There was no statistically significant difference between groups and sites of injury.

Regarding IL-1:

Panel A shows the number of IL-1 immunopositive neurons following ICH. The number of positive cells were significantly higher in the ipsilateral side ($p<0,05$). Panel B shows the number of IL-1 immunopositive neurons one day following ICH. There was no statistically significant difference between groups and sites of injury.

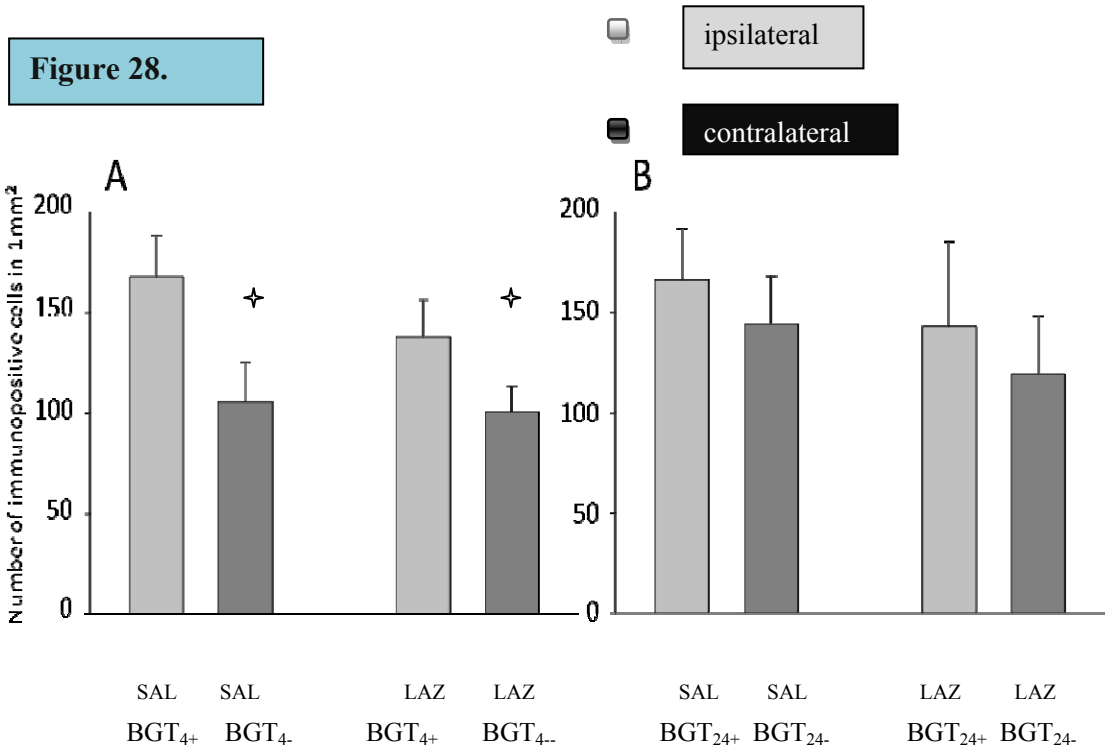


Figure 28.

Panel A shows the number of IL-1 immunopositive cells 4 hrs following ICH. The number of positive cells were significantly higher in the ipsilateral side (*$p<0,05$). Panel B shows the number of IL-1 neurons one day following ICH. There was no statistically significant difference between groups and sites of injury.

ii. DISCUSSION.

The employed model of ICH is known to induce perihematomal oxidative stress (Wagner, 2007), and has already been studied in terms of its effect in AChE activity (Bimpis, Papalois et al., 2012), and also regarding the modulation of crucial adenosinetriphosphatase activities (Bimpis, Papalois et al., 2013).

Proinflammatory cytokines are known to play a very important role in the pathophysiology of several brain pathologies, amongst them in ICH also. TNF-a is a very important cytokine. Seems that results in brain damage from the studies available in TBI and stroke (Barone, Feuerstein, 1999; Hallenbeck, 2002). The mechanisms of action seems to be those of BBB disruption (Yang et al., 1994; Wagner et al., 1999; Xi et al., 2001a) neutrophils and monocytes activation (Gong et al., 2000; Xi et al., 2001a) and apoptosis (Matsushita et al., 2000). Data that is available regarding especially the role of this cytokine, suggests that in in vitro models of rats and pigs, TNF-α increases after ICH, triggering complement activation (Hua Y et al., 1998; Xi et al., 2001a, b). In other studies, inhibition of TNF-α, finally reduced brain injury (Mayne et al., 2001). Other investigators emphasized in the TACE enzyme, which is known to be a very important enzyme in activation of TNF-α and early increase in his levels suggests TNF-α expression (Hua, 2006b). They observed that TACE was increased within one hour in their study, suggesting relevance to TNF-α activation. In the TNF-α knock-out mouse, TNF-α was absent and the neurological deficit was reduced in comparison, the oedema also. In other similar studies, blocking TNF-α results in less brain damage (Barone et al., 1997). Clinically, Castillo et al. (2002), reported relevance in the plasma TNF-α levels and oedema and particularly early, at 5 hrs after the onset.

Controversy exists regarding the timeline of TNF-α, and IL-1, after ICH in experimental models. As early as 2001, Qureshi et al. found that there is not an early increase in the TNF-α levels after ICH in a dog model of ICH. Hua Ya et al. (2006), showed on the opposite that there is an early increase in TNF-α after ICH in mice. Further to that starts at hour two when it is maximum and then decreases rapidly so as by the first 24 hrs returns approximately to normal levels and by the hour 72, approximates finally the baseline.

Regarding the role of IL-1, Wagner et al. (2004, 2006), showed that there is an early increase of IL-1 levels in intracerebral haemorrhage, in their study observed that the levels of interleukin 1, increases in the perihaematomal white matter as early as 1-2 hrs after the haematoma induction. Other authors have studied in excess the proinflammatory contribution of IL-1 in various brain pathologies such as those of brain injury, neurodegeneration, and conditions of increased blood brain barrier pathologies (Holmin , Mathiesen, 2000; Allan,

Rothwell, 2003; Wagner et al. 2004; Aronowski, Hall, 2005, Wagner et al.2006). In another study, Masada et al. (2001), observed that an IL-1 receptor antagonist (IL-1ra), when overexpressed, reduced oedema caused by thrombin stimulation in the perihaematomal area. Lu et al. (2006), showed that there is a continuous upregulation of IL-1, expression over the first 24 hrs. IL-1, in perihaematomal white matter, at 24 hrs after the haematoma induction, is found especially to astrocytes and microglial cells (Wagner et al., 2004).

Conclussion

In our study, we observed that there is a time course of TNF-α, in a porcine model of ICH, during the first 24 hrs of intracerebral haematoma induction. Our findings, showed that there is an increased number of immunopositive neurons 4 hrs following ICH, which was significantly higher in the control group than in the treated with antioxidant U-74389G group (*p<0,05). A significant difference between ipsilateral and contralateral sites was also observed. After 24 hrs there was no statistically significant difference between groups and sites of injury. These findings suggest that there is a timeline of TNF-α expression that is evident at hour 4 after the haematoma induction and decreases 24 hrs after the haematoma induction. These findings are similar to those of Ya Hua et al. that showed there is a timeline in TNF-α levels after ICH. In their study in mice, there was an early increase in TNF-α, at hour 2, persisting at hour 4 and start decreasing at hour 6, returning gradually at baseline at hour 24 and 72. In the same study, the overall levels of TNF-α, in the ipsilateral hemisphere were statistically significantly higher in comparison to contralateral hemisphere. These findings suggest that there is an early increase in TNF-α immunopositive cells that is very important regarding the secondary injury given the crucial role of this proinflammatory cytokine in brain damage after ICH. Further to that, the observation in our study that the groups treated with the antioxidant U-74389G, had statistically significant lower levels of TNF-α, regarding the control groups at hour 4 after the haematoma induction returning approximately to baseline 24 hrs after the haematoma induction, suggests a neuroprotective role of U-74389G in our porcine model of intracerebral haemorrhage. Regarding the changes in IL-1 immunopositive cells, there were similar findings that are in accordance with the findings of other previous studies regarding the upregulation of IL-1 after intracerebral haematoma induction. In our study there was a statistically significant increase of IL-1 immunopositive cells 4 hrs after the haematoma induction which wasn't present after 24 hrs of onset, between sites and experimental groups. Our findings suggest that there is an early increase in IL-1, immunopositive cells that decrease 24 hrs after the haematoma induction. The group treated with antioxidant had less immunopositive cells than the group treated with saline but the result was not statistically significant.

The finding that there is a statistically significant difference in TNF-α between those treated with U-74389G and those treated with saline at hour 4, suggests that the lazaroid U-74389G, is a potent antioxidant and could be considered as a treatment option early after the haematoma induction. This observation, carries the known from the past discussion regarding the differences between laboratory and bedside for most of the possible antioxidant treatments and the limitations of many agents studied in the past and found to be effective for their antioxidant role in preclinical models but failed the clinical trials, such as the lazaroid U-74006 (tirilazad mesylate) in the past.

Overall, our results, suggest that there is an early (at hour 4), increase of proinflammatory cytokines (TNF-α, IL-1), in our porcine model of ICH which is statistically significant and there is also a statistically significant difference when compare the groups treated with the antioxidant U-74389G regarding TNF-α. This result could suggest the potent antioxidant role of U-74389G, taking into account the differences between bench and bedside that have been observed in many studies in the past. Further investigations are needed in order to determine more precisely the changes and the role of these proinglammatory cytokines in ICH and moreover the role of a possible antioxidant treatment early after ICH occurrence.

Abstract

Spontaneous intracerebral haemorrhage (ICH) represents a partially understood cerebrovascular disease of high incidence, morbidity and mortality, accounting for 10-15% of all strokes. In my study, I investigated the pathophysiological changes that follow intracerebral haematoma induction on a porcine model of ICH. The study focused on the determination of changes in tumour necrosis factor α (TNF-α) and interleukin 1 (IL-1) the first 4 and 24 hrs of ICH as well as the changes of the crucial enzymes adenosinetriphosphatases (ATPases), in combination with a study of the effectiveness of the lazaroid antioxidant U-74389G. Regarding both TNF-α and IL-1, statistically significant increase was observed in the ipsilateral side of the haematoma as early as 4 hrs and decrease again 24 hrs after the onset. U-74389G when administered resulted in statistically significant decrease of TNF-α, (at the heamatoma site in comparison to the controls) and non statistically significant decrease of IL-1 when compared to the controls. Regarding the ATPases, my study demonstrated that the examined ICH model does not cause a decrease in Na^+,K^+-ATPase activity (the levels of which are responsible for very large part of neuronal energy expenditure) in the perihaematomal basal ganglia territory, nor a change in the activity of Mg^{2+}-ATPase. The administration of U-74389G (an established neuroprotectant) in this ICH model revealed an injury specific type of behavior, that could be considered as neuroprotective.

I also investigated the role of acetylocholinesterase (AChE; a crucial membrane bound enzyme involved in cholinergic neurotransmission) in the same porcine model of spontaneous ICH, with a focus on the first 4 and 24 hrs following the lesion's induction, in combination with a study of the effectiveness of the lazaroid antioxidant U- 74389G administration. My study demonstrates the activation of AChE activity following U-74389G administration. The lazaroid U-74389G seems to be an established neuroprotectant and this is the first report of its supporting role in the enhancement of cholinergic response to the induction of ICH.

Keywords: intracerebral haemorrhage; porcine model; lazaroid; U-74389G. acetylocholinesterase. AChE , adenosinetriphosphatases; Na^+,K^+-ATPase; Mg^{2+}-ATPase; activity; Tumour Necrosis Factor-α, Interleukin-1.

Περίληψη

Η αυτόματη ενδοεγκεφαλική αιμορραγία (AEE), είναι μια μερικώς κατανοητή αγγειακή εγκεφαλική νόσος, χαρακτηριζόμενη από υψηλή επίπτωση θνητότητα και θνησιμότητα , αποτελεί δε το 10-15% όλων των αγγειακών εγκεφαλικών επεισοδίων.Στη παρούσα μελέτη, εξέτασα παθοφυσιολογικές μεταβολές που έπονται της πρόκλησης ενδοεγκεγαλικού αιματώματος σε πειραματικό πρότυπο ενδοεγκεφαλικού αιματώματος σε χοίρο. Η μελέτη επικεντρώθηκε στον προσδιορισμό των αλλαγών στον παράγοντα νέκρψσης όγκου α (TNF-α) και στην ιντερλευκίνη 1, τις πρώτες 4 και 24 ώρες από την πρόκληση του αιματώματος όπως και στις μεταβολές των σημαντικών ενζύμων αδενοτριφωσφατασες (ATPases), σε συνδιασμό με τη μελέτη της αντιοξειδωτικής αποτελεσματικότητας του Λαζαροειδούς U-74389G. Όσον αφορά τόσο τον TNF-α όσο και την IL-1, στατιστικά σημαντική αύξηση παρατηρήθηκε στο ομόπλευρο του αιματώματος ημισφαίριο στις 4 ώρες από την πρόκληση του αιματώματος και μείωση στο εικοσιτετράωρο. Ο παράγοντας U-74389G προκάλεσε στατιστικά σημαντική μείωση του TNF-α στην περιοχη του αιματώματος σε σχέση με την ομάδα ελέγχου και μείωση (αλλά όχι στατιστικά σημαντική) της IL-1 σε σύγκριση με τις ομάδες ελέγχου. Όσον αφορά στις ATPάσες, η μελέτη κατέδειξε ότι δεν προκαλείτε μείωση στη δραστικότητα της Na^+,K^+-ATPασης ούτε της Mg^{2+}-ATPάσης στην περί του αιματώματος περιοχή. Η χορήγηση του U-74389G (με γνωστή νευροπροστατευτική δράση) παρουσίασε στο μοντέλο συμπεριφορά που θα μπορούσε να θεωρηθεί νευροπροστατευτική.

Στη μελέτη, εξετάστηκε επίσης ο ρόλος της ακετυλοχολινεστεράσης (AChE,σημαντικού μεμβρανικού ενζύμου που εμπλέκεται στη νευροδιαβίβαση) στο ίδιο μοντέλο, με επικέντρωση στις πρώτες 4 και 24 ώρες από την πρόκληση του αιματώματος σε συνδιασμό με τη μελέτη της επίδρασης του αντιοξειδωτικού λαζαροειδούς U- 74389G.Η μελέτη κατέδειξε την ενεργοποίηση της ακετυλοχολινεστεράσης μετά τη χορήγηση του παράγοντα U- 74389G. Το λαζαροειδές U-74389G φαίνεται να ασκεί νευροπροστατευτική δράση και αυτή είναι η πρώτη αναφορά του ρόλου του στην ενεργοποίηση του χολινεργικού συστήματος ως απάντηση στην πειραματική πρόκληση ενδοεγκεφαλικού αιματώματος.

Λέξεις κλειδιά: ενδοεγκεφαλικό αιμάτωμα, μοντέλο χοίρου, λαζαροειδές, U-74389G, ακετυλοχολινεστεράση(Ache), αδενοτριφωσφατάσες; Na^+,K^+-ATPαση; Mg^{2+}-ATPαση δραστικότητα, παράγοντας νέκρωσης όγκου-α , ιντερλευκίνη-1.

References

Abilleira S, Montaner J, Molina CA, Monasterio J, Castillo J, Alvarez-Sabin J. Matrix metalloproteinase-9 concentration after spontaneous intracerebral hemorrhage. J Neurosurg 2003; 99:65–70.

Abbott RD, Yin Y, Reed DM, Yano K. Risk of stroke in male cigarette smokers.N Engl J Med. 1986 Sep 18; 315 (12):717-20.

Acheson RM and Williams DRR (1983) Does consumption of fruit and vegetables protect against stroke? *Lancet* 8335:1191–1193

Aggarwal SK, Williams V, Levine SR, et al. (1996). Cocaineassociated intracranial hemorrhage: absence of vasculitis in 14 cases. Neurology 46: 1741–1743.

Alemany Ripoll M, Gustafsson O, Siosteen B, Olsson Y, Raininko R. MR follow-up of small experimental intracranial haemorrhages from hyperacute to subacute phase. Acta Radiol 2002; 43(1):2–9.

Albers GW, Sherman DG, Gress DR, et al. (1991). Stroke prevention in nonvalvular atrial fibrillation: a review of prospective randomized trials. Ann Neurol 30: 511–518

Alberts MJ, Davis JP, Graffagnino C, McClenny C, Delong D, Granger C, *et al*. Endoglin gene polymorphism as a risk factor for sporadic intracerebral hemorrhage. Ann Neurol 1997;41:683-6

Allan SM, Rothwell NJ. Inflammation in central nervous system injury.
Philos Trans R Soc Lond B Biol Sci. 2003 Oct 29;358(1438):1669-77. Review.

Alvarez-Sabin J, Delgado P, Abilleira S, Molina CA, Arenillas J, Ribo M, Santamarina E, Quintana M, Monasterio J, Montaner J. Temporal profile of matrix metalloproteinases and their inhibitors after spontaneous intracerebral hemorrhage: relationship to clinical and radiological outcome. Stroke 2004;35:1316–1322.

Ames BN, Cathcart R, Schweisrs E, and Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant and radical caused aging and cancer: a hypothesis. *Proc Natl Acad Sci USA* **73:**6858–6862

Anderson, D. K., Braughler, J. M., Hall, E. D., Waters, T. R., McCall, J.M., and Means, E. D.: Effects of treatment with U-74006F on neurological outcome following experimental spinal cord injury. J. Neurosurg. **69:** 562–567, 1988

Anderson DK, Hall ED, Braughler JM, McCall JM, and Means ED (1991) Effects of delayed administration of U-74006F (tirilazad mesylate) on recovery of locomotor function following experimental spinal cord injury. *J. Neurotrauma* 8:187–192.

Anderson MF, Blomstrand F, Blomstrand C, Eriksson PS, Nilsson M. Astrocytes and stroke: networking for survival? Neurochem Res 2003;28: 293–305.

Anik I, Secer HI, Anik Y, Duz B, Gonul E. Meta-analyses of intracerebral hematoma treatment.Turk Neurosurg. 2011 Jan;21(1):6-14

Anish Bhardwaj, Nabil J. Alkayed, Jeffrey R. Kirsch,Richard J. Traystman., "acute stroke bench to bedside", eds. Chapter 15, p 164 Informa Healthcare, USA, Newyork, 2007.

Antithrombotic Trialists' Collaboration (2002). Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 324: 71–86.

Aoki T, Sumii T, Mori T, Wang X, Lo EH. Blood-brain barrier disruption and matrix metalloproteinase-9 expression during reperfusion injury. Mechanical versus embolic focal ischemia in spontaneously hypertensive rats. Stroke. 2002;33:2711–2717

Arakawa S, Saku Y, Ibayashi S, et al. (1998). Blood pressure control and recurrence of hypertensive brain hemorrhage.Stroke 29: 1806–1809.

Ariesen MJ, Claus SP, Rinkel GJ, Algra A. Risk factors for intracerebral hemorrhage in the general population: a systematic review Stroke. 2003 Aug; 34(8):2060-5. Epub 2003 Jul 3.

Ardizzone TD, Lu A, Wagner KR, Tang Y, Ran R, Sharp FR. Glutamate receptor blockade attenuates glucose hypermetabolism in perihematomal brain after experimental intracerebral hemorrhage in rat. Stroke. 2004 Nov;35(11):2587-91. Epub 2004 Sep 16.

Aronowski J, Zhao X. Molecular pathophysiology of cerebral hemorrhage: secondary brain injury.Stroke. 2011 Jun; 42(6):1781-6. doi: 10.1161/STROKEAHA.110.596718. Epub 2011 Apr 28. Review.

Aronowski J, Hall CE. New horizons for primary intracerebral hemorrhage treatment: experience from preclinical studies. *Neurol Res*. 2005; 27:268–279.

Aronowski J, Zhao X (2011) Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. Stroke 42:1781-1786.

Asano T, Takakura K, Sano K, Kikuchi H, Nagai H, Saito I, Tamura A, Ochiai C, Sasaki T. Effects of a hydroxyl radical scavenger on delayed ischemic neurological deficits following aneurysmal subarachnoid hemorrhage: results of a multicenter, placebo-controlled double-blind trial. J Neurosurg. 1996 May;84(5):792-803.

Ashrafian H. Familial stroke 2700 years ago. Stroke. 2010 Apr;41(4):e187; author reply e188.

Astrup J (1982) Energy-requiring cell functions in the ischemic brain. Theircritical supply and possible inhibition in protective therapy. J Neurosurg 56:482-497

Atrial Fibrillation Investigators (1994). Risk factors for stroke and efficacy of antithrombotic therapy in atrial fibrillation. Analysis of pooled data from five randomized controlled trials. Arch Intern Med 154: 1449–1457

Auer LM, Deinsberger W, Niederkorn K, Gell G, Kleinert R, Schneider G, Holzer P, Bone G, Mokry M, Körner E, et al. Endoscopic surgery versus medical treatment for spontaneous intracerebral hematoma: a randomized study.J Neurosurg. 1989 Apr;70(4):530-5.

Auer LM. Endoscopic evacuation of intracerebral haemorrhage. High-tec-surgical treatment--a new approach to the problem? Acta Neurochir (Wien). 1985;74(3-4):124-8.

Aurell M, Hood B. Cerebral hemorrhage in a population after a decade of active antihypertensive treatment. Acta Med Scand. 1964 Sep;176: 377-83.

Aydin MD, Erdogan AR, Cevli SC, Gundogdu C, Dane S, Diyarbakirli S. Ganglionary mechanisms of spasticity and ileus in cerebral hemorrhage: an experimental study. Int J Dev Neurosci 2006;24(7):455–9.

Back T, Schüler OG. The natural course of lesion development in brain ischemia. Acta Neurochir Suppl. 2004;89:55-61. Review.

Bae H, Jeong D, Doh J, Lee K, Yun I, Byun B. Recurrence of bleeding in patients with hypertensive intracerebral hemorrhage. Cerebrovasc Dis 1999;9: 102-8.

Bagley, C.: Spontaneous cerebral hemorrhage. Arch. Neurol. & Psychiat.,1932, 27, 1133.

Bailey RD, Hart RG, Benavente O, Pearce LA. Recurrent brain hemorrhage is more frequent than ischemic stroke after intracranial hemorrhage. Neurology 2001;56:773-7.

Balestrino M, Rebaudo R, and Lunardi G (1999) Exogenous creatine delays anoxic depolarization and protects from hypoxic damage: dose effect relationship. *Brain Res* 816:124–130.

Bamford J, Sandercock P, Dennis M, et al. (1988). A prospective study of acute cerebrovascular disease in the community: the Oxfordshire Community Stroke Project 1981–86. 1. Methodology, demography and incident cases.

Barnhart, R.ed. *The Barnhart Concise Dictionary of Etymology* (1995).

Barone FC, Feuerstein GZ: Inflammatory mediators and stroke: New opportunities for novel therapeutics. J Cereb Blood Flow Metab 19:819–834, 1999.

Barr TL, Latour LL, Lee KY, Schaewe TJ, Luby M, Chang GS, El-Zammar Z, Alam S, Hallenbeck JM, Kidwell CS, Warach S. Blood-brain barrier disruption in humans is independently associated with increased matrix metalloproteinase-9. Stroke 2010;41:e123–e128.

