ASPECTS OF RETINAL AND OPTIC NERVE PATHOLOGY AFTER EXCITOTOXIC RETINAL INJURY

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This thesis is dedicated to those

who fight blindness with courage and dignity

& my husband, Hiren, whose love I cherish.

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ABSTRACT

A large body of evidence supports the notion that excitotoxicity plays a major role in the pathogenesis of a number of neurological diseases, including central nervous system (CNS) ischaemia, Alzheimer's disease, motor neurone disease, and glaucoma. In the global population 60 years of age and over, these diseases are among the leading causes of mortality and morbidity. Although the site of excitotoxic injury is principally at the level of the cell body (perikaryal), understanding the secondary effects on the neuronal axon is important because axonopathy is a documented early feature of these common neurological conditions; hence, an understanding of the pattern and mechanisms of secondary axonal degeneration after excitotoxic perikaryal injury could provide novel detection and treatment strategies in the early phase of neurological disease. The retina and optic nerve, as approachable regions of the CNS, provide a unique anatomical substrate to investigate axonal degeneration after perikaryal excitotoxic injury.

Spatiotemporal changes in the retina and optic nerve were studied after injection of 20nM of N-methyl-D-Aspartate (NMDA) in the left eye of the rat with the saline-injected right eye serving as the control. Temporal changes in the morphology of retina and optic nerve were studied by light and electron microscopy. Progressive retinal damage beginning at 72 hrs, seen as thinning of the inner retina and cell loss in the ganglion cell layer, showed strong correlation ($R= 0.949$) with degenerative changes in the optic nerve; the distal optic nerve segment displayed significantly more axon loss, axon swellings and myelin damage than the proximal segment ($p<0.05$), suggestive of a 'dying-back type degeneration'. Beginning at 24 hrs, electron microscopy demonstrated various features of necrosis in retinal ganglion cells (RGCs): mitochondrial and endoplasmic reticulum swelling, disintegration of polyribosomes, rupture of membranous organelle and formation of myelin bodies. Ultrastructural damage in the optic nerve, which began at 72 hrs, mimicked the changes of Wallerian degeneration, where early nodal-paranodal disturbances were followed by the appearance of three major morphological variants: watery

degeneration, dark degeneration, and demyelination. Features suggestive of RGC regeneration in the form of dendritic sprouting after acute excitotoxic injury were also demonstrated at day 7.

Immunohistochemistry revealed glial cell responses and changes to the axon transport system. Excitotoxic injury resulted in progressive activation of macroglia (Müller cells and astrocytes) and microglial cells in the retina and optic nerve as demonstrated by increased glial-fibrillary-acidic protein (GFAP) and ED-1 immunolabelling as early as 72 hrs. Interxonal glial cells in the optic nerve also showed increased β-amyloid precursor protein (β-APP) beginning at 72 hrs. Impairment of slow axonal transport at 72 hrs resulted in decrease anterograde transport of neurofilament-light (NF-L) to the axon terminal and hence their accumulation in proximal neuron (seen as NF-L rich spheroids).

This fundamental research revealed a pathological picture of Wallerian-like degeneration after perikaryal excitotoxic injury in the CNS. This novel finding is consistent with recent evidence of a labile axonal "survival" factor, nicotinamide mononucleotide adenylyltransferase 2,(Nmnat2) produced by the neuronal cell body. Further study is required to test the hypothesis that a lack of Nmnat2 is the mechanism by which axons degenerate after excitotoxic perikaryal injury.

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 to Dr. Sarabjit Kaur Saggu and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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SARABJIT KAUR SAGGU

PUBLICATIONS/ PRESENTATIONS RELATED TO THESIS

Publication

Sarabjit K Saggu, Hiren P Chotaliya, Peter C Blumbergs, Robert J Casson. Wallerian-like axonal degeneration in the optic nerve after excitotoxic retinal insult: an ultrastructural study. BMC Neuroscience 2010, 11:97. Copyright © 2010 Saggu et al; licensee BioMed Central Ltd.

Saggu SK, Chotaliya HP, Cai Z, Blumbergs P, Casson RJ. The spatiotemporal pattern of somal and axonal pathology after perikaryal excitotoxic injury to retinal ganglion cells: A histological and morphometric study. Experimental Neurology, 211 (2008), 52-58. Copyright © 2007 Elsevier Inc.

Saggu SK, Chotaliya HP, Manavis J, Blumbergs P, Casson RJ. The spatial and temporal pattern of optic nerve pathology after excitotoxic retinal injury. (abstract) Clinical and experimental ophthalmology, 34 (Suppl 1) 2006. Copyright © Blackwell Publishing Asia

Presentations

Saggu SK, Chotaliya HP, Ghabriel MN. Will prevention of retinal excitotoxicity modulate optic nerve degeneration? An essay presented for Sir Grafton Elliot Smith Award, 2005 at the Annual Australian Neuroscience Society meeting: 31st January- 3rd February, 2006: Sydney, Australia.

