Toll-like receptor 4-dependent barrier dysfunction and its impact on irinotecan-induced gut toxicity and pain

A thesis submitted in fulfillment for degree of

DOCTOR OF PHILOSOPHY

in

The Discipline of Anatomy and Pathology

School of Medicine

The University of Adelaide

by

Hannah Rose Wardill

22/08/16
Declaration

“This work contains no material that has been accepted for the award of any other degree or diploma in any university or 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.”

I give consent for this copy of my thesis, when deposited in the University Library, being made available for loan and photocopy.

Hannah Rose Wardill

22/08/16
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Publications arising from this thesis


* denotes invited review
Contributions made by co-authors

Professor Rachel J Gibson


Professor Rachel J Gibson was my co-principal supervisor (with Dr Joanne M Bowen) and has therefore been listed on all publications arising from this thesis. Rachel helped design and interpret results as well as being responsible for obtaining funding for this project. She was also involved in drafting all manuscripts in preparation for publication.
Dr Joanne M Bowen


Dr Joanne M Bowen was my co-principal supervisor (with Professor Rachel J Gibson) and has therefore been listed on all publications arising from this thesis. Joanne provided significant technical advice for much of the experimental work and also helped design and interpret results. She was also responsible for obtaining funding for this project and drafting all manuscripts in preparation for publication.
Contributions made by co-authors

Professor Richard M Logan


Professor Richard M Logan is my third supervisor. He has been listed as co-author on all publications from this thesis. Richard was involved in the original clinical study from which archival tissue samples were obtained and used for one of my studies (Chapter 3). He has also revised many drafts and provided assistance in gaining independent funding to support my research.
Contributions made by co-authors

Ms Ysabella ZA Van Sebille


Ms Ysabella ZA Van Sebille is a member of the Cancer Treatment Toxicities group. Ysabella contributed to several publications arising from this thesis by assisting with laboratory and animal work, as well as reading draft manuscripts.
Ms Kimberley A Mander


Ms Mander is part of the Adelaide Centre for Neuroscience Research. She has a keen interest in implications for blood brain barrier disruption in various disease states. Kim was involved in two reviews that were completed during my candidature, reading drafts and providing information about blood brain barrier regulation.
Ms Kate R Secombe


Ms Kate R Secombe was an honours student and research assistant in the Cancer Treatment Toxicities group during my candidature. During her honours degree, Kate helped with animal and laboratory work and has therefore been listed on the two publications arising from this study. During her time as a research assistant, she provided assistance in maintaining cell culture lines and stocks.
Dr Janet K Coller


Dr Janet K Coller was responsible for obtaining funding to conduct my animal project. She also contributed significant time in reading draft manuscripts in preparation for publication.
Ms Imogen E Ball (nee White)


Ms Imogen E Ball is a research assistant in the Cancer Treatment Toxicities Group. Imogen was listed as co-author on the two publications based on her contribution to the animal study.
Professor Mark R Hutchinson


Professor Mark R Hutchinson was listed as co-author on the first publication arising from my animal study as he sourced and provided the BALB/c-Tlr4<sup>±/±</sup> billy mice. Mark is highly specialised in the area of neuroimmunology, and he therefore provided advice regarding my analysis of neuroinflammation. He was also involved in preparing this manuscript for publication, reading several drafts.
Ms Vicky Staikopoulos


Ms Vicky Staikopoulos was a research officer in the Neuroimmunopharmacology Laboratory during my candidature. She was involved in training me on many techniques required for my animal study. These techniques were not available within the Cancer Treatment Toxicities Group. Vicky also assisted in analysis of glial immunohistochemistry and read several draft manuscripts.
Mr Jim Manavis


Mr Jim Manavis is head of histology in the School of Medicine, University of Adelaide. He has experience in immunohistochemical analysis of immune cells within the central nervous system as well as detecting changes in the blood brain barrier. Jim provided substantial advice regarding my analysis of central nervous system pathologies, an area my laboratory has little experience with. He provided technical assistance and read drafts of the manuscript.
Contributions made by co-authors

Ms Romany Stansborough


Ms Romany Stansborough was an undergraduate during my candidature. Romany was involved in conducting parts of the laboratory work that contributed to the publication listed. She is now a member of the Cancer Treatment Toxicities Group.
Ms Joseph Shirren


Ms Joseph Shirren was an undergraduate during my candidature. Joseph was involved in conducting parts of the laboratory work that contributed to the publication listed. He is now a member of the Cancer Treatment Toxicities Group.
Dr Emma Bateman


