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Chemotherapy-induced mucositis: The role of gastrointestinal microflora and mucins in the luminal environment

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Mucositis manifesting as diarrhoea is a common side effect of chemotherapy which remains poorly understood. It is one of a number of manifestations of alimentary mucositis, which affects the entire gastrointestinal tract. The exact number of patients that are affected by diarrhoea as a result of treatment is uncertain, although it is believed that approximately 10% of patients with advanced cancer will be afflicted. Despite advances in the understanding of oral and small intestinal mucositis over recent years, large intestinal mucositis, including diarrhoea, has not been well defined and the underlying mechanisms of the condition are yet to be established. The majority of the literature available concerning diarrhoea is based on clinical observations, with very little basic research existing. However, from the research conducted, it is likely that the intestinal microflora and mucins play a role in the development of chemotherapy-induced diarrhoea. This thesis will examine in detail what is known about the mechanisms of chemotherapy-induced diarrhoea (CID). Furthermore it will explore the potentially important relationship between intestinal microflora, the luminal environment and the subsequent development of chemotherapy-induced mucositis and diarrhoea.

5-Fluorouracil (5-FU) is a commonly used chemotherapy agent in clinical oncology practice. Two of its major side effects are mucositis and diarrhoea. The structure of mucins offers mucosal protection, and allows maintenance of intestinal flora by providing attachment sites and preventing bacterial overgrowth and/or penetration. Following treatment with 5-FU, we showed decreases in *Clostridium spp.*, *Lactobacillus spp.* and *Streptococcus spp.*, and an increase in Escherichia spp. in the jejunum. In the colon, 5-FU caused decreases in *Enterococcus spp.*, *Lactobacillus spp.* and *Streptococcus spp.* Real time PCR of faecal samples showed decreasing trends in *Lactobacillus spp.* and *Bacteroides spp.*, and an increasing trend in *E. coli*. Significant increases ($p<0.05$) were seen in *Clostridium spp.* and *Staphylococcus spp.* at 24 h. Goblet cell numbers decreased significantly in the jejunum from 24-72 h, with a significant increase in the percentage of cavitated goblet cells, suggesting 5-FU

treatment causes significant changes in intestinal flora and mucin secretion in rats. These changes could result in systemic effects, and in particular may contribute to the development of chemotherapy-induced mucositis.

Irinotecan causes cholinergic and delayed onset diarrhoea in patients, in which β-glucuronidase produced by gut bacteria is thought to be involved. Diarrhoea was observed in treated rats, as expected, following irinotecan treatment. β-glucuronidase expression increased in the jejunum and colon. Faecal flora changed quantitatively after treatment also, with increases in *E. coli*, *Staphylococcus spp.*, and *Clostridium spp.* (all β-glucuronidase producing), and decreases in *Lactobacillus spp.*, *Bifidobacterium spp.* (both beneficial bacteria), and *Bacteroides spp.* (β-glucuronidase producing, major component of intestinal flora), suggesting that irinotecan-induced diarrhoea may be caused by an increase in β-glucuronidase producing bacteria. However, the increase in bacteria may also be caused by irinotecan, further exaggerating the toxicity of the drug, and emphasising the need for these specific bacteria to be therapeutically targeted for successful treatment regimens to be accomplished.

Mucus production appears to be increased after irinotecan treatment, which may contribute to the development of diarrhoea. Goblet cells were demonstrated to decrease significantly after irinotecan treatment. However, mucin secretion increased. Mucin expression changed significantly after treatment. Muc2 and Muc4 decreased significantly in the villi of the jejunum after treatment, Muc2 and Muc4 decreased significantly in the crypts. Muc2 decreased significantly in the colon. This indicates that irinotecan causes an increase in mucin secretion and a net decrease in mucin-producing goblet cells, and the expression of Muc2 and Muc4 in the gastrointestinal tract is altered following treatment. Increased mucin secretion is likely to be related to altered mucin expression, and may contribute to chemotherapy-induced diarrhoea.

To determine if the changes to the intestinal microflora caused by chemotherapy could be translated to the clinic, a pilot clinical study was carried out. Sixteen patients experiencing CID were recruited to the study with two control subjects. A large proportion of patients (75%) demonstrated a reduced anaerobic component of their faecal microflora. A reduced diversity of species was also observed in patients. The majority of patients exhibited decreases in *Clostridium spp.*, *Lactobacillus spp.* and *Bifidobacterium spp.*, whilst all patients exhibited decreases in *Bacteroides spp.* and *Enterococcus spp.* Patients receiving antibiotics did not exhibit any marked differences to patients not receiving antibiotics. This indicates that the results observed in the animal studies are clinically relevant, and further research into this area should be undertaken. CID is associated with marked changes in the intestinal microflora. These changes may result in diminished bacterial functions within the gut, altering gut function and initiating intestinal damage, resulting in the onset of diarrhoea.

In conclusion, there is clear evidence demonstrating chemotherapy treatment results in changes to the intestinal microflora and mucin secretion, which may be responsible in part for the development of severe mucositis and diarrhoea. Irinotecan toxicity may be compounded by the increase in β-glucuronidase producing bacteria. The intestinal flora of cancer patients experiencing CID is also noticeably different to that of healthy subjects. Irinotecan causes changes to mucin secretion, and the specific expression of Muc2, Muc4 and Klf4, suggesting that secretory control by the enteric nervous system may also be affected by chemotherapy. This research has extended the understanding of chemotherapy-induced mucositis and diarrhoea, complex side effects of chemotherapy. However, new areas for future research have also been identified.