Barrett RJ, Hussain R, Coplin WM, Berry S, Keyl PM, Hanley DF, Johnson RR, Carhuapoma JR. Frameless stereotactic aspiration and thrombolysis of spontaneous intracerebral hemorrhage.Neurocrit Care. 2005;3:237-45.

Barron KD, Fergusson G (1959). Intracranial hemorrhage as a complication of anticoagulant therapy. Neurology 9:447–455.

Batjer HH, Reisch JS, Allen BC, Plaizier LJ, Su CJ. Failure of surgery to improve outcome in hypertensive putaminal hemorrhage. A prospective randomized trial.Arch Neurol. 1990 Oct;47(10):1103-6.

Back T. and Schuler OG. The natural course of lesion development in brain ischemia. Acta Neurochir (2004) [Suppl] 89: 55-619.

Beilharz EJ , Williams CE , Dragunow M , Sirimanne ES , Gluckman PD. Mechanisms of delayed celldeath following hypoxic-ischemic injury in the immature rat: evidence for apoptosis during selective neuronal loss . Brain Res Mol Brain Res 1995 ; 29 : 1 – 14 .

Bellander BM, von Holst H, Fredman P, Svensson M. Activation of the complement cascade and increase of clusterin in the brain following a cortical contusion in the adult rat.J Neurosurg. 1996 Sep;85(3):468-75.

Bendich A (1993) Biological functions of dietary carotenoids. *Ann NY Acad Sci* 691:61–67.

Benveniste H, Dreger J, Schousboe A, and Diemer NH (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. *J Neurochem* 43:1369–1374.

Benzie IFF and Strain JJ (1996) Uric acid: friend or foe. *Redox Report* 2:231–234.

Bernstein M, Fleming JF, Deck JH. Cerebral hyperperfusion after carotid endarterectomy: a cause of cerebral hemorrhage. Neurosurgery. 1984 Jul;15(1):50-6.

Beschorner R, Adjodah D, Schwab JM, Mittelbronn M, Pedal I, Mattern R, Schluesener HJ, Meyermann R. Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. Acta Neuropathol (Berl) 2000;100:377–384.

Bestue-Cardiel M, Martin-Martinez J, Iturriaga-Heras C, Ara-Callizo JR, Oliveros-Juste A. Leukocytes and primary intracerebral hemorrhage. Rev Neurol 1999;29:968–971.

Beyer CE, Steketee JD, and Saphier D (1998) Antioxidant properties of melatonin—an emerging mystery. *Biochem Pharmacol* **56:**1265–1272.

Bhardwaj A, Ulatowski JA. Cerebral Edema: Hypertonic Saline Solutions. Curr Treat Options Neurol. 1999 Jul;1(3):179-188.

Bimpis A, Papalois A, Tsakiris S, Zarros A, Kalafatakis K, Botis J, Stolakis V,Zissis KM, Liapi C (2012) Activation of acetylcholinesterase after U-74389G administration in a porcine model of intracerebral hemorrhage. Metab Brain Dis 27:221-225.

Bimpis A, Papalois A, Tsakiris S, Kalafatakis K, Zarros A, Gkanti V, Skandali N, Al-Humadi H, Kouzelis C, Liapi C. Modulation of crucial adenosinetriphosphatase activities due to U-74389G administration in a porcine model of intracerebral hemorrhage.Metab Brain Dis. 2013 Jan 24.

Blackshear JL, Kopecky SL, Litin SC, Safford RE, Hammill SC. Management of atrial fibrillation in adults: prevention of thromboembolism and symptomatic treatment. Mayo Clin Proc. 1996 Feb;71(2):150-60. Review.

Bogdanski DF, Tissari A, Brodie BB (1968) Role of sodium, potassium, ouabain and reserpine in uptake, storage and metabolism of biogenic amines in synaptosomes. Life Sci 7:419-428

Bose R, Schnell CL, Pinsky C, and Zitko V (1992) Effects of excitotoxins on free radical indices in mouse brain. *Toxicol Lett* 60:211–219

Bowler K, Tirri R (1974) The temperature characteristics of synaptic membrane ATPases from immature and adult rat brain. J Neurochem 23:611-613

Bracken MB, Shepard MJ, Holford TR, et al. for the National Acute Spinal Cord Injury Study (1997) Administration of methylprednisolone for 24 or 48 hours or tirilazad mesylate for 48 hours in the treatment of acute spinal cord injury: results of the Third National Acute Spinal Cord Injury Randomized Controlled Trial. *JAMA (J Am Med Assoc)* 277:1597–1604.

Brahmachari S, Fung YK, Pahan K. Induction of glial fibrillary acidic protein expression in astrocytes by nitric oxide. J Neurosci 2006;26:4930–4939.

Braughler, J. M., Burton, P. S., Chase, J. F., Pregenzer, J. F., Jacobsen, E. J. Vandoornik, F. J. Tustin, J. M., Ayer, D. E., and Bundy, G. L.: Novel membrane localized iron chelators as inhibitors of iron-dependent lipid peroxidation. Biochem. Pharmacol. 37: 3853–3860, 1988a.

Braughler, J. M., Duncan, L. A., and Chase, R. L.: The involvement of iron in lipid peroxidation: Importance of ferric to ferrous ratios in initiation.J. Biol. Chem. 261: 10282–10289, 1986

Braughler, J. M., and Pregenzer, J. F.: The novel 21-aminosteroid inhibitors of lipid peroxidation: reactions with lipid peroxyl and phenoxy radicals. Free Radic. Biol. Med. 7: 125–130, 1989.

Briani S, Dallo Ore G. [Angiographic aspects of spontaneous intracerebral hematomas].Minerva Neurochir. 1958 Jul-Sep;2(3):163-70. Italian. No abstract available.

Broderick JP. Stroke trends in Rochester, Minnesota, during 1945 to 1984.Ann Epidemiol. 1993a Sep;3(5):476-9.

Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. Volume of intracerebral hemorrhage. A powerful and easy-to-use predictor of 30-day mortality.Stroke. 1993b Jul;24(7):987-93.

Broderick JP, Brott T, Tomsick T, et al. (1992). The risk of subarachnoid and intracerebral hemorrhages in blacks as compared with whites. N Engl J Med 326: 733–736.

Bromont C, Marie C, and Bralet J (1989) Increased lipid peroxidation in vulnerable brain regions after transient forebrain ischemia. *Stroke* 20:918–924.

Brott T , Broderick J , Kothari R , et al. Early hemorrhage growth in patients with intracerebral hemorrhage. Stroke 1997 ; 28 : 1 – 5 .

Brott T, Thalinger K, Hertzberg V (1986). Hypertension as a risk factor for spontaneous intracerebral hemorrhage.Stroke 17: 1078–1083.

Brown RC, Mark KS, Egleton RD, Huber JD, Burroughs AR, Davis TP. Protection against hypoxia-induced increase in blood-brain barrier permeability: role of tight junction proteins and NFkappaB. J Cell Sci. 2003 Feb 15;116(Pt 4):693-700.

Brundin P, Pogarell O, Hagell P, Piccini P, Widner H, Schrag A, Kupsch A, Crabb L, Odin P, Gustavii B, Björklund A, Brooks DJ, Marsden CD, Oertel WH, Quinn NP, Rehncrona S, Lindvall O. Bilateral caudate and putamen grafts of embryonic mesencephalic tissue treated with lazaroids in Parkinson'sdisease.Brain. 2000 Jul;123 (Pt 7):1380-90.

Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: effect on regional cerebral blood flow. Surg Neurol 1988;29(2):101–7.

Bullock R, Mendelow AD, Teasdale GM, Graham DI. Intracranial haemorrhage induced at arterial pressure in the rat. Part 1: Description of technique, ICP changes and neuropathological findings. Neurol Res 1984;6(4):184–8.

Bullock R, Zauner A, Woodward J, and Young HF (1995) Massive persistent release of excitatory amino acids following human occlusive stroke. *Stroke* 26:2187–2189.

Bundy GL, Ayer DE, Banitt LS, Belonga KL, Mizsak SA, Palmer JR, Tustin JM, Chin JE, Hall ER, Linseman KL, et al. (1995) Synthesis of novel 2,4 diaminopyyrrolo-[2,3-d] pyrimidines with antioxidant neuroprotective and antiasthma activity.*J Med Chem* 38:4161–4163.

Burns J , Lisak R , Schut L , et al. Recovery following brainstem hemorrhage . Ann Neurol 1980 ; 7 (2) : 183 – 184 .

Butcher KS , Baird T , MacGregor L , Desmond P , Tress B , Davis S . Perihematomal edema in primary intracerebral hemorrhage is plasma derived . Stroke 2004 ; 35 (8) : 1879 – 1885 .

Campo GM, Squadrito F, Altavilla D, Squadrito G, Avenoso A, Canale P, Ioculano M, Sperandeo A, Caputi AP. Protection of ischemic and reperfused rat myocardium by the nonglucocorticoid 21-aminosteroid U-74389G, a new inhibitor of lipid peroxidation. J Pharmacol Exp Ther. 1996 Apr;277(1):333-40.

Cao X and Phillis JW (1995). The free radical scavenger alpha-lipoic acid protects against cerebral ischemia-reperfusion injury in gerbils. *Free Radic Res* 23**:**365– 370.

Carageorgiou H, Tzotzes V, Pantos C, Mourouzis C, Zarros A, Tsakiris S (2004) In vivo and in vitro effects of cadmium on adult rat brain total antioxidant status, acetylcholinesterase, (Na+, K+)-ATPase and Mg2+-ATPase activities: protection by L-cysteine. Basic Clin Pharmacol Toxicol 94:112–118

Carhuapoma JR , Wang PY , Beauchamp NJ , Keyl PM , Hanley DF , Barker PB . Diffusion-weighted MRI, and proton MR: spectroscopic imaging in the study of secondary neuronal injury after intracerebral hemorrhage. Stroke 2000 ; 31 : 726 – 732 .

Carriedo SG, Yin HZ, Sensi SL, Weiss JH. Rapid Ca2+ entry through Ca2+-permeable AMPA/Kainate channels triggers marked intracellular Ca2+ rises and consequent oxygen radical production.J Neurosci. 1998 Oct 1;18(19):7727-38.

Castellanos M, Leira R, Serena J, Pumar JM, Lizasoain I, Castillo J, Davalos A. Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. Stroke 2003;34:40–46. [PubMed: 12511748]

Castellanos M, Sobrino T, Millan M, Garcia M, Arenillas J, Nombela F, Brea D, Perez de la Ossa N, Serena J, Vivancos J, Castillo J, Davalos A. Serum cellular fibronectin and matrix metalloproteinase-9asscreening biomarkers for the prediction of parenchymal hematoma after thrombolytic therapy in acute ischemic stroke: a multicenter confirmatory study. Stroke2007;38: 1855–1859.

Castellazzi M, Tamborino C, De Santis G, Garofano F, Lupato A, Ramponi V, Trentini A, Casetta I, Bellini T, Fainardi E. Timing of serum active MMP-9 and MMP-2 levels in acute and subacute phases after spontaneous intracerebral hemorrhage. Acta Neurochir Suppl 2010;106: 137–140.

Castillo J , Davalos A , Alvarez-Sabin J , et al. Molecular signatures of brain injury after intracerebral hemorrhage . Neurology 2002; 58 : 624 – 629 .

Catto A, Carter AM, Barrett JH, Stickland M, Bamford J, Davies JA, *et al*. Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular disease. Stroke 1996; 27: 435-40.

Catto AJ, Kohler HP, Bannan S, Stickland M, Carter A, Grant PJ. Factor XIII Val 34 Leu: A novel association with primary intracerebral hemorrhage. Stroke 1998; 29: 813-6.

Cazevielle C, Muller A, Meynier F, and Bonne C (1993) Superoxide and nitric oxidecooperation in hypoxia/reoxygenation-induced neuron injury. *Free Radic Biol Med*14:389–395

Cervera A, Amaro S, Chamorro A. Oral anticoagulant-associated intracerebral hemorrhage.J Neurol. 2012 Feb;259 (2):212-24. doi: 10.1007/s00415-011-6153-3. Epub 2011 Jul 5. Review.

Chambers IR , Banister K , Mendelow AD . Intracranial pressure within a developing intracerebral haemorrhage. Br J Neurosurg 2001; 15 (2): 140– 141 .

Chan PH (2001) Reactive oxygen radicals in signaling and damage in the ischemic brain. *J Cereb Blood Flow Metab* 21:2–14.

Chang CY, Lai YC, Cheng TJ, Lau MT, and Hu ML (1998) Plasma levels of antioxidant vitamins, selenium, total sulfhydryl groups and oxidative products in ischemic-stroke patients as compared to matched controls in Taiwan. *Free Radic Res* 28:15–24.

Chen CM, Liu SH, Lin-Shiau SY (2007) Honokiol, a neuroprotectant against mouse cerebral ischaemia, mediated by preserving Na+,K+-ATPase activity and mitochondrial functions. Basic Clin Pharmacol Toxicol 101:108-116

Chen XL, Dodd G, Thomas S, Zhang X, Wasserman MA, Rovin BH, Kunsch C. Activation of Nrf2/ARE pathway protects endothelial cells from oxidant injury and inhibits inflammatory gene expression.Am J Physiol Heart Circ Physiol. 2006 May;290(5):H1862-70. Epub 2005 Dec 9.

Chen XL, Kunsch C. Induction of cytoprotective genes through Nrf2/ antioxidant response element pathway: a new therapeutic approach for the treatment of inflammatory diseases. Curr Pharm Des 2004; 10:879–891.

Chen M, Regan RF. Time course of increased heme oxygenase activity and expression after experimental intracerebral hemorrhage: correlation with oxidative injury. J Neurochem 2007;103: 2015–2021.

Chen RJ, Jinn TR, Chen YC, Chung TY, Yang WH, Tzen JT (2011) Active ingredients in Chinese medicines promoting blood circulation as Na+/K+-ATPase inhibitors. Acta Pharmacol Sin 32:141-151

Cheung RT, Zou LY. Use of the original, modified, or new intracerebral hemorrhage score to predict mortality and morbidity after intracerebral hemorrhage. Stroke. 2003 Jul;34(7):1717-22. Epub 2003 Jun 12.

Chen ST , Chen SD , Hsu CY , et al. Progression of hypertensive intracerebral hemorrhage . Neurology 1989 ; 39 (11) : 1509 – 1514 .

Cho KH, Kim BC, Kim MK, Shin BA. No association of factor XIII Val34Leu polymorphism with primary intracerebral hemorrhage and healthy controls in Korean population.J Korean Med Sci. 2002 Apr;17(2):249-53.

Cho SO, Ban JY, Kim JY, Ju HS, Lee IS, Song KS, Bae K, Seong YH (2009) Anti-ischemic activities of *Aralia cordata* and its active component oleanolic acid. Arch Pharm Res 32:923-932

Clark JF, Loftspring M, Wurster WL, Beiler S, Beiler C, Wagner KR, Pyne-Geithman GJ. Bilirubin oxidation products, oxidative stress, and intracerebral hemorrhage.Acta Neurochir Suppl. 2008;105:7-12.

Clark WM, Hazel JS, and Coul BM (1995) CNS pharmacology and current research. *Drugs* 50:971–983.

Clausen T, Van Hardeveld C, Everts ME (1991) Significance of cation transport in control of energy metabolism and thermogenesis. Physiol Rev 71:733-774

Cohen, G.: Oxygen radicals and Parkinson's disease. *In* Oxygen Radicals andTissue Injury, ed. by E. D. Halliwell, vol. 135, pp. 130–135, FASEB, Bethesda, Maryland, 1988.

Cole A, Aube M: late onset migraine with intracerebral haemorrhage: A recognizable syndrome. Neurology 37S1:238, 1987

Cole FM , Yates PO . Pseudo-aneurysms in relationship to massive cerebral hemorrhage . J Neurol NeurosurgPsychiatry 1967 ; 30 : 61 – 66 .

Corral J, Iniesta JA, González-Conejero R, Villalón M, Rivera J, Vicente V. Factor XIII Val34Leu polymorphism in primary intracerebral haemorrhage. Hematol J 2000;1:269-73.

Corral J, Iniesta JA, González-Conejero R, Villalón M, Vicente V. Polymorphisms of clotting factors modify the risk for primary intracranial hemorrhage. Blood 2001;97:2979-82.

Cousar JL, Lai Y, Marco CD, Bayir H, Adelson PD, Janesko-Feldman KL, Kochanek PM, Clark RS. Heme oxygenase 1 in cerebrospinal fluid from infants and children after severe traumatic brain injury. Dev Neurosci 2006;28:342–347.

Coyle JT and Puttfarcken P (1993) Oxidative stress, glutamate and neurodegenerative disorders. Science (Wash DC) 262:689–695.

Craig, W. M., and Adson, A. W.: Spontaneous intracerebral hemorrhage. Arch. Neurol. & Psychiat., 1936, 35, 701.

Danton GH, Dietrich WD. The search for neuroprotective strategies in stroke.AJNR Am J Neuroradiol. 2004 Feb;25(2):181-94. Review.

Dardiotis E, Jagiella J, Xiromerisiou G, Dardioti M, Vogiatzi C, Urbanik A, *et al*. Angiotensin-converting enzyme tag single nucleotide polymorphisms in patients with intracerebral hemorrhage. Pharmacogenet Genomics 2011;21:136-41.

Davalos A, Castillo J, Serena J, and Noya M (1997) Duration of glutamate release after acute ischemic stroke. *Stroke* 28:708–710.

Daverat P, Castel JP, Dartigues JF, Orgogozo JM. Death and functional outcome after spontaneous intracerebral hemorrhage. A prospective study of 166 cases using multivariate analysis.Stroke. 1991 Jan;22(1):1-6.

Daviglus ML, Orencia AJ, Dyer AR, Liu K, Morris DK, Persky V, Chavez N, Goldberg J, Drum M, Shekelle RB, Stamler J. Dietary vitamin C, beta-carotene and 30-year risk of stroke: results from the Western Electric Study.Neuroepidemiology. 1997;16(2):69-77.

Dawson I, van Bockel JH, Ferrari MD, et al. (1993). Ischemic and hemorrhagic stroke in patients on oral anticoagulants after reconstruction for chronic lower limb ischemia. Stroke 24: 1655–1663.

D'Ambrosio AL, Sughrue ME, Yorgason JG, Mocco JD, Kreiter KT, Mayer SA, McKhann GM 2nd, Connolly ES Jr. Decompressive hemicraniectomy for poor-grade aneurysmal subarachnoid hemorrhage patients with associated intracerebral hemorrhage: clinical outcome and quality of life assessment.Neurosurgery. 2005;56(1):12-9; dicussion 19-20.

Deinsberger W, Hartmann M, Vogel J, et al. Local fibrinolysis and aspiration of intracerebral hematomas in rats. An experimental study using MR monitoring. Neurol Res 1998;20(4): 349–52.

de Haan P, Kalkman CJ, Vanicky I, Jacobs MJ, Drummond JC (1998) Effect of mild hypothermia and the 21-aminosteroid U-74389G on neurologic and histopathologic outcome after transient spinal cord ischemia in the rabbit. J Neurosurg Anesthesiol 10:86–93.

De Kumar A and Rukmini D (1988) Physiological antioxidants and antioxidative enzymes in vitamin E-deficient rats. *Toxicol Lett* 44:47–54.

De la Ossa NP, Sobrino T, Silva Y, Blanco M, Millan M, Gomis M, Agulla J, Araya P, Reverte S, Serena J, Davalos A. Iron-related brain damage in patients with intracerebral hemorrhage. Stroke 2010;41:810–813.

Delanty N, Dichter MA. Antioxidant therapy in neurologic disease. Arch Neurol. 2000 Sep;57(9):1265-70. Review.

Demirkaya S, Topcuoglu MA, Aydin A, Ulas UH, Isimer AI, and Vural O (2001)Malondialdehyde, glutathione peroxidase and superoxide dismutase in peripheral blood erythrocyres of patients with acute cerebral ischemia. *Eur J Neurol* 8:43–51

Dempsey PW, Fearon DT. Complement: instructing the acquired immune system through the CD21/CD19 complex. Res Immunol. 1996 Feb;147(2):71-5; discussion 119-20.

Dereeper E, Berré J, Vandesteene A, Lefranc F, Vincent JL. Barbiturate coma for intracranial hypertension: clinical observations.J Crit Care. 2002 Mar;17(1):58-62.

Di Carlo A. Human and economic burden of stroke.Age Ageing. 2009 Jan;38(1):4-5

Dietz V. and Colombo G. Recovery from spinal cord injury- underlying mechanisms and efficacy of rehabilitation. Acta Neurochir (2004) [Suppl] 89:95-100

Diener HC, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke.J Neurol Sci. 1996 Nov;143(1-2):1-13.

Dinkel K, Dhabhar FS, Sapolsky RM. Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. Proc Natl Acad Sci U S A 2004;101:331–336.

Diringer MN. Intracerebral hemorrhage: pathophysiology and management. Crit Care Med. 1993 Oct;21(10):1591-603. Review.

Diringer MN , Edwards DF . Admission to a neurologic / neurosurgical intensive care unit is associated with reduced mortality rate after intracerebral hemorrhage . Crit Care Med 2001 ; 29 (3) : 635 – 640 .

Diringer MN, Edwards DF, Zazulia AR. Hydrocephalus: a previously unrecognized predictor of poor outcome from supratentorial intracerebral hemorrhage.Stroke. 1998 Jul;29(7):1352-7.

Dixon AA, Holness RO, Howes WJ, Garner JB. Spontaneous intracerebral haemorrhage: an analysis of factors affecting prognosis. Can J Neurol Sci. 1985 Aug;12(3):267-71.

Djuric□ić BM, Mićić DV, Mršulja BB (1984) Phasic recognition of edema caused by ischemia. In: Go KG, Baethmann A (eds). Recent Progress in the Study and Therapy of Brain Edema. Plenum Press, New York, pp 491-498.

Domenica Altavilla, Francesco Squadrito, Micaela Serrano, Giuseppe M. Campo,Giovanni Squadrito, Mariarita Arlotta, Giuseppe Urna, Aurora Sardella, Antonino Saitta &Achille P. Caputi Inhibition of tumour necrosis factor and reversal of endotoxin-induced shock by U-83836E, a `second generation'lazaroid in rats. British Journal of Pharmacology (1998) 124, 1293 – 1299.

Douglas HA, Callaway JK, Sword J, Kirov SA, Andrew RD (2011) Potent inhibition of anoxic depolarization by the sodium channel blocker dibucaine. J Neurophysiol 105:1482-1494.

Drapkin A, Merskey C (1972). Anticoagulant therapy after acute myocardial infarction. Relation of therapeutic benefit to patient's age, sex, and severity of infarction. JAMA 222: 541– 548.

Drew PD, Xu J, Storer PD, Chavis JA, Racke MK. Peroxisome proliferator-activated receptor agonist regulation of glial activation: relevance to CNS inflammatory disorders. Neurochem Int. 2006;49:183–189.

Drury I, Whisnant JP, Garraway WM. Primary intracerebral hemorrhage: impact of CT on incidence. Neurology. 1984 May;34(5):653-7.

