Cellular Pathology and Apoptosis in Experimental and Human Acute and Chronic Compressive Myelopathy

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June 2010

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in the University library, being made available for loan and copying.

Signed: Date

Rowena Elizabeth Anne Newcombe

Acknowledgements

I acknowledge the invaluable and expert teaching, encouragement, patience and wisdom of my supervisors, Professor Peter Blumbergs, Professor Robert Vink, Dr John Finnie, Professor Peter Reilly, and Professor Nigel Jones. In particular I thank Professor Blumbergs who first enabled me this opportunity, providing unwavering guidance until its completion.

I sincerely thank Mr Jim Manavis for his technical expertise, enthusiasm and assistance over many years.

I am ever grateful for the encouragement and insights provided by my dear husband Matthew, my wise mother Carolyn, and my family and friends throughout this process of learning. I could not have achieved this without you.

I make special mention of my father, Doctor Raymond Newcombe, to whom I dedicate this thesis, for his careful, inspiring and excellent mentoring. He is exemplary of those qualities desired in any doctor of medicine; integrity, perseverance, compassion, precise clinical acumen, curiosity, and an unyielding belief in the human spirit.

„He who has health has hope, and he who has hope has everything."

- Arabian proverb

Publications and Presentations

Newcombe REA, Vink R, Finne R, Reilly P, Blumbergs PC. Functional and pathologic studies of experimental chronic compressive spinal cord injury and the effects of decompression. J Neurotrauma.2009;A2-A101.

Newcombe REA, Blumbergs PC, Sarvestani G, Manavis J, Jones NR. Caspase-3-mediated Proteolysis of Amyloid Precursor Protein and the Production of Amyloid-beta in Human Acute and Chronic Compressive Myelopathy. J Bone Joint Surg Br.2004;86-B: 462.

Newcombe REA, Blumbergs PC, Sarvestani G, Manavis J, Jones NR. Apoptosis in Human Acute and Chronic Compressive Myelopathy. In: Proceedings of the Australian Neuroscience Society. 2004.

Newcombe REA, Blumbergs PC, Sarvestani G, Manavis J, Jones NR. A Human Study of Apoptosis in Acute and Chronic Compressive Myelopathy. In: Proceedings of the Spine Society of Australia.2003.

Prizes and Scholarships

2009 International Neurotrauma Society Student Travel Grant
2007 National Health and Medical Research Council Training Postgraduate Fellowship
2004 Spine Society of Australia Award for Spinal Research
2003 The Douglas Hardy Research Project Prize – The University of Adelaide
2003 Australian Medical Students‟ Association Research Scholarship
2003 John Curtin School of Medical Research Summer Scholarship
2003 Spine Society of Australia Award for Spinal Research

Abbreviations

AD	Alzheimer‟s Disease
ADP	Adenosine Diphosphate
AHC	Anterior Horn Cell
ALA	Anterolateral White Matter Area
AIF	Apoptosis Inducing Factor
ANOVA	Analysis of Variance
APP	Amyloid Precursor Protein
ATP	Adenosine Triphosphate
aC3	Active Caspase-3
BACE	β-site APP-cleaving Enzyme
Bak	Bcl-2 Antagoinist Killer
Bax	B-Cell Lymphoma-Associated X
BBB	Blood Brain Barrier
BBB Score	Beattie Basso Bresnahan Score
Bcl-2/x	B-cell Lymphoma 2/x
BDNF	Brain-derived Neurotrophic Factor
C3	Caspase-3
C9	Caspase-9
cDNA	Complementary Deoxyribonucleic Acid
CCA	Caspase-3 and Caspase-mediated Cleavage of APP
cm	Centimetre(s)
CMAP	Caspase-3-mediated APP Proteolytic Peptide Antibody
CNPase	Cyclic Nucleotide Phosphodiesterase
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CSM	Cervical Spondylotic Myelopathy
CT	Computed Tomography
CV	Coefficient of Variance
DAB	Diaminobenzidine
DNA	Deoxyribonucleic Acid
DNA-PK	DNA-dependent Protein Kinase
DNA-PKcs	DNA-dependent Protein Kinase Catalytic Subunit
DR3/6	Death Receptor 3/6
e.g.	Exempli Gratia (for example)
EDAR	Ectodermal Dysplasia Receptor

