Alimentary Tract Mucositis: NF-κB and Pro-Inflammatory Cytokines in the Tissues and Serum Following Chemotherapy

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Thesis submitted for the degree of Doctor of Philosophy

March 2008

Declaration

This work contains no material which has been accepted for the award of any 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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March 2008

Table of Contents

Chapter 2

Chapter 3

Chapter 5

Chapter 6

Abstract

Mucositis refers to the widespread damage of mucosal surfaces throughout the length of the alimentary tract (AT) that can occur during cancer treatment. Its development is an important clinical problem that complicates and limits treatment options as well as adversely affecting the quality of life and treatment outcomes for patients. Recent studies directed at determining the pathobiology of mucositis have indicated increasing evidence for the role of transcription factors, such as nuclear factor-κB (NF-κB), and certain pro-inflammatory cytokines, for example tumour necrosis factor (TNF), interleukin-1β (IL-1β) and interleukin-6 (IL-6), in its development. This thesis developed from an initial clinical investigation in which the expression of NF-κB and COX-2 in oral mucosa was investigated in patients undergoing chemotherapy. Increased levels of NF-κB were demonstrated in the buccal mucosa following chemotherapy.

It is well established that mucositis occurs in different sites of the AT. The aims of this research, therefore, were to compare and contrast the changes that do occur at different sites of the AT following chemotherapy in an established animal model (Dark Agouti (DA) rat). Furthermore, the studies were conducted to determine whether changes in tissue and serum levels of NF-κB and pro-inflammatory cytokines occurred following chemotherapy and, with respect to tissue levels, identify whether there were differences in expression at different sites throughout the AT. The final aim was to examine whether the histological changes and changes in pro-inflammatory cytokines were affected by the type of chemotherapy drug used.

The effects of three chemotherapy drugs with different mechanisms of action (irinotecan, methotrexate and 5-fluorouracil) were investigated, all of which can cause mucositis in the clinical setting.

The thesis is divided into a *Literature Review* (Chapter 1) followed by 4 research papers: Chapter 2 – "*Nuclear factor- κB (NF- κB) and cyclooxygenase-2 (COX-2) expression in the oral mucosa following cancer chemotherapy*" Chapter 3 – "*Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: Implications for the pathobiology of mucositis*" Chapter 4 – "*Is the pathobiology of chemotherapy-induced alimentary tract mucositis influenced by the type of mucotoxic drug administered*?", Chapter 5 – "*Serum levels of NF-κB and pro-inflammatory cytokines following administration of mucotoxic drugs*". Chapter 6 provides an overall summary and discussion of the results.

Previous research has indicated that following administration of chemotherapeutic agents there may be subclinical changes occurring in the mucosa prior to obvious clinical manifestations. The results presented in this thesis also demonstrate this in both humans and animals following administration of chemotherapy. Immunohistochemical analysis of tissue taken from the oral cavity, jejunum and colon from the DA rats following chemotherapy demonstrated that changes in NF-κB and the pro-inflammatory cytokines, TNF, IL-1β and IL-6, occurred at all sites over a 72 hour time period. This was evident before severe histological evidence of mucositis were observed such as epithelial atrophy in the oral mucosa, atrophy, blunting and fusion of the villi in the jejunum and crypt ablation in the jejunum and colon. Furthermore, each of the three drugs caused different patterns of NF-κB and pro-inflammatory cytokine expression in the tissues; in spite of this, however, histological features of damage were similar. With respect to serum levels of NF-κB and pro-inflammatory cytokines, differences were observed between the serum and tissue levels. Generally, serum changes followed initial histological changes in the tissues, or occurred simultaneously with histological changes. The mechanisms behind this are unclear; however it may be that elevated cytokines in the tissues "overflow" into the serum as tissue damage

increases. Furthermore, the use of serum cytokine level measurement to predict mucosal damage is limited because of the differences in timing and short time intervals between changes in the serum and tissues.