Ducruet AF, Zacharia BE, Hickman ZL, Grobelny BT, Yeh ML, Sosunov SA, Connolly ES Jr. The complement cascade as a therapeutic target in intracerebral hemorrhage. Exp Neurol. 2009 Oct;219(2):398-403. doi: 10.1016/j.expneurol.2009.07.018. Epub 2009 Jul 24. Review.

Dunn L, Gifford H, et al. Spontaneous cerebral hemorrhage in a patient with essential hypertension Ellis RJ, Olichney JM, Thal LJ, et al. (1996). Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV. Neurology 46:1592–1596.

Durmaz R, Kanbak G, Akyuz F, Isiksoy S, Yucel F, Inal M, Tel E (2003) Lazaroid attenuates edema by stabilizing ATPase in the traumatized rat brain. Can J Neurol Sci 30:143-149.

Ebadi M, Srinivasan SK, and Baxi MD (1996) Oxidative stress and antioxidanttheory in Parkinson's disease. *Prog Neurobiol* 48:1–19.

Egashira T, Goto H, Takeda H, Takada K, Matsumiya T (1999) Alterations in neurotransmitter, amino acid and free radical related substances in cerebrospinal fluid in patients with cerebrovascular diseases. Nippon Ronen Igakkai Zasshi 36:256–261.

Ellis RJ, Olichney JM, Thal LJ, Mirra SS, Morris JC, Beekly D, Heyman A. Cerebral amyloid angiopathy in the brains of patients with Alzheimer's disease: the CERAD experience, Part XV.Neurology. 1996 Jun;46(6):1592-6.

Ellman GL, Courtney KD, Andres V Jr, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Emily Sena, Bsc; Philippa Wheble, Bsc; Peter Sandercock, MD; Malcolm Macleod, PhD Systematic Review and Meta-Analysis of the Efficacy of Tilizarad in Experimental Stroke. Stroke. 2007; 38: 338-394.

Endler G, Funk M, Haering D, Lalouschek W, Lang W, Mirafzal M, *et al*. Is the factor XIII 34Val/Leu polymorphism a protective factor for cerebrovascular disease? Br J Haematol 2003;120:310-4

Fabian RH, Dewitt DS, Kent TA (1998) The 21-aminosteroid U-74389G reduces cerebral superoxide anion concentration following fluid percussion injury of the brain. J Neurotrauma 15:433–440

Faden AI, Demediuk P, Panter SS, and Vink R (1989) The role of excitatory aminoacids and NMDA receptors in traumatic head injury. *Science (Wash DC)* 244:798–800.

Fainardi E, Borrelli M, Saletti A, Sarubbo S, Roversi G, Bernardoni A, Latini F, Azzini C, Borgatti L, De Vito A, Cavallo M, Ceruti S, Chieregato A. Temporal changes in perihematomal apparent diffusion coefficient values during the transition from acute to subacute phases in patients with spontaneous intracerebral hemorrhage.Neuroradiology. 2012 Sep 18. [Epub ahead of print]

Fagni L, Lafon-Cazal M, Roundouin G, Manzoni O, Lerner-Natoli M, and BockaertJ (1994) The role of free radicals in NMDA-dependent neurotoxicity. *Prog BrainRes* 103:381–390.

Fan R, Xu F, Previti ML, Davis J, Grande AM, Robinson JK, Van Nostrand WE. Minocycline reduces microglial activation and improves behavioral deficits in a transgenic model of cerebral microvascular amyloid. J Neurosci 2007;27:3057–3063.

Fang HY , Lin CY , Ko WJ . Hematology and coagulation parameters predict outcome in Taiwanese patients with spontaneous intracerebral hemorrhage . Eur J Neurol 2005 ; 12 (3) : 226 – 232 .

Farbiszewski R, Chwiecko M, Ustymowicz J (1994) The 21- aminosteroid U-74389G protects the antioxidant enzymes in the ischemia/reperfusion-induced rat brain damage. Eur J Pharmacol 270:263–265

Feigin VL, Anderson N, Rinkel GJ, Algra A, van Gijn J, Bennett DA. Corticosteroids for aneurysmal subarachnoid haemorrhage and primary intracerebral haemorrhage. Cochrane Database Syst Rev. 2005 Jul 20;(3):CD004583. Review.

Feigin VL, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review.Lancet Neurol. 2009 Apr;8(4):355-69. doi: 10.1016/S1474-4422(09)70025-0. Epub 2009 Feb 21. Review.

Félix B, Léger ME, Albe-Fessard D, Marcilloux JC, Rampin O, Laplace JP (1999) Stereotaxic atlas of the pig brain. Brain Res Bull 49:1–137

Fieschi C, Carolei A, Fiorelli M, Argentino C, Bozzao L, Fazio C, Salvetti M, Bastianello S. Changing prognosis of primary intracerebral hemorrhage: results of a clinical and computed tomographic follow-up study of 104 patients.Stroke. 1988 Feb;19(2):192-5.

Fihn SD, McDonell M, Martin D, Henikoff J, Vermes D, Kent D, White RH. Risk factors for complications of chronic anticoagulation. A multicenter study. Warfarin Optimized Outpatient Follow-up Study Group. Ann Intern Med. 1993 Apr 1;118(7):511-20.

Fisher CM. Pathological observations in hypertensive cerebral hemorrhage.J Neuropathol Exp Neurol. 1971 Jul;30(3):536-50.

FitzMaurice E, Wendell L, Snider R, Schwab K, Chanderraj R,Kinnecom C, et al. Effect of statins on intracerebral hemorrhageoutcome and recurrence. Stroke. 2008;39:2151–2154.

Fernandes HM , Gregson B , Siddique S , Mendelow AD . Surgery in intracerebral hemorrhage . The uncertainty continues . Stroke 2000; 31 (10): 2511– 2516 .

Fernandez MP, Belmonte A, Meizoso MJ, Garcia Novio M, Garcia Iglesias E. Effect of tirilazad on brain nitric oxide synthase activity during cerebral ischaemia in rats. Pharmacology 1997; 54: 108-12

Fleishaker JC, Hulst-Pearson LK, and Peters GR (1995) Effect of gender and menopausal status on the pharmacokinetics of tirilazad mesylate in healthy subjects. *Am J Ther* 2:553–560.

Fleishaker JC, Peters GR, Cathcart KS. Evaluation of the pharmacokinetics and tolerability of tirilazad mesylate, a 21-aminosteroid free radical scavenger: I – single dose administration. J Clin Pharmacol 1993; 33: 175-81.

Fleishaker JC, Peters GR, Cathcart KS, Steenwyck RC. Evaluation of the pharmacokinetics and tolerability of tirilazad mesylate, a 21-aminosteroid free radical scavenger: II – multiple dose administration. J Clin Pharmacol 1993; 33:182-90

Flibotte JJ , Hagan N , O'Donnell J , et al. Warfarin, hematoma expansion, and outcome of intracerebral hemorrhage . Neurology 2004 ; 63 (6) : 1059 – 1064.

Forsmark AP, Lee CP, Dallner G, and Ernster L (1997) Lipid peroxidation and changes in the ubiquinone content and the respiratory chain enzymes of submitochodrial particles. *Free Radic Biol Med* 22:391–400

Forsting M , Mattle HP , Huber P . Anticoagulation-related intracerebral hemorrhage . Cerebrovasc Dis 1991 : 97 .

Forsyth KD, Levinsky RJ. Fibronectin degradation: an in-vitro model of neutrophil mediated endothelial cell damage. J Pathol. 1990;161: 313–319

Fujii Y, Takeuchi S, Sasaki O, et al. (1998). Multivariate analysis of predictors of hematoma enlargement in spontaneous intracerebral hemorrhage. Stroke 29: 1160–1166

Fujii Y , Tanaka R , Takeuchi S , Koike T , Minakawa T , Sasaki O . Hematoma enlargement in spontaneous intracerebral hemorrhage . J Neurosurg 1994 ; 80 : 51 – 57 .

Fung C, Murek M, Z'graggen WJ, Krähenbühl AK, Gautschi OP, Schucht P, Gralla J, Schaller K, Arnold M, Fischer U, Mattle HP, Raabe A, Beck J. Decompressive hemicraniectomy in patients with supratentorial intracerebral hemorrhage. Stroke. 2012 Dec;43(12):3207-11.

Furlan AJ, Whisnant JP, Elveback LR. The decreasing incidence of primary intracerebral hemorrhage: a population study.Ann Neurol. 1979 Apr;5(4):367-73.

Fulkerson CV, Young BD, Jackson ND, Porter B, Levine JM. MRI characteristics of cerebral microbleeds in four dogs. Vet Radiol Ultrasound. 2012 Jul Aug;53(4):389-93. doi: 10.1111/j.1740-8261.2011.01910.x. Epub 2012 Jan 11.

Gaetani P, Pasqualin A, Rodriguez YB, Borasio E, and Marzatico F (1998) Oxidative stress in the human brain after subarachnoid hemorrhage. *J Neurosurg* 89:748–754.

Gao Z, Wang J, Thiex R, Rogove AD, Heppner FL, Tsirka SE. Microglial activation and intracerebral hemorrhage. Acta Neurochir Suppl 2008;105:51–53.

Gårde A, Böhmer G, Seldén B, Neiman J. 100 cases of spontaneous intracerebral haematoma. Diagnosis, treatment and prognosis. Eur Neurol. 1983;22(3):161-72.

Garibi J, Bilbao G, Pomposo I, Hostalot C. Prognostic factors in a series of 185 consecutive spontaneous supratentorial intracerebral haematomas. Br J Neurosurg. 2002 Aug;16(4):355-61.

Garraway WM, Whisnant JP, Drury I. The continuing decline in the incidence of stroke.Mayo Clin Proc. 1983 Aug;58(8):520-3.

Garraway WM, Whisnant JP, Furlan AJ, Phillips LH 2nd, Kurland LT, O'Fallon WM. The declining incidence of stroke.N Engl J Med. 1979 Mar 1;300(9):449-52.

Gary H. Danton and W. Dalton Dietrich The Search for Neuroprotective Strategies in Stroke *AJNR Am J Neuroradiol* 25:181–194, February 2004 3241.

Gatchev O, Rastam L, Lindberg G, et al. (1993). Subarachnoid hemorrhage, cerebral hemorrhage, and serum cholesterol concentration in men and women. Ann Epidemiol 3: 403–409.

Gebel JM Jr, Jauch EC, Brott TG, et al. (2002a). Natural history of perihematomal edema in patients with hyperacute spontaneous intracerebral hemorrhage. Stroke 33:2631–2635.

Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immuneffector cell of the brain.Brain Res Brain Res Rev. 1995 Mar;20(3):269-87. Review.

Gemmati D, Serino ML, Ongaro A, Tognazzo S, Moratelli S, Resca R, *et al*. A common mutation in the gene for coagulation factor XIII-A (VAL34Leu): A risk factor for primary intracerebral hemorrhage is protective against atherothrombotic diseases. Am J Hematol 2001;67:183-8

Gennarelli TA (1997) The pathobiology of traumatic brain injury. *The Neuroscientist* 3:73–81.

Gey KF, Stähelin HB, Eichholzer M. Poor plasma status of carotene and vitamin C is associated with higher mortality from ischemic heart disease and stroke: Basel Prospective Study.Clin Investig. 1993 Jan;71(1):3-6.

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 2010;11:87–99.

Gilles C, Brucher JM, Khoubesserian P, Vanderhaeghen JJ. Cerebral amyloid angiopathy as a cause of multiple intracerebral hemorrhages. Neurology. 1984 Jun;34(6):730-5.

Ginsberg, MDAdventures in the Pathophysiology of Brain Ischemia:Penumbra, Gene Expression, Neuroprotection. The 2002 Thomas Willis Lecture (*Stroke*. 2003; 34:214-223.)

Giroud M, Gras P, Chadan N, et al. (1991). Cerebral haemorrhage
in a French prospective population study. J Neurol Neurosurg Psychiatry 54: 595–598

Gong C, Boulis N, Qian J, Turner DE, Hoff JT, Keep RF. Intracerebral hemorrhage-induced neuronal death.Neurosurgery. 2001 Apr;48(4):875-82; discussion 882-3.

Gong C, Hoff JT, Keep RF. Acute inflammatory reaction following experimental intracerebral hemorrhage in rat. Brain Res 2000;871:57–65.

Gonçalves JF, Fiorenza AM, Spanevello RM, Mazzanti CM, Bochi GV, Antes FG, Stefanello N, Rubin MA, Dressler VL, Morsch VM, Schetinger MR (2010) N-Acetylcysteine prevents memory deficits, the decrease in acetylcholinesterase activity and oxidative stress in rats exposed to cadmium. Chem Biol Interact 186:53–60.

Gore JM, Sloan M, Price TR, et al. (1991). Intracerebral hemorrhage, cerebral infarction, and subdural hematoma after acute myocardial infarction and thrombolytic therapy in the Thrombolysis in Myocardial Infarction Study. Thrombolysis in Myocardial Infarction, Phase II, pilot and clinical trial. Circulation 83: 448–459.

Grasso G, Graziano F, Sfacteria A, Carletti F, Meli F, Maugeri R, et al. Neuroprotective effect of erythropoietin and darbepoetin alfa after experimental intracerebral hemorrhage. Neurosurgery. 2009;65:763–769.

Greenberg Mark S ed. (7th Edition 2010 Thieme Medical Publishers, New York).

Greenberg SM, Briggs ME, Hyman BT, Kokoris GJ, Takis C, Kanter DS, Kase CS, Pessin MS. Apolipoprotein E epsilon 4 is associated with the presence and earlier onset of hemorrhage in cerebral amyloid angiopathy Stroke. 1996 Aug;27(8):1333-7.

Gregson BA, Broderick JP, Auer LM, Batjer H, Chen XC, Juvela S, Morgenstern LB, Pantazis GC, Teernstra OP, Wang WZ, Zuccarello M, Mendelow AD. Individual patient data subgroup meta-analysis of surgery for spontaneous supratentorial intracerebral hemorrhage.Stroke. 2012 Jun;43(6):1496-504. doi: 10.1161/STROKEAHA.111.640284. Epub 2012 Apr 17.

Greisenegger S, Weber M, Funk M, Endler G, Lang W, Ferrari J, *et al*. Polymorphisms in the coagulation factor VII gene and risk of primary intracerebral hemorrhage. Eur J Neurol 2007;14:1098-101

Grieb P, Ryba MS, Sawicki J, and Chrapusta SJ (1997) Oral coenzyme Q10 administration prevents the development of ischemic brain lesions in rabbit model of symptomatic vasospasm. *Acta Neuropathol* 94:363–368.

Grilli M, Memo M. Possible role of NF-kappaB and p53 in the glutamate-induced pro-apoptotic neuronal pathway. *Cell Death Differ*. 1999;6: 22–27.

Gross J, Lapiere CM. Collagenolytic activity in amphibian tissues: a tissue culture assay. Proc Natl Acad Sci U S A. 1962 Jun 15;48:1014-22.

Grossetete M, Rosenberg GA. Matrix metalloproteinase inhibition facilitates cell death in intracerebral hemorrhage in mouse. J Cereb Blood Flow Metab 2008;28:752–763.

Gu W, Zhao H, Yenari MA, Sapolsky RM, Steinberg GK. Catalase over-expression protects striatal neurons from transient focal cerebral ischemia.Neuroreport. 2004 Mar 1;15(3):413-6.

Gu Y, Hua Y, He Y, Wang L, Hu H, Keep RF, Xi G. Iron accumulation and DNA damage in a pig model of intracerebral hemorrhage. Acta Neurochir Suppl. 2011;111:123-8.

Gu Y, Hua Y, Keep RF, Morgenstern LB, Xi G. Deferoxamine reduces intracerebral hematomainduced iron accumulation and neuronal death in piglets. Stroke 2009;40:2241–2243

Gueders MM, Foidart JM, Noel A, Cataldo DD. Matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs in the respiratory tract: potential implications in asthma and other lung diseases. Eur J Pharmacol 2006;533:133–144.

Guidelines for Severe Head Injury Mangement - J Neurotrauma 2000;17(6-7):471-8

Guo FQ, Li XJ, Chen LY, Yang H, Dai HY, Wei YS, Huang YL, Yang YS, Sun HB, Xu YC, Yang ZL. Study of relationship between inflammatory response and apoptosis in perihematoma region in patients with intracerebral hemorrhage.

Gustafsson O, Rossitti S, Ericsson A, Raininko R. MR imaging of experimentally induced intracranial hemorrhage in rabbits during the first 6 hours. Acta Radiol 1999;40(4):360–8.

Gutteridge JM. Hydroxyl radicals, iron, oxidative stress, and neurodegeneration. Ann N Y Acad Sci 1994;738:201–213.

Hagberg H, Lehmann A, Sandberg M, Nystrom B, Jacobson I, and Hamberger A (1985) Ischemia-induced shift to inhibitory and excitatory amino acids from intrato extra-cellular compartments. *J Cereb Blood Flow Metab* 5:413–419.

Haley EC Jr, Kassell NF, Alves WM, Weir BK, Apperson-Hansen C. Phase II trial of tilizarad in aneurysmal subarachnoid hemorrhage. J Neurosurg 1995; 82: 786-90.

Haley EC Jr, Kassell NF, Apperson-Hanson C, Maile MH, Alves WM. A randomised double-blind, vehicle controlled trial of tirilarad mesylate in patients with aneurysmal subarachnoid haemorrhage: a cooperative study in North America. J Neurosurgery 1997; 86: 467-74.

Hall ED (1988a) Effects of the 21-aminosteroid U-74006f on experimental headinjury in mice. *J Neurosurg* 68:456–461.

Hall ED (1988b) Effects of the 21-aminosteroid U-74006f on posttraumatic spinalcord ischemia in cats. *J Neurosurg* 68:462–465

Hall ED, Andrus PK, Smith SL, Fleck TJ, Scherch HM, Lutzhe BS, Sawada GA, Althans JS, Vonvoigtlander PF, Padbury GE, et al. (1997) Pyrrolopyrimidnes novel brain penetrating antioxidants with neuroprotective activity in brain injury and ischemia models. *J Pharmacol Exp Ther* 281:895–904.

Hall ED, Andrus PK, Yonkers PA, et al. Generation and detection of hydroxyl radicals following experimental head injury. Ann N Y Acad Sci 1994; 738: 15-24

Hall ED, Braughler JM. Central nervous system trauma and stroke. II. Physiological and pharmacological evidence for involvement of oxygen radicals and lipid peroxidation.Free Radic Biol Med. 1989;6(3):303-13. Review.

Hall, E. D., McCall, J. M., and Means, E. D.: Therapeutic potential of the Lazaroids (21-aminosteroids) in acute central nervous system trauma, ischemia and subarachnoid haemorrhage. Adv. Pharmacol. 28: 221–268, 1994.

Hall ED, Pazara KE, Braughler JM. Effect of tirilazad mesylate on postischaemic brain lipid peroxidation and recovery of extracellular calcium in gerbils. Stroke 1991; 22: 361-6.

Hall ED, Yonkers PA, Horan KL, Braughler JM. Correlation between attenuation of post traumatic spinal cord ischaemia and preservation of vitamin E by the 21-aminosteroid U-74006: evidence for an in vivo antioxidant effect. J Neurotrauma 1989; 6: 169-76.

Hall, E. D., Yonkers, P. A., McCall, J. M., and Braughler, J. M.: Effects of the 21-aminosteroid U-74006F on experimental head injury in mice. J. Neurosurg.68: 456–461, 1988b.

Hall NC et al. Protein oxidation and enzyme susceptibility in white and gray matter with in vitro oxidative stress: relevance to brain injury from intracerebral hemorrhage.Cell Mol Biol (Noisy-le-grand). (2000).

Hallenbeck JM: The many faces of tumor necrosis factor in stroke. Nat Med 8:1363–1368, 2002.

Halliwell B and Gutteridge JMC (1989) *Free Radicals in Biology and Medicine*, 2nd ed, Clarendon Press, Oxford.

Halliwell, B., and Gutteridge, J. M. C.: Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219: 1–14, 1984.

Halliwell, B., and Gutteridge, J. M. C.: Role of free radicals and catalytic metal ions in human disease: an overview. Brain Injury 6: 203–212, 1992.

Hamann GF, Okada Y, del Zoppo GJ. Hemorrhagic transformation and microvascular integrity during focal cerebral ischemia/reperfusion. *J Cereb Blood Flow Metab*. 1996;16:1373–1378.

Hanger HC, Wilkinson TJ, Fayez-Iskander N, Sainsbury R. The risk of recurrent stroke after intracerebral haemorrhage. J Neurol Neurosurg Psychiatry 2007;78:836-40.

Handley AJ, Emerson PA, Fleming PR (1972). Heparin in the prevention of deep vein thrombosis after myocardial infarction. BMJ 2: 436–438.

Harat M, Kochanowski J (1999) Effect of treatment with 21- aminosteroid U-74389G and glucocorticoid steroid methylprednisolone on somatosensory evoked potentials in rat spinal cord during mild compression. J Neurotrauma 16:187–193.

Hart RG, Benavente O, Pearce LA. Increased risk of intracranial hemorrhage when aspirin is combined with warfarin: A meta-analysis and hypothesis.Cerebrovasc Dis. 1999 Jul-Aug;9(4):215-7.

Hart RG, Boop BS, Anderson DC (1995). Oral anticoagulants and intracranial hemorrhage. Facts and hypotheses. Stroke 26: 1471–1477.

Hart RG, Pearce LA (1993). In vivo antithrombotic effect of aspirin: dose versus nongastrointestinal bleeding. Stroke 24: 138–139.

Hasegawa Y, Yamaguchi T, Omae T, Woodward M, Chalmers J; PROGRESS CT Substudy Investigators. Effects of perindopril-based blood pressure lowering and of patient characteristics on the progression of silent brain infarct: the Perindopril Protection against Recurrent Stroke Study (PROGRESS) CT Substudy in Japan. Hypertens Res. 2004 Mar;27(3):147-56. Erratum in: Hypertens Res. 2004 Jun;27(6):447.

Hayashi M, Hasegawa T, Kobayashi H, Munemoto S, Yamamoto S. [Aspiration of hypertensive intracerebral hematoma by stereotactic technique (author's transl)].No Shinkei Geka. 1981 Nov;9(12):1365-71. Japanese.

Hayashi N, Nishimura S, Numagami Y, Murakimi K, Inoue T, Obara H, Nishijima M. [Retrospective analysis of effects and complications in cases treated with endoscopic evacuation ofintracerebral hemorrhage]. No Shinkei Geka. 2006 Dec;34(12):1233-8. Japanese.

Hayberry KA, Pou S, and Eccles CU (1992) Potentiation of quinolinate-induced hippocampal lesions by inhibition of NO synthesis. *Neurosci Lett* 146:187–190.

He J, Whelton PK, Vu B, et al. (1998). Aspirin and risk of hemorrhagic stroke: a meta-analysis of randomized controlled trials. JAMA 280: 1930–1935

Helweg-Larsen S, Sommer W, Strange P, Lester J, Boysen G. Prognosis for patients treated conservatively for spontaneous intracerebral hematomas. Stroke. 1984 Nov-Dec;15(6):1045-8.