EDTA	Ethylenediamine Tetra-acetic Acid
ELISA	Enzyme-linked Immunosorbent Assay
et al.	Et Alii (and others)
FasL	Fas Receptor Ligand
FasR	Fas Receptor
FADD	Fas Associated Death Domain
g	Gram(s)
GFAP	Glial Fibrillary Acidic Protein
GM	Grey Matter
GM-CSF	Granulocyte Macrophage-colony Stimulating Factor
H&E	Haematoxylin and Eosin
hr	Hour(s)
i.e.	Id Est (that is to say)
IAP	Inhibitors of Apoptosis
Iba1	Ionised Calcium Binding Adaptor Molecule 1
IL	Interleukin
IHC	Immunohistochemistry
LPC	Lysophosphatidylcholine
LCST	Lateral Corticospinal Tract
m	Metre(s)
MBP	Myelin Basic Protein
MCP-1	Macrophage Chemotactic Protein-1
min	Minute(s)
MIP-1α	Macrophage Inflammatory Protein-1α
MLS	Mitochondrial Localisation Sequence
Mm	Millimetre(s)
MOMP	Mitochondrial Outer Membrane Permeability
MRI	Magnetic Resonance Imaging
NA	Numerical Aperture
NAD	Nicotinamide Adenine Dinucleotide
NAPO	Negative in Apoptosis Marker
NeuN	Neuronal Nuclei Antibody
NGF	Nerve Growth Factor
NF-κB	Nuclear Factor-kappaB
ng	Nanogram(s)
NHS	Normal Horse Serum
nm	Nanometre(s)

NMDA	N-methyl-D-aspartate
nNOS	Neuronal Nitric Oxide Synthase
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NT-3	Neurotrophin-3
Olig2	Oligodendrocyte Transcription Factor 2
p	Probability
PAR	Poly (ADP-ribose)
PARG	Poly (ADP-ribose) Glycohydrolase
PARP	Poly (ADP-ribose) Polymerase
PBS	Phosphate Buffered Saline
PCA	Posterior Column Area
PCD	Programmed Cell Death
PMT	Photomultiplier Tube(s)
PNS	Peripheral Nervous System
PS-1	Presenelin-1
RPM	Revolutions Per Minute
ROS	Reactive Oxygen Species
SCI	Spinal Cord Injury
SEM	Standard Error of the Mean
Smac/DIABLO	Second Mitochondria-derived Activator of Caspases/Direct IAP Binding Protein with Low PI
TBI	Traumatic Brain Injury
TBS	Tris-buffered saline
TMRM	Tetramethyl Rhodamine Methyl Ester
TNF	Tumour Necrosis Factor
TNF-α	Tumour Necrosis Factor-α
TPA	Tissue Polypeptide Antigen
TRAIL-R1/2	TNF-related Apoptosis Inducing Ligand Receptor 1/2
TUNEL	Terminal in situ Nick-end Labelling
µl	Microlitre(s)
UPLAPO	Universal Plan Apochromatic Objectives
WM	White Matter
XRCC1	X-ray Repair Cross-complimenting Group 1
^{o}C	Degrees Celsius
3-AB	3-aminobenzamide
5-AIQ	5-aminoisoquinolinone

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Page

Re: Thesis emendations for PhD thesis by Dr Rowena Newcombe

During printing of the thesis a variation occurred in page numbering between the electronic and the printed copies. Subsequently, Examiner 1's emendations are 19-20 pages in advance of the correct numbering. In addition, Examiner 1 identified that the list of corrections given was incomplete, and that further review of spelling and grammatical errors be made, further altering the page structure. Thus, the actual page at which the correction is located in the thesis correlates to the page number and line set in bold at the end of the noted correction.

The following emendations have been made to the thesis as recommended by the examiners.

Examiner 1

<u>Minor changes that would enhance the thesis</u>

P48 Description of the model used in the studies in comparison to previous models might be better in the discussion than in the introduction, unless the model was previously used and published.

