DETECTION OF SMALL INTESTINAL MUCOSITIS UTILISING THE NON-INVASIVE $^{13}$C-SUCROSE BREATH TEST

BY

KATIE LOUISE TOOLEY

BHSc (Sc Hons)

A thesis submitted for the degree of Doctor of Philosophy

The Centre for Paediatric and Adolescent Gastroenterology
Children, Youth & Women’s Health Services

And

Discipline of Physiology, School of Molecular and Biological Sciences
University of Adelaide, South Australia, Australia.
# TABLE OF CONTENTS

ABSTRACT..............................................................................................................vii

DECLARATION.........................................................................................................ix

ACKNOWLEDGEMENTS.........................................................................................1

LIST OF ABBREVIATIONS.....................................................................................xii

PUBLICATIONS AND PRESENTATIONS...............................................................xv

PART I: INTRODUCTION.........................................................................................1

CHAPTER 1: INTRODUCTION, LITERATURE REVIEW AND AIMS.............................2

1.1 INTRODUCTION...............................................................................................2

1.2 THE SMALL INTESTINE..................................................................................3

1.2.1 Small Intestinal Cell Kinetics.................................................................4

1.2.2 Crypt Cells...............................................................................................6

1.2.3 Cell Death...............................................................................................7

1.3 CHEMOTHERAPY AND THE SMALL INTESTINE............................................7

1.3.1 Anti-metabolite Chemotherapy Agents................................................11

1.3.2 DNA Topoisomerase Inhibitors..............................................................13

1.3.3 Anthracycline Chemotherapy Agents..................................................17

1.3.4 Alkylating Agents..................................................................................18

1.4 METHODS FOR ASSESSING SMALL INTESTINAL FUNCTION....................19

1.4.1 Small Intestinal Permeability.................................................................20

1.4.2 Oro-Caecal Transit Time.......................................................................21

1.4.3 Hydrogen Breath Test...........................................................................23
1.4.4 $^{13}$C-Sucrose Breath Test ........................................ 24

1.5 ANTI-MUCOSITIS THERAPIES ........................................... 28

1.5.1 Growth Factors and Mucositis ........................................ 30

1.5.1.1 Keratinocyte Growth Factor ..................................... 30

1.5.1.2 Insulin-Like Growth Factor-I .................................. 32

1.5.1.3 Epidermal Growth Factor ....................................... 33

1.5.1.4 Whey-Derived Growth Factor Extracts .......................... 33

1.5.2 Glutamine and Mucositis ............................................. 34

1.5.3 Zinc and Mucositis ...................................................... 35

1.5.4 Folinic Acid (Folinate) ................................................ 35

1.6 EMERGING ANTI-MUCOSITIS THERAPIES ............................. 36

1.6.1 Probiotics ................................................................. 36

1.6.2 Lygeinol ................................................................. 39

1.7 SUMMARY ................................................................. 41

1.8 PhD STUDY AIMS: .......................................................... 42

Assessment of the Sucrose Breath Test in rats .......................... 42

Assessment of the Sucrose Breath Test in paediatric cancer patients ... 42

PART II: NON-INVASIVE ASSESSMENT OF SMALL INTESTINAL
FUNCTION IN RATS .......................................................... 43

CHAPTER 2: DETERMINATION OF THE OPTIMAL DOSE OF SUCROSE FOR APPLICATION
OF THE SBT TO THE DARK AGOUTI RAT .............................. 44

2.1 INTRODUCTION ............................................................ 44

2.2 MATERIALS & METHODS ................................................ 45

2.3 RESULTS ................................................................. 51
PART III: SMALL INTESTINAL FUNCTION IN PAEDIATRIC CANCER

PATIENTS ................................................................................................................. 159

CHAPTER 7: ASSESSING SMALL INTESTINAL DAMAGE IN CHILDREN WITH CANCER . 160

7.1 INTRODUCTION ................................................................................................. 160
7.2 PATIENTS & METHODS ..................................................................................... 161
7.3 RESULTS .............................................................................................................. 170
7.4 DISCUSSION ........................................................................................................ 175

