

Application of Fluorescent Tracer Agent Technology to Point-of-Care Gastrointestinal Permeability Measurement

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ABSTRACT

Gut dysfunction, often accompanied by increased mucosal permeability to gut contents, frequently accompanies a variety of human intestinal inflammatory conditions. These disorders include inflammatory bowel diseases (e.g., Crohn's Disease) and environmental enteropathy and enteric dysfunction, a condition strongly associated with childhood malnutrition and stunting in resource poor areas of the world. The most widely used diagnostic assay for gastrointestinal permeability is the lactulose to mannitol ratio (L:M) measurement. These sugars are administered orally, differentially absorbed by the gut, and then cleared from the body by glomerular filtration in the kidney. The amount of each sugar excreted in the urine is measured. The larger sugar, lactulose, is minimally absorbed through a healthy gut. The smaller sugar, mannitol, in contrast, is readily absorbed through both a healthy and injured gut. Thus a higher ratio of lactulose to mannitol reflects increased intestinal permeability. However, several issues prevent widespread use of the L:M ratio in clinical practice. Urine needs to be collected over time intervals of several hours, the specimen then needs to be transported to an analytical laboratory, and sophisticated equipment is required to measure the concentration of each sugar in the urine.

In this presentation we show that fluorescent tracer agents with molecular weights similar to those of the sugars, selected from our portfolio of biocompatible renally cleared fluorophores, mimic the L:M ratio test for gut permeability. This fluorescent tracer agent detection technology can be used to overcome the limitations of the L:M assay, and is amenable to point-of-care clinical use.

- **Keywords:** Fluorescent tracer agent, optical monitoring, gastrointestinal permeability, enteropathy, lactulose to mannitol ratio measurement, pyrazine, fluorescence

1. INTRODUCTION

Gastrointestinal Permeability (GIP) is a leading indicator of gut inflammatory disease presence and activity, and has been particularly useful in screening for, and monitoring, Crohn's Disease (CD). The utility of evaluating GIP in cases of Ulcerative Colitis (UC) is also emerging. The implications of earlier detection and proactive disease management in both of these illnesses ("treat to target") are substantial.¹ However, current disease monitoring strategies for inflammatory bowel disease control are expensive (colonoscopies), or are cumbersome (usually involving stool tests).

The prevalence of CD and UC (collectively comprising Inflammatory Bowel Disease (IBD)) in the US is estimated to affect 1.2 million people. Estimated total annual direct economic burden of CD and UC in the US is at least \$6.3 billion. In addition the indirect costs of CD and UC combined have been estimated at \$3.6 billion in lost productivity. In the developing world gut inflammation (generally referred to as Tropical or Environmental Enteropathy (EE)) is an important contributor to malnutrition and stunting.

The most widely used GIP test entails determination of the Lactulose to Mannitol ratio (L:M). Sugars (i.e., lactulose and mannitol) are administered orally, differentially absorbed through the gut, and then cleared from the body by glomerular filtration in the kidney. The amount of each sugar excreted in the urine is measured. The larger sugar, lactulose, is

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minimally absorbed through a healthy gut. The smaller sugar, mannitol, in contrast, is readily absorbed through both a healthy and injured gut. Thus a higher L: M ratio is an indication of higher GIP.² However, several issues prevent widespread use of the L:M technique for clinical practice. Urine needs to be collected over time intervals of several hours, the urine needs to be transported to an analytical laboratory, and sophisticated equipment needs to be employed to measure each sugar concentration. In addition, the sugars can be degraded post-collection but pre-analysis, and presence of mannitol in the body from food confound the results.

To overcome the deficiencies of the dual sugar ratio test, we queried our portfolio of biocompatible fluorescent tracer agents that are also cleared from the body via glomerular filtration. For example, our compound MB-102 is currently in clinical trials as a point-of-care glomerular filtration agent a fluorescent tracer.^{3,4} Indeed we have a fluorophore of similar molecular weight as mannitol with fluorescence emission at one wavelength (MB-301), and have a fluorophore of similar molecular weight as lactulose with fluorescence emission at a different wavelength (MB-402). Thus the hypothesis was that upon oral administration of both fluorophores, a measurement of the ratio of the fluorescence emissions would yield similar information as the dual sugar test, but in real time at the point-of-care, thus overcoming the negative aspects of the dual sugar test.

2. MATERIALS AND METHODS

2.1. Fluorescent Tracer Agents.

MB-301 and MB-402 are fluorescent compounds belonging to the general class of compounds known as pyrazines. The chemical structures are shown in Figure 1. The chemical name of MB-301 is 3,6-diaminopyrazine-2,5-dicarboxylic acid. MB-301 has a molecular weight of 198, with light absorption and emission maxima at 405 nm and 540 nm, respectively. The chemical name of MB-402 is 3,6-bis((2,3-dihydroxypropyl)amino)pyrazine-2,5-dicarboxamide. MB-402 has a molecular weight of 422, with light absorption and emission maxima at 500 nm and 620 nm, respectively.

