Chemotherapy-induced mucositis: the role of matrix metalloproteinases and the extracellular matrix

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Chemotherapeutic agents, including irinotecan hydrochloride, are highly effective in the treatment of a range of cancers; however, they cause a variety of unwanted toxicities. Mucositis is the term used to describe the damage caused by cytotoxic agents to mucous membranes of the alimentary tract (AT). This condition affects 40-100% of patients depending on dose regimen. There is currently no effective treatment and the underlying molecular mechanisms are not fully understood. Previous research has shown that mucositis encompasses changes in stress response gene expression and subsequently activation of tissue injury and inflammation mediators.

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases; which have been shown to play a role in tissue injury and inflammation in many gastrointestinal disorders. Furthermore, MMPs mediate these phenomena through the regulation of the extracellular matrix (ECM). This work aims to elucidate whether MMPs contribute to the pathogenesis of mucositis and whether these can be used as biomarkers for mucositis development or be targeted for future treatment strategies. To investigate these aims, studies were performed in an animal model of irinotecan-induced mucositis. A pilot clinical study was also conducted.

To investigate the role of MMPs in mucositis pathogenesis, a time-course model of irinotecan-induced mucositis was utilised. Rats were administered with 200mg/kg irinotecan intraperitoneally at 0h and killed 30, 60, 90 min, 2, 6, 12, 24, 48, 72, 144h post-treatment. Sections were embedded in paraffin or frozen for further analysis. To ensure the accuracy of the molecular investigations in this thesis, the appropriateness of a range of housekeeping genes for normalisation of RT-PCR methods was investigated for the first time in this model. Findings indicated that the most suitable combination of genes

to use is Ywhaz/UBC in the jejunum and UBC/β-actin in the colon *or* UBC if restricted to a single housekeeping gene. Subsequent molecular and histological assessments demonstrated a significant alteration in gene expression and tissue levels of MMPs and their inhibitors (TIMPs) following irinotecan ($p<0.05$). The increase in MMP-2, -3, -9 and -12 levels was associated with inflammatory infiltrate and maximum tissue damage. In contrast, MMP-1 expression correlated with tissue restitution. Furthermore, histological techniques illustrated a substantial increase in total collagen deposits around crypts from 24h in the jejunum and colon. Fibronectin expression decreased significantly in both regions from 6-24h following treatment. Irinotecan induced a significant alteration in epithelial cell kinetics in both the jejunum and colon ($p<0.05$) and this correlated with changes in ECM components.

To determine if systemic MMP levels are useful markers of impending toxicity, a pilot clinical study was carried out. Eight patients receiving a variety of chemotherapy regimens were recruited. The most reported toxicity following treatment was diarrhoea. Analysis of patient serum samples revealed a 5.74-fold increase in systemic MMP-3 and a 2-fold increase in systemic MMP-12 levels following the administration of chemotherapy. Analysis of MMP-3 levels with patient symptoms revealed a correlation.

Findings from this thesis provide clear evidence demonstrating a role for MMPs and ECM components in the pathogenesis of irinotecan-induced mucositis. Alterations in total collagen deposits and fibronectin levels in the AT following treatment may underlie the dysregulated cell kinetics following treatment hence leading to toxicity. Furthermore, preliminary findings from the pilot clinical study suggest that circulating MMPs are potential biomarkers of gastrointestinal toxicity induced by specific chemotherapy agents.

All thanks to God for providing me with this opportunity and helping me achieve my PhD for without His assistance and guidance none of this would have been possible.

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Dr Rachel Gibson

Dr Rachel Gibson was my principal supervisor and therefore listed as a co-author on all publications arising from this thesis. She assisted in the development of my original research proposal and provided funding for the work that was completed during my candidature. In addition she read through many drafts of the individual papers as well as this thesis.

Professor Dorothy Keefe

Professor Dorothy Keefe was my co-supervisor and therefore listed as a co-author on all publications arising from this thesis. She assisted in the development of my original research proposal and provided funding for the work that was completed during my candidature. In addition she read through many drafts of the individual papers as well as this thesis.

Dr Joanne Bowen

Dr Joanne Bowen was my co-supervisor and therefore listed as a co-author on all publications arising from this thesis. She assisted in the development of my original research proposal and provided funding for the work that was completed during my candidature. In addition she read through many drafts of the individual papers as well as this thesis.

Associate Professor Richard Logan

Associate Professor Richard Logan is a member of the Mucositis Research Group. He assisted with all of the animal experiments undertaken in this study. He also read numerous drafts of the individual papers making up this thesis.

Dr Andrea Stringer

Dr Andrea Stringer is a member of the Mucositis Research Group. She assisted with all of the animal experiments undertaken in this study. She also read numerous drafts of the individual papers making up this thesis.

During my candidature, I was involved in several other studies, not presented in this thesis. These have resulted in primary authorship or co-authorship on several manuscripts. I am first author on an invited review. I have also contributed an invited book chapter on a newly emerging field in mucositis research.

Al-Dasooqi N, Gibson RJ, Bowen JM & Keefe D (2011). HER2 targeted therapy-induced gastrointestinal toxicity: from the clinical experience to possible molecular mechanisms. In: Whitehouse, D and Rapley, R *Molecular and Cellular Therapeutics* (xx). UK: John Wiley & Son (invited book chapter)

Al-Dasooqi N, Gibson RJ, Bowen JM & Keefe D (2009). HER2 targeted therapies for Cancer and the Gastrointestinal Tract. *Current Drug Targets*, 10(6): 537-542 IF 4.19 (invited review)

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Stringer A, Gibson R, Bowen J, Logan R, Ashton K, Yeoh A, **Al-Dasooqi N**, Keefe D (2008). Irinotecan-induced mucositis manifesting as diarrhoea corresponds with an amended intestinal flora and mucin profile. *International Journal of Experimental Pathology,* 90(5): 489-99 IF 1.89

This thesis is composed of 6 chapters: literature review, four distinct research chapters, followed by a general discussion. During the course of my candidature, four chapters were published, with a further one under review. Accordingly, each research chapter is written as a publication complete with introduction, materials and methods, results and discussions. Some minor editing of the chapters has been made to ensure a consistent format of the chapters, to avoid significant repetition and to include relevant data omitted from publications. Unavoidable repetition has occurred only as necessary due to the format of the paper.

The animal studies conducted were approved by the Animal Ethics Committee of The Institute of Medical and Veterinary Sciences and of The University of Adelaide. They complied with the National Health and Medical Research Council (Australia) Code of Practice for Animal Care in Research and Training (2004). Due to the potentially severe nature of the diarrhoea caused by irinotecan, animals were monitored four times daily and if any animals showed certain criteria (as defined by the Animal Ethics Committee) they were euthanased. These criteria included a dull ruffled coat with accompanying dull and sunken eyes, coolness to touch with no spontaneous movement, and a hunched appearance.

The clinical study was approved by the Ethics of Human Research Committee of the Royal Adelaide Hospital and was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient prior to enrolment in the study. All patients were de-identified.