PART IV: FINAL CONCLUSIONS ................................................................................. 185

CHAPTER 8: THESIS OUTCOMES, DISCUSSION AND FUTURE DIRECTIONS ............... 186

8.1 INTRODUCTION ................................................................................................. 186
8.2 SPECIFIC OUTCOMES ....................................................................................... 187
8.3 DISCUSSION ........................................................................................................ 189
8.4 CONCLUDING REMARKS .................................................................................... 198

PART V: APPENDICES ................................................................................................ 200

APPENDIX 1: SYNTHETICALLY ENRICHED 13C-SUCROSE AND THE SBT IN A MODEL OF MODERATE CHEMOTHERAPY-INDUCED DAMAGE; A PILOT STUDY ................................................. 201

A1.1 INTRODUCTION ................................................................................................. 201
A1.2 MATERIALS & METHODS .............................................................................. 202
A1.3 RESULTS ........................................................................................................... 204
A1.4 CONCLUSIONS ................................................................................................. 209

APPENDIX 2: THE EFFECT OF MULTIPLE CHEMOTHERAPY REGIMENS ON SMALL INTESTINAL FUNCTION IN CHILDREN UNDERGOING CANCER TREATMENT ................. 210
ABSTRACT

Mucositis is a common side-effect of chemotherapy, which is characterised by ulceration to the epithelium lining the gastrointestinal tract. This epithelium is susceptible to damage due to its rapid cellular turn-over rate and the inability of the treatment to distinguish between tumour and normal tissue. Assessment of small intestinal chemotherapy-induced mucositis has largely been hindered due to the unavailability of a suitable non-invasive diagnostic tool. Recently, a non-invasive breath test, the $^{13}$C-sucrose breath test (SBT), has been developed and applied as a biomarker to detect small intestinal damage associated with methotrexate (MTX)-induced mucositis in rats.

This thesis extended this work, firstly optimising the sucrose dose (0.25 g/mL) for the application of the SBT in the rat versus the previously reported 1.0 g/mL dose. The SBT was shown to provide non-invasive monitoring of the time-course of MTX-induced small intestine damage in rodents, where maxima damage occurred 72 h post-MTX, with repair commencing 96 h post-MTX. In conjunction with biochemical sucrase activity analyses, the SBT quantified the capacity of the small intestine to adapt in response to damage, where ileal sucrase activity was elevated 144 h post-MTX to compensate for damage to the proximal small intestine. This phenomenon may have been due to elevated luminal carbohydrate levels in the ileum due to duodenal damage. The SBT was also successfully applied as a non-invasive biomarker of small intestinal health and integrity in rats receiving chemotherapy with other classes of agents. Here, the SBT demonstrated strong concordance with biochemical sucrase activity. The SBT was also capable of determining the efficacy of Streptococcus thermophalus (TH-4), a
potential probiotic, in attenuating MTX-induced mucositis. Specifically, TH-4 attenuated damage to the proximal jejunum as shown by the SBT, and corroborated by sucrase and myeloperoxidase activities, and histological analyses. In contrast, TH-4 treatment in a tumour-bearing model of MTX-induced mucositis did not protect the small intestine. This may be due to elevated pro-inflammatory circulating cytokines or the dose of TH-4 may need to be increased.

The SBT and the small intestinal permeability (SIP) test were applied in a cohort of paediatric cancer patients undergoing chemotherapy to determine small intestinal function. It was demonstrated that the SBT was a superior biomarker of small intestinal function capable of detecting small intestinal changes in patients diagnosed with mucositis, characterised by a significantly lower SBT, whereas SIP was not significantly altered throughout a cycle of chemotherapy. A small pilot study was performed to determine the capability of the SBT to monitor small intestinal function throughout multiple regimes of chemotherapy, where it was demonstrated that there was a gradual decline in small intestinal integrity due to multiple cycles of chemotherapy.

In conclusion, the non-invasive SBT is a biomarker of small intestinal function that can be applied easily and cost-effectively, in both animals and humans, to monitor gut function in relation to chemotherapy agents and/or potential anti-mucositis treatments. This thesis has illustrated the important potential application of the SBT in the arena of supportive cancer care, where new chemotherapy and anti-mucositis agents can be assessed in relation to small intestinal toxicity.