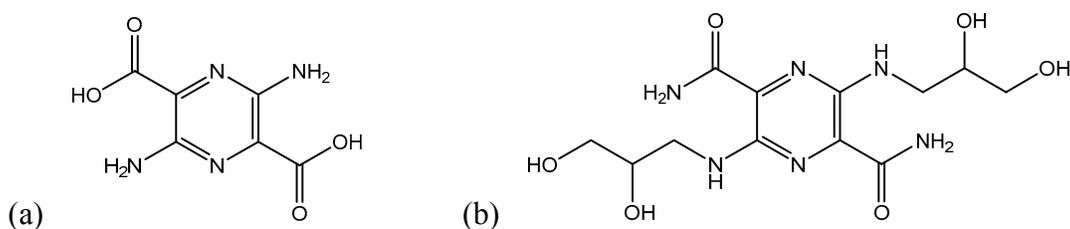


Figure 1. (a) Structure of MB-301, (b) Structure of MB-402

2.2. Animal Model.

The model consisted of exclusively female Sprague-Dawley rats of approximate weight ~235 g. Induced gut dysfunction was by administration of indomethacin at 20mg/kg, 18-20 hours prior to the start of an experiment. These are designated “treated” or “challenged” rats. “Control” rats were not given indomethacin. Chow but not water was withheld prior to experiment.

2.3. Procedure.

The animals are anesthetized with isoflurane and a catheter is inserted in the bladder. The MB-301 solution was prepared at 8 mg/mL in DMSO/water, and the MB-402 solution was prepared at 32 mg/mL also in DMSO/water. The solutions are combined and 1 mL of the combined solution was gavage-administered via feeding tube to the rats. Urine was collected at time intervals of 30, 60, 120, 180, 240, 300, 360, 420, and 480 minutes oral post-administration. Urine samples were frozen upon collection, transported to our analytical laboratory and concentration of each fluorophore determined by HPLC analysis.

3. RESULTS

3.1 Experiment 1

Figure 2 contains the data from the first experiment comparing results from a treated rat with that of a control rat. As the oral dose eventually moves into the gut and is absorbed, the amount of the higher molecular weight fluorophore (MB-402) with respect to that of the lower molecular weight fluorophore (MB-301) grows in the treated rat but not in the control rat. This indicates that the gut is more permeability in the treated rat with respect to the control rat. Thus this methodology does in fact mimic the dual sugar test.

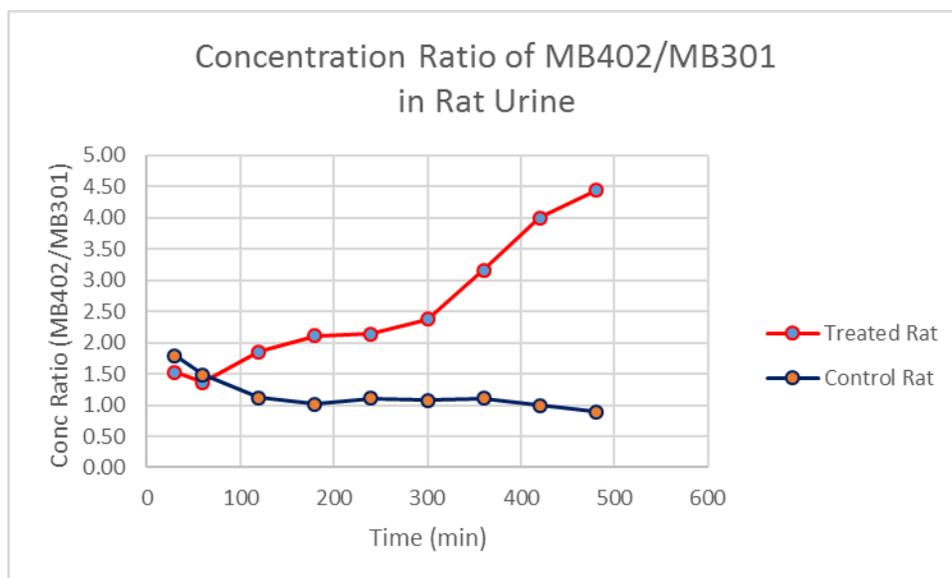


Figure 2. Ratio of the measured concentrations in urine of MB-402 to MB-301. The circles are the measured concentration ratio of MB-402 to MB-302 at each time point and the line is a guide for the eye.

3.2 Experiment 2

A repeat of the above experiment was performed employing 2 treated animals and 2 control animals. The results are shown in Figure 3. Again, we see the same distinctive signature. The treated animals have a porous gut and the higher molecular weight fluorophore passes through and appears in the urine at a greater ratio with respect to the lower molecular weight fluorophore than in the control animals.