Hemphill JC 3rd, Bonovich DC, Besmertis L, Manley GT, Johnston SC. The ICH score: a simple, reliable grading scale for intracerebral hemorrhage. Stroke. 2001 Apr;32(4):891-7.

Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease.N Engl J Med. 1996 May 2;334(18):1145-9.

Hernandez J (1987) Brain Na+,K+-ATPase activity possibly regulated by a specific serotonin receptor. Brain Res 408:399-402.

Hickenbottom SL, Grotta JC, Strong R, Denner LA, Aronowski J. Nuclear factor-kappaB and cell death after experimental intracerebral hemorrhage in rats. *Stroke*. 1999;30:2472–2477.

Hijioka M, Matsushita H, Hisatsune A, Isohama Y, Katsuki H (2011).Therapeutic effect of nicotine in a mouse model of intracerebral hemorrhage. J Pharmacol Exp Ther 338:741–749

Hinzmann JS, Mc Kenna RL, Pierson TS, Han F, Kenzy FJ, Epps DE. Interactions of antioxidants with depth dependant fluoresence quenchers and energy transfer probes in lipid bilayers. Chem Phys Lipids 1992; 62: 123-38.

Hirose G , Kosoegawa H , Saeki M , et al. The syndrome of posterior thalamic hemorrhage . Neurology 1985 ; 35 (7) : 998 – 1002 .

Hiroshi, Katsuki. Exploring Neuroprotective Drug Therapies for Intracerebral Hemorrhage J Pharmacol Sci 114, 366 – 378 (2010).

Horimoto C, Yamaga S, Toba T, Tsujimura M. [Stereotactic evacuation of massive hypertensive intracerebral hemorrhage].[Article in Japanese] No Shinkei Geka. 1993 Jun;21(6):509-12.

Horstmann S, Kalb P, Koziol J, Gardner H, Wagner S. Profiles of matrix metalloproteinases, their inhibitors, and laminin in stroke patients: influence of different therapies. Stroke. 2003 Sep;34(9):2165-70. Epub 2003 Aug 7.

Holtzman D, Togliatti H, Khait I, and Jensen F (1998) Creatine increases survival and suppress seizures in the hypoxic immature rat. *Pediatr Res* 44:410–414.

Hwang BY, Appelboom G, Ayer A, Kellner CP, Kotchetkov IS, Gigante PR, Haque R, Kellner M, Connolly ES. Advances in neuroprotective strategies: potential therapies for intracerebral hemorrhage.Cerebrovasc Dis. 2011;31(3):211-22. doi: 10.1159/000321870. Epub 2010 Dec 21. Review.

Hua Y, de Courten-Myers GM, Myers RE, Wagner KR: Ultra-early induction of tumor necrosis factor-_ in perihematomal edematous white matter following experimental intracerebral hemorrhage. Soc Neurosci Abstr 24:1230,1998.

Hua Y, Keep RF, Hoff JT, Xi G. Brain injury after intracerebral hemorrhage: the role of thrombin and iron.Stroke. 2007 Feb;38(2 Suppl):759-62. Review.

Hua Y, Nakamura T, Keep RF, Wu J, Schallert T, Hoff JT, Xi G. Long-term effects of experimental intracerebral hemorrhage: the role of iron. J Neurosurg 2006a;104:305–312.

Hua Y, Wu J, Keep RF, Nakamura T, Hoff JT, Xi G. Tumor necrosis factor-alpha increases in the brain after intracerebral hemorrhage and thrombin stimulation.Neurosurgery. 2006b Mar;58(3):542-50; discussion 542-50.

Hua Y, Xi G, Keep RF, Hoff JT. Complement activation in the brain after experimental intracerebral hemorrhage.J Neurosurg. 2000 Jun;92(6):1016-22.

Huang FP, Xi G, Keep RF, Hua Y, Nemoianu A, Hoff JT. Brain edema after experimentalintracerebral hemorrhage: role of hemoglobin degradation products. J Neurosurg 2002;96: 287–293.

Hulst LK, Fleishaker JK, Peters GR, Harry JD, Wright DM, Ward P. Effect of age and gender on tirilazad pharmacokinetics in humans. Clin Pharmacol Ther 1994; 55: 378-84

Hylek EM, Singer DE (1994). Risk factors for intracranial hemorrhage in outpatients taking warfarin. Ann Intern Med 120: 897–902.

Inagawa T. Recurrent primary intracerebral hemorrhage in Izumo City, Japan. Surg Neurol 2005;64:28-35; discussion 35-6.

Iniesta JA, Corral J, González-Conejero R, Piqueras C, Vicente V. Polymorphisms of platelet adhesive receptors: Do they play a role in primary intracerebral hemorrhage? Cerebrovasc Dis 2003;15:51-5.

Iribarren C, Jacobs DR, Sadler M, et al. (1996). Low total serum cholesterol and intracerebral hemorrhagic stroke: is the association confined to elderly men? The Kaiser Permanente Medical Care Program. Stroke 27: 1993–1998.

Iso H, Jacobs DR Jr, Wentworth D, et al. (1989). Serum cholesterol levels and six-year mortality from stroke in 50,977 men screened for the multiple risk factor intervention trial. N Engl J Med 320: 904–910.

Iwamoto N, Kusaka M, Tsurutani T, Kameda H, Ito H. Ultrasound imaging for stereotactic evacuation of hypertension-associated intracerebralhematomas with aqua-stream and aspiration. Stereotact Funct Neurosurg. 1993;60(4):194-204.

Iwata-Ichikawa E, Kondo Y, Miyazaki I, Asanuma M, and Ogawa N (1999) Glial cells protect neurons against oxidative stress via transcriptional up-regulation of glutathione synthesis. *J Neurochem* 72:2344–2344.

James C. Grotta, MD Acute Stroke Therapy at the Millennium: Consummating The Marriage Between the Laboratory and Bedside.The Feinberg Lecture (*Stroke*. 1999; 30:1722-1728.)

James JH, Fang CH, Schrantz SJ, Hasselgren PO, Paul RJ, Fischer JE (1996) Linkage of aerobic glycolysis to sodium-potassium transport in rat skeletal muscle. Implications for increased muscle lactate production in sepsis. J Clin Invest 98:2388-2397.

Janny P , Papo I , Chazal J , Colnet G , Barretto LC . Intracranial hypertension and prognosis of spontaneous intracerebral haematomas: a correlative study of 60 patients . Acta Neurochir (Wien) 1982; 61: 181– 186 .

Jenner P and Olnaw CW (1996) Oxidative stress and the pathogenesis of Parkinson's disease. *Neurology* 47 (Suppl):S161–S176.

Jenkins A , Mendelow AD , Graham DI , Nath F , Teasdale GM . Experimental intracranial hematoma: the role of blood constituents in early ischemia . Br J Neurosurg 1990 ; 4 : 45 – 51 .

Jiang Y, Wu J, Hua Y, Keep RF, Xiang J, Hoff JT, Xi G. Thrombin-receptor activation and thrombin-induced brain tolerance.J Cereb Blood Flow Metab. 2002 Apr;22(4):404-10.

Joice SL, Mydeen F, Couraud PO, Weksler BB, Romero IA, Fraser PA, Easton AS. Modulation of blood-brain barrier permeability by neutrophils: in vitro and in vivo studies. Brain Res 2009;1298:13–23.

Johshita H, Sasaki T, Matsui T, Hanamura T, Masayasu H, Asano T, and Takakura K (1990) Effects of ebselen (PZ51) on ischemic brain edema after focal ischemia in cats. *Acta Neurochir* 51 (Suppl):239–241.

Joseph Kwan, MD, MRCP Helen C. Roberts, FRCP Nicola Englyst, PhD. Do We Really Understand the Pathophysiology and Clinical Impact of Poststroke Infection? *Stroke*. 2006; 37:1656.

Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke. 2004;35:1744–1749.

Jung KH, Chu K, Lee ST, Kim SJ, Song EC, Kim EH, et al. Blockade of AT1 receptor reduces apoptosis, inflammation, and oxidative stress in normotensive rats with intracerebral hemorrhage.
J Pharmacol Exp Ther. 2007;322:1051–1058.

Jung YW, Choi IJ, Kwon TH (2007) Altered expression of sodium transporters in ischemic penumbra after focal cerebral ischemia in rats. Neurosci Res 59:152-159.

Juvela S, Hillbom M, Palomäki H. Risk factors for spontaneous intracerebral hemorrhage.Stroke. 1995 Sep;26(9):1558-64.

Juvela S, Kuurne T. [Surgical management of cerebral hemorrhage].
Duodecim. 1989;105(7):705-9. Review. Finnish. No abstract available.

Kalita J, Misra UK, Bindu IS, Kumar B, Mittal B. Angiotensin-converting enzyme (rs4646994) and alpha ADDUCIN (rs4961) gene polymorphisms' study in primary spontaneous intracerebral hemorrhage. Neurol India 2011;59:41-6.

Kamal AK , Dyke JP, et al. Temporal evolution of diffusion after spontaneous supratentorial intracranial hemorrhage . Am J Neuroradiol 2003 ; 24 : 895 – 901.

Kanaya H , Kuroda K. Development in neurosurgical approaches to hypertensive intracerebral hemorrhage in Japan. In: Kaufman HH (ed): Intracerebral Hematomas: Etiology, Pathophysiology, Clinical Presentation and Treatment. New York: Raven, 1992: 197–210.

Kase CS, O'Neal AM, Fisher M, et al. (1990). Intracranial hemorrhage after use of tissue plasminogen activator for coronary thrombolysis. Ann Intern Med 112: 17–21.

Kase CS, Robinson RK, Stein RW, et al. (1985). Anticoagulantrelated intracerebral hemorrhage. Neurology 35: 943–948.

Kase CS, Williams JP, Wyatt DA, Mohr JP. Lobar intracerebral hematomas: clinical and CT analysis of 22 cases.Neurology. 1982 Oct;32(10):1146-50.

Kassel NF, Haley EC Jr, Apperson-Hansen C, and Alves WM (1996) Randomized, double blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurismal subarachnoid hemorrhage: a cooperative study in Europe, Australia, and New Zealand. *J Neurosurg* 84:221–228.

Katsuki H (2010) Exploring neuroprotective drug therapies for intracerebral hemorrhage. J Pharmacol Sci 114:366–378.

Katsuki H, Kurimoto E, Takemori S, Kurauchi Y, Hisatsune A, Isohama Y, et al. Retinoic acid receptor stimulation protects midbrain dopaminergic neurons from inflammatory degeneration via BDNF-mediated signaling. J Neurochem. 2009;110:707–718.

Karki K, Knight RA, Han Y, Yang D, Zhang J, Ledbetter KA, et al. Simvastatin and atorvastatin improve neurological outcome after experimental intracerebral hemorrhage. Stroke. 2009;40:3384–3389.

Katz SM, Sun S, Schechner RS, Tellis VA, Alt ER, Greenstein SM. Improved small intestinal preservation after lazaroid U74389G treatment and cold storage in University of Wisconsin solution. Transplantation. 1995 Mar 15;59(5):694-8.

Kavanagh R.J. and P.C.A.Kam. Lazaroids: Efficacy and mechanism of action of the 21- aminosteroids in neuroprotection. Br J Anaesth 2001; 86: 110-19.

Kawai N, McCarron RM, Spatz M (1996) Effect of hypoxia on Na+-K+-Clcotransport in cultured brain capillary endothelial cells of the rat. J Neurochem 66:2572-2579.

Kawamata T, Takeshita M, Kubo O, Izawa M, Kagawa M, Takakura K.Management of intracranial hemorrhage associated with anticoagulant therapy.Surg Neurol. 1995 Nov;44(5):438-42; discussion 443.

Kazui S , Naritomi H , Yamamoto H , Sawada T , Yamaguchi T . Enlargement of spontaneous intracerebral hemorrhage: incidence and time course . Stroke 1996 ; 27 : 1783 – 178.

Kazui S, Minematsu K, Yamamoto H, Sawada T, Yamaguchi T. Predisposing factors to enlargement of spontaneous intracerebral hematoma. Stroke. 1997 Dec;28(12):2370-5.

Kaufman HH, Pruessner JL, Bernstein DP, Borit A, Ostrow PT, Cahall DL (1985) A rabbit model of intracerebral hematoma. Acta Neuropathol 65:318–321.

Keep RF, Xi G, Hua Y, Hoff JT. The deleterious or beneficial effects of different agents in intracerebral hemorrhage: think big, think small, or is hematoma size important? Stroke 2005;36:1594–1596.

Keith W. Muir, MD, MRCP. Heterogeneity of Stroke Pathophysiology and Neuroprotective Clinical Trial Design Stroke 2002; 33:1545-1550.

Keli SO, Hertog MG, Feskens EJ, Kromhout D. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Arch Intern Med. 1996 Mar 25;156(6):637-42.

Keller JN, Germeyer A, Begley JG, Mattson MP (1997) 17Beta-estradiol attenuates oxidative impairment of synaptic Na+/K+-ATPase activity, glucose transport, and glutamate transport induced by amyloid beta-peptide and iron. J Neurosci Res 50:522-530.

Kenneth R. Wagner. Modeling Intracerebal Hemorrhage: Glutamate, Nuclear Factor-{kappa}BSignaling and Cytokines*Stroke* 2007;38;753-758.

Kevser Erol and Ruhi Uyar. MgSO4 and lazaroid (U-83836E) partially protects glioma cells against glutamate toxicity *in vitro* Acta Neurobiol Exp 2004, 64: 461-466.

Kidwell CS, Chalela JA, Saver JL, Starkman S, Hill MD, Demchuk AM, Butman JA, Patronas N, Alger JR, Latour LL, Luby ML, Baird AE, Leary MC, Tremwel M, Ovbiagele B, Fredieu A, Suzuki S, Villablanca JP, Davis S, Dunn B, Todd JW, Ezzeddine MA, Haymore J, Lynch JK, Davis L, Warach S. Comparison of MRI and CT for detection of acute intracerebral hemorrhage. JAMA. 2004 Oct 20;292(15):1823-30.

Kidwell CS , Saver JL , Mattiello J , et al. Diffusion-perfusion MR evaluation of perihematomal injury in hyperacute intracerebral hemorrhage . Neurology 2001 ; 57 (9) : 1611 – 1617 .

Kilic E, Ozdemir YG, Bolay H, Kelestimur H, and Dalkara T (1999) Pinealectomy aggravates and melatonin administration attenuates brain damage in focal ischemia.*J Cereb Blood Flow Metab* 19:511–516.

Kim IS, Son BC, Lee SW, Sung JH, Hong JT. Comparison of frame-based and frameless stereotactic hematoma puncture and subsequent fibrinolytic therapy for the treatment of supratentorial deep seated spontaneous intracerebral hemorrhage. Minim Invasive Neurosurg. 2007 Apr;50(2):86-90.

Kim MH, Song JH, Kim SH, Park DB, Shin KM. A new trend in operative technique for intracerebral hemorrhage: a comparative study of stereotactic endoscopic removal and stereotactic catheter drainage.Neurosurg Focus. 1996 Oct 15;1(4):e2; discussion e2.

Kim MH, Kim EY, Song JH, Shin KM. Surgical options of hypertensive intracerebral hematoma: stereotactic endoscopic removal versus stereotactic catheter drainage.J Korean Med Sci. 1998 Oct;13(5):533-40.

Kingman TA, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral mass: description of model, intracranial pressure changes and neuropathology. J Neuropathol Exp Neurol 1988;47(2):128–37.

Kingman TA, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral mass: time-related effects on local cerebral blood flow. J Neurosurg. 1987 Nov;67(5):732-8.

Kirkeby OJ, Rise IR, Risöe C. Local variations in the cerebral microcirculatory response to hypercapnia and haemorrhage.Acta Neurochir (Wien). 1997;139(10):9

Kitajima I, Soejima Y, Takasaki I, Beppu H, Tokioka T, Maruyama I. Ceramide-induced nuclear translocation of NF-kappa B is a potential mediator of the apoptotic response to TNF-alpha in murine clonal osteoblasts. Bone. 1996 Sep;19(3):263-70.

Klatsky AL, Friedman GD, Sidney S, Kipp H, Kubo A, Armstrong MA. Risk of hemorrhagic stroke in Asian American ethnic groups. Neuroepidemiology. 2005;25(1):26-31. Epub 2005 Apr 25.

Kobari M, Gotoh F, Tomita M, Tanahashi N, Shinohara T, Terayama Y, Mihara B. Bilateral hemispheric reduction of cerebral blood volume and blood flow immediately after experimental cerebral hemorrhage in cats. Stroke. 1988 Aug;19(8):991-6.

Kobayashi S, Sato A, Kageyama Y, Nakamura H, Watanabe Y, Yamaura A. Treatment of hypertensive cerebellar hemorrhage--surgical or conservative management? Neurosurgery. 1994 Feb;34(2):246-50; discussion 250-1.

Koeppen AH, Dickson AC, Smith J. Heme oxygenase in experimental intracerebral hemorrhage: the benefit of tin-mesoporphyrin. J Neuropathol Exp Neurol 2004;63:587–597.

Koeppen AH, Dickson AC, McEvoy JA. The cellular reactions to experimental intracerebral hemorrhage. J Neurol Sci 1995; 134(Suppl):102–12.

Kollmar R, Juettler E, Huttner HB, Dörfler A, Staykov D, Kallmuenzer B, Schmutzhard E, Schwab S, Broessner G; CINCH investigators. Cooling in intracerebral hemorrhage (CINCH) trial: protocol of a randomized German-Austrian clinical trial.Int J Stroke. 2012 Feb;7(2):168-72.

Kothari RU, Brott T, Broderick JP, Barsan WG, Sauerbeck LR, Zuccarello M, Khoury J. The ABCs of measuring intracerebral hemorrhage volumes. Stroke. 1996 Aug;27(8):1304-5.

Kraft AD, Johnson DA, Johnson JA. Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. J Neurosci. 2004 Feb 4;24(5):1101-12.

Kubota M, Yamaura A, Ono J, et al. (1997). Is family history an independent risk factor for stroke? J Neurol Neurosurg Psychiatry 62: 66–70.

Küker W, Thiex R, Rohde I, Rohde V, Thron A. Experimental acute intracerebral hemorrhage. Value of MR sequences for a safe diagnosis at 1.5 and 0.5 T. Acta Radiol. 2000 Nov;41(6):544-52.

Kumon Y, Sakaki S. [Indication of stereotactic aspiration for hypertensive intracerebral hematoma]. Nihon Rinsho. 1993 Dec;51 Suppl:77-84. Review. Japanese. No abstract available.

Kumral E, Kocaer T, Ertübey NO, Kumral K. Thalamic hemorrhage. A prospective study of 100 patients.Stroke. 1995 Jun;26(6):964-70.

Kunitz SC, Gross CR, Heyman A, Kase CS, Mohr JP, Price TR, Wolf PA. The pilot Stroke Data Bank: definition, design, and data.Stroke. 1984 Jul-Aug;15(4):740-6.

Kurauchi Y, Hisatsune A, Isohama Y, Sawa T, Akaike T, Shudo K, et al. Midbrain dopaminergic neurons utilize nitric oxide/ cyclic GMP signaling to recruit ERK that links retinoic acid receptor stimulation to up-regulation of BDNF. J Neurochem. In press.

Kurth T, Kase CS, Berger K, Gaziano JM, Cook NR, Buring JE. Smoking and risk of hemorrhagic stroke in women. Stroke. 2003a Dec;34(12):2792-5. Epub 2003 Nov 13.

Kurth T, Kase CS, Berger K, Schaeffner ES, Buring JE, Gaziano JM. Smoking and the risk of hemorrhagic stroke in men.Stroke. 2003b May;34(5):1151-5. Epub 2003 Mar 27.

Kwiatkowski T, Libman R, Tilley BC, Lewandowski C, Grotta JC, Lyden P, Levine SR, Brott T; National Institute of Neurological Disorders and StrokeRecombinant Tissue PlasminogenActivator Stroke Study Group. The impact of imbalances in baseline stroke severity on outcome in the National Institute of Neurological Disorders and Stroke Recombinant Tissue Plasminogen Activator Stroke Study. Ann Emerg Med. 2005 Apr;45(4):377-84.

Laizure SC, Franklin LK, Kaiser DG, Williams CL, Stevens RC, Sanders PL, Miller M. Disposition of tirilazad (U74006F), a 21-aminosteroid, in the plasma, heart, brain, and liver of the rat.Drug Metab Dispos. 1993 Sep-Oct;21(5):951-4.

Landefeld CS, Goldman L (1989). Major bleeding in outpatients treated with warfarin: incidence and prediction by factors known at the start of outpatient therapy. Am J Med 87: 144–152.

Lanzino G, Kassell NF, Dorsch NW, et al. Double-blind, randomized, vehicle-controlled study of high-dose tilizarad mesylate in women with aneurysmal subarahnoid hemorrhage. Part I. A cooperative study in Europe, Australia, New Zealand, and South Africa. J Neurosurg 1999a; 90: 1011-7.

Lanzino G, Kassell NF, Double-blind, randomized, vehicle-controlled study of high-dose tilizarad mesylate in women with aneurysmal subarahnoid hemorrhage. Part II. A cooperative study in North America. J Neurosurg 1999b; 90: 1018-1024.

Lapchak PA, Chapman DF, Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. Stroke 2000;31:3034–3040.

Larsson A, Orrenius S, Holngren A, and Mannervik B (1983) *Functions of Glutathione.Biochemical Physiological Toxicological and Clinical Aspects*, Raven Press,New York.

Laurent JP, Molinari GF, Oakley JC. Primate model of cerebral hematoma. J Neuropathol Exp Neurol 1976;35(5):560–8.

Lebel, C. P., and Bondy, S. C.: Oxygen radicals: common mediators of neurotoxicity.Neurotoxicol. Teratol. 13: 341–346, 1991.

Lee JM, Yin KJ, Hsin I, Chen S, Fryer JD, Holtzman DM, Hsu CY, Xu J. Matrix metalloproteinase-9 and spontaneous hemorrhage in an animal model of cerebral amyloid angiopathy. Ann Neurol 2003;54:379–382.

Lee KR, Betz AL, Kim S, Keep RF, Hoff JT. The role of the coagulation cascade in brain edema formation after intracerebral hemorrhage. Acta Neurochir (Wien). 1996;138(4):396-400; discussion 400-1.

Lee KR , Betz AL , Keep RF , Chenevert TL , Kim S , Hoff JT . Intracerebral infusion of thrombin as a cause of brain edema . J Neurosurg 1995 ; 83 : 1045 – 1050 .

Lee KR, Colon GP, Betz AL, Keep RF, Kim S, Hoff JT. Edema from intracerebral hemorrhage: the role of thrombin. J Neurosurg. 1996a Jan;84(1):91-6.

Lee KR, Kawai N, Kim S, Sagher O, Hoff JT. Mechanisms of edema formation after intracerebral hemorrhage: effects of thrombin on cerebral blood flow, blood-brain barrier permeability, and cell survival in a rat model. J Neurosurg. 1997b Feb;86(2):272-8.

Lee ST, Chu K, Sinn DI, Jung KH, Kim EH, Kim SJ, et al. Erythropoietin reduces perihematomal inflammation and cell death with eNOS and STAT3 activations in experimental intracerebral hemorrhage. J Neurochem. 2006;96:1728–1739.