Response: A model of chronic compressive myelopathy as used by Kim et al. 2004 is described within the introduction. This is a partial reference which is expanded in more detail in the discussion, in agreement with the referee's comments **Page 29 Line 23**

P48 Syringomyelia is not really a form of cord compression in the usual sense. Although it may result in some compression of cord tissue, the pathology is likely to be quite different to forms of external compression. Although it is valid to include the studies of syringomyelia in this work, it would be preferable if this distinction were clarified in the text.

Response: The probable differing mechanism of syringomyelia and spondylotic myelopathy is noted **Page 24 Line 2**

P89: what pressure was used for perfusion fixation?

Response: A pressure of 80-120mmHg was used for perfusion fixation **Page 71 Line 11**

P125 '...a subtype of oligodendrocytes was identified,' is not clear. What was the subtype?

Response: The use of the term 'subtype' was removed from the text to simply describe the cell 'oligodendrocyte' throughout the thesis. **Page 103**

It would be preferable to reduce the number of significant figures used for the cord cross-sectional area results, BBB score results, and rotarod times. For example, reporting mean BBB scores to 2 decimal places has no meaning.

Response: The significant figures were rounded to a consistent 2 decimal places.

Table 112 ‘Communicating syrinx’ is usually used to refer to cysts that communicate with the fourth ventricle rather than the central canal. Did these syrinxes really communicate with the fourth ventricle? (It would be highly unusual if associated with Chiari I malformations).

Response: There is no table 112, and it is suspected that the Examiner may be referring to Table 28, in which communicating syrinx was correctly defined as a communication with the central canal.

Grammatical and spelling errors that require correction

P23: “during **the** 1952”

As recommended this was changed to, ‘during 1952’. **Page 4 Line 5**

P23: ‘canalicular’

As recommended this was changed to, ‘cannalicular’. **Page 5 Line 18**

P28: ‘An association between osteophytes and concave, load baring areas within the spine were recognised early’ should be ‘An association between osteophytes and concave, load bearing areas within the spine **was** recognised early...’.

A correction was made as recommended. **Page 9 Line 4**

P35:

‘Multiple forms of programmed cell death (PCD) and classified as types I, II, and III PCD’ Should this be, ‘Multiple forms of programmed cell death (PCD) **are** classified as types I, II, and III PCD.’

A correction was made as recommended. **Page 17 Line 6**

P36: The ontology of apoptosis was given twice.

This was corrected as appropriate. **Page 17 Line 21**

P47: The words ‘central canal’ should be ‘spinal canal’

This was corrected **Page 28 Line 14**

P50: ‘This current study aims assess the effects of decompression in an experimental model of mild chronic cord compression’ should be ‘This current study aims **to** assess the effects of decompression in an experimental model of mild chronic cord compression.’

A correction was made as recommended. **Page 31 Line 6**

P50 ‘…a minimum of approximately 10% of axons’. Should this be ‘…a **maximum** of approximately 10% of axons’?

No change was necessary as the sentence reads correctly **Page 32 Line 15**

P66: ‘transaction’ was corrected to ‘transection’.

This was corrected **Page 48 Line 1**

P66 Tables 86-92 are out of order and not referenced in the text or the table of tables.

This was corrected **Page 69 Line 1**

P66 ‘Processotomy’ is an unusual term. Would ‘removal of the spinous process and laminectomy’ be better?

The term, ‘processotomy’ is consistent with surgical terminology to describe full resection of the spinous process to expose the dura, and thus the term was retained. **Page 57 Line 7**

P91: The Gracile fasciculus was labelled. **Page 73**

P110: ‘wee k’ was changed to ‘week’. **Page 92 line 5**

‘tetromethylbenzidine’ was changed to ‘tetramethylbenzidine’. **Page 92 Line 15**

‘data is’ was changed to ‘data are’ as recommended. **Page 93 Line 8**

P113: ‘emersion’ was corrected to ‘immersion’. **Page 95 Line 15**

‘A complete representation of pathological and apoptotic changes in human cases are documented...’ should be ‘A complete representation of pathological and apoptotic changes in human cases **is** documented...’