Saggu SK, Chotaliya HP, Blumbergs P, Manavis J, Casson RJ. Optic nerve pathology after excitotoxic retinal injury. Presented at Australia and New Zealand Glaucoma Investigator Group meeting; 21-22nd July, 2006; Sydney, Australia.

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Australasian Winter Conference on Brain Research; 26-30th August, 2006; Queentown, New Zealand.

Saggu SK, Chotaliya HP, Blumbergs P, Manavis J, Casson RJ. The spatial pattern of retinal ganglion cell axonal degeneration after intraocular excitotoxic injury. Presented at 38th Annual Scientific Congress of the Royal Australian and New Zealand College of Ophthalmologists; 4-8th November, 2006; Sydney, Australia.

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Casson RJ, **Saggu SK**, Childlow G, Wood J, Cai Z, Blumbergs P. Dying back-like axonopathy and early neurofilament degeneration in the optic nerve after perikaryal excitotoxic injury to retinal ganglion cells. Presented at the ARVO 2008 annual meeting; April-May 2008; Fort Lauderdale, Florida, USA.

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ACRONYMS

AD	Alzheimer's Disease
ADP	Adenosine Di-Phosphate
AIF	Apoptosis Inducing Factor
ALS	Amyotrophic Lateral Sclerosis
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	Adenosine Tri-Phosphate
ATPase	Adenosine Tri-Phosphatase
AUD	Australian Dollars
B-APP	Beta-Amyloid Precursor Protein
BBB	Blood Brain Barrier
BL	Basal Lamina
CNS	Central Nervous System
CysLT1	Cysteinyl Leukotriene receptor 1
DAB	DiaminoBenzidine
DDSA	DoDecyl Succinic Anhydride
DMP	DiMethylaminomethyl Phenol
DNA	Deoxyribo Nucleic Acid
EDTA	Ethylene DiamineTetracetic Acid
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ER	Endoplasmic Reticulum
GA	Golgi apparatus
GCL	Ganglion Cell Layer
GFAP	Glial Fibrillary Acidic Protein
GTP	Guanosine Tri-Phosphate

H_2O	Di-hydrogen oxide (Water)
H&E	Haematoxylin & Eosin
HIER	Heat Induced epitope Retrieval
HIV	Human Immunodeficiency Virus
HRP	HorseRadish Peroxidase
HSP	Hereditary Spastic Paraplegia
INL	Inner Nuclear Layer
IOP	Intra-Ocular Pressure
IPL	Inner Plexiform Layer
IRT	Inner Retinal Thickness
JNK	Jun N-terminal Kinase
LHON	Leber's Hereditary Optic Neuropathy
LIF	Leukaemia Inhibitory Factor
LM	Light Microscopy
LSAB	Labelled Streptavidin-Biotin
MAP	Microtubule-associated Proteins
MAPK	Mitogen Activated Protein Kinase
MCP-1	Monocyte Chemoattractant Protein-1
MHC-II	Major Histocompatibility Complex-II
MNA	Methyl Nadic Anhydride
MND	Motor Neuron Disease
mRNA	messenger Ribo Nucleic Acid
MTL	Myelin Terminal Loop
MTP	Mitochondrial Transition Pore
Na^+-K^+ATPase	Sodium-Potassium-Adenosine Tri-Phosphatase
NF-H	Neurofilament-heavy

NF-L	Neurofilament- light
NF-M	Neurofilament-medium
NGF	Nerve Growth Factor
NHS	Normal Horse Serum
NMDA	N-Methyl-D-Aspartate
NO	Nitric Oxide
NOS	Nitrous Oxide Synthase
NR1	NMDA receptor subunit 1
NR2	NMDA receptor subunit 2
NR3	NMDA receptor subunit 3
NT-3	Neurotrophin-3
OFL	Optic Fibre Layer
ONL	Outer Nuclear Layer
OPL	Outer Plexiform Layer
PARP-1	Poly (ADP-Ribose) Polymerase-1
PBS	Phosphate Buffer Saline
PGE2	Prostaglandin E2
PNS	Peripheral Nervous System
RGC	Retinal Ganglion Cell
rER	Rough Endoplasmic reticulum
ROS	Reactive Oxygen Species
SCa	Slow Component a
SCb	Slow Component b
Sec	Second
SPC	Streptavidin Peroxidase Conjugate
STAT	Signal Transducers and Activators of Transcritption

TdT	Terminal deoxynucleotidyl Tranferase
TEM	Transmission Electron Microscopy
TLR	Toll-like Receptor
TNF-α	Tumour Necrosis Factor-α
TUNEL	TdT-mediated dUTP-biotin Nick End Lebeling
UTP	Uridine TriPhosphate
WD	Wallerian Degeneration

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