The aspirin augmented standardized lactulose mannitol test as a measure of the 'health' of the gastrointestinal tract

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

at

MASSEY UNIVERSITY

Palmerston North, New Zealand

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2015

DEDICATED TO

My father, Roosevelt Franklin Sequeira

30.04.1947 – 23.02.2014

For teaching me to never give up.

I miss you every day.

ABSTRACT

In this thesis, I studied the 'classical' lactulose mannitol test for intestinal permeability that has been used to measure the integrity of the intestinal mucosa and thus to provide an index of recovery from inflammatory bowel disease (IBD) and from autoimmune diseases such as coeliac disease. Perusal of the literature indicates that the protocol for the test has not been standardized and a variety of different test protocols have been used. Hence there are differences in the duration of urinary sampling, the doses of the two test probes, the volumes of fluid consumed during the test and the administration of the test during the fasted or fed state. There is therefore a need for a standardized test.

The bulk of the research conducted in this thesis was to develop an optimal protocol with a standardized osmolarity (720 osmol l^{-1}) for the test solution that contained 10 g of lactulose and 5 g of mannitol dissolved in 100 ml of water. Similarly the total fluid intake was standardized to 700 ml. The volumes of fluid consumed over the experimental period were also standardized in order to control for any osmolar effects of the test drink and to hydrate the subjects sufficiently to enable them to produce half-hourly urine samples of a reasonable volume.

The rates of excretion and the timings of the peaks in the excretion of mannitol and lactulose were found to vary over time in healthy subjects. Hence the rate of mannitol excretion peaked during the first two hrs whilst the rate of lactulose excretion peaked at four hrs. The correlation between urinary excretion with intestinal transit times were confirmed using a wireless motility capsule. The work with the wireless motility capsule indicated that the probe sugars were in the small intestine from 2½ - 4 hrs and in the proximal colon from 4½ - 6 hrs following dosage with the test solution. Hence a sample

collected during the 2½ - 4 hr period is best for assessing permeability of the small intestinal mucosa in healthy subjects. The wireless motility capsule also confirmed that the standardized dose of the lactulose mannitol did not influence gastric transit time or that through the small intestine and large intestine. These findings confirmed that the standardized test was determining absorption during transit of the test sugars through the small and the large intestine.

The effect of co-dosage with 600 mg of aspirin in the standardized test was then examined as a means of assessing the effect of a reproducible noxious stimulus on the absorption of the sugar probes. This agent augmented small intestinal permeability to lactulose and decreased its permeability to mannitol. Furthermore dosage with aspirin amplified the effect of a pre-existing adverse stimulus such as smoking. Hence the aspirin augmented test could conceivably be used to 'unearth' sub-clinical inflammation. Further work explored the effect of an antioxidant, ascorbic acid, on mucosal permeability. The results showed that, rather than mitigating the adverse effects of aspirin, ascorbic acid augmented intestinal permeability.

In summary the work in this thesis has enabled the development of a standardized test that optimizes the ability of the lactulose mannitol test to detect clinical disorders of absorption. Further, augmenting the test with a single dose of aspirin may be useful as an index of gut health or robustness.

ACKNOWLEDGEMENTS

'The only limits are, as always, those of vision' - James Broughton

I take this opportunity to present an unedited gratuitous expression to a few people that deserve special mention for their role in the accomplishment of my thesis.

My 'academic parents' here at Massey, Prof. Roger Lentle and Prof. Marlena Kruger, for caring and believing in me even when I doubted myself - your unfailing expectations, unwavering support and guidance helped fuel my determination.

Roger, my 'gastro *guru',* has always believed in my abilities. His passion and enthusiasm was often contagious. In reviewing my work and writing, he offered painstaking comments and always respected my voice. His comments and suggestions often evoked a mixture of gratitude (for the detailed attention to my arguments), exhilaration (as he always dramatically improved it!) and awe ("why didn't I think of that?"). Thank you Roger for 'damaging my brain', for always reiterating 'don't you worry lass' and for the constant kind words that motivated me to do better.

Marlena has been the calming influence throughout my work. Had it not been for the consideration that she gave to my application in 2009, a PhD in New Zealand would not have become a reality. As the voice of reason and a solid sounding board she often came up with issues that I would not have otherwise identified. I express my deep gratitude to her for thinking me worthy of this opportunity, for her encouragement and continuous support.

I gratefully acknowledge Dr. Roger Hurst and the New Zealand Institute for Plant and Food Research for the funding received during the course of this project. Dr. Roger

Hurst's advice and guidance during the project has been invaluable. Routine meetings with both Rogers' in attendance have never been dull!

Chris Booth and Michelle McGrath have been more than lab managers associated with the project, but friends whom I could count on and 'go to' when work got the better of me. Also Corrin, Shampa and others that gathered during morning tea, I appreciate the constant encouragement and healthy doses of caffeine you've shared with me. Dr. Gordon Reynolds, Prof. Patrick Morel and Dr. Fran Wolber, whose brain I picked to often review my writing, thank you for your patience with reading and offering me invaluable advice.