This was corrected as suggested. **Page 95, Line 12**

‘A similar panel of apoptotic markers were used...’ should be ‘A similar panel of apoptotic markers **was** used...’

A correction was made as suggested **Page 95 Line 21**
P114: ‘A subset of enlarged axons were immunopositive...’ should be ‘A subset of enlarged axons **was** immunopositive...’.

A correction was made as suggested. **Page 97 Line 4**

P123: ‘Rare of occasional immunopositive glia was seen...’ should be ‘Rare or occasional immunopositive glia **were** seen...’.

A correction was made as suggested. **Page 105 Line 19**

P124: ‘At 9 week,...’ was changed to, ‘At 9 weeks,...’ as suggested. **Page 106 Line 12**

P125: ‘...frequent glial staining was seen in the majority cases...’ was changed to, ‘...frequent glial staining was seen in the majority **of** cases...’ as recommended. **Page 107 Line 12**

P126: ‘...TUNEL was either absent of rarely present,’ was changed to, ‘...TUNEL was either absent or rarely present,’ as recommended. **Page 108 Line 17**

P130: ‘APP axonal immunopositivity was rare or occasionally present in compression groups but were frequently present...’ should be ‘APP axonal immunopositivity was rare or occasionally present in compression groups but **was** frequently present...’

A correction was made as recommended. **Page 112 Line 11**

P131: ‘...the ratio or posterior to anterolateral white matter at the site was comparable to controls...’ should be ‘...the ratio **of** posterior to anterolateral white matter at the site was comparable to controls...’.

A correction was made as recommended. **Page 113 Line 14**

P140: ‘Statistically, the rotarod results were compared between the seven groups using a Cox proportional hazards model. A value of 120 second was considered to be right censored’ was repeated.

A correction was made as recommended. **Page 122 Line 7**

P184: It was recommended that, ‘In **experimental chronic compressive myelopathy**, caspase-9, PARP and aC3 staining was found...’ be changed to, ‘In **experimental chronic compressive myelopathy**, caspase-9, PARP and aC3 staining **were** found...’

A correction was made as recommended. **Page 166 Line 13**

P184: As recommended, ‘Although our data...’ was changed to, ‘Although our results...’. **Page 169 Line 1**

P202: As recommended, ‘Tissue from human cases was also studies for apoptosis...’ was changed to, ‘Tissue from human cases was also **studied** for apoptosis...’. **Page 185 Line 3**

As recommended, ‘ Glial positivity to TUNEL, the gold standard biochemical marker of apoptosis, TUNEL, was seen at 24 hours and at 1 week post-injury,’ was changed to, ‘Glial positivity to TUNEL, the “gold standard” biochemical marker of apoptosis, was seen at 24 hours and at 1 week post-injury. **Page 185 Line 10**

Examiner 2

Minor grammatical changes and errors:

Page 2

2nd and 3rd paragraph – suggest moving first 2 sentences of the third paragraph to before the 2nd sentence of the 2nd paragraph.

A correction was made as recommended. **Page 2 Line 18**

Page 9

Paragraph starting ‘…Theories vary…’ suggest putting a ‘comma’ after ‘syringomyelia’ and delete the word ‘where’ before ‘the Venturi.

A correction was made as recommended. **Page 10 Line 10 and 11**

Page 15

Paragraph starting ‘Principal modes of cell death…’, Line 4 - change ‘apoptotic forms’ to ‘apoptotic process’.

A correction was made as recommended. **Page 17 Line 5**

Page 49

2nd paragraph, Line 1 ‘suggest’ should be ‘suggests’.

A correction was made as recommended. **Page 51 Line 10**

Page 120 In the 1st paragraph Line 1 should read ‘The rotarod score was used in **the** assessment of…’

A correction was made as recommended. **Page 122 Line 2**

Page 163

In the 2nd paragraph it was recommended to remove ‘comma’ after ‘a’ and the inverted commas around ‘percentage of apoptotic cells.

