PROTEOMICS OF
THE HUMAN
ALCOHOLIC
BRAIN:

Implications for the pathophysiology
of alcohol-related brain damage


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This thesis is submitted in the fulfilment of the requirements for
the degree of Doctor of Philosophy in Medicine.

November, 2007

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Statement of Originality

I certify that this thesis describes original research work, the majority of which was undertaken in the Discipline of Pathology, the Faculty of Medicine at the University of Sydney.

These results have not been previously submitted and will not be submitted for any other degree or qualification.

Unless otherwise specified in the text, the author performed all studies reported within this thesis.

Ethical approval for the use of human brain tissue was obtained from the Ethics committee of the Central Sydney Area Health Service (Protocol No.X03-00285) and the University of Sydney.

Signed,

Kimberley L. Alexander-Kaufman

SID: 200005705

November, 2007
Acknowledgements

It is with some reticence that I complete this thesis, for it signifies truly the end of a veritable kinship with the most special people I will continue to know and love.

Clive Harper is an inspiration. I thank the day I gathered the courage to approach you – “what is this thing called brain banking?” Oceans of gratitude and respect, Uncle Cliff! The gems in the pathology crown, Irina and Therese, how I’m going to survive without our midday interludes, I cannot imagine! Irina, you have given me such invaluable guidance in and out of the laboratory. Thank you both so very much for your advice, your patience, your coffee and most of all, your friendship.

Thank you to the past and present members of the Molecular Pathology Lab, namely, Izuru Matsumoto, Danielle Clark, Gabriel James, Kashem Abul, Sara ten Have, Haruka Matsuda and Takeshi Iwasaki. Thanks to the TRC ladies, past and present, Maria, Donna, Lisa, Alisa, Helen, Cheryl, Yen, Juliette, Rebecca, Nina and Claire. The APAF whizzes Dr Stuart Cordwell and Dr Ben Crossett, whose technical advice has simply been invaluable, a big thanks. The Molecular Psychiatry Lab at RIKEN Brain Science Institute Tokyo, where I had the most rewarding experience and made some wonderful tomodachis, Dr Yoshikawa, Dr Ohnishi, Dr Hattori, Yoshimi, Mizuho, Tomoko and Hisako, domo arigato gozaimasu! Thank you A/Prof Peter Wilce from the University of Queensland and the Pathology Department Staff and Students

To my wonderful, newly extended family for your unwavering love and support and to the many friends who’ve frequently endured my rants, a myriad of thanks.

Financial support was provided by grants from the NSW Government BioFirst Award and Brewers’ Foundation (awarded to A/Prof Matsumoto). K. Alexander-Kaufman is an Australia Postgraduate Award (APA) recipient. Human brain tissues were provided by the NSW Tissue Resource Centre, which is supported by the University of Sydney, Neuroscience Institute of Schizophrenia and Allied Disorders, National Institute of Alcohol Abuse and Alcoholism and NSW Department of Health.

Only one animal was harmed during the production of this thesis, My Polish cat, Oskar (aka Ascaris lumbricoides). Sorry about the leg little man.
This thesis is dedicated to Alex,

My friend. my love. my home.
Publications

2005


2006


Conference Attendance

• Australian Neuroscience Society, Melbourne, 2004
• Australian Neuroscience Society, Perth, 2005
• International Neuroscience Society, Innsbruck, Austria, 2005
• Australian Neuroscience Society, Sydney, 2006
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• Japanese Society for Neuropathology, Tokyo, 2007
• Research Society on Alcoholism, Chicago, 2007

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• Australian Postgraduate Award
• Bercovici Prize for Excellence in Medical Research for the publication – *K. Alexander-Kaufman, et al, Molecular Psychiatry, 2006*

(Awarded to the postgraduate student with the most outstanding publication at The University of Sydney, Australia)

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Summary

Proteomics is rapidly achieving recognition as a complimentary and perhaps superior approach to examine global changes in protein abundance in complex biological systems and the value of these techniques in neuropsychiatry is beginning to be acknowledged. Characterizing the brain’s regional proteomes provides a foundation for the detection of proteins that may be involved in disease-related processes. Firstly, optimal conditions were achieved for the application of two dimensional-gel electrophoresis (2D-GE)-based proteomics with postmortem human brain tissue. These optimized techniques were then applied to soluble fractions of adjacent grey and white matter of a single cytoarchitecturally defined area (Brodmann area 9; BA9) and of two adjacent regions of frontal white matter (BA9 and CC body) from healthy individuals. These normative proteomic comparisons highlighted the importance of correct tissue sampling, i.e. proper separation of regional white matter, as heterogeneity in the respective proteomes was demonstrated. Furthermore, they stressed the necessity for future molecular brain mapping studies.