Lee ST, Chu K, Jung KH, Kang KM, Kim JH, Bahn JJ, Jeon D, Kim M, Lee SK, Roh JK (2010) Cholinergic anti-inflammatory pathwayin intracerebral hemorrhage. Brain Res 1309:164–171.

Lew KH, Ludwig EA, Milad MA, et al. Gender based effects on methylprednisolone pharmacokinetics and pharmacodynamics. Clin pharmacol Ther 1993; 54; 402-14.

Lees KR . Cerestat and other NMDA antagonists in ischemic stroke . Neurology 1997 ; 49 : S66 – S69 .

Leira R, Dávalos A, Silva Y, Gil-Peralta A, Tejada J, Garcia M, Castillo J; Stroke Project, Cerebrovascular Diseases Group of the Spanish Neurological Society. Early neurologic deterioration in intracerebral hemorrhage: predictors and associated factors.Neurology. 2004 Aug 10;63(3):461-7.

Leist M, Nicotera P. Apoptosis, excitotoxicity, and neuropathology. Exp Cell Res. 1998 Mar 15;239(2):183-201. Review.

Lema PP, Girard C, Vachon P. Evaluation of dexamethasone for the treatment of intracerebral hemorrhage using a collagenaseinduced intracerebral hematoma model in rats. J Vet Pharmacol Ther. 2004;27:321–328.

Levine SR, Brust JC, Futrell N, et al. (1990). Cerebrovascular complications of the use of the “crack” form of alkaloidal cocaine. N Engl J Med 323: 699–704.

Lewen A, Matz P, and Chan PH (2000) Free radical pathway in CNS injury. *J Neurotrauma* 17:871–890.

Li Z, Sun L, Zhang H, Liao Y, Wang D, Zhao B, *et al*. Elevated plasma homocysteine was associated with hemorrhagic and ischemic stroke, but methylenetetrahydrofolate reductase gene C677T polymorphism was a risk factor for thrombotic stroke: A Multicenter Case-Control Study in China. Stroke 2003;34:2085-90.

Lillehei KO, Chandler WF, Knake JE. Real time ultrasound characteristics of the acute intracerebral hemorrhage as studied in the canine model. Neurosurgery 1984;14(1):48–51.

Lim YH, Jeong YS, Kim SK, Kim DH, Yun DH, Yoo SD, *et al*. Association between TGFBR2 gene polymorphism (rs2228048, Asn389Asn) and intracerebral hemorrhage in Korean population. Immunol Invest 2011;40:569-80.

Lin KI, Lee SH, Narayanan R, Baraban JM, Hardwick JM, Ratan RR. Thiol agents and Bcl-2 identify an alphavirus-induced apoptotic pathway that requires activation of the transcription factor NF-kappa B. J Cell Biol. 1995 Dec;131(5):1149-61.

Lindsberg PJ, Strbian D, Karjalainen-Lindsberg ML. Mast cells as early responders in the regulation of acute blood-brain barrier changes after cerebral ischemia and hemorrhage.J Cereb Blood Flow Metab. 2010 Apr;30(4):689-702. doi: 10.1038/jcbfm.2009.282. Epub 2010 Jan 20. Review.

Lindenstrom E, Boysen G, Nyboe J (1994). Influence of total cholesterol, high density lipoprotein cholesterol, and triglycerides on risk of cerebrovascular disease: the Copenhagen City Heart Study. BMJ 309: 11–15.

Linn F, Seo K, Hossmann KA (1989) Experimental transplantation gliomas in the adult cat brain. 3. Regional biochemistry. Acta Neurochir (Wien) 99:85-93.

Lipton SA. Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. Nat Rev Drug Discov. 2006 Feb;5(2):160-70. Review.

Lippitz BE, Mayfrank L, Spetzger U, Warnke JP, Bertalanffy H, Gilsbach JM. Lysis of basal ganglia haematoma with recombinant tissue plasminogen activator (rtPA) after stereotactic aspiration: initial results. Acta Neurochir (Wien). 1994;127(3-4):157-60.

Little JR , Tubman DE , Ethier R . Cerebellar hemorrhage in adults. Diagnosis by computerized tomography . J Neurosurg 1978 ; 48 (4) : 575 – 579 .

Liu B, Zhang L, Yang Q. Genetics of intracerebral hemorrhage: Insights from candidate gene approaches. Neurol India. 2012 Jan-Feb;60(1):3-8. doi: 10.4103/0028-3886.93581.

Lofgren Fo. Carotid angiography in the diagnosis of spontaneous intracerebral haemorrhage.Acta radiol. 1953 Aug-Sep;40(2-3):173-81. No abstract available.

Love S (1999) Oxidative stress in brain ischemia. *Brain Pathol* 9:119–131.

Lovelock CE, Molyneux AJ, Rothwell PM; Oxford Vascular Study. Change in incidence and aetiology of intracerebral haemorrhage in Oxfordshire, UK, between 1981 and 2006: a population-based study.LancetNeurol. 2007 Jun;6(6):487-93. Erratum in: Lancet Neurol. 2007 Sep;6(9):757.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.

Lucas James Michael Z, WarnerDS, Daniel Z, Laskowitz T. Preclinical Models of Intracerebral Hemorrhage: A Translational Perspective Neurocrit Care (2008) 9:139–152.

Lucas San-Marie, Rothwell Nancy J & Gibson Rosemary.The role of inflammation in CNS injury and disease. British Journal of Pharmacology (2006) 147, S232–S240.

Lou M, Lieb K, Selim M. The relationship between hematoma iron content and perihematoma edema: an MRI study. Cerebrovasc Dis 2009;27:266–271.

Lu A, Tang Y, Ran R, Ardizzone TL, Wagner KR, Sharp FR. Brain genomics of intracerebral hemorrhage. J Cereb Blood Flow Metab 2006;26:230–252.

Lyden PD, Shuaib A, Lees KR, Davalos A, Davis SM, Diener HC, et al. Safety and tolerability of NXY-059 for acute intracerebral hemorrhage: the CHANT Trial. Stroke. 2007;38:2262–2269.

Ma J, Li H, You C, Huang S, Ma L, Ieong C. Accuracy of computed tomography angiography in detecting the underlying vascular abnormalities for spontaneous intracerebral hemorrhage: a comparative study and meta-analysis. Neurol India. 2012 May-Jun;60(3):299-303.

Ma Q, Khatibi NH, Chen H, Tang J, Zhang JH. History of preclinical models of intracerebral hemorrhage. Acta Neurochir Suppl. 2011;111:3-8.

MacKenzie JM, Clayton JA. Early cellular events in the penumbra of human spontaneous intracerebral hemorrhage. J Stroke Cerebrovasc Dis 1999;8:1–8.

Malhotra AK, Schweitzer JB, Fox JL, Fabian TC, Proctor KG. Cerebral perfusion pressure elevation with oxygen-carrying pressor after traumatic brain injury and hypotension in Swine.J Trauma. 2004 May;56(5):1049-57.

Mandybur TI. Cerebral amyloid angiopathy: the vascular pathology and complications.J Neuropathol Exp Neurol. 1986 Jan;45(1):79-90.

Manley GT, Rosenthal G, Lam M, Morabito D, Yan D, Derugin N, Bollen A, Knudson MM, Panter SS. Controlled cortical impact in swine: pathophysiology and biomechanics.J Neurotrauma. 2006 Feb;23(2):128-39.

Manno EM. Update on intracerebral hemorrhage. Continuum (Minneap Minn). 2012 Jun;18(3):598-610. Review.

Mark S Greenberg (7^{th} Edition 2010).

Marshall LF, Maas AI, Marshall SB, et al. A multicenter trial on the efficacy of using tirilazad mesylate in cases of head injury. J. Neurosurgery 1998; 89: 519-525.

Massaro AR, Sacco RL, Scaff M, Mohr JP. Clinical discriminators between acute brain hemorrhage and infarction: a practical score for early patient identification. Arq Neuropsiquiatr. 2002 Jun;60(2-A):185-91.

Mata M, Fink DJ, Gainer H, Smith CB, Davidsen L, Savaki H, Schwartz WJ, Sokoloff L (1980) Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. J Neurochem 34:213-215.

Matsushita H, Hijioka M, Hisatsune A, Isohama Y, Shudo K, Katsuki H. A retinoic acid receptor agonist Am80 rescues neurons, attenuates inflammatory reactions, and improves behavioral recovery after intracerebral hemorrhage in mice.J Cereb Blood Flow Metab. 2011 Jan;31(1):222-34. Epub 2010 Jun 16.

Matsushita K, Meng W, Wang X, Asahi M, Asahi K, Moskowitz MA, Lo EH. Evidence for apoptosis after intercerebral hemorrhage in rat striatum. J Cereb Blood Flow Metab 2000;20:396–404.

Mayer SA , Brun NC , Begtrup K , et al. Recombinant activated factor VII for acute intracerebral hemorrhage. N Engl J Med 2005; 352 (8): 777– 785.

Mayer SA , Lignelli A , Fink ME , et al. Perilesional blood fl ow and edema formation in acute intracerebral hemorrhage: a SPECT study . Stroke 1998 ; 29 : 1791 – 1798 .

Mayer SA, Rincon F. Treatment of intracerebral haemorrhage.
Lancet Neurol. 2005 Oct;4(10):662-72. Review.

Mayfrank L, Kim Y, Kissler J, Delsing P, Gilsbach JM, Schröder JM, Weis J. Morphological changes following experimental intraventricular haemorrhage and intraventricular fibrinolytic treatment with recombinant tissue plasminogen activator.Acta Neuropathol. 2000 Nov;100(5):561-7.

Mayne M, Ni W, Yan HJ, Xue M, Johnston JB, Del Bigio MR, Peeling J, Power C: Antisense oligodeoxynucleotide inhibition of tumor necrosis factor-alpha expression is neuroprotective after intracerebral hemorrhage. Stroke 32:240–248, 2001.

Mayr U, Bauer P, Fischer J. Non-traumatic intracerebral haemorrhage. Prognostic implications of neurological and computer-tomographical findings in 100 consecutive patients.Neurochirurgia (Stuttg). 1983 Mar;26(2):36-41.

Matsushita H, Hijioka M, Hisatsune A, Isohama Y, Shudo K, Katsuki H. A retinoic acid receptor agonist Am80 rescues neurons, attenuates inflammatory reactions, and improves behavioral recovery after intracerebral hemorrhage in mice. J Cereb Blood Flow Metab. in press.

Mattson MP, and Meffert MK. Roles for NF-jB in nerve cell survival, plasticity,and disease Cell Death Differ. 2006 May;13(5):852-60.

Matz PG, Weinstein PR, Sharp FR. Heme oxygenase-1 and heat shock protein 70 induction in glia andneurons throughout rat brain after experimental intracerebral hemorrhage. Neurosurgery 1997;40:152–160. discussion 160–2.

McCall JM, Braughler JM, Hall ED. A new class of compounds for stroke and trauma: effects of 21-aminosteroids on lipid peroxidation.Acta Anaesthesiol Belg. 1987;38(4):417-20.

McDonald C, Carter BS. Medical management of increased intracranial pressure after spontaneous intracerebral hemorrhage. Neurosurg Clin N Am. 2002 Jul;13(3):335-8.

Mckissock W , Richardson A , Taylor J . Primary intracerebral hæmorrhage: a controlled trial of surgical and conservative treatment in 180 unselected cases . Lancet 1961 ; 278 : 221 – 226 .

Mehdiratta M, Kumar S, Hackney D, Schlaug G, Selim M. Association between serum ferritin level and perihematoma edema volume in patients with spontaneous intracerebral hemorrhage. Stroke 2008;39:1165–1170.

Mendelow AD , Gregson BA , Fernandes HM , et al. STICH Investigators . Early surgery versus initial conservative treatment in patients with spontaneous supratentorial intracerebral haematomas in the International Surgical Trial in Intracerebral Haemorrhage (STICH): a randomised trial. Lancet 2005 ; 365 (9457) : 387 – 397 .

Miao J, Xu F, Davis J, Otte-Holler I, Verbeek MM, Van Nostrand WE. Cerebral microvascular amyloid beta protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotropic mutant amyloid beta precursor protein. Am JPathol 2005;167:505–515.

Min KJ, Yang MS, Kim SU, Jou I, Joe EH. Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation. J Neurosci 2006;26:1880–1887.

Misra UK, Kalita J, Somarajan BI, Kumar B, Das M, Mittal B. Do ACE (rs4646994) and aADDUCIN (rs4961) gene polymorphisms predict the recurrence of hypertensive intracerebral hemorrhage? Neurol Sci 2011 [In press].

Miller CM, Vespa P, Saver JL, Kidwell CS, Carmichael ST, Alger J, Frazee J, Starkman S, Liebeskind D, Nenov V, Elashoff R, Martin N. Image-guided endoscopic evacuation of spontaneous intracerebral hemorrhage.Surg Neurol. 2008 May;69(5):441-6; discussion 446.

Miller G. Neuroscience. The dark side of glia. Science 2005;308:778–781.

Minotti, G., and Aust, S. D.: The role of iron in oxygen radical mediated lipid peroxidation. Chem. Biol. Interact. 71: 1–19, 1989.

Miot-Noirault E, Barantin L, Akoka S, Le Pape A. T2 relaxation time as a marker of brain myelination: experimental MR study in two neonatal animal models. J Neurosci Methods. 1997 Mar;72(1):5-14.

Mohadjer M, Braus DF, Myers A, Scheremet R, Krauss JK. CT-stereotactic fibrinolysis of spontaneous intracerebral hematomas.Neurosurg Rev. 1992;15(2):105-10.

Mohr JP, Caplan LR, Melski JW, Goldstein RJ, Duncan GW, Kistler JP, Pessin MS, Bleich HL. The Harvard Cooperative Stroke Registry: a prospective registry. Neurology. 1978 Aug;28(8):754-62.

Moldeus P, Cotgreune IA, and Berggren M (1986) Lung protection by a thiol containing antioxidant *N*-acetylcysteine. *Respiration* 50:531–542.

Montaner J, Alvarez-Sabin J, Molina C, Angles A, Abilleira S, Arenillas J, Gonzalez MA, Monasterio J. Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 2001a;32:1759–1766.

Montaner J, Alvarez-Sabin J, Molina CA, Angles A, Abilleira S, Arenillas J, Monasterio J. Matrix metalloproteinase expression is related to hemorrhagic transformation after cardioembolic stroke. Stroke 2001b;32:2762–2767.

Montaner J, Molina CA, Monasterio J, Abilleira S, Arenillas JF, Ribo M, Quintana M, Alvarez-Sabin J. Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation 2003;107:598–603.

Morgenstern LB, Hemphill JC 3rd, Anderson C, Becker K, Broderick JP, Connolly ES Jr, Greenberg SM, Huang JN, MacDonald RL, Messé SR, Mitchell PH, Selim M, TamargoRJ; American HeartAssociation Stroke Council and Council on Cardiovascular Nursing. Guidelines for the management of spontaneous intracerebral hemorrhage: a guideline for healthcare professionalsfromthe American Heart Association/American Stroke Association.Stroke. 2010 Sep;41(9):2108-29. Epub 2010 Jul 22.

Moro MA, Almeida A, Bolanos JP, Lizasoain I (2005) Mitochondrial respiratory chain and free radical generation in stroke. Free Radic Biol Med 39:1291-1304.

Morris JH . The nervous system . In: Cotran RS , Kumar V , Robbin SL , eds. Pathologic Basis of Disease . 3rd ed . Philadelphia : W.B. Saunders Co ., 1999 : 1385 – 1450.

Mrsulja BB, Djuricić BM, Cvejić V, Mrsulja BJ, Abe K, Spatz M, Klatzo I (1980) Biochemistry of experimental ischemic brain edema. Adv Neurol28:217-230.

Mukai H, Yamashita J, Kitamura A, Ito H. Stereotactic Aqua-Stream and Aspirator in the treatment of intracerebral hematoma. An experimental study. Stereotact Funct Neurosurg. 1991;57(4):221-7.

Muller A, Cadenas E, Graf P, and Sies H (1984) A novel biologically active selenoorganic compound. I. Glutathione peroxidase like activity in vitro and antioxidant capacity of PZ51 (Ebselen). *Biochem Pharmacol* 33:3235–3239.

Multicenter Acute Stroke Trial—Europe Study Group (1996). Thrombolytic therapy with streptokinase in acute ischemic stroke. N Engl J Med 335: 145–150.

Mun-Bryce S, Roberts L, Bartolo A, Okada Y. Transhemispheric depolarizations persist in the intracerebral hemorrhage swine brain following corpus callosal transection. Brain Res 2006;1073–1074:481–90.

Mun-Bryce S, Roberts LJ, Hunt WC, Bartolo A, Okada Y. Acute changes in cortical excitability in the cortex contralateral to focal intracerebral hemorrhage in the swine. Brain Res 2004b; 1026(2):218–26.

Mun-Bryce S, Rosenberg GA. Matrix metalloproteinases in cerebrovascular disease.J Cereb Blood Flow Metab. 1998 Nov;18(11):1163-72. Review.

Mun-Bryce S, Wilkerson A, Pacheco B, Zhang T, Rai S, Wang Y, Okada Y. Depressed cortical excitability and elevated matrix metalloproteinases in remote brain regions following intracerebral hemorrhage. Brain Res 2004a; 1026:227–234.

Mun-Bryce S, Wilkerson A, Pacheco B, et al. Depressed cortical excitability and elevated matrix metalloproteinases in remote brain regions following intracerebral hemorrhage. Brain Res 2004;1026(2):227–34.

Mun-Bryce S, Wilkerson AC, Papuashvili N, Okada YC. Recurring episodes of spreading depression are spontaneously elicited by an intracerebral hemorrhage in the swine. Brain Res 2001;888(2):248–55.

Mutlu N , Berry RG , Alpers BJ . Massive cerebral hemorrhage: clinical and pathological correlations . Arch Neurol 1963 ; 8 : 644 – 661 .

Nakamura T, Keep RF, Hua Y, Hoff JT, Xi G (2005) Oxidative DNA injury
after experimental intracerebral hemorrhage. Brain Res 1039:30-36.

Nakamura T, Keep RF, Hua Y, Schallert T, Hoff JT, Xi G. Deferoxamine-induced attenuation of brain edema and neurological deficits in a rat model of intracerebral hemorrhage. J Neurosurg 2004a; 100:672–678.

Nakamura T, Kuroda Y, Yamashita S, Zhang X, Miyamoto O,Tamiya T, et al. Edaravone attenuates brain edema and neurologic deficits in a rat model of acute intracerebral hemorrhage. Stroke.2008;39:463–469.

Nakamura T, Xi G, Park JW, Hua Y, Hoff JT, Keep RF. Holo-transferrin and thrombin can interact to cause brain damage. Stroke. 2005 Feb;36(2):348-52. Epub 2005 Jan 6.

Nakano T, Ohkuma H. Surgery versus conservative treatment for intracerebral haemorrhage--is there an end to the long controversy? Lancet. 2005 Jan 29-Feb 4;365(9457):361-2. No abstract available.

Nakashima K , Yamashita K , Uesugi S , Ito H . Temporal and spatial profi le of apoptotic cell death in transient intracerebral mass lesion of the rat . J Neurotrauma 1999 ; 16 : 143 – 151 .

Nakaso K, Kitayama M, Mizuta E, Fukuda H, Ishii T, Nakashima K, Yamada K. Co-induction ofheme oxygenase-1 and peroxiredoxin I in astrocytes and microglia around hemorrhagic region in the rat brain. Neurosci Lett 2000;293:49–52.

Narayan RK, Narayan TM, Katz DA, Kornblith PL, Murano G. Lysis of intracranial hematomas with urokinase in a rabbit model. J Neurosurg 1985;62(4):580–6.

Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM. Early hemodynamic changes in experimental intracerebral hemorrhage. J Neurosurg 1986;65(5):697–703.

Nath FP , Kelly PT , Jenkins A , Mendelow AD , Graham DI , Teasdale GM . Effects of experimental intracerebral hemorrhage on blood fl ow, capillary permeability, and histochemistry. J Neurosurg 1987; 66: 555–562 .

Nath FP, Nicholls D, Fraser RJ. Prognosis in intracerebral haemorrhage. Acta Neurochir (Wien). 1983;67(1-2):29-35.

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Naval NS, Abdelhak TA, Zeballos P, Urrunaga N, Mirski MA, Carhuapoma JR. Prior statin use reduces mortality in intracerebral hemorrhage. Neurocrit Care. 2008a;8:6–12.

Naval NS, Abdelhak TA, Urrunaga N, Zeballos P, Mirski MA, Carhuapoma JR. An association of prior statin use with decreased perihematomal edema. Neurocrit Care. 2008b;8(1):13-8.

Nehls DG , Mendelow AD , Graham DI , Sinar EJ , Teasdale GM . Experimental intracerebral hemorrhage: progression of hemodynamic changes after production of a spontaneous mass lesion . Neurosurgery1988 ; 23 : 439 – 444 .

Nehls DG , Mendelow DA , Graham DI , Teasdale GM . Experimental intracerebral hemorrhage: early removal of a spontaneous mass lesion improves late outcome . Neurosurgery 1990 ; 27 (5) : 674 – 682 ;discussion 682 .

Newman GC. Clarification of abc/2 rule for ICH volume.
Stroke. 2007 Mar;38(3):862. Epub 2007 Jan 25. No abstract available.

Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J Biol Chem 2009;284:13291–13295.

Nguyen HX, O'Barr TJ, Anderson AJ. Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. J Neurochem2007;102:900–912.

Niclou S, Suidan HS, Brown-Luedi M, Monard D. Expression of the thrombin receptor mRNA in rat brain. Cell Mol Biol (Noisy-le-grand). 1994 May;40(3):421-8.

Niizuma H, Otsuki T, Johkura H, Nakazato N, Suzuki J. CT-guided stereotactic aspiration of intracerebral hematoma--result of a hematoma-lysis method using urokinase.Appl Neurophysiol. 1985;48(1-6):427-30.

Niizuma H, Yonemitsu T, Jokura H, Nakasato N, Suzuki J, Yoshimoto T. Stereotactic aspiration of thalamic hematoma. Overall results of 75 aspirated and 70 nonaspirated cases. Stereotact Funct Neurosurg. 1990;54-55:438-44.

Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005;308:1314–1318.

Ning M, Furie KL, Koroshetz WJ, Lee H, Barron M, Lederer M, Wang X, Zhu M, Sorensen AG, Lo EH, Kelly PJ. Association between tPA therapy and raised early matrix metalloproteinase-9 in acute stroke. Neurology 2006;66:1550–1555.

Nishihara T, Nagata K, Tanaka S, Suzuki Y, Izumi M, Mochizuki Y, Akabane A, Ochiai C. Newly developed endoscopic instruments for the removal of intracerebral hematoma.Neurocrit Care. 2005;2(1):67-74.

Noack H, Kube U, and Augustin W (1994) Relations between tocopherol depletion and coenzyme Q during lipid peroxidation in rat liver mitochondria. *Free RadicRes* 20:375–386.

Nolte KB, Brass LM, Fletterick CF (1996). Intracranial hemorrhage associated with cocaine abuse: a prospective autopsy study. Neurology 46: 1291–1296.

