

Potential peripheral biomarkers for chronic pain

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The University of Adelaide

June 2014

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

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Abstract

Chronic pain biomarkers can assist clinicians to diagnose patients, identify underlying mechanisms of disease, reduce the time and cost to reach a decision in early clinical trials and guide personalized pain treatments. Unfortunately, to date, there is no validated biomarker for chronic pain due to the difficulty in accessing the central nervous system. However, emerging literature has consistently provided evidence for the involvement of the immune system to play a substantial role in the modulation of chronic pain. Thus, this thesis examines components of the immune system such as Toll like receptor (TLR) signalling, peripheral immune cells and pro-inflammatory cytokine as an accessible source which may mirror similarities in brain immune cells and capture the changes in chronic pain state.

Therefore, the purpose of this thesis was to examine the use of peripheral immune cell reactivity as a potential biomarker for chronic pain. The first study was conducted in heterogeneous chronic pain and pain-free cohorts. Peripheral blood mononuclear cells (PBMCs) were isolated and stimulated with various TLR agonists to generate a pro-inflammatory cytokine interleukin-1 β (IL-1 β) concentration response curve. Chronic pain patients displayed significantly enhanced expression of IL-1 β compared with the pain-free cohort hence the TLR responsiveness demonstrated face validity as a chronic pain biomarker.

The second study demonstrated the translatability of the importance of TLR responsiveness in a preclinical neuropathic pain model. IL-1 β levels were quantified from basal and TLR2/4 agonist stimulated isolated rat PBMCs and spinal cord tissues, and together with the behaviour responses were used to generate statistical models. The main findings of this study were the inclusion of basal and TLR agonist stimulated outputs were required to predict the presence of pain and severity of allodynia with high sensitivity and specificity and that peripherally collected outputs correlated with the outputs from the spinal cord, suggesting the ability of peripheral outputs to give insight into

central signalling. In addition, a mathematical model developed from rat studies using peripheral and central tissues was able to identify chronic pain patients by their peripheral blood response with high accuracy.

The final study assessed TLR responsiveness of isolated PBMCs collected from a cohort of medication overuse headache (MOH) patients to determine the efficacy of a novel treatment for headache (ibudilast). This study consisted of MOH patients on 8 weeks of either placebo or ibudilast treatments. After 8 weeks of treatment, both groups did not experience a change in their headache frequency or intensity. However, a significant reduction in the TLR responsiveness occurred in the ibudilast group. These data provide the first evidence of a biomarker for ibudilast treatment.

In sum, this thesis provides evidence that peripheral cells are a good source to be biomarker for chronic pain and the importance to assess TLR signalling as an approach to capture the dysregulated immune system as a result of chronic pain. The discovered biomarkers require further replication and validation before than can be routinely used. However, the present finding can assist with the development of future cellular biomarkers for chronic pain.

Declaration

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Kwok YH, Tuke J, Nicotra LL, Grace PG, Rolan PE, Hutchinson MR (2013) TLR 2 and 4 Responsiveness from Isolated Peripheral Blood Mononuclear Cells from Rats and Humans as Potential Chronic Pain Biomarkers. *PLoS ONE* 8(10), e77799.

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Date

Acknowledgments

I would like to sincerely thank my supervisors Professor Paul Rolan and Professor Mark Hutchinson for giving me the opportunity to undertake such an exciting project. Their enthusiasm and immense knowledge really formed a concrete picture in my mind as to what “being passionate” is really about and I strive to be as passionate a researcher as them one day. Their ongoing encouragement, patience and guidance over the years are greatly appreciated and I could not have wished for better supervisors.

The research presented in this thesis would have been impossible without the financial support from the Faculty of Health Sciences Divisional PhD Scholarship as well as support from the Pain and Anaesthesia Research Clinic. The opportunities to present my work at numerous national and international conferences were generously supported by the Australasian Society for Clinical and Experimental Pharmacologists and Toxicologists Travel Grant, Australian Pain Society PhD Travel grant, Faculty of Health Sciences Postgraduate Travelling Fellowship, The School of Medical Sciences Postgraduate Travel Award and Professor Paul Rolan.

I would also like to acknowledge the work and express my gratitude to the following people: Melanie Gentgall and all the staffs at the Pain and Anaesthesia Research Clinic for teaching me how to run clinical studies as well as providing their clinical expertise; Dr Jonathan Tuke and Prof Mark Hutchinson for teaching me how to use and develop scripts for the statistical programming of R; Jacinta Johnson and Nicole Sumracki for their collaborations with the clinical studies; Lauren Nicotra for the collaboration with the preclinical study; Dr Janet Coller and Dr Dan Barratt for assistance with genotyping; I would like to thank Dr Dan Barratt again for his patience in training me on extracting RNA and providing insightful troubleshooting microarray advices; I'm also very grateful to Perona Ho who helped proofread my thesis; I would like to express my thanks to Gordon Crabbe and Karen Nunes-Vraz for their administrative support; I would like to thank all past and present members of the

Discipline of Pharmacology as they have provided me with valuable feedback throughout the years, which will be remembered; I would like to thank all the past and present students in the Discipline of Pharmacology and particularly to my colleagues in N529a and N511. They have been amazing and I will never forget the fun times we shared.

Furthermore, I am thankful to my friends for being so supportive and understanding over the years, whether it was chatting over lunch/dinner, hiking, watching the latest movies, singing karaoke or just hanging out -those moments have kept my life in balance!

Last but not least, I would like to thank my family, especially my parents. They have been my pillars of support both emotionally and financially and their constant encouragement really helped me get to where I am today and for that I am infinitely grateful.

Abbreviations

ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CCI	Chronic constriction injury
CCL	Chemokine (C-C) motif ligand
CNS	Central nervous system
COX	Cyclooxygenase
DAMPs	Danger-associated molecular patterns
DRG	Dorsal root ganglia
ELISA	Enzyme-linked immunosorbent assay
FCS	Fetal calf serum
FDA	Food and Drug Administration
fMRI	Functional magnetic resonance imaging
GABA	γ -aminobutyric acid
GFAP	Glial fibrillary acidic protein
KO	Knockout
IL	Interleukin
I κ B- α	NF κ B inhibitor α
iNOS	Nitric oxide synthase
IRAK	IL-1 receptor-associated kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MOH	Medication overuse headache
MyD88	Myeloid differentiation primary response gene 88
MSU crystals	Monosodium urate crystals

NGF	Nerve growth factor
NF- κ B	Nuclear factor- κ B
NMDA	N-methyl-D-aspartate glutamate
NNT	Number needed to treat
NO	Nitric oxide
Nox2	NADPH oxidase 2
NRS	Numeric rating scale
NSAIDS	Nonsteroidal anti-inflammatory drugs
P2X4	Purinergic receptor
PAMPs	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
SSRIs	Selective serotonin reuptake inhibitors
StEP	Standardized Evaluation of Pain
SDSN	Supernatant of damaged sensory neurons
TCA	Tricyclics antidepressants
TIR	Toll/IL-1 receptor
TGF- β 1	Transforming growth factor beta
TLR	Toll-like receptor
TNF- α	Tumour necrosis factor alpha
TRAF6	Tumour necrosis factor receptor 6
TRIF	TIR-domain containing adapter-inducing interferon- β
VAS	Visual analogue scale
VRS	Verbal rating scale