Dr Emma Bateman is the laboratory manager of the Mucositis Research Group. Emma was involved in conducting the original animal study from which archival tissue samples were sought for the study listed. She also read several drafts of the manuscript and has therefore been listed as a co-author.
Ms Masooma Sultani


Ms Masooma Sultani was a member of the Mucositis and Gut Microbiome Research Groups during my candidature. She was involved in conducting the original animal study from which archival tissue samples were sought for the study listed.
Additional studies and publications

During my honours degree and PhD candidature, I published several first author reviews/primary research papers that are not presented in my thesis. These publications are listed below:


**Wardill HR, Logan RM, Bowen JM, and Gibson RJ (2015)** Chemotherapy causes tight junction defects in the oral cavity of patients, which coincide with elevated proinflammatory cytokines and MMPs. *Australian Dental Journal*. 60(4): S20.


* denotes invited review
Co-authored publications

During my candidature, I was also involved in several other studies investigating intestinal toxicity. This involvement resulted in co-authorship of several manuscripts. These publications are not presented in my thesis, and are listed below:


Van Sebille YZA, Gibson RJ, **Wardill HR** and Bowen JM, ErbB small molecule tyrosine kinase inhibitor (TKI) induced diarrhoea: chloride secretion as a mechanistic hypothesis. *Cancer Treatment Reviews*. 41(7): 646-52.


* denotes invited review
The format of my thesis is as follows: a general introduction, a literature review, two research chapters, a second literature review, three research chapters, a general discussion and references. During my candidature, I made significant effort to publish my research findings. Each research chapter is presented in its original publication format. This may result in slight repetition between chapters arising from the same study.

My thesis has three distinct themes relating to the pathobiology of chemotherapy-induced gut toxicity. The first aims to characterise the extent of tight junction disruption in the alimentary tract following chemotherapy treatment (clinically and preclinically), giving rise to the first two research chapters (Chapter 2 and 3). The first publication (Chapter 2) was completed early in my candidature (2013). The second publication (Chapter 3) arose from independent research funding I obtained from the Australian Dental Research Foundation. Together, these chapters formed the scope and theme for my PhD, and are therefore followed by two literature reviews and the remaining four research chapters. The second theme relates to involvement of innate immune regulation in the development of chemotherapy-induced gut toxicity and barrier dysfunction, giving rise to an additional two primary research chapters (Chapter 6 and 7). The third aim of this thesis was to develop a high throughput in vitro model for the study of chemotherapy-induced mucosal injury and targeted therapeutic approaches. This is summarised in Chapters 8 and 9.

During my candidature, I had the opportunity to work with Professor Stephen Sonis from Dana-Farber Harvard Cancer Centre, Harvard University, Boston. After presenting my work at the Multinational Association for Supportive Care in Cancer in 2014 (Miami, USA), Professor Sonis and I developed the hypothesis that gut-derived inflammation affects central neurological functions. This formed the basis for my secondary literature review (Chapter 5) as well as an additional literature review on cytokine-mediated blood brain barrier permeability and its involvement in chemotherapy-induced cognitive decline. The latter literature review is not included as a chapter in this thesis, but as an appendix in its original publication format (PDF).
Nomenclature

This thesis contains variations in terminology relating to chemotherapy-induced gastrointestinal toxicity. This reflects failure in the field to accurately define this pathology. Inconsistencies within this thesis are due to requests made by reviewers during peer-review of each publication. I did not change the terminology from what was used in the original publications to avoid altering their content.

Please review the following nomenclature.

**Alimentary tract**: any region from mouth to anus

**Alimentary toxicity/mucositis**: ulceration/inflammation of the mouth or gastrointestinal tract

**Oral toxicity/mucositis**: ulceration and inflammation of the mouth

**Gastrointestinal/gut toxicity/mucositis**: ulceration/inflammation of the small or large intestine, rectum and anus

In addition, publications from within this thesis conform to standard nomenclature for naming genetically modified mice as per the guidelines outlined in International Committee on Standardised Genetic Nomenclature for Mice. Wild-type mice on a BALB/c background will be referred to as wild-type (WT). Toll-like receptor 4 knockout mice on a BALB/c background will be referred to as BALB/c-\(Tlr4^{-/-}\). Reference to the process of deleting a gene will be preferred to as knockout or \(-/-\), e.g. \(Tlr4^{-/-}\).