O'Donnell HC, Rosand J, Knudsen KA, Furie KL, Segal AZ, Chiu RI, *et al*. Apolipoprotein E genotype and the risk of recurrent lobar intracerebral hemorrhage. N Engl J Med 2000;342:240-5.

O'Donnell MJ, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, Rangarajan S, Islam S, Pais P, McQueen MJ, Mondo C, Damasceno A, Lopez-Jaramillo P, Hankey GJ, Dans AL, Yusoff K, Truelsen T, Diener HC, Sacco RL, Ryglewicz D, Czlonkowska A, Weimar C, Wang X, Yusuf S; INTERSTROKE investigators. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study.Lancet. 2010 Jul 10;376(9735):112-23. Epub 2010 Jun 17.

Ogawa N, Tsukamoto S, Hirose Y and Kuroda H (1986) Survival effect of coenzyme Q10, and naloxone on experimental stroke. *Pharmacol Biochem Behav* 24:315–317.

Ogawa A, Yoshimoto T, Kikuchi H, Sano K, Saito I, Yamaguchi T and Yasuhara H(1999). Ebselen in acute middle cerebral artery occlusion: a placebo-controlled double-blind clinical trial. *Cerebrovas Dis* 9:112–118

Ojemann RG, Heros RC (1983). Spontaneous brain hemorrhage. Stroke 14: 468–475.

Okada H, Horibe H, Yoshiyuki O, et al. (1976). A prospective study of cerebrovascular disease in Japanese rural communities, Akabane and Asahi. Part 1: Evaluation of risk factors in the occurrence of cerebral hemorrhage and thrombosis. Stroke 7: 599–607.

Okada Y, Kloiber O, Hossmann KA (1992) Regional metabolism in experimental brain tumors in cats: relationship with acid/base, water, and electrolyte homeostasis. J Neurosurg 77:917-926

Okauchi M, Hua Y, Keep RF, Morgenstern LB, Xi G. Effects of deferoxamine on intracerebral hemorrhage-induced brain injury in aged rats. Stroke 2009b;40:1858–1863.

Okauchi M, Xi G, Keep RF, Hua Y. Tissue-type transglutaminase and the effects of cystamine on intracerebral hemorrhage-induced brain edema and neurological deficits. Brain Res. 2009a;1249: 229–236.

Okimura Y, Ono J, Iwadate Y, Karasudani H, Suda S, Isobe K. [Evaluation of CT-guided stereotactic hematoma aspiration in mild cases with putaminal hemorrhage].No Shinkei Geka. 1991 Jul;19(7):611-7. Japanese.

Oliver CN, Starke-Reed PE, Stadtman ER, Liu GJ, Carney JM, and Floyd RA (1990) Oxidative damage to brain proteins, loss of glutamine synthetase activity, and production of free radicals during ischemia/reperfusion-induced injury to gerbil brain. *Proc Natl Acad Sci USA* 87:5144–5157

Olson JD. Mechanisms of hemostasis. Effect on intracerebral hemorrhage.
Stroke. 1993 Dec;24(12 Suppl):I109-14. Review.

Orakcioglu B, Uozumi Y, Kentar MM, Santos E, Unterberg A, Sakowitz OW. Evidence of spreading depolarizations in a porcine cortical intracerebral hemorrhage model.Acta Neurochir Suppl. 2012;114:369-72. doi: 10.1007/978-3-7091-0956-4 71.

Oselkin M, Tian D, Bergold PJ (2010) Low-dose cardiotonic steroids increase sodium-potassium ATPase activity that protects hippocampal slice cultures from experimental ischemia. Neurosci Lett 473:67-71

Ott KH , Kase CS , Ojemann RG , et al. Cerebellar hemorrhage: diagnosis and treatment . A review of 56 cases . Arch Neurol 1974 ; 31 (3) : 160 – 167 .

Paciaroni Maurizio, Bogousslavsky Julien. Chapter 1 The history of stroke and cerebrovascular disease,Handbook of Clinical Neurology,Volume 92, 2008, Pages 3–28 Stroke.

Packer L (1998) alpha-Lipoic acid: a metabolic antioxidant which regulates NF antioxidants in central nervous system injury

Padh H (1990) Cellular functions of ascorbic acid. *Biochem Cell Biol* 68:1166–1173.

Palmer C, Roberts RL, and Bero C (1994) Deferoxamine post-treatment reduces ischemic brain injury in neonatal rats. *Stroke* 25:1039–1045.

Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges.Lancet Neurol. 2010 Jul;9(7):689-701.

Papo I , Janny P , Caruselli G , Colnet G , Luongo A . Intracranial pressure time course in primary intracerebral hemorrhage . Neurosurgery 1979 ; 4 : 504 – 511.

Parfenova H, Leffler CW. Cerebroprotective functions of HO-2. Curr Pharm Des 2008;14:443–453.

Pari L, Murugavel P (2007) Diallyl tetrasulfide improves cadmium induced alterations of acetylcholinesterase, ATPases and oxidative stress in brain of rats. Toxicology 234:44–50.

Park CK and Hall ED (1994) Dose response analysis of the tirilazad mesylate (U-74006F) upon neurological outcome and ischemic brain damage in permanent focal cerebral ischemia. *Brain Res* 645:157–163.

Park HK, Chu K, Lee ST, Jung KH, Kim EH, Lee KB, et al. Granulocyte colony-stimulating factor induces sensorimotor recovery in intracerebral hemorrhage. Brain Res. 2005;1041: 125–131.

Pathak EB, Sloan MA. Recent racial/ethnic disparities in stroke hospitalizations and outcomes for young adults in Florida, 2001-2006. Neuroepidemiology. 2009;32(4):302-11. doi: 10.1159/000208795. Epub 2009 Mar 13.

Paul RJ, Bauer M, Pease W (1979) Vascular smooth muscle: aerobic glycolysis linked to sodium and potassium transport processes. Science 206:1414-1416.

Payne HA , Maravilla KR , Levinstone A , et al. Recovery from primary pontine hemorrhage . Ann Neurol 1978 ; 4 (6) : 557 – 558 .

Peck G, Smeeth L, Whittaker J, Casas JP, Hingorani A, Sharma P. The genetics of primary haemorrhagic stroke, subarachnoid haemorrhage and ruptured intracranial aneurysms in adults. PLoS One 2008;3:E3691.

Peeling J, Yan HJ, Chen SG, Campbell M, Del Bigio MR. Protective effects of free radical inhibitors in intracerebral hemorrhage in rat. Brain Res. 1998;795:63–70.

Peeling J, Del Bigio MR, Corbett D, Green AR, Jackson DM. Efficacy of disodium 4-[(*tert*-butylimino)methyl]benzene-1,3- disulfonate *N*-oxide (NXY-059), a free radical trapping agent, in a rat model of hemorrhagic stroke. Neuropharmacology. 2001; 40:433–439.

Pellegrini-Giampeitro DE, Cherici G, Alesiani M, Caria V, and Moroni F (1990) Excitatory amino acids release and free radical formation may cooperate in the genesis of ischemia-induced neuronal damage.*J Neurosci* 10:1035–1041.

Penfield, W.: The operative treatment of spontaneous intracerebral hemorrhage. Canad. Med. Asso. J., 1933, 28, 369.

Pera J, Slowik A, Dziedzic T, Pulyk R, Wloch D, Szczudlik A. Glutathione peroxidase 1 C593T polymorphism is associated with lobar intracerebral hemorrhage. Cerebrovasc Dis 2008;25:445-9

Peterson PL, Roszler M, Jacobs I, et al. (1991). Neurovascular complications of cocaine abuse. J Neuropsychiatry Clin Neurosci 3: 143–149.

Pfefferkorn T, Rosenberg GA. Closure of the blood-brain barrier by matrix metalloproteinase inhibition reduces rtPA-mediated mortality in cerebral ischemia with delayed reperfusion. Stroke 2003;34:2025–2030.

Phan TG, Koh M, Vierkant RA, Wijdicks EF. Hydrocephalus is a determinant of early mortality in putaminal hemorrhage. Stroke. 2000 Sep;31(9):2157-62.

Piepgras U , Riegerand P . Thalamic bleeding: diagnosis, course and prognosis . Neuroradiology 1981 ; 22 (2) : 85 – 91 .

Pizzi M, Spano P. Distinct roles of diverse nuclear factor-kappaB complexes in neuropathological mechanisms. *Eur J Pharmacol*. 2006;545: 22–28.

Pleasure David, et., al, section editor. Antioxidant Therapy in Neurologic Disease ARCH NEUROL/VOL 57, SEP 2000.

Portenoy RK, Lipton RB, Berger AR, Lesser ML, Lantos G. Intracerebral haemorrhage: a model for the prediction of outcome.J Neurol Neurosurg Psychiatry. 1987 Aug;50(8):976-9.

Power C, Henry S, Del Bigio MR, Larsen PH, Corbett D, Imai Y, Yong VW, Peeling J. Intracerebral hemorrhage induces macrophage activation and matrix metalloproteinases. Ann Neurol2003;53:731–742.

Poungvarin N. Steroids have no role in stroke therapy.
Stroke. 2004 Jan;35(1):229-30. Epub 2003 Dec 18. No abstract available.

Poungvarin N, Bhoopat W, Viriyavejakul A, Rodprasert P, Buranasiri P, Sukondhabhant S, Hensley MJ, Strom BL. Effects of dexamethasone in primary supratentorial intracerebral hemorrhage.N Engl J Med. 1987 May 14;316(20):1229-33.

Powers WJ, Zazulia AR, Videen TO, et al. Autoregulation of cerebral blood flow surrounding acute (6–22 h) intracerebral hemorrhage. Neurology 2001;57(1):18–24.

Prange HW. Pathophysiology, therapy and prognosis of hypoxic-ischemic brain damage.Z Kardiol. 1994;83 Suppl 6:127-34. Review. German.

Prasad K, Mendelow AD, Gregson B. Surgery for Primary Supratentorial Intracerebral Hematoma. A Meta-Analysis of 10 Randomized Controlled Trials. Stroke. 2009 Sep 24. [Epub ahead of print] No abstract available

Prasad K, Mendelow AD, Gregson B. Surgery for primary supratentorial intracerebral haemorrhage. Cochrane Database Syst Rev. 2008 Oct 8;(4):CD000200. doi: 10.1002/14651858.CD000200.pub2.

Premkumar DR, Cohen DL, Hedera P, Friedland RP, Kalaria RN. Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. Am J Pathol 1996;148:2083-95.

Priorities for clinical research in intracerebral hemorrhage:report from a National Institute of Neurological Disorders andStroke workshop. Stroke 2005;36(3):e23–41.

Pulsinelli WA (1992) Pathophysiology of stroke. *Lancet* 339:533–536.

Pyo H, Yang MS, Jou I, Joe EH. Wortmannin enhances lipopolysaccharide-induced inducible nitric oxide synthase expression in microglia in the presence of astrocytes in rats. Neurosci Lett 2003;346:141–144.

Qureshi AI, Ali Z, Suri MF, Shuaib A, Baker G, Todd K, Guterman LR, Hopkins LN. Extracellular glutamate and other amino acids in experimental intracerebral hemorrhage: an in vivo microdialysis study. Crit Care Med. 2003 May;31(5):1482-9.

Qureshi AI, Ling GS, Khan J, et al. Quantitative analysis of injured, necrotic, and apoptotic cells in a new experimental model of intracerebral hemorrhage. Crit Care Med 2001a;29(1):152–7.

Qureshi AI, Mendelow AD, Hanley DF (2009) Intracerebral haemorrhage.Lancet 373:1632-1644

Qureshi AI, Safdar K, Patel M, Janssen RS, Frankel MR. Stroke in young black patients. Risk factors, subtypes, and prognosis.Stroke. 1995 Nov;26(11):1995-8.

Qureshi AI, Safdar K, Weil J, Barch C, Bliwise DL, Colohan AR, Mackay B, Frankel MR. Predictors of early deterioration and mortality in black Americans with spontaneous intracerebral hemorrhage.Stroke. 1995 Oct;26(10):1764-7.

Qureshi AI , Suri MF , Ling GS , Khan J , Guterman LR , Hopkins LN . Absence of early proinfl amatory cytokine expression in experimental intracerebral hemorrhage . Neurosurgery 2001b ; 49 : 416 – 421 .

Qureshi AI, Suri MF, Ling GS, Khan J, Guterman LR, Hopkins LN: Absence
of early proinflammatory cytokine expression in experimental intracerebral hemorrhage. Neurosurgery 49:416–420.

Qureshi AI, Suri MF, Ostrow PT, Kim SH, Ali Z, Shatla AA, Guterman LR, Hopkins LN. Apoptosis as a form of cell death in intracerebral hemorrhage.Neurosurgery. 2003b May;52(5):1041-7; discussion 1047-8.

Qureshi AI, Suri MF, Ringer AJ, Guterman LR, Hopkins LN. Regional intraparenchymal pressure differences in experimental intracerebral hemorrhage: effect of hypertonic saline. Crit Care Med 2002;30(2):435–41.

Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF: Spontaneous intracerebral hemorrhage. N Engl J Med 344:1450–1460, 2001b.

Qureshi AI, Wilson DA, Traystman RJ. Treatment of elevated intracranial pressure in experimental intracerebral hemorrhage: comparison between mannitol and hypertonic saline.Neurosurgery. 1999 May;44(5):1055-63; discussion 1063-4.

Raabe A, Krug U. Migraine associated bilateral intracerebral haemorrhages. Clin Neurol Neurosurg. 1999 Sep;101(3):193-5.

Rachel C. Brown, PhD; Thomas P. Davis, PhD. Calcium Modulation of Adherens and Tight Junction Function. A Potential Mechanism for Blood-Brain Barrier Disruption After Stroke. Stroke, June 2002:1706- 1711.

Radberg JA, Olsson JE, Radberg CT (1991). Prognostic parameters in spontaneous intracerebral hematomas with special reference to anticoagulant treatment. Stroke 22: 571–576.

Ramazan Durmaz, Selda Deliorman, Serap IªIKSOY, Ruhi Uyar, Kevser Erol, Eºref TEL1. Antiproliferative Properties of the Lazaroids U-83836E and U-74389G on Glioma Cells In Vitro. PATHOLOGY ONCOLOGY RESEARCH Vol 5, No 3, 1999.

Raney RB, Raney AA. The contribution of cerebral angiography in diagnosis. Calif Med. 1950 Oct;73(4):342-9.

Reiner AP, Kumar PN, Schwartz SM, Longstreth WT Jr, Pearce RM, Rosendaal FR, *et al*. Genetic variants of platelet glycoprotein receptors and risk of stroke in young women. Stroke 2000;31:1628-33.

Reiner AP, Schwartz SM, Frank MB, Longstreth WT Jr, Hindorff LA, Teramura G, *et al*. Polymorphisms of coagulation factor XIII subunit A and risk of nonfatal hemorrhagic stroke in young white women. Stroke 2001;32:2580-7.

Report from the Joint Commitee for Stroke Facilities -- XII. Computed Tomography in the Managemnt of Cerebrovascular Disease *Stroke.* 1975;6:103-107.

Reuter B, Bugert P, Stroick M, Bukow S, Griebe M, Hennerici MG, *et al*. Timp-2□gene polymorphism is associated with intracerebral hemorrhage. Cerebrovasc Dis 2009;28:558-63.

Rieger A, Rainov NG, Sanchin L, Ebel H, Furka I, Görömbey Z, Burkert W. Is it useful to measure supratentorial ICP in the presence of a posterior fossa lesion? Absence of transtentorial pressure gradients in an animal model. Br J Neurosurg. 1999 Oct;13(5):454-8.

Rincon F, Mayer SA. The Epidemiology of Intracerebral Hemorrhage in the United States from 1979 to 2008.Neurocrit Care. 2012 Oct 26.

Rise IR, Risöe C, Kirkeby OJ. Effect of hemorrhage on cerebral microcirculation during normal and high cerebrospinal fluid pressure in pigs. J Neurosurg Anesthesiol. 1998 Jan;10(1):49-54.

Roberto Federico Villa and Antonella Gorini. Pharmacology of Lazaroids and Brain Energy Metabolism: A ReviewPHARMACOLOGICAL REVIEWS Vol. 49, No. 1, 1997.

Robinson, G. W.: Encapsulated brain hemorrhages. Arch. Neurol. & Psychiat.,1932, 27, 1441.

Rodrigues CM, Sola S, Nan Z, Castro RE, Ribeiro PS, Low WC, et al. Tauroursodeoxycholic acid reduces apoptosis and protects against neurological injury after acute hemorrhagic stroke in rats. Proc Natl Acad Sci U S A. 2003;100:6087–6092.

Rohde V, Rohde I, Thiex R, Ince A, Jung A, Dückers G, Gröschel K, Röttger C, Küker W, Müller HD, Gilsbach JM. Fibrinolysis therapy achieved with tissue plasminogen activator and aspiration of the liquefied clot after experimental intracerebral hemorrhage: rapid reduction in hematoma volume but intensification of delayed edema formation.J Neurosurg. 2002 Oct;97(4):954-62.

Rohde V, Rohde I, Thiex R, Küker W, Ince A, Gilsbach JM. The role of intraoperative magnetic resonance imaging for the detection of hemorrhagic complications during surgery for intracerebral lesions an experimental approach.Surg Neurol. 2001 Oct;56(4):266-74; discussion 274-5.

Rohde V, Uzma N, Thiex R, Samadani U. Management of delayed edema formation after fibrinolytic therapy for intracerebral hematomas: preliminary experimental data.Acta Neurochir Suppl. 2008;105:101-4.

Ropper AH , Davis KR . Lobar cerebral hemorrhages: acute clinical syndromes in 26 cases . Ann Neurol 1980 ; 8 (2) : 141 – 147 .

Rosand J , Eckman MH , Knudsen KA , et al. The effect of warfarin and intensity of anticoagulation on outcome of intracerebral hemorrhage . Arch Intern Med 2004 ; 164 (8) : 880 – 884 .

Rosand J , Eskey C , Chang Y , Gonzalez RG , Greenberg SM , Koroshetz WJ . Dynamic single-section CT demonstrates reduced cerebral blood fl ow in acute intracerebral hemorrhage . Cerebrovasc Dis 2002 ;14 : 214 – 220 .

Rosell A, Ortega-Aznar A, Alvarez-Sabin J, Fernandez-Cadenas I, Ribo M, Molina CA, Lo EH, Montaner J. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. Stroke 2006;37:1399–1406.

Rosenberg GA, Cunningham LA, Wallace J, Alexander S, Estrada EY, Grossetete M, Razhagi A, Miller K, Gearing A. Immunohistochemistry of matrix metalloproteinases in reperfusion injury to rat brain: activation of MMP-9 linked to stromelysin-1 and microglia in cell cultures. Brain Res 2001;893:104–112.

Rosenberg GA, Estrada E, Wesley M, Kyner WT. Autoradiographic patterns of brain interstitial fluid flow after collagenaseinduced haemorrhage in rat. Acta Neurochir Suppl (Wien)1990;51:280–2.

Rosenberg GA, Kornfeld M, Estrada E, Kelley RO, Liotta LA, Stetler-Stevenson WG. TIMP-2 reduces proteolytic opening of blood-brain barrier by type IV collagenase.Brain Res. 1992 Apr 3;576(2):203-7.

Rosenberg GA, Navratil M. Metalloproteinase inhibition blocks edema in intracerebral hemorrhage in the rat. Neurology 1997;48:921–926.

Rosenblum WI. Miliary aneurysms and "fibrinoid" degeneration of cerebral blood vessels.Hum Pathol. 1977 Mar;8(2):133-9.

RussellDA, GoughMJ.Intracerebral haemorrhage following carotid endarterectomy. Eur J Vasc Endovasc Surg. 2004 Aug;28(2):115-23.

Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radic Biol Med 2000;28:289–309.

Saito I, Asano T, Takakura K, Abe H, Yoshimoto T, Kikuchi H, Ohta T, and Ishibashi S (1998) Neuroprotective efficacy of an antioxidant ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. *Neurosurgery* 42:269–277.

Salahudeen A, Badr K, Morrow J, Roberts J 2nd. Hydrogen peroxide induces 21-aminosteroid-inhibitable F2-isoprostane production and cytolysis in renal tubular epithelial cells. J Am Soc Nephrol. 1995 Oct;6(4):1300-3.

Salerni A, Wald S, Flanagan M. Relationships among cortical ischemia, infarction, and hemorrhage in eclampsia. Neurosurgery. 1988 Feb;22(2):408-10.

Sanada T, Nakamura T, Nishmura MC, Isayama K, and Pitts LH (1993). Effect of U-74006F on neurological function and brain edema after fluid percussion injury in rats. *J Neurotrauma* 10:65–71.

Sandrzadeh SM, Anderson DK, Panter SS, Hallaway PE, and Eaton JW (1993) Hemoglobin potentiates nervous system damage. *J Clin Invest* 79:662–664.

Sansing LH , Kaznatcheeva EA , Perkins CJ , Komaroff E , Gutman FB , Newman GC . Edema after intracerebral hemorrhage: correlations with coagulation parameters and treatment. J Neurosurg 2003 ; 98 : 985 – 992.

Santos MS, Moreno AJ, Carvalho AP (1996) Relationships between ATP depletion, membrane potential, and the release of neurotransmitters in rat nerve terminals. An *in vitro* study under conditions that mimic anoxia, hypoglycemia, and ischemia. Stroke 27:941-950.

Sanui H, Rubin H (1982) The role of magnesium in cell proliferation and transformation. In: Boynton AL, McKochan WL, Whitfield JP (eds). Ions, Cell Proliferation and Cancer. Academic Press, New York, 517-537.

Sarco DP, Becker J, Palmer C, Sheldon RA, and Ferriero DM (2000) The neuroprotective effect of deferoxamine in the hypoxic-ischemic immature mouse brain. *Neurosci Lett* 17:113–116.

Sastry BS, Phillis JW (1977) Antagonism of biogenic amine-induced depression of cerebral cortical neurones by Na+,K+-ATPase in inhibitors. Can J Physiol Pharmacol 55:170-179.

Sato PH, Hall ED. Tirilazad mesylate protects vitamic C and E in brain ischaemia reperfusion injury. J Neurochem 1992; 58: 2263-8.

Savard C, Lema PP, Hélie P, Vachon P. Effects of timing of dexamethasone treatment on the outcome of collagenase-induced intracerebral hematoma in rats. Comp Med. 2009;59:444–448.

Savill, J.; Haslett, C. Resolution of inflammation. In: Ley, K., editor. Physiology of Inflammation. New York: Oxford University Press; 2001. p. 496-525.

Savman K, Nilsson UA, Blennow M, Kjellmer I, Whitelaw A. Non-protein-bound iron is elevated in cerebrospinal fluid from preterm infants with post-hemorrhagic ventricular dilatation. Pediatr Res 2001;49:208–212.

Sazci A, Ergul E, Tuncer N, Akpinar G, Kara I. Methylenetetrahydrofolate reductase gene polymorphisms are associated with ischemic and hemorrhagic stroke: Dual effect of MTHFR polymorphisms C677T and A1298C. Brain Res Bull 2006;71:45-50.

