Probiotic-Derived Factors for the Treatment and Prevention of 5-Fluorouracil-Induced Intestinal Mucositis

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Contents

Declaration

This thesis contains no material that has been accepted for the award of any other 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.

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

Date

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Dedication

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Publications derived from thesis

PRISCIANDARO, L. D., GEIER, M. S., CUMMINS, A.G., BUTLER, R.N., HOWARTH, G.S. 2009. Probiotics and their derivatives as a treatment for inflammatory bowel disease? *Inflammatory Bowel Disease*s, 15(12):1906-14.

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Abstract

5-fluorouracil (5-FU) is one of the most commonly prescribed anti-neoplastic drugs in modern cancer treatment. Although the drug is effective at destroying cancer cells, its administration is accompanied by serious, dose-limiting side effects, amongst the most prevalent of which is intestinal mucositis. This disorder is characterised by ulceration and inflammation of the small intestine, and sufferers often experience severe abdominal pain, nausea and diarrhoea. Despite its predominance, there are currently no definitive treatments for intestinal mucositis.

Probiotics are defined as live bacteria which are able to exert beneficial physiological or therapeutic effects. Strains can be sourced from either food or the human microbiota, but must meet specific requirements prior to being officially recognised as probiotics. The mechanisms of probiotic action are highly species and strain specific, however, a number of strains have been shown to exert beneficial effects which may be suited to the treatment of intestinal mucositis. These include; inhibition of pathogenic bacterial growth and inflammation, maintenance of cell cycling and strengthening of the intestinal barrier. While the majority of probiotic research has focused on the use of live bacteria, there has been a recent interest in bioactive factors that are secreted by the bacterial cells into the cell-free supernatant (SN). There are a range of benefits to using SNs in preference to live bacteria, such as reduced risk of sepsis and greater quality control during production. This thesis represents the first detailed examination into the efficacy of probiotic-based SNs in the treatment of 5-FU-induced intestinal mucositis.

Firstly, four different probiotic SNs were investigated *in vitro* for their ability to maintain cell growth following administration of 5-FU. The two strains deemed most effective where then assessed in an *in vivo* model of intestinal mucositis. Rats were treated with SNs both before and after 5-FU administration. Improvement was reported in some indicators of intestinal damage in rats following SN administration. However, the overall effects were less pronounced than expected, given the extent of improvement reported in the *in vitro* model. These findings suggested that a different screening method was required prior to *in vivo* examination, and that the current *in vivo* treatment protocol required review.

As mucositis occurs only following chemotherapy administration, there is opportunity to administer therapeutic compounds prior to the onset of the disorder with the aim of preventing its development, rather than treating the damage. Two strains, *Lactobacillus rhamnosus* GG (LGG) and *Escherichia coli* Nissle 1917 (EcN), were examined for their ability to prevent 5-FU-induced reduction in intestinal barrier function and increased epithelial cell apoptosis in an *in vitro* model. Both SNs inhibited 5-FU-induced changes to barrier function and apoptosis. The success of these strains in a preventative treatment regime warranted further investigation *in vivo.* However, in the rat model of 5-FU-induced mucositis, no significant protective effects were observed. These findings highlighted inconsistencies between *in vitro* and *in vivo* models. One reason for this disagreement may have been due to the degradation of active compounds during gut transit. In order to determine if acidic or proteinase-rich conditions (two characteristics of the gastric environment) altered the efficacy of LGG and EcN SNs, a small *in vitro* pilot study was performed. All SNs not exposed to either acidic

or proteinase-rich conditions were effective in maintaining cell proliferation following 5-FU administration, but the efficacy of LGG SN was significantly reduced following protease- and acid-treatment. However, neither treatment diminished the efficacy of EcN SN. These results suggested a requirement for new administration techniques to allow the SNs to reach their target area.

In summary, this thesis explores the potential use of probiotic-derived factors to treat 5-FU-induced intestinal mucositis. It describes the capacity for LGG and EcN SNs to improve parameters of chemotherapy-induced damage *in vitro*. These strains were less effective *in vivo*, however, further investigations into effective delivery methods are warranted to ensure that the active compounds reach the small intestine. This thesis provides support for future investigations into the use of probiotic SNs for the treatment of intestinal mucositis.

Abbreviations

5-FU	5-fluorouracil
ANOVA	Analysis of variance
BR11	*Lactobacillus fermentum* BR11
CEC	CytoScan electron carrier
CM	Culture media
DMEM	Dulbecco's modified eagle medium
DPBS	Dulbecco's phosphate buffered saline
DSS	Dextran Sulphate Sodium
EcN	*Escherichia coli* Nissle 1917
EGCG	Epigallocatechin gallate
EGF	Epidermal growth factor
GLP-2	Glucagon-like peptide-2
HID-AB	High iron diamine-alcian blue
IFN	Interferon
IL	Interleukin
JI	Jejunum-ileum junction
LGG	*Lactobacillus rhamnosus* GG
LPS	Lipopolysaccharide
MCP	Monocyte chemotactic protein
MPO	Myeloperoxidase
MRS	de Man rogosa and sharpe
MTX	Methotrexate

NF-κB	Nuclear factor-κB
PAS	Periodic acid-schiff
ROS	Reactive oxygen species
SN	Supernatant
TEER	Transepithelial electrical resistance
TH-4	*Streptococcus thermophilus* TH-4
TNBS	Trinitrobenzene sulphonic acid
TNF	Tumour necrosis factor
TSB	Tryptic soy broth
ZO	Zonula occluden