



THE UNIVERSITY
of ADELAIDE

**The Role of Matrix Metalloproteinases
and Their Inhibitors in Irinotecan-
Induced Oral Mucositis;
An Animal Model**

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*Thesis submitted for the degree of Doctor of Clinical Dentistry
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Declaration

This thesis is submitted for the fulfilment of the Doctor of Clinical Dentistry (Oral Pathology). I declare that it contains no material which has been accepted for the award of any other degree or diploma in any university and that, to the best of my knowledge and belief, the Thesis contains no material previously published or written by another person, except where due reference is made in the text.

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Abstract

Background

Chemotherapy-induced oral mucositis is defined as damage of oral mucosa caused by unwanted detrimental effects of the cytotoxic chemotherapy. Oral mucositis presents as widespread painful ulcerations and erythematous eruptions and occurs in between 40-100% of all patients undergoing chemotherapy. Currently, there are no standard treatments available to prevent oral mucositis and the consequences on health care systems remain extensive.

Research into the pathogenesis of oral mucositis has shown a complex underlying process, involving several overlapping biological events in the epithelium and submucosal layers. However, this pathogenesis is still not fully understood. A group of proteolytic enzymes called matrix metalloproteinases (MMPs) have been recently postulated to play a part in mediating the damaging process seen in oral mucositis. It is well established that MMPs and their naturally existing inhibitors (tissue inhibitors of metalloproteinases; TIMPs) maintain a balanced level in normal physiological status of oral mucosa. Their dysregulated balance underlies some pathophysiological aspects of several diseases including some diseases of the oral and gastrointestinal mucosae. These mucosal diseases include ulcerative colitis, Crohn's disease, recurrent aphthous stomatitis and oral lichen planus. However, as MMPs and TIMPs have not been well studied in oral mucositis, this formed the basis of this thesis.

Hypothesis and Aims of the thesis

If MMPs and TIMPs are involved in the pathogenesis of oral mucositis, their tissue levels will change following the administration of cytotoxic chemotherapy. This may correlate to any histopathological changes in the oral mucosa. This thesis aimed to investigate the morphological changes and the tissue expression levels of MMP-2, -3 -9 and TIMP-1 within the oral cavity in a well-established pre-clinical animal model of chemotherapy-induced oral mucositis.

Results and Discussion

The findings presented in this thesis demonstrate epithelium thickness reduces without obvious ulceration in the oral mucosa very early after chemotherapy administration. Maximum atrophy is observed 60 min following chemotherapy in both dorsal and ventral surfaces of the tongue. This reduction in epithelial thickness is associated with significant up-regulation of MMP-2, -3 and -9 and down-regulation of TIMP-1 in all layers of the oral mucosa. MMP-9 is also up-regulated at later time point.

These findings support previous evidence that oral mucositis involves changes in the submucosa before it is clinically evident. The early reduction in epithelial thickness confirms similar findings reported in earlier studies of oral mucositis in rat buccal mucosa. The up-regulation of MMP-2, -3, -9 and down-regulation of TIMP-1 coincided with the previously described early up-regulation of transcription factors and pro-inflammatory cytokines in oral mucosa, suggesting a relationship between their up-regulation/down-regulation and the release of these factors and cytokines. Furthermore, the different patterns of expression demonstrated by MMP-2, -3, -9 suggest that these

MMPs are involved in various aspects of the 5-phase model of OM pathophysiology including initiation of inflammatory response and tissue injury, recruitment of other mediators of OM and restoration of oral mucosa to normal physiological status.

Conclusion

This thesis has provided evidence that MMPs play a key role in the aetiology of oral mucositis. Research in this area needs to be directed towards studying other relevant MMPs and also towards interventional therapies aiming to target MMP-2, -3 and -9 to prevent or reduce the severity of oral mucositis, as well as to promote faster healing of OM lesions. This will help provide an optimum treatment outcomes provided to cancer patients and improve the quality of life during and after their treatment.

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Explanation of the Thesis

This thesis is entitled „The Role of Matrix Metalloproteinases and Their Inhibitors in Irinotecan-Induced Oral Mucositis; An Animal Model”. It is composed of 7 Chapters presented in the conventional thesis format. Chapter 1 is an introductory chapter that provides a background to the research area undertaken for fulfilment of the Doctorate of Clinical Dentistry (Oral Pathology). The next Chapter 2 is the “Literature Review” that addresses the published literature related to the impact of chemotherapy on the normal function and structure of oral mucosa, matrix metalloproteinases and their inhibitors and their role in mediating various diseases, focussing on the potential role they may play in oral mucositis and interventional opportunities targeting them. Chapter 3 describes the materials and methods used in this study. The results of this study are outlined in Chapter 4. Chapter 5 discusses the major findings of the study, comparing or correlating them to previous studies. Chapter 6 is the “Conclusions and Future Directions”: it outlines the significance of the findings obtained from this study and provides recommendations for future research directions. The list of references is provided in the Bibliography Chapter (7) at the end. All relevant work and list of publications during candidature are included in the Appendices section.

Animal Ethics

The established animal model used in this study was approved by the Animal Ethics Committee of The University of Adelaide and of The Institute of Medical and Veterinary Sciences. 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 euthanized. These criteria included a dull ruffled coat with accompanying dull and sunken eyes, coolness to touch with no spontaneous movement, and a hunched appearance.

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List of Abbreviations

Commonly used abbreviations in the thesis

Abbreviation	Term
5-FU	5-fluorouracil
AE	Apical layer of epithelium
AT	Alimentary tract
BE	Basal layer of epithelium
CT	Chemotherapy
DA	Dark Agouti (rats)
DT	Dorsal surface of tongue
ECM	Extracellular matrix
H&E	Haematoxylin and Eosin
IHC	Immunohistochemistry
GI	Gastrointestinal
LP	Lamina propria layer
MMP/s	Matrix Metalloproteinase/s
NF	Nuclear factor
OM	Oral mucositis
TIMP/s	Tissue inhibitor/s of metalloproteinases
RT	Radiotherapy
SM	Submucosal layer
VT	Ventral surface of tongue

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