The correction was made as recommended. **Page 165 Line 12**

Page 217

Paragraph starting ‘In similarity’ suggest change to more common word usage, Eg ‘Similar to chronic compressive…’

A correction to, ‘Similar to chronic compressive…’ was made. **Page 221 Line 1**

Page 227

Paragraph starting with ‘The principal aims…’ this 1st sentence is too long and should be changed to 2 sentences at least.

The change was made as recommended. **Page 229 Line 10**

Paragraph starting, ‘The experimental model…’ Line 1 the word ‘newly’is inappropriate and should be changed. Eg. delete and leave sentence as is or use ‘recently’ instead.

The word ‘newly’ was deleted. **Page 229 Line 22**

Page 228

Paragraph starting with, ‘Our studies…’ again the word ‘newly’ is inappropriate and should be deleted and rework the sentence or change the word to a more appropriate word.

The word ‘newly’ was deleted. **Page 230 Line 18**

Page 233

Point 1: Line 3 - the word ‘in’ should read ‘at.

The correction was made as recommended. **Page 235 Line 3**

References

The recommendation was to be consistent with use of a ‘stop’ and a ‘space’ after the Journal listed.

This was amended for each reference to consistent use of the ‘stop’ and ‘space’.

Page 241, 3rd reference – no ‘year’ is noted in this reference.

This was corrected. **Page 243 Line 9**

Appendix

Page 261 Point ‘1’ Fullstop be used after the word ‘right’.

Page 271 Line 5 sentence should read, ‘There was a past…right arm **and right** leg weakness…’

The corrections were made as recommended **Page 263 Line 15, 273 Line 6.**

Figures

Page

Tables

ABSTRACT

Evidence suggests that apoptosis of neurons and glia may play an important role in the pathophysiology and functional outcome of spinal cord compression. In the current thesis, chronic and acute rodent experimental models analysed the functional, cellular and apoptotic marker changes produced by compression and subsequent surgical decompression.

In experimental mild chronic compression there was a loss of posterior white matter maximal at the compression site. Total cross-sectional area decreased with a longer duration of compression (3 weeks) but resolved with decompression (e.g. 3 week group mean 3.05mm^2 increasing following decompression at 3 weeks to 5.75 mm^2). A significant increase in posterior white matter area was found above and below the site at 3 weeks. Caspase-9, PARP, AIF and active caspase-3 staining was found in glia at, above and below the site in all groups. Caspase-3 was greater expressed in the 24 hour (mean 0.32, $p = 0.01$) and 3 week (mean 0.31, $p = 0.02$) decompression groups when compared with the 9 week compression group (mean 0.19). APP axonal immunopositivity was frequently seen after decompression.

Following experimental acute compression, central necrosis was seen, surrounded by axonal swellings and inflammatory infiltrate. Glial positivity using TUNEL occurred at 24 hours and 1 week post-injury. PARP, DNA-PKcs and AIF immunopositivity occurred in glia at, above and below the site. APP immunopositivity was present in axonal swellings.

In human chronic compression, axonal swellings, loss of anterior horn cells, and cystic change were seen in severe cases. TUNEL, DNA-PKcs, PARP and AIF immunopositivity in glia wereas seen at, above and below the compression. APP immunopositivity was seen in axonal swellings.

In human acute compression, the central cord showed haemorrhagic necrosis and inflammatory cells. TUNEL, DNA-PKcs and PARP immunopositive glia were found at, above and below the site. Axonal swellings, a subset of which were APP immunopositive,

occurred in the penumbra. APP immunopositive axonal swellings were found above and below the site of compression, indicating widespread changes in fast axoplasmic transport.

We conclude that mild, chronic, fixed posterior compression results in a potentially reversible reduction of white matter at the site and increased white matter above and below the site of compression. This, combined with evidence of axonal injury, may indicate altered axoplasmic transport. Decompressive surgery results in increased immunostaining for apoptotic markers and increased axonal injury despite restoration of spinal cord anatomy. These studies provide novel evidence that neuronal and glial apoptosis occurs in acute and chronic compressive myelopathy at various time points of compression, maximal at the site of injury.