Two methods of biomarker discovery: applications in neuropathic pain and pharmacotherapy

Peter M. Grace BHSc (Hons)

Discipline of Pharmacology, School of Medical Sciences
(Faculty of Health Sciences)
The University of Adelaide

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Abstract

Biomarkers have potential utility in the treatment of pain as diagnostics and for quantification of drug efficacy and safety. A qualified biomarker will capture overlapping disease mechanisms and will be responsive to treatment. The necessity for these strict requirements renders it difficult to discover new biomarkers, particularly one that is reliable, practical and non-invasive, and simple for routine utilisation. This thesis demonstrates that two approaches may be useful to overcome these challenges: bottom-up and top-down biomarker discovery and development. Current animal models of neuropathic pain are inadequate to develop biomarkers as they only cover ‘no pain’ and ‘high pain’: not the heterogeneity that exists between these extremes. Therefore, a novel rat model of graded neuropathic pain was developed by advancing the existing chronic constriction injury model. Sciatic nerve and subcutaneous chromic gut sutures were varied, resulting in ‘dose-dependent’ behavioural allodynia. Allodynia was correlated with microglial activation marker expression in the ipsilateral lumbar dorsal horn of the spinal cord, suggesting that changes in behaviour are associated with disease mechanisms. A literature review of the pathophysiological mechanisms of pain, filtered by the criterion for accessible biomarkers, revealed that the peripheral immune system was the ideal target for the bottom-up approach. As such, the graded model was then used to explore peripheral immune mechanisms in order to begin the process of construct validation of potential neuropathic pain biomarkers. It was demonstrated that peripheral immune cells significantly contribute to chronic constriction injury-induced allodynia, as adoptive transfer of splenocytes or peripheral blood mononuclear cells from high pain donors to low pain recipients potentiates allodynia. Intrathecal transfer of high pain immune cells to low pain recipients potentiated allodynia, confirming that infiltrating immune cells are not passive bystanders, but actively contribute to nociceptive hypersensitivity in the lumbar spinal cord. The graded transcriptome of dorsal horn of the ipsilateral lumbar spinal cord was compared with that in the blood, identifying chemokines and transcription factors as potential blood-borne biomarkers of neuropathic pain. The top-down approach
explored the utility of saccadic eye movements as an objective, functional biomarker of sedation, an adverse effect associated with opioid treatment of pain. This study compared the interaction between sleep deprivation and opioids on opioid-naïve with opioid-tolerant participants. The naive-participant study evaluated the effects of sleep deprivation alone, morphine alone and the combination; the tolerant-participant study compared day-to-day effects of alternate-daily-dosed buprenorphine and the combination of buprenorphine on the dosing day with sleep deprivation. Psychomotor impairment was measured using saccadic eye movements, other oculomotor measures and an alertness visual analogue scale (VAS). Saccadic eye movements demonstrated an additive interaction between acute opioids and sleep deprivation, however the nature of the interaction between chronic buprenorphine and sleep deprivation remained unclear. This study revealed greater saccadic eye movement, but not VAS impairment in tolerant versus naive participants, suggesting that chronically dosed patients may not become tolerant to the sedative effects of opioids. These findings open up a number of new opportunities for pain biomarker development within the peripheral immune system, identify potential pain biomarker candidates, as well as further validating saccadic eye movement analysis as a biomarker of sedation. This thesis highlights that bottom-up and top-down approaches are appropriate methods for biomarker discovery and development.
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 has been made in the text.

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Peter Michael Grace 1 November 2010
Statement of Authorship


Impact Factor: 2.295

Mr. Grace had a major input in the experimental design, performed most surgeries, most behavioural testing, tissue collection immunohistochemistry imaging and densitometry, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

Signed 1 November 2010

Dr. Hutchinson was involved in the experimental design, performed some surgeries and conducted some behavioural testing, assisted with tissue collection, contributed to the data interpretation and preparation of the manuscript.

Signed 1 November 2010

Mr. Manavis conducted the immunohistochemistry and contributed to the preparation of the manuscript, including writing much of the immunohistochemistry section of the Methods.

Signed 1 November 2010

Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed 1 November 2010

Impact Factor: 5.061

Mr. Grace had a major input in the experimental design, conducted all experimental procedures except intrathecal injections, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

Signed 1 November 2010

Dr. Hutchinson was involved in the experimental design, performed intrathecal injections, assisted with tissue collection, contributed to the data interpretation and preparation of the manuscript.

Signed 1 November 2010

Mr. Bishop assisted with the flow cytometry and contributed to the preparation of the manuscript.

Signed 1 November 2010

Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Assoc. Prof. Mayrhofer was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Mr. Grace had major input in the experimental design, performed surgeries, behavioural testing and RNA purification, statistical analysis and graphical presentation of the data collected, and prepared the manuscript for submission.

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Prof. Somogyi was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed 1 November 2010

Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

Signed 1 November 2010

Dr. Hutchinson was involved in the experimental design, contributed to the data interpretation, assisted with statistical analyses and preparation of the manuscript.

