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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


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.

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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
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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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5. Sequeira IR, Lentle RG, Kruger MC, Hurst RD. Ascorbic acid may exacerbate aspirin induced increase in intestinal permeability. Basic and Clinical Pharmacology and Toxicology 2015. doi: 10.1111/bcpt.12388

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2. Doctoral student gains international recognition.
   Manawatu Standard. February 25th 2014

3. Contains sweeteners.
   Massey defining NZ. July 14th 2014
   (http://definingnz.com/contains-sweeteners)

   Our Changing World. Radio NZ.
   (http://www.radionz.co.nz/national/programmes/ourchangingworld#audio-20161296)
## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AJC</td>
<td>Adhesive junctional complex</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AQP</td>
<td>Aquaporin</td>
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<tr>
<td>ATL</td>
<td>Aspirin triggered lipoxin</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine tri phosphate</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under curve</td>
</tr>
<tr>
<td>CLO</td>
<td>Camphylobacter like organism</td>
</tr>
<tr>
<td>COX</td>
<td>Cycloxygenase</td>
</tr>
<tr>
<td>Cr-EDTA</td>
<td>Chromium labelled ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cell</td>
</tr>
<tr>
<td>DPPC</td>
<td>Dipalmitoyl-phosphotidyl choline</td>
</tr>
<tr>
<td>DHA</td>
<td>Dehydroascorbic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>FABP</td>
<td>Fatty acid binding protein</td>
</tr>
<tr>
<td>FOS</td>
<td>Fructo-oligosaccharide</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>GALT</td>
<td>Gut associated lymphoid tissue</td>
</tr>
<tr>
<td>GHP</td>
<td>Glutathione peroxidise</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GLUT</td>
<td>Glucose transporter</td>
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<td>GOS</td>
<td>Galacto-oligosaccharide</td>
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<tr>
<td>GST</td>
<td>Glutathione s-transferase</td>
</tr>
<tr>
<td>HETE</td>
<td>Hydroxyeicosatetraenoic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<tr>
<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
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<tr>
<td>IBS-D</td>
<td>Irritable bowel syndrome with diarrhoea</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>Ig</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>JAM</td>
<td>Junctional adhesion molecules</td>
</tr>
<tr>
<td>LAL</td>
<td>Limulus amebocyte lysate</td>
</tr>
<tr>
<td>LI</td>
<td>Large intestine</td>
</tr>
<tr>
<td>LMR</td>
<td>Lactulose mannitol ratio</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LTB</td>
<td>Leukotriene B</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen activated phospho/protein kinase</td>
</tr>
<tr>
<td>MCT</td>
<td>Monocarboxylic acid transport</td>
</tr>
<tr>
<td>MD</td>
<td>Molecular dynamic</td>
</tr>
<tr>
<td>MLCK</td>
<td>Myosin light chain kinase</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>NFκB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>NHE3</td>
<td>Sodium-hydrogen antiporter 3</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOD</td>
<td>Nucleotide-binding oligomerization domain receptors</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>O/E</td>
<td>Observed to expected</td>
</tr>
<tr>
<td>OTC</td>
<td>Over the counter</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphatidylcholine</td>
</tr>
<tr>
<td>PDA</td>
<td>Photodiode array</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PG</td>
<td>Prostaglandin</td>
</tr>
<tr>
<td>PGE2</td>
<td>Prostaglandin E2</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PGHS</td>
<td>Prostaglandin endoperoxidase synthase</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PRR</td>
<td>Pattern recognition receptor</td>
</tr>
<tr>
<td>RID</td>
<td>Refractive index detector</td>
</tr>
<tr>
<td>RMA</td>
<td>Reduced major axis</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
<tr>
<td>SGLT1</td>
<td>Sodium glucose transporter 1</td>
</tr>
<tr>
<td>SI</td>
<td>Small intestine</td>
</tr>
<tr>
<td>SIBO</td>
<td>Small intestinal bacterial overgrowth</td>
</tr>
<tr>
<td>SLR</td>
<td>Simple linear regression</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>SVCT</td>
<td>Sodium dependent secondary active transport</td>
</tr>
<tr>
<td>Tc-DPTA</td>
<td>Technetium-99-labelled diethylenediaminepeptolytic acid</td>
</tr>
<tr>
<td>TEER</td>
<td>Transepithelial electrical resistance</td>
</tr>
<tr>
<td>TJ</td>
<td>Tight junction</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptors</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor α</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative Colitis</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>UWL</td>
<td>Unstirred water layer</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>ZO</td>
<td>Zona occludins</td>
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