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Publications arising from this thesis

1. **Stringer AM,** Gibson RJ, Bowen JM, Logan RM, Yeoh, ASJ, Keefe DMK (2007). Chemotherapy-induced mucositis: the role of the gastrointestinal microflora and mucins. *J Support Oncol* **5**(6):259-267.
2. **Stringer AM**, Gibson RJ, Bowen JM, Logan RM, Burns J and Keefe DMK (2007). Chemotherapy-induced diarrhoea is associated with changes in the luminal environment in the DA rat. *Exp Biol Med* **232**(1):96-106.
3. **Stringer AM,** Gibson RJ, Logan RM, Bowen JM, Yeoh ASJ and Keefe DMK (2008). Faecal microflora and β-glucuronidase expression are altered in an irinotecan-induced diarrhoea model in rats. Accepted *Cancer Biol Ther* (September 8, 2008).
4. **Stringer AM,** Gibson RJ, Logan RM, Bowen JM, Laurence J and Keefe DM (2008). Irinotecan-induced mucositis is associated with changes in intestinal mucins. Accepted *Cancer Chemother Pharmacol* (October 11, 2008).

Publications: Submitted for publication or in preparation

1. **Stringer AM,** Gibson RJ, Logan RM, Bowen JM, Yeoh ASJ and Keefe DMK (2008). Gastrointestinal microflora and mucins play a role in the development of 5-Fluorouracil-induced gastrointestinal mucositis in rats. Submitted to *Exp Biol Med*.
2. **Stringer AM,** Gibson RJ, Bowen JM, Ashton K, Logan RM, Al-Dasooqi N, Yeoh ASJ and Keefe DMK (2008). Irinotecan-induced mucositis manifesting as diarrhoea corresponds with an amended intestinal flora and mucin profile. Submitted to *Int J Exp Pathol*.
3. **Stringer AM,** Gibson RJ, Yeoh ASJ and Keefe DMK (2008). Chemotherapy-induced diarrhoea is associated with changes to the microbiome in cancer patients. Submitted to *Int J Cancer.*

Professor Dorothy 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.

Dr Rachel Gibson

Dr Gibson 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 Richard Logan

Dr 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 Joanne 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.

Ms Ann Yeoh

Ms Yeoh is a member of the Mucositis Research Group. She assisted with all of the animal experiments undertaken in this study.

During my candidature, I was involved in several other studies, not presented in this thesis. These have resulted in co-authorship of several other manuscripts. I am first author on an invited review, and have been co-author on several other studies within the laboratory and reviews on a variety of mucositis-related topics.

1. **Stringer AM,** Gibson RJ, Bowen JM and Keefe DMK (2008). Chemotherapy-induced changes to microflora: Evidence and implications of change. *Curr Drug Metab* (invited review).
2. Logan RM, **Stringer AM**, Bowen JM, Gibson RJ, Sonis ST and Keefe DMK (2008). Serum levels of Serum levels of NFkappaB and pro-inflammatory cytokines following administration of mucotoxic drugs. *Cancer Biol Ther* 2008 **7**(7):1139-45.
3. Logan RM, **Stringer AM**, Bowen JM, Gibson RJ, Sonis ST and Keefe DM (2008). Is the pathobiology of chemotherapy-induced alimentary tract mucositis influenced by the type of mucotoxic drug administered? *Cancer Chemother Pharmacol* accepted March 20, 2008 *in press*.
4. Logan RM, Gibson, RJ, Bowen, JM, **Stringer, AM**, Sonis, ST, Keefe, DM (2007). Characterisation of mucosal changes in the alimentary tract following administration of irinotecan: implications for the pathobiology of mucositis. *Cancer Chemother Pharmacol* **62**(1):33-41
5. Yeoh AS, Gibson RJ, Yeoh EE, Bowen JM, **Stringer AM**, Giam KA, Keefe DM (2007). A novel animal model to investigate fractionated radiotherapy-induced alimentary mucositis: the role of apoptosis, p53, nuclear factor-kappaB, COX-1, and COX-2, *Mol Cancer Ther* **6**(8):2319-27.
6. Bowen JM, Gibson RJ, Tsykin A, **Stringer AM**, Logan RM and Keefe DMK (2007). Gene expression analysis of multiple gastrointestinal regions following cytotoxic chemotherapy by oligonucleotide microarrays. *Int J Cancer* **121**(8):1847-56.
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12. Yeoh A, Gibson R, Yeoh E, Bowen J, **Stringer A**, Giam K, Logan R, Keefe D (2006). Radiation therapy-induced mucositis: relationships between fractionated radiation, NF-kappaB, COX-1, and COX-2, *Cancer Treat Rev*. **32**(8):645-51.

This thesis is composed of eight chapters: literature review, six distinct research chapters, followed by general discussion. During the course of my candidature, four chapters were published, with a further three under review at various journals. 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 avoid significant repetition and to include relevant data omitted from the publications. Unavoidable repetition has occurred only as necessary due to the format of the papers.

The animal studies 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.

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.