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Mr. Grace had a major input in the experimental design, recruited all participants, conducted all experimental procedures, some statistical analysis and all graphical presentation of the data collected, and prepared the manuscript for submission.

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Mr. Stanford performed the majority of statistical analyses and contributed to the preparation of the manuscript, including writing much of the statistics section of the Methods.

Signed 1 November 2010

Mrs. Gentgall was responsible for the clinical trial logistics and provided editorial assistance.

Signed 1 November 2010

Prof. Rolan was involved in the experimental design, contributed to the data interpretation and preparation of the manuscript.

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Abbreviations

5-HT  
AMP A  
ASIC  
ATP  
AVAS  
BDNF  
BK  
BOLD  
CB  
CCI  
CGRP  
CIP  
CNS  
COX  
CSF  
CSGAAS  
DA  
DLF  
DRG  
DSST  
EBN  
ECF  
EOG  

5-hydroxytryptamine/ serotonin
α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
Acid-sensing Ion Channel
Adenosine triphosphate
Alertness visual analogue scale
Brain derived neurotrophic factor
Bradykinin
Blood oxygenation level dependent
Cannabinoid
Chronic constriction injury
Calcitonin gene related peptide
Compact integrated pupillograph
Central nervous system
Cyclooxygenase
Cerebrospinal fluid
Cardiff saccades generating and analysis system
Dark Agouti
Dorsolateral funiculus
Dorsal root ganglion
Digit symbol substitution test
Excitatory burst neuron
Extracellular fluid
Electro-oculography
EW  Edinger Westphal
FEF  Frontal eye field
fMRI  Functional magnetic resonance imaging
GABA  γ-aminobutyric acid
GFAP  Glial fibrillary acidic protein
GKO  Gene knockout
IASP  International Association for the Study of Pain
IBN  Inhibitory burst neuron
IFN  Interferon
IL  Interleukin
IN  Internuclear neuron
i.p.  Intraperitoneal
i.t.  Intrathecal
LC  Locus coeruleus
LDI  Laser Doppler imaging
LIP  Lateral intraparietal area
LPS  Lipopolysaccharide
MAPK  Mitogen activated protein kinase
medRF  Medullary reticular formation
MHC  Major histocompatibility complex
MVN  Medial vestibular nuclei
N  Neuronal
NA  Noradrenaline
NFκB  Nuclear factor κB
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>NGF</td>
<td>Nerve growth factor</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NNT</td>
<td>Number needed to treat</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NPH</td>
<td>Nuclei prepositus hypoglossi</td>
</tr>
<tr>
<td>NRS</td>
<td>Normal rat serum</td>
</tr>
<tr>
<td>OIH</td>
<td>Opioid induced hyperalgesia</td>
</tr>
<tr>
<td>OPN</td>
<td>Omnidirectional pause neurons</td>
</tr>
<tr>
<td>PAG</td>
<td>Periaqueductal grey</td>
</tr>
<tr>
<td>PAMP</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>Prostaglandin</td>
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<td>PNL</td>
<td>Partial nerve ligation</td>
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<td>QST</td>
<td>Quantitative sensory testing</td>
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<td>ra</td>
<td>Receptor antagonist</td>
</tr>
<tr>
<td>rCBF</td>
<td>Regional cerebral blood flow</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>----------</td>
<td>-----------------------------------------------------------------------------</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RPD</td>
<td>Resting pupil diameter</td>
</tr>
<tr>
<td>RVM</td>
<td>Rostral ventromedial medulla</td>
</tr>
<tr>
<td>S</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>S1, 2</td>
<td>Somatosensory cortex, primary, secondary</td>
</tr>
<tr>
<td>SC</td>
<td>Superior colliculus</td>
</tr>
<tr>
<td>SD</td>
<td>Sprague Dawley</td>
</tr>
<tr>
<td>SEF</td>
<td>Supplementary eye fields</td>
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<tr>
<td>SEMs</td>
<td>Saccadic eye movements</td>
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<tr>
<td>SG</td>
<td>Substantia gelatinosa</td>
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<tr>
<td>SNL</td>
<td>Spinal nerve ligation</td>
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<tr>
<td>SSRI</td>
<td>Selective serotonin reuptake inhibitors</td>
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<tr>
<td>TASK</td>
<td>Tandem of P domains in a Weak Inward rectifying K⁺ channel-related acid-sensitive K⁺</td>
</tr>
<tr>
<td>TCA</td>
<td>Tricyclic antidepressant</td>
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<tr>
<td>Tₜ</td>
<td>Helper T cell</td>
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<td>TLR</td>
<td>Toll like receptor</td>
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<td>TNF</td>
<td>Tumour necrosis factor</td>
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<td>trkA</td>
<td>Tyrosine kinase receptor A</td>
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<td>TRPA</td>
<td>Transient receptor potential subfamily A</td>
</tr>
<tr>
<td>TRPV</td>
<td>Transient receptor potential vanilloid</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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*Peter M. Grace, PhD Thesis 2010 xxiv*