The main focus of this thesis however, was to examine the proteomes of brain regions specifically vulnerable to alcohol-induced damage underlying cognitive dysfunction. Alcoholic patients commonly experience mild to severe cognitive decline. It is postulated that cognitive dysfunction is caused by an alcohol-induced region selective brain damage, particularly to the prefrontal cortex. The cerebellum is increasingly recognized for its role in various aspects of cognition and alcohol–induced damage to the cerebellar vermis could indirectly affect neurocognitive functions attributed to the frontal lobe. We used a 2D-GE-based proteomics approach to compare protein
abundance profiles of BA9 grey and white matter and the cerebellar vermis from human alcoholics (neurologically uncomplicated and alcoholics complicated with liver cirrhosis) and healthy control brains. Among the protein level changes observed are disturbances in the levels of a number of thiamine-dependent enzymes. A derangement in energy metabolism perhaps related to thiamine deficiency seems to be important in all regions analysed, even where there are no clinical or pathological findings of Wernicke-Korsakoff Syndrome. Evidence of oxidative changes was also seen in all regions and effects of liver dysfunction in the vermis found. However, overall, these results highlight the complexity of this disease process in that a number of different proteins from different cellular pathways appear to be affected. By identifying changes in protein abundance levels in the prefrontal grey and white matter and the cerebellar vermis, hypotheses may draw upon more mechanistic explanations as to how chronic ethanol consumption causes the structural and functional alterations associated with alcohol-related brain damage. Furthermore, by comparing these results, we may be able to isolate disturbances in molecular pathways specific to the brain damage caused by alcohol, severe liver dysfunction and thiamine deficiency.
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**Abbreviations**

2D-GE, 2 dimensional gel electrophoresis; 2DIGE, 2 dimensional differential gel electrophoresis

**A**
δALAD, δ-Aminolevulinic acid dehydratase; AC, Adenyl cyclase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; ANOVA, Analysis of variance; APAF, Australian proteome analysis facility

**B**
BA9, Brodmann area 9; BAL, Blood alcohol level; BSA, Bovine serum albumin

**C**
CA, Complicated alcoholics; CA-2, Carbonic anhydrase-2; cAMP, cyclic adenosine 3’, 5’-monophosphate CC, Corpus callosum; CNS, Central nervous system; CT, Computerised tomography;

**D**
DALYS, Disability adjusted life years; dlPFC, Dorsolateral prefrontal cortex; DRP, Dihydropyrimidinase-related protein; DSM-IV, Diagnostic and statistical manual of mental disorders, 4th ed.; DTI, Diffusion tensor imaging; DTT, Dithiothreitol

**E**
E-MS, Electrospray mass spectrometry

**F**
FDR, False discovery rate

**G**
GABA, γ-Aminobutyric acid; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; GDH-1, glutamate dehydrogenase 1; GDP, Guanosine Diphosphate; GFAP, Glial fibrillary acidic protein; GM, Grey matter; GRPs, Glucose regulated proteins; GTP, Guanosine triphosphate

**H**
H₂O₂, Hydrogen peroxide; HE, Hepatic encephalopathy; hNP22, Human neuronal protein 22; HSP, Heat shock protein

**I**
ICAT, isotope coded affinity tag; IEF, Isoelectric focusing; IEGs, Immediate early genes; IPA, Ingenuity pathway analysis; IPGs, Immobilised pH gradient strips;

**K**
KP, Korsakoff’s psychosis

**M**
MALDI-TOF, Mass Absorption/Desorption Ionisation Time of Flight; MRI, Magnetic resonance Imaging; MRS, Magnetic resonance spectroscopy; MS, Mass spectrometry; MS/MS, Tandom mass spectrometry; MW, Molecular weight
N
NAA, N-acetylaspartate; NF-L, Neurofilament light protein; NMDA, N-methyl-D-aspartate; NO, Nitric oxide; nNOS, Neuronal nitric oxide synthase, NSF, N ethylmaleimide sensitive factor; NSW TRC, New South Wales tissue resource centre

P
PA, Phosphatidic acid; PFC, Prefrontal cortex; PE, Phosphatidylethanolamine; PEBP, Phosphatidylethanolamine-binding protein; PEth, phosphatidylethanol; PI, phosphoinositol; PICS, Pericerebral space; PIMT, Protein-L-isoaspartate O-methyl transferase; PMI, Post-mortem interval; PLC, Phospholipase C; PLD, Phospholipase D; PLP, pyridoxal-5-phosphate; PPP, Pentose phosphate pathway

R
Rho GDI 1, Rho GDP-dissociation inhibitor 1; ROS, Reactive oxygen species;

S
SDS-PAGE. Sodium dodecyl sulphate polyacrylamide gel electrophoresis; SNAP-β, Beta-soluble NSF attachment protein; SSS, Standard stock solution

T
TCA, Tricarboxylic acid cycle; TPP, Thiamine pyrophosphate;

U
UA, Uncomplicated alcoholics

V
V-ATPase, Vacuolar H+ -ATP synthase

W
WE, Wernicke’s encephalopathy; WKS, Wernicke-Korsakoff’s syndrome; WM, White matter; WHO, World health organization;