

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

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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 chronic 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

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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.

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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.

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Abbreviations

5-HT	5-hydroxytryptamine/ serotonin
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
ASIC	Acid-sensing Ion Channel
ATP	Adenosine triphosphate
AVAS	Alertness visual analogue scale
BDNF	Brain derived neurotrophic factor
BK	Bradykinin
BOLD	Blood oxygenation level dependent
CB	Cannabinoid
CCI	Chronic constriction injury
CGRP	Calcitonin gene related peptide
CIP	Compact integrated pupillograph
CNS	Central nervous system
COX	Cyclooxygenase
CSF	Cerebrospinal fluid
CSGAAS	Cardiff saccades generating and analysis system
DA	Dark Agouti
DLF	Dorsolateral funiculus
DRG	Dorsal root ganglion
DSST	Digit symbol substitution test
EBN	Excitatory burst neuron
ECF	Extracellular fluid
EOG	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
NF κ B	Nuclear factor κ B

NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase
NPH	Nuclei prepositus hypoglossi
NRS	Normal rat serum
OIH	Opioid induced hyperalgesia
OPN	Omnidirectional pause neurons
PAG	Periaqueductal grey
PAMP	Pathogen-associated molecular patterns
PAT	Pupil adaptation test
PBMC	Peripheral blood mononuclear cell
PET	Positron emission tomography
PG	Prostaglandin
PLR	Pupil light reflex
PNL	Partial nerve ligation
PNS	Peripheral nervous system
PO	Postoperative
PPRF	Paramedian pontine reticular formation
PSV	Peak saccadic velocity
QST	Quantitative sensory testing
ra	Receptor antagonist
rCBF	Regional cerebral blood flow

ROS	Reactive oxygen species
RPD	Resting pupil diameter
RVM	Rostral ventromedial medulla
S	Subcutaneous
S1, 2	Somatosensory cortex, primary, secondary
SC	Superior colliculus
SD	Sprague Dawley
SEF	Supplementary eye fields
SEMs	Saccadic eye movements
SG	Substantia gelatinosa
SNL	Spinal nerve ligation
SSRI	Selective serotonin reuptake inhibitors
TASK	Tandem of P domains in a Weak Inward rectifying K ⁺ channel-related acid-sensitive K ⁺
TCA	Tricyclic antidepressant
T _H	Helper T cell
TLR	Toll like receptor
TNF	Tumour necrosis factor
TREK	Tandem of P domains in a Weak Inward rectifying K ⁺ channel -related K ⁺ channel
trkA	Tyrosine kinase receptor A
TRPA	Transient receptor potential subfamily A
TRPV	Transient receptor potential vanilloid
VAS	Visual analogue scale