This thesis has provided additional important information on mucositis pathobiology and highlights its complexity. In particular, it has provided new evidence supporting the notion that mucositis is not restricted to the oral cavity and that other sites of the AT are also affected. Furthermore, these results confirm previous data indicating that subclinical changes occur in the mucosa prior to the development of obvious histological damage or clinical manifestations of mucositis. Contrary to previous reports, these studies have indicated that, although the clinical and histological changes may be similar, the alterations in NF-κB and pro-inflammatory cytokines in the tissues are affected by the type of drug used. This has important implications in the management and prevention of mucositis in the clinical setting particularly when multi-drug or chemotherapy-radiotherapy regimens are used. A common pathway that leads to mucosal damage is yet to be determined. The fact that serum levels appear to reflect the "global" nature of the effects of chemotherapy, highlights the fact that ongoing research needs to be directed, not necessarily at specific side effects, but rather how side effects of chemotherapy are interrelated so that better patient management can be achieved and ultimately provide optimum treatment and better survival for patients with cancer.

Acknowledgements

I would particularly like to acknowledge the support of my two supervisors, Professor Dorothy Keefe and Professor Stephen Sonis. Their enthusiastic encouragement and always constructive comments have been invaluable throughout my candidature.

To the members of the Adelaide Mucositis Research Group, Dr. Rachel Gibson, Dr. Joanne Bowen, Andrea Stringer and Ann Yeoh, I would like to thank them for their assistance with the animal work, advice regarding laboratory techniques and their friendship over the last few years.

I also wish to acknowledge the following people for their assistance with this work: Sandie Hughes and Marjorie Quinn, Discipline of Oral Pathology, The University of Adelaide for laboratory advice and assistance with tissue sectioning and staining; Thomas Sullivan, Discipline of Public Health, The University of Adelaide and Professor Grant Townsend and Dr Toby Hughes, School of Dentistry, The University of Adelaide for assistance with the statistical analyses.

Thanks must also go to my family, in particular my Mum and Dad, Graham and Margaret Logan, who have always supported and encouraged me throughout my career. I would also like to thank my parents-in-law, Avi and Lala Chandiok, who have also provided encouragement and always shown interest in my work.

Finally, I am most grateful to my wife, Mika, and sons, James and Callum, for putting up with my early mornings, working in the evenings as well as weekends spent in the laboratory; their support and belief in me has made the task of undertaking and completing this PhD possible.

Co-author Contributions

Professor Dorothy M. K. Keefe

Professor Keefe 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 Stephen T. Sonis

Professor Sonis was my co-supervisor who, in spite of being on the opposite side of the world, was crucial in helping to develop my research project. He read drafts of each paper and this thesis.

Dr Rachel J. Gibson

Dr Gibson is a member of the Mucositis Research Group. She helped plan and provided valuable assistance with the initial irinotecan animal experiment and provided advice on laboratory techniques. Dr Gibson also read numerous drafts of the individual papers that make up this thesis.

Dr Joanne M. Bowen

Dr Bowen is a member of the Mucositis Research Group. She assisted with all of the animal experiments undertaken in this study and provided advice on laboratory techniques. She also read numerous drafts of the individual papers that make up this thesis.

Andrea M. Stringer

Ms Stringer is a fellow PhD student and member of the Mucositis Research Group. She assisted with all of the animal experiments and provided advice on laboratory techniques. She also read numerous drafts of the individual papers that make up this thesis.

Ann S-J. Yeoh

Ms Yeoh is a fellow PhD student and member of the Mucositis Research Group. She read and commented on numerous drafts of the literature review for which she is listed as a co-author.

Explanation of the Thesis

This thesis is composed of 6 chapters: a literature review, four distinct research papers and finally, summary and conclusions. During the course of my candidature the literature review and 3 research papers were published or accepted for publication (Appendix II); Chapter 5 has been submitted for publication. Accordingly, each research chapter is written as a publication complete with introduction, materials and methods, results and discussion. Some minor editing of the chapters has been made to avoid significant repetition; the chapters have also been updated since publication and, in some instances, additional figures have been added. Unavoidable repetition has occurred only as necessary due to the format of the papers.

Animal Ethics

The animal studies (Chapters 3-5) were approved by the Animal Ethics Committees 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 that can be induced by irinotecan, animals were monitored four times daily and if any animal showed certain criteria (as defined by the Animal Ethics Committee) they were euthanised. These criteria included a dull ruffled coat with accompanying dull and sunken eyes, coolness to touch with no spontaneous movement, and a hunched appearance.