Schaller C, Rohde V, Meyer B, Hassler W. Stereotactic puncture and lysis of spontaneous intracerebral hemorrhage using recombinant tissue-plasminogen activator.Neurosurgery. 1995 Feb;36(2):328-33; discussion 333-5.

Schallreuter KU, Elwary SM, Gibbons NC, Rokos H, Wood JM (2004) Activation/deactivation of acetylcholinesterase by H2O2: more evidence for oxidative stress in vitiligo. Biochem Biophys Res Commun 315:502–508.

Schuit SC, de Jong FH, Stolk L, Koek WN, van Meurs JB, Schoofs MW, *et al*. Estrogen receptor alpha gene polymorphisms are associated with estradiol levels in postmenopausal women. Eur J Endocrinol 2005;153:327-34

Schmidek H H, Sweet W H(eds).Operative neurosurgical techniques. 1st ed., Grune and Stratton, New York, 1982.

Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, and SchwaningerM (1999) NF_B is activated and promotes cell death in focal cerebral ischemia. *Nat Med* 5:554–559.

Shadnia S, Dasgar M, Taghikhani S, Mohammadirad A, Khorasani R, Abdollahi M (2007) Protective effects of alpha-tocopherol and Nacetyl- cysteine on diazinon-induced oxidative stress and acetylcholinesterase inhibition in rats. Toxicol Mech Methods 17:109– 115.

Siesjo BK (1992a) Pathophysiology and treatment of focal cerebral ischemia. Part I: Pathophysiology. *J Neurosurg* 77:169–184.

Siesjo BK (1992b) Pathophysiology and treatment of focal cerebral ischemia. Part II: Mechanism of damage and treatment. *J Neurosurg* 77:337–354.

Siesjo BK (1993) Basic mechanisms of traumatic brain damage. *Ann Emerg Med* 22:959–969.

Sills C , Villar-Cordova C , Pasteur W , et al. Demonstration of hypoperfusion surrounding intracerebral hematoma in humans . J Stroke Cerebrovasc Dis 1996 ; 6 : 17 – 24 .

Silva Y, Leira R, Tejada J, Lainez JM, Castillo J, Dávalos A; Stroke Project, Cerebrovascular Diseases Group of the Spanish Neurological Society. Molecular signatures of vascular injury are associated with early growth of intracerebral hemorrhage. Stroke. 2005 Jan;36(1):86-91. Epub 2004 Nov 18.

Silva Y, Leira R, Tejada J, Lainez JM, Castillo J, Davalos A. Molecular signatures of vascular injury are associated with early growth of intracerebral hemorrhage. Stroke 2005;36:86–91.

Silverstein A . Primary pontile hemorrhage . A review of 50 cases . Confi n Neurol 1967 ; 29 (1) : 33 – 46 .

Simonian NA and Coyle JT (1996) Oxidative stress in neurodegenerative diseases.*Annu Rev Pharmacol Toxicol* l 36:83–106.

Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: effects of a temporary mass lesion. J Neurosurg 1987;66(4):568–76.

Singh, J. P., and Bonin, P. D.: Inhibition of proliferation of fibroblast by lazaroids (21-aminosteroids). Life Sci. 49: 2053–2058, 1991.

Sinn DI, Kim SJ, Chu K, Jung KH, Lee ST, Song EC, et al. Valproic acid-mediated neuroprotection in intracerebral hemorrhage via histone deacetylase inhibition and transcriptional activation. Neurobiol Dis. 2007a;26:464–472.

Sinn DI, Lee ST, Chu K, Jung KH, Kim EH, Kim JM, et al. Proteasomal inhibition in intracerebral hemorrhage: neuroprotective and anti-inflammatory effects of bortezomib. Neurosci Res. 2007b;58:12–18.

Segal AZ, Chiu RI, Eggleston-Sexton PM, et al. (1999). Low cholesterol as a risk factor for primary intracerebral hemorrhage: a case-control study. Neuroepidemiology 18: 185–193.

Seifert T, Lechner A, Flooh E, Schmidt H, Schmidt R, Fazekas F. Lack of association of lobar intracerebral hemorrhage with apolipoprotein E genotype in an unselected population. Cerebrovasc Dis 2006;21:266-70

Sean G. Carriedo, Hong Zhen Yins, Stefano L. Sensi, and John H. Weiss Rapid Ca21 Entry through Ca21-Permeable AMPA/Kainate Channels Triggers Marked Intracellular Ca21 Rises and Consequent Oxygen Radical Production.

Selda Kabadere, Pýnar Öztopçu, Seval Korkmaz, Seyfried D, Han Y, Lu D, Chen J, Bydon A, Chopp M. Improvement in neurological outcome after administration of atorvastatin following experimental intracerebral hemorrhage in rats.J Neurosurg. 2004 Jul;101(1):104-7.

Sen CK (1998) Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem Pharmacol* 55:1747–1758.

Senant J, Samson M, Proust B, Szeibert J, Onnient Y. A multi-factorial approach in the vital prognosis of spontaneous intracerebral hematoma]. Rev Neurol (Paris). 1988;144(4):279-83. Review. French.

Shigeno T, Asano T, Mima T, Takakura K (1989) Effect of enhanced capillary activity on the blood-brain barrier during focal cerebral ischemia in cats. Stroke 20:1260-1266

Shimoda LA, Norins NA, Madden JA. Responses to pulsatile flow in piglet isolated cerebral arteries.Pediatr Res. 1998 Apr;43(4 Pt 1):514-20.

Shivakumar BR, Kolluri SV, and Ravindranath V (1995) Glutathione and protein thiol homeostasis in brain during reperfusion after cerebral ischemia. *J Pharmacol Exp Ther* 274:1167–1173

Slowik A, Turaj W, Dziedzic T, Haefele A, Pera J, Malecki MT, *et al*. DD genotype of ACE gene is a risk factor for intracerebral hemorrhage. Neurology 2004;63:359-61.

Slowik A, Dziedzic T, Pera J, Figlewicz DA, Szczudlik A. Coagulation factor xiii vai34□leu polymorphism in patients with small vessel disease or primary intracerebral hemorrhage. Cerebrovasc Dis 2005;19:165-70

Smeeton NC, Heuschmann PU, Rudd AG, McEvoy AW, Kitchen ND, Sarker SJ, Wolfe CD. Incidence of hemorrhagic stroke in black Caribbean, black African, and white populations: the South London stroke register, 1995-2004. Stroke. 2007 Dec;38(12):3133-8. Epub 2007 Oct 25.

Smith EE, Rosand J, Knudsen KA, et al. (2002). Leukoaraiosis is associated with warfarin-related hemorrhage following ischemic stroke. Neurology 59: 193–197.

Sobrino T, Arias S, Rodríguez-González R, Brea D, Silva Y, de la Ossa NP, et al. High serum levels of growth factors are associated with good outcome in intracerebral hemorrhage. J Cereb Blood Flow Metab. 2009;29:1968–1974.

Somarajan BI, Kalita J, Mittal B, Misra UK. Evaluation of MTHFR C677T polymorphism in ischemic and hemorrhagic stroke patients. A case-control study in a Northern Indian population. J Neurol Sci 2011;304:67-70.

Song S, Hua Y, Keep RF, Hoff JT, Xi G. A new hippocampal model for examining intracerebral hemorrhage-related neuronal death: effects of deferoxamine on hemoglobin-induced neuronal death. Stroke 2007;38:2861–2863.

Staykov D, Wagner I, Volbers B, Hauer EM, Doerfler A, Schwab S, Bardutzky J. Natural course of perihemorrhagic edema after intracerebral hemorrhage. Stroke. 2011 Sep;42(9):2625-9.

Stein M, Misselwitz B, Hamann GF, Scharbrodt W, Schummer DI, Oertel MF. Intracerebral hemorrhage in the very old: future demographic trends of an aging population.Stroke. 2012 Apr;43(4):1126-8.

Stein RW , Kase CS , Hier DB , et al. Caudate hemorrhage . Neurology 1984 ; 34 (12) : 1549 – 1554 .

Steiner I, Gomori JM, Melamed E. The prognostic value of the CT scan in conservatively treated patients with intracerebral hematoma. Stroke. 1984 Mar-Apr;15(2):279-82.

Steiner L, Lofgren J, Zwetnow NN. Characteristics and limits of tolerance in repeated subarachnoid hemorrhage in dogs. Acta Neurol Scand 1975;52(4):241–67.

Steiner T, Petersson J, Al-Shahi Salman R, Christensen H, Cordonnier C, Csiba L, Harnof S, Krieger D, Mendelow D, Molina C, Montaner J, Overgaard K, Roine RO, Schmutzhard E, Tatlisumak T, Toni D, Stapf C; European Research Network on Intracerebral Haemorrhage. European research priorities for intracerebral haemorrhage.Cerebrovasc Dis. 2011;32(5):409-19. doi: 10.1159/000330653. Epub 2011 Oct 8. Review.

Strand M, Söderström I, Wiklund PG, Hallmans G, Weinehall L, Söderberg S, *et al*. Estrogen receptor alpha gene polymorphisms and first-ever intracerebral hemorrhage. Cerebrovasc Dis 2007;24:500-8.

Straw RN, Means ED, Kitt TM, et al. Preliminary Efficacy and Safety results from protocol P/2700/0017: Tirilazad mesylate in the treatment of acute head injury (North American study)- Technical report. Kalamazoo, MI: Pharmacia & Upjohn Co.; 1995.

Strbian D, Tatlisumak T, Ramadan UA, Lindsberg PJ. Mast cell blocking reduces brain edema and hematoma volume and improves outcome after experimental intracerebral hemorrhage. J Cereb Blood Flow Metab 2007;27: 795–802.

Striggow F, Riek M, Breder J, Henrich-Noack P, Reymann KG, Reiser G: The protease thrombin is an endogenous mediator of hippocampal neuroprotection against ischemia at low concentrations but causes degeneration at high concentrations. Proc Natl Acad Sci U S A 97:2264–2269, 2000.

Sudlow C, Martinez Gonzalez NA, Kim J, Clark C. Dose apolipoprotein E genotype influence the risk of ischemic stroke, intracerebral hemorrhage, or subarachnoid hemorrhage? Systematic review and meta-analyses of 31 studies among 5961 cases and 17,965 controls. Stroke 2006;37:364-70.

Sugi T, Fujishima M, Omae T. Lactate and pyruvate concentrations, and acid-base balance of cerebrospinal fluid inexperimentally induced intracerebral and subarachnoid hemorrhage in dogs. Stroke 1975;6(6):715–9.

Sullivan PG, Geiger JD, Mattson MP, and Scheff SW (2000) Dietary supplement creatine protects against traumatic brain injury. *Ann Neurol* 48:723–729.

Sun L, Li Z, Zhang H, Ma A, Liao Y, Wang D, *et al*. Pentanucleotide TTTTA repeat polymorphism of apolipoprotein(a) gene and plasma lipoprotein(a) are associated with ischemic and hemorrhagic stroke in Chinese: A multicenter case-control study in China. Stroke 2003;34:1617-22

Sussman BJ, Barber JB, Goald H. Experimental intracerebral hematoma. Reduction of oxygen tension in brain and cerebrospinal fluid. J Neurosurg. 1974 Aug;41(2):177-86.

Sutherland GR, Auer RN. Primary intracerebral hemorrhage. J Clin Neurosci. 2006 Jun;13(5):511-7. Review.

Suttner DM, Dennery PA. Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron. Faseb J 1999;13:1800–1809.

Suzuki J , Ebina T . Sequential changes in tissue surrounding ICH . In: Pia HW , Longmaid C , Zierski J , eds. Spontaneous Intracerebral Hematomas . Berlin : Springer-Verlag , 1980 : 121 – 128 .

Swann AC (1984) (Na+,K+)-adenosine triphosphatase regulation by the sympathetic nervous system: effects of noradrenergic stimulation and lesions *in vivo*. J Pharmacol Exp Ther 228:304-311.

Szymanska A, Biernaskie J, Laidley D, Granter-Button S, Corbett D. Minocycline and intracerebral hemorrhage: influence of injury severity and delay to treatment. Exp Neurol. 2006;197:189–196.

Tagaya M, Mastsumoto M, Kitagawa K, Niinobe M, Ohtsuki T, Hata R, Ogawa S, Hanada N, Mikoshiba K, and Kamada T (1992) Recombinant human superoxide dismutase can attenuate ischemic neuronal damage in gerbils. *Life Sci* 51:253–259.

Takahashi T, Morita K, Akagi R, Sassa S. Heme oxygenase-1: a novel therapeutic target in oxidative tissue injuries. Curr Med Chem 2004;11:1545–1561.

Takashaki E, Sasaki N, Takeda J, Ito H. The geographic distribution of cerebral hemorrhage and hypertension in Japan. Hum Biol. 1957 May; 29(2):139-66.

Takasugi S, Ueda S, Matsumoto K. Chronological changes in spontaneous intracerebral hematoma—an experimental and clinical study. Stroke 1985;16(4):651–8.

Tamura S, Tamura Y, Tsuka T, Uchida K. Sequential magnetic resonance imaging of an intracranial hematoma in a dog. Vet Radiol Ultrasound. 2006 Mar-Apr;47(2):142-4.

Tanaka H, Ueda Y, Hayashi M, et al. (1982). Risk factors for cerebral hemorrhage and cerebral infarction in a Japanese rural community. Stroke 13: 62–73.

Tanaka Y , Furuse M , Iwasa H , et al. Lobar intracerebral hemorrhage: etiology and a long-term followup study of 32 patients . Stroke 1986 ; 17 (1) : 51 – 57 .

Tang J, Liu J, Zhou C, Alexander JS, Nanda A, Granger DN, Zhang JH. Mmp-9 deficiency enhances collagenase-induced intracerebral hemorrhage and brain injury in mutant mice. J Cereb Blood Flow Metab 2004;24:1133–1145.

Tang J, Liu J, Zhou C, Ostanin D, Grisham MB, Neil Granger D, Zhang JH. Role of NADPH oxidase in the brain injury of intracerebral hemorrhage. J Neurochem. 2005 Sep;94(5):1342-50. Epub 2005 Jul 11.

Taylor BM, Fleming WE, Benjamin CW, Wu Y, Mathews WR and Sun FF. The mechanism of cytoprotective action of lazaroids I: Inhibition of reactive oxygen species formation and lethal cell injury during periods of energy depletionJ Pharmacol Exp Ther. 1996 Mar;276(3):1224-31.

Teernstra OP , Evers SM , Lodder J , Leffers P , Franke CL , Blaauw G . Multicenter randomized controlled trial (SICHPA) . Stereotactic treatment of intracerebral hematoma by means of a plasminogen activator: a multicenter randomized controlled trial (SICHPA) . Stroke 2003 ; 34 (4) : 968 – 974 .

Tejima E, Zhao BQ, Tsuji K, Rosell A, van Leyen K, Gonzalez RG, Montaner J, Wang X, Lo EH.Astrocytic induction of matrix metalloproteinase-9 and edema in brain hemorrhage. J Cereb Blood Flow Metab 2007;27:460–468.

Thai QA, Pradilla G, Legnani FG, Kretzer RM, Hsu W, Tamargo RJ. Lysis of intracerebral hematoma with stereotactically implanted tissue plasminogen activator polymers in a rabbit model. J Neurosurg 2006;105(3):424–9.

Thiex R, Küker W, Jungbluth P, Kayser C, Müller HD, Rohde I, Gilsbach JM, Rohde V. Minor inflammation after surgical evacuation compared with fibrinolytic therapy of experimental intracerebral hemorrhages. NeurolRes. 2005 Jul;27(5):493-8.

Thiex R, Rohde V, Rohde I, Mayfrank L, Zeki Z, Thron A, Gilsbach JM, Uhl E. Frame-based and frameless stereotactic hematoma puncture and subsequent fibrinolytic therapy for the treatment of spontaneous intracerebral hemorrhage.J Neurol. 2004 Dec;251(12):1443-50.

Thimmulappa RK, Lee H, Rangasamy T, Reddy SP, Yamamoto M, Kensler TW, Biswal S. Nrf2 is a critical regulator of the innate immune response and survival during experimental sepsis.J Clin Invest. 2006 Apr;116(4):984-95.

The American-Canadian Co-OperativeStudy group. Persantine Aspirin Trial in cerebral ischemia. Part II: Endpoint results. Stroke. 1985 May-Jun;16(3):406-15.

The Canadian CooperativeStudy Group. A randomized trial of aspirin and sulfinpyrazone in threatened stroke. [No authors listed] N Engl J Med. 1978 Jul 13;299(2):53-9.

The ESPS Group. The European Stroke Prevention Study (ESPS). Principal end-points. The ESPS Group. Lancet. 1987 Dec 12;2(8572):1351-4.

The RANTTAS investigators. High dose tilizarad mesylate in patients with acute stroke (RANTTAS). Stroke 1996; 27: 1453-8

The RANTTASII investigators. High dose tirilazad for acute stroke (RANTTAS II). Stroke 1998; 29:1256-7

Thomas DG, Nouby RM. Experience in 300 cases of CT-directed stereotactic surgery for lesion biopsy and aspiration of haematoma. Br J Neurosurg. 1989;3(3):321-5.

Thompson JE. The evolution of surgery for the treatment and prevention of stroke. The Willis Lecture.Stroke. 1996 Aug;27(8):1427-34.

Thompson KJ, Shoham S, Connor JR. Iron and neurodegenerative disorders. Brain Res Bull 2001;55:155–164.

Thrift AG, McNeil JJ, Forbes A, et al. (1998). Three important subgroups of hypertensive persons at greater risk of intracerebral hemorrhage. Melbourne Risk Factor Study Group. Hypertension 31: 1223–1229

TIMI Study Group (1989). Comparison of invasive and conservative strategies after treatment with intravenous tissue plasminogen activator in acute myocardial infarction.Results of the thrombolysis in myocardial infarction (TIMI) phase II trial. N Engl J Med 320: 618–627.

Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders andStroke rt-PA Stroke Study Group. N Engl J Med. 1995 Dec 14;333(24):1581-7.

Titova E, Ostrowski RP, Kevil CG, Tong W, Rojas H, Sowers LC, Zhang JH, Tang J. Reduced brain injury in CD18-deficient mice after experimental intracerebral hemorrhage. J Neurosci Res 2008;86:3240–3245.

Tomita H, Ito U, Ohno K, Hirakawa K. Chronological changes in brain edema induced by experimental intracerebral hematoma in cats. Acta Neurochir Suppl (Wien) 1994;60:558–60.

Travis J, Salvesen GS. Human plasma proteinase inhibitors. Annu Rev Biochem 1983;52:655-709.

Tuhrim S, Dambrosia JM, Price TR, Mohr JP, Wolf PA, Hier DB, Kase CS. Intracerebral hemorrhage: external validation and extension of a model for prediction of 30-day survival.Ann Neurol. 1991 Jun;29(6):658-63.

Tuhrim S, Dambrosia JM, Price TR, Mohr JP, Wolf PA, Heyman A, Kase CS. Prediction of intracerebral hemorrhage survival. Ann Neurol. 1988 Aug;24(2):258-63.

Tuhrim S , Horowitz DR , Sacher M , et al. Volume of ventricular blood is an important determinant of outcome in supratentorial intracerebral hemorrhage . Crit Care Med 1999 ; 27 (3) : 617 – 621 .

Tuhrim S , Yang WC , Rubinowitz H , et al. Primary pontine hemorrhage and the dysarthria-clumsy hand syndrome . Neurology 1982 ; 32 (9) : 1027 – 1028 .

Tsakiris S (2001) Effects of L-phenylalanine on acetylcholinesterase and Na+,K+-ATPase activities in adult and aged rat brain. Mech Ageing Dev122:491-501.

Tzaan WC, Lee ST, Lui TN. Combined use of stereotactic aspiration and intracerebral streptokinase infusion in the surgical treatment of hypertensive intracerebral hemorrhage. J Formos Med Assoc. 1997 Dec;96(12):962-7.

Tzourio C, Arima H, Harrap S, Anderson C, Godin O, Woodward M, *et al*. APOE genotype, ethnicity, and the risk of cerebral hemorrhage. Neurology 2008;70:1322-8.

Back T. and Schuler OG. The natural course of lesion development in brain ischemia. Acta Neurochir (2004) [Suppl] 89: 55-619.

Ueda K, Omae T, Hirota Y, Takeshita M, Katsuki S, Tanaka K, Enjoji M. Decreasing trend in incidence and mortality from stroke in Hisayama residents, Japan.Stroke. 1981 Mar-Apr;12(2):154-60.

Uemura K , Shishido F , Higano S , et al. Positron emission tomography in patients with a primary intracerebral hematoma . Acta Radiol Suppl 1986 ; 369 : 426 – 428

United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: interim results. UK-TIA Study Group. Br Med J (Clin Res Ed). 1988 Jan 30;296(6618):316-20.

Van Asch CJ, Luitse MJ, Rinkel GJ, van der Tweel I, Algra A, Klijn CJ. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis.Lancet Neurol. 2010 Feb;9(2):167-76. doi: 10.1016/S1474-4422(09)70340-0. Epub 2010 Jan 5. Review.

Van Neerven S, Kampmann E, Mey J. RAR/RXR and PPAR/ RXR signaling in neurological and psychiatric diseases. Prog Neurobiol. 2008;85:433–451.

Van Rossum D, Hanisch UK. Microglia. Metab Brain Dis 2004;19:393–411.

Vaughan PJ, Pike CJ, Cotman CW, Cunningham DD: Thrombin receptor activation protects neurons and astrocytes from cell death produced by environmental insults. J Neurosci 15:5389–5401, 1995.

Vespa P, McArthur D, Miller C, O'Phelan K, Frazee J, Kidwell C, Saver J, Starkman S, Martin N. Frameless stereotactic aspiration and thrombolysis of deep intracerebral hemorrhage is associated with reduction of hemorrhage volume and neurological improvement.Neurocrit Care. 2005;2(3):274-81.

Vignes JR, Hugon J. In vitro efficacy of three lazaroids in a model of acute chemical neuronal hypoxia. Neurosci Lett. 2006 Oct 23; 407(2):171-5.

Vinters HV. Cerebral amyloid angiopathy. A critical review. Stroke. 1987 Mar-Apr;18(2):311-24. Review. No abstract available.

Viswanathan A, Rakich SM, Engel C, Snider R, Rosand J, Greenberg SM, Smith EE. ntiplatelet use after intracerebral hemorrhage Neurology. 2006 Jan 24;66(2):206-9.

Volpin L, Cervellini P, Colombo F, Zanusso M, Benedetti A. Spontaneous intracerebral hematomas: a new proposal about the usefulness and limits of surgical treatment. Neurosurgery. 1984 Nov;15(5):663-6.

Vonsattel JP, Myers RH, Hedley-Whyte ET, Ropper AH, Bird ED, Richardson EP Jr.Cerebral amyloid angiopathy without and with cerebral hemorrhages: a comparative histological study.Ann Neurol. 1991 Nov;30(5):637-49.

Vlkolinský R, Stolc S, Ross A (1999) Effect of stobadine, U-74389G trolox and melatonin on resistance of rat hippocampal slices to oxidative stress. Life Sci 65:1969–1971.

Waga S, Fujimoto K, Okada M, Miyazaki M, Tanaka Y. Caudate hemorrhage.Neurosurgery. 1986 Apr;18(4):445-50.