In recognition of the lasting friendships that I have made; Merrin, Kenneth, Zirsha, Jerry, Mel, Samuel, Alex, Julia, Matthew and Wensheng thank you for the immense support, constant encouragement and for being there when I needed to let off steam.

My parents, Roosevelt and Lorena, brother Delano and god father Carl have been my pillars of strength and greatest support. Thank you for nurturing my goals and ambitions and supporting me during my strife. Much love to you.

Thank you all for being a part of my journey. The stress, excitement, hard work, sleepless nights and sheer joy at finally feeling like I have made a 'discovery' is unimaginable.

A special mention to 'Damien', the HPLC system, he was steadfast and rock solid when there were very many samples that required analysis!

To all the participants without whom none of the studies would have been possible, I am very grateful. Approval for all research described in this thesis has been obtained from the Massey University Human Ethics Committee Southern A.

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LIST OF PUBLICATIONS

Published manuscripts

1. Sequeira IR, Lentle RG, Kruger MC, Hurst RD. The effect of aspirin and smoking on urinary excretion profiles of lactulose and mannitol in young women: toward a dynamic, aspirin augmented, test of gut mucosal permeability. Neurogastroenterology & Motility 2012; 24:e401-e11.
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LIST OF CONFERENCE PRESENTATIONS

2012

I. R. Sequeira, R. G. Lentle, M. C. Kruger, R. Hurst. (2012) The effect of aspirin and smoking on excretion of lactulose and mannitol in fit young women: Towards an aspirin augmented test of gut permeability. Oral presentation at the *Human Nutrition and Physiology Symposium* Massey University, Palmerston North New Zealand

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MEDIA ARTICLES

2014

1. Massey research improves gut permeability test.

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4. Using sugars to test gut health.

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ABBREVIATIONS

Å	Angstrom
ADP	Adenosine diphosphate
AJC	Adhesive junctional complex
ANOVA	Analysis of variance
AQP	Aquaporin
ATL	Aspirin triggered lipoxin
ATP	Adenosine tri phosphate
AUC	Area under curve
CLO	Camphylobacter like organism
COX	Cyclooxygenase
Cr-EDTA	Chromium labelled ethylenediamaine tetra-acetic acid
CV	Coefficient of variation
DC	Dendritic cell
DPPC	Dipalmitoyl-phosphotidyl choline
DHA	Dehydroascorbic acid
ELISA	Enzyme linked immunosorbent assay
FABP	Fatty acid binding protein
FOS	Fructo-oligosaccharide
FTIR	Fourier transform infrared spectroscopy
GALT	Gut associated lymphoid tissue
GHP	Glutathione peroxidise
GI	Gastrointestinal
GLUT	Glucose transporter
GOS	Galacto-oligosaccharide
GST	Glutathione s-transferase
HETE	Hydroxyeicosatetraenoic acid
HPLC	High performance liquid chromatography
IBD	Inflammatory bowel disease

IBS-D	Irritable bowel syndrome with diarrhoea
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
iNOS	Inducible nitric oxide synthase
JAM	Junctional adhesion molecules
LAL	Limulus amebocyte lysate
LI	Large intestine
LMR	Lactulose mannitol ratio
LOD	Limit of detection
LPS	Lipopolysaccharide
LTB	Leukotriene B
MAPK	Mitogen activated phospho/protein kinase
MCT	Monocarboxylic acid transport
MD	Molecular dynamic
MLCK	Myosin light chain kinase
MS	Mass spectrometry
NFκB	Nuclear factor kappa B
NHE3	Sodium-hydrogen antiporter 3
NO	Nitric oxide
NOD	Nucleotide-binding oligomerization domain receptors
NSAID	Non-steroidal anti-inflammatory drug
O/E	Observed to expected
OTC	Over the counter
PC	Phosphatidylcholine
PDA	Photodiode array
PEG	Polyethylene glycol
PG	Prostaglandin
PGE2	Prostaglandin E2

PGHS	Prostaglandin endoperoxidase synthase
PKC	Protein kinase C
PRR	Pattern recognition receptor
RID	Refractive index detector
RMA	Reduced major axis
ROS	Reactive oxygen species
SD	Standard deviation
SE	Standard error
SGLT1	Sodium glucose transporter 1
SI	Small intestine
SIBO	Small intestinal bacterial overgrowth
SLR	Simple linear regression
SOD	Superoxide dismutase
SVCT	Sodium dependent secondary active transport
Tc-DPTA	Technetium-99-labelled diethylenediaminepeptolytic acid
TEER	Transepithelial electrical resistance
TJ	Tight junction
TLC	Thin layer chromatography
TLR	Toll-like receptors
TNF-α	Tumour necrosis factor α
UC	Ulcerative Colitis
UV	Ultraviolet
UWL	Unstirred water layer
WHO	World Health Organization
ZO	Zona occludins

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