Waga S, Miyazaki M, Okada M, Tochio H, Matsushima S, Tanaka Y. Hypertensive putaminal hemorrhage: analysis of 182 patients. Surg Neurol. 1986b Aug;26(2):159-66.

Wang CX, Shuaib A. Involvement of inflammatory cytokines in central nervous system injury.Prog Neurobiol. 2002 Jun;67(2):161-72. Review.

Wang J, Dore S. Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. Brain 2007a;130:1643–1652.

Wang J, Doré S. Inflammation after intracerebral hemorrhage. J Cereb Blood Flow Metab 2007b;27:894–908.

Wang J, Dore S. Heme oxygenase 2 deficiency increases brain swelling and inflammation after intracerebral hemorrhage. Neuroscience 2008;155:1133–1141.

Wang J, Zhuang H, Doré S. Heme oxygenase 2 is neuroprotective against intracerebral hemorrhage.Neurobiol Dis 2006b;22:473–476.

Wang J, Fields J, Dore S. The development of an improved preclinical mouse model of intracerebral hemorrhage using double infusion of autologous whole blood. Brain Res 2008;1222:214–221.

Wang J, Portbury S, Thomas MB, Barney S, Ricca DJ, Morris DL, Warner DS, Lo DC (2006a) Cardiac glycosides provide neuroprotection against ischemic stroke: discovery by a brain slice-based compound screening platform. Proc Natl Acad Sci U S A 103:10461-10466

Wang J, Rogove AD, Tsirka AE, Tsirka SE. Protective role of tuftsin fragment 1–3 in an animal model of intracerebral hemorrhage. Ann Neurol 2003;54:655–664.

Wang J, Tsirka SE. Contribution of extracellular proteolysis and microglia to intracerebral hemorrhage. Neurocrit Care 2005a;3:77–85.

Wang J, Tsirka SE. Neuroprotection by inhibition of matrix metalloproteinases in a mouse model of intracerebral haemorrhage. Brain 2005b;128:1622–1633.

Wang J, Tsirka SE. Tuftsin fragment 1–3 is beneficial when delivered after the induction of intracerebral hemorrhage. Stroke 2005c;36:613–618.

Wang KC, Chen CP, Yang YC, Wang KG, Hung FY, Su TH. Stroke complicating pregnancy and the puerperium.Zhonghua Yi Xue Za Zhi (Taipei). 1999 Jan;62(1):13-9.

Wang X. and Feuerstein G.Z..The Janus face of inflammation in ischemic brain injury. Acta Neurochir (2004) [Suppl] 89: 49-548.

Wagner KR. Modeling intracerebral hemorrhage: glutamate, nuclear factor-kappa B signaling and cytokines. Stroke 2007;38:753–758.

Wagner KR, Beiler S, Dean C, Hall CL, Knight J, Packard BA, Smulian AG, Linke MJ, de Courten-Myers GM. NFkB activation and pro-inflammatory cytokine gene upregulation in white matter following porcine intracerebral hemorrhage. In: Krieglstein J, Klumpp S, es. *Pharmacology of Cerebral Ischemia 2004.* Stuttgart: Medpharm Scientific Publishers; 2004: 185–194.

Wagner KR, Broderick JP. Hemorrhagic stroke: pathophysiological mechanisms and neuroprotective treatments. In: Lo EH, Marwah J, eds. N*europrotection.* Scottsdale: Prominent Press; 2001: 471–508.

Wagner KR, Dean C, Beiler S, Bryan DW, Packard BA, Smulian AG, Linke MJ, de Courten-Myers GM. Plasma infusions into porcine cerebral white matter induce early edema, oxidative stress, pro-inflammatory

cytokine gene expression and DNA fragmentation: implications for white matter injury with increased blood-brain-barrier permeability.Curr Neurovasc Res. 2005 Apr;2(2):149-55.

Wagner KR, Hua Y, de Courten-Myers GM, Broderick JP, Nishimura RN, Lu SY, Dwyer BE. Tinmesoporphyrin,a potent heme oxygenase inhibitor, for treatment of intracerebral hemorrhage: in vivo and in vitro studies. Cell Mol Biol (Noisy-le-grand) 2000;46:597–608.

Wagner KR, Packard BA, Hall CL, Smulian AG, Linke MJ, De Courten-Myers GM, Packard LM, Hall NC. Protein oxidation and heme oxygenase-1 induction in porcine white matter following intracerebral infusions of whole blood or plasma. Dev Neurosci 2002;24:154–160.

Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF (2003) Heme and iron metabolism: role in cerebral hemorrhage. J Cereb Blood Flow Metab 23:629-15652.

Wagner KR, Xi G, Hua Y, et al. Lobar intracerebral hemorrhage model in pigs: rapid edema development in perihematomal white matter. Stroke 1996;27(3):490–7.

Wagner KR, Xi G, Hua Y, Kleinholz M, de Courten-Myers GM, Myers RE (1998) Early metabolic alterations in edematous perihematomal brain regions following experimental intracerebral hemorrhage. J Neurosurg 88:1058-1065

Wagner KR, Xi G, Hua Y, Zuccarello M, de Courten-Myers GM, Broderick JP, Brott TG. Ultra-early clot aspiration after lysis with tissue plasminogen activator in a porcine model of intracerebral hemorrhage: edema reduction and blood-brain barrier protection. J Neurosurg. 1999 Mar;90(3):491-8.

Walshe TM, Davis KR, Fisher CM. Thalamic hemorrhage: a computed tomographic-clinical correlation.Neurology. 1977 Mar;27(3):217-22.

Ware ML, Nemani VM, Meeker M, Lee C, Morabito DJ, Manley GT. Effects of 23.4% sodium chloride solution in reducing intracranial pressure in patients with traumatic brain injury: a preliminary study. Neurosurgery 2005;57(4):727–36; discussion -36.

Warkentin LM, Auriat AM, Wowk S, Colbourne F. Failure of deferoxamine, an iron chelator, to improve outcome after collagenase-induced intracerebral hemorrhage in rats. Brain Res. 2010 Jan 14;1309:95-103. doi: 10.1016/j.brainres.2009.10.058. Epub 2009 Oct 30.

Warner DS, Huaxin Sheng and Ines Batinic´-Haberle. Oxidants, antioxidants and the ischemic brain the Journal of Experimental Biology 207, 3221n3231 2004.

Wasserman JK, Zhu X, Schlichter LC. Evolution of the inflammatory response in the brain following intracerebral hemorrhage and effects of delayed minocycline treatment. Brain Res 2007;1180:140–154.

Wasserman JK, Schlichter LC. Minocycline protects the blood-brain barrier and reduces edema following intracerebral hemorrhage in the rat. Exp Neurol 2007a;207:227–237.

Wasserman JK, Schlichter LC. Neuron death and inflammation in a rat model of intracerebral hemorrhage: effects of delayed minocycline treatment. Brain Res 2007b;1136:208–218.

Wei X, Wang G, Jiang C, Li D, Zhao G. Association between hypertensive cerebrovascular stroke and renin-angiotensin system gene polymorphism from Chinese cohort in Shanghai. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2000;17:256-8.

Weimar C, Weber C, Wagner M, Busse O, Haberl RL, Lauterbach KW, Diener HC; German Stroke Data Bank Collaborators. Management patterns and health care use after intracerebral hemorrhage. a cost-of-illness study from a societal perspective in Germany.Cerebrovasc Dis. 2003;15(1-2):29-36.

Weiner L, Kreimer D, Roth E, Silman I (1994) Oxidative stress transforms acetylcholinesterase to a molten-globule-like state. Biochem Biophys Res Commun 198:915–922

Weinkers LC, Steenwyck RC, Hauer MJ, Freishaker JC, Pearson PG. Biotransformation of tirilazad in human: Tirilazad A-ring reduction by human liver microsomal 5 alpha reductase type I and type 2. J pharmacol Exp Ther 1998; 287: 583-9.

Weisberg LA . Subcortical lobar intracerebral haemorrhage: clinical-computed tomographic correlations. J Neurol Neurosurg Psychiatry 1985 ; 48 (11) : 1078 – 1084.

Weisberg LA . Thalamic hemorrhage: clinical-CT correlations . Neurology 1986a ; 36 (10) : 1382 – 1386 .

Weisberg LA . Primary pontine haemorrhage: clinical and computed tomographic correlations . J Neurol Neurosurg Psychiatry 1986b ; 49 (4) : 346 – 352 .

Weinstein JR, Gold SJ, Cunningham DD, Gall CM. Cellular localization of thrombin receptor mRNA in rat brain: expression by mesencephalic dopaminergic neurons and codistribution with prothrombin mRNA. J Neurosci. 1995 Apr;15(4):2906-19.

Wells JE, Biernaskie J, Szymanska A, Larsen PH, Yong VW, Corbett D. Matrix metalloproteinase(MMP)-12 expression has a negative impact on sensorimotor function following intracerebral haemorrhage in mice. Eur J Neurosci 2005;21:187–196.

Wessmann A, Chandler K, Garosi L. Ischaemic and haemorrhagic stroke in the dog.Vet J. 2009 Jun;180(3):290-303. doi: 10.1016/j.tvjl.2007.12.023. Epub 2008 Jun 24.

White BC, Aust SD, Arforts KE, and Aronson LD (1984) Brain injury by ischemicanoxia: hypothesis extension—a tale of two ions? *Ann Emerg Med* 13:862–867.

White SH, Brisson CD, Andrew RD (2012) Examining protection from anoxic depolarization by the drugs dibucaine and carbetapentane using whole cell recording from CA1 neurons. J Neurophysiol 107:2083-2095

Wienkers LC, Steenwyck rc, Mizsak SA, Pearson PG. In vitro metabolism of tirilazad mesylate in male and female rats: contribution of cytochrome P4502CI land Δ4-5-alpha reductase . Drug Met Dispos 1995; 23: 383-92.

Wintzen AR, de Jonge H, Loeliger EA, et al. (1984). The risk of intracerebral hemorrhage during oral anticoagulant treatment: a population study. Ann Neurol 16: 553–558.

Wiggins WS, Moody DM, Toole JF, Laster DW, Ball MR. Clinical and computerized tomographic study of hypertensive intracerebral hemorrhage. Arch Neurol. 1978 Dec;35(12):832-3.

Willmot M , Leonardi-Bee J , Bath PM . High blood pressure in acute stroke and subsequent outcome: a systematic review . Hypertension 2004 ; 43 (1) : 18 – 24 .

Wisniewski H. The pathogenesis of some cases of cerebral hemorrhage (a morphologic study of the margins of hemorrhagic foci and areas of the brain distant form the hemorrhage). Acta Med Pol1961;2:379–390.

Witlin AG, Mattar F, Sibai BM. Postpartum stroke: a twenty-year experience. Am J Obstet Gynecol. 2000 Jul;183(1):83-8.

Woltz P and Krieglstein J (1996) Neuroprotective effects of alpha-lipoic acid and its enantiomers demonstrated in rodent models of focal cerebral ischemia. *Neuropharmacology* 35:369–375.

Woo D, Sauerbeck LR, Kissela BM, Khoury JC, Szaflarski JP, Gebel J, Shukla R, Pancioli AM, Jauch EC, Menon AG, Deka R, Carrozzella JA, Moomaw CJ, Fontaine RN, Broderick JP. Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study. Stroke. 2002 May;33(5):1190-5.

Wu G, Zhong W. Effect of minimally invasive surgery for cerebral hematoma evacuation in different stages on motor evoked potential and thrombin in dog model of intracranial hemorrhage. Neurol Res. 2010 Mar;32(2):127-33.

Wu H, Zhang Z, Li Y, Zhao R, Li H, Song Y, Qi J, Wang J. Time course of upregulation of inflammatory mediators in the hemorrhagic brain in rats: correlation with brain edema. Neurochem Int 2010b;57:248–253.

Wu H, Cong Y, Wang D, Zhao R, Qi J. Correlation of macrophage inflammatory protein-2 expression and brain edema in rats after intracerebral hemorrhage. Int J Clin Exp Pathol 2009a;2:83–90.

Wu H, Zhang Z, Hu X, Zhao R, Song Y, Ban X, Qi J, Wang J. Dynamic changes of inflammatory markers in brain after hemorrhagic stroke in humans: a postmortem study. Brain Res 2010a;1342:111–117.

Wu H, Zhao R, Qi J, Cong Y, Wang D, Liu T, Gu Y, Ban X, Huang Q. The expression and the role of protease nexin-1 on brain edema after intracerebral hemorrhage. J Neurol Sci 2008;270:172–183.

Wu J, Yang S, Xi G, Fu G, Keep RF, Hua Y. Minocycline reduces intracerebral hemorrhage-induced brain injury. Neurol Res.2009;31:183–188.

Wu J, Hua Y, Keep RF, Nakamura T, Hoff JT, Xi G. Iron and iron-handling proteins in the brain after intracerebral hemorrhage. Stroke 2003;34:2964–2969.

Wyse AT, Stefanello FM, Chiarani F, Delwing D, Wannmacher CM, Wajner M (2004) Arginine administration decreases cerebral cortex acetylcholinesterase and serum butyrylcholinesterase probably by oxidative stress induction. Neurochem Res 29:385–389

Xi G, Keep RF, Hoff JT. Mechanisms of brain injury after intracerebral haemorrhage. Lancet Neurol 2006;5:53–63.

Xi G, Keep RF, Hua Y, Xiang JM, Hoff JT: Attenuation of thrombin-induced brain edema by cerebral thrombin preconditioning. Stroke 30:1247–1255,1999.

Xi G, Hua Y, Keep RF, Younger JG, Hoff JT: Systemic complement depletion diminishes perihematomal brain edema. Stroke 32:162–167, 2001b.

Xi G, Hua Y, Bhasin RR, Ennis SR, Keep RF, Hoff JT: Mechanisms of edema formation after intracerebral hemorrhage: Effects of extravasated red blood cells on blood flow and blood-brain barrier integrity. Stroke 32:2932–2938, 2001a.

Xi G, Hua Y, Keep RF, Younger JG, Hoff JT. Brain edema after intracerebral hemorrhage: the effects of systemic complement depletion. Acta Neurochir Suppl. 2002;81:253-6.

Xi G, Wagner KR, Keep RF, Hua Y, de Courten-Mayers G, Broderick JP, Brott TG, Hoff JT. Role of blood clot formation on early edema development after experimental intracerebral hemorrhage. Stroke. 1998;29:2580 –2586.

Xia J, Yang QD, Yang QM, Xu HW, Liu YH, Zhang L, *et al*. Apolipoprotein H gene polymorphisms and risk of primary cerebral hemorrhage in a Chinese population. Cerebrovasc Dis 2004;17:197-203

Xue M, Del Bigio MR. Intracerebral injection of autologous whole blood in rats: time course of inflammation and cell death. Neurosci Lett 2000;283:230–232.

Xue M, Fan Y, Liu S, Zygun DA, Demchuk A, Yong VW. Contributions of multiple proteases to neurotoxicity in a mouse model of intracerebral haemorrhage. Brain 2009a;132:26–36.

Xue M, Hollenberg MD, Demchuk A, Yong VW. Relative importance of proteinase-activated receptor-1 versus matrix metalloproteinases in intracerebral hemorrhage-mediated neurotoxicity in mice. Stroke 2009b;40:2199–2204.

Xue M, Hollenberg MD, Yong VW. Combination of thrombin and matrix metalloproteinase-9 exacerbates neurotoxicity in cell culture and intracerebral hemorrhage in mice. J Neurosci2006;26:10281–10291.

Xue M, Mikliaeva EI, Casha S, Zygun D, Demchuk A, Yong VW. Improving outcomes of neuroprotection by minocycline: guides from cell culture and intracerebral hemorrhage in mice. Am J Pathol. 2010;176:1193–1202.

Xue D, Slivka A, and Buchan AM (1992) Tirilazad reduces cortical infarction after transient, but not permanent focal cerebral ischemia in rats. *Stroke* 23:894–899.

Yamada Y, Metoki N, Yoshida H, Satoh K, Ichihara S, Kato K, *et al*. Genetic risk for ischemic and hemorrhagic stroke. Arterioscler Thromb Vasc Biol 2006;26:1920-5.

Yamani MH, Starling RC, Cook DJ, Tuzcu EM, Abdo A, Paul P, Powell K, Ratliff NB, Yu Y, McCarthy PM, Young JB. Donor spontaneous intracerebral hemorrhage is associated with systemic activation of matrix metalloproteinase-2 and matrix metalloproteinase-9 and subsequent development of coronary vasculopathy in the heart transplant recipient.Circulation. 2003 Oct 7;108(14):1724-8. Epub 2003 Sep 15

Yamanaka R, Satoh S. Comparison of stereotactic aspiration, craniotomy, and conservative treatment for putaminal hemorrhage. Neurol Med Chir (Tokyo). 1988 Oct;28(10):986-90. Review. No abstract available.

Yamanaka R, Sato S, Kawasaki S, Sekiguchi K, Sato I, Mori S, Watanabe M, Nishizawa E, Morii K,Takahama H, et al. [Clinical study of primary pontine hemorrhage].No Shinkei Geka. 1988 Jan;16(1):57-64. Japanese.

Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT: Experimental intracerebral hemorrhage: Relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. J Neurosurg 81:93–102, 1994.

Yang GY , Betz AL , Chenevert TL , Brunberg JA , Hoff JT . Experimental intracerebral hemorrhage: relationship between brain on 18F-fl uoromisonidazole PET after intracerebral hemorrhage . Neurology 1999; 53: 2179 .

Yang Y, Zhang XJ, Yin J, Li LT (2008) Brain damage related to hemorrhagic transformation following cerebral ischemia and the role of KATP channels. Brain Res 1241:168-175

Yano K, Reed DM, MacLean CJ (1989). Serum cholesterol and hemorrhagic stroke in the Honolulu Heart Program. Stroke 20: 1460–1465.

Yochum LA, Folsom AR, Kushi LH. Intake of antioxidant vitamins and risk of death from stroke in postmenopausal women.Am J Clin Nutr. 2000 Aug;72(2):476-83.

Yong VW, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. Lancet Neurol 2004;3:744–751.

Yong WH, Robert ME, Secor DL, Kleikamp TJ, Vinters HV. Cerebral hemorrhage with biopsy-proved amyloid angiopathy. Arch Neurol 1992;49:51–58.

Yoshida H, Imaizumi T, Fujimoto K, Itaya H, Hiramoto M, Yoshimizu N, *et al*. A mutation in plasma platelet-activating factor acetylhydrolase (Val279Phe) is a genetic risk factor for cerebral hemorrhage but not for hypertension. Thromb Haemost 1998;80:372-5

Yossi Gilgun-Sherki, Ziv Rosenbaum, Eldad Melamed, and Daniel Offen.Antioxidant therapy in Acute Central Nervous System Injury: Current State. PHARMACOLOGICAL REV. 54: 271-284, 2002.

Youdim, M. B. H., and Ben-Schaschar, D.: The neurotoxic component inParkinson's disease may involve iron-melanin interaction and lipid peroxidation in the substantia nigra. N. Vistas Drug Res. 1: 111–122, 1988.7.

Young B, Runge JW, Waxman KS, Harrington T, Wilberger J, Muizelaar JP, Boddy A, and Kupiec JW (1996) Effects of pegorgotein on neurologic outcome of patients with severe head injury: a multicenter, randomized controlled trial. *JAMA* 276:538–543.

Young WB , Lee KP , Pessin MS , et al. Prognostic signifi cance of ventricular blood in supratentorial hemorrhage: a volumetric study . Neurology 1990 ; 40 (4) : 616 – 619 .

Yu ZF, Bruce-Keller AJ, Goodman Y, and Mattson MP (1998) Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury in vivo. *J Neurosci Res* 53:613–625

Zazulia AR , Diringer MN , Videen TO , et al. Hypoperfusion without ischemia surrounding acute intracerebral hemorrhage . J Cereb Blood Flow Metab 2001 ; 21 : 804 – 810 .

Zecca L, Youdim MB, Riederer P, Connor JR, Crichton RR. Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 2004;5:863–873.

Zhan C, Yang J (2006) Protective effects of isoliquiritigenin in transient middle cerebral artery occlusion-induced focal cerebral ischemia in rats. Pharmacol Res 53:303-309.

Zhang D, Hu X, Qian L, O'Callaghan JP, Hong JS. Astrogliosis in CNS pathologies: is there a role for microglia? Mol Neurobiol. 2010 Jun;41(2-3):232-41.

Zhang D, Hu X, Qian L, Wilson B, Lee C, Flood P, Langenbach R, Hong JS. Prostaglandin E2 released from activated microglia enhances astrocyte proliferation in vitro. Toxicol Appl Pharmacol 2009;238:64–70.

Zhang R, Shohami E, Beit-Yannai, Bass R, Trembovler V, and Samuni A (1998) Mechanism of brain protection by nitroxide radicals in experimental model of closed head injury. *Free Radic Biol Med* 24:332–340.

Zhang X, Li H, Hu S, Zhang L, Liu C, Zhu C, Liu R, Li C. Brain edema after intracerebral hemorrhage in rats: the role of inflammation. Neurol India. 2006 Dec;54(4):402-7.

Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, Grotta JC, Aronowski J. Hematoma resolution as a target for intracerebral hemorrhage treatment: Role for peroxisome proliferatoractivated receptor gamma in microglia/macrophages. Ann Neurol 2007b;61:352–362.

Zhao X, Zhang Y, Strong R, Grotta JC, Aronowski J. 15d–Prostaglandin J2 activates peroxisomeproliferator-activated receptor-gamma, promotes expression of catalase, and reduces inflammation, behavioral dysfunction, and neuronal loss after intracerebral hemorrhage in rats. J Cereb Blood Flow Metab 2006c;26:811–820.

Zhao X, Sun G, Zhang J, Strong R, Dash PK, Kan YW, Grotta JC, Aronowski J. Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. Stroke 2007a; 38:3280–3286.

Zhou JF, Zhou YH, Zhang L, Chen HH, Cai D (2003) 3,4-methylenedioxymethamphetamine (MDMA) abuse markedly inhibits acetylcholinesterase activity and induces severe oxidative damage and liperoxidative damage. Biomed Environ Sci 16:53–61.

Zhou X, Lü FQ, Chen L, Feng C, Li B, Yang L, Tang J, Li TS. [An experimental study of contrast enhanced ultrasound diagnosis of cerebral intraparenchymalhemorrhage in dogs].2012 May;24(5):302-5. Chinese.

Zhu H, Wang Z, Shi W. Keyhole endoscopic hematoma evacuation in patients. Turk Neurosurg. 2012; 22(3):294-9.

Zhu HL, Luo WQ, Wang H (2008) Iptakalim protects against hypoxic brain injury through multiple pathways associated with ATP-sensitive potassium channels. Neuroscience 157:884-894.

Zhu XL, Chan MS, Poon WS. lliterature. Stroke. 1997 Jul;28(7):1406-9. Review.

Zodpey SP, Tiwari RR, Kulkarni HR (2000). Risk factors for haemorrhagic stroke: a case-control study. Public Health 114: 177–182.

Zuccarello, M., and Anderson, D. K.: Interaction between free radicals and excitatory amino acids in the blood-brain barrier disruption after iron injury in the rat. J. Neurotrauma. 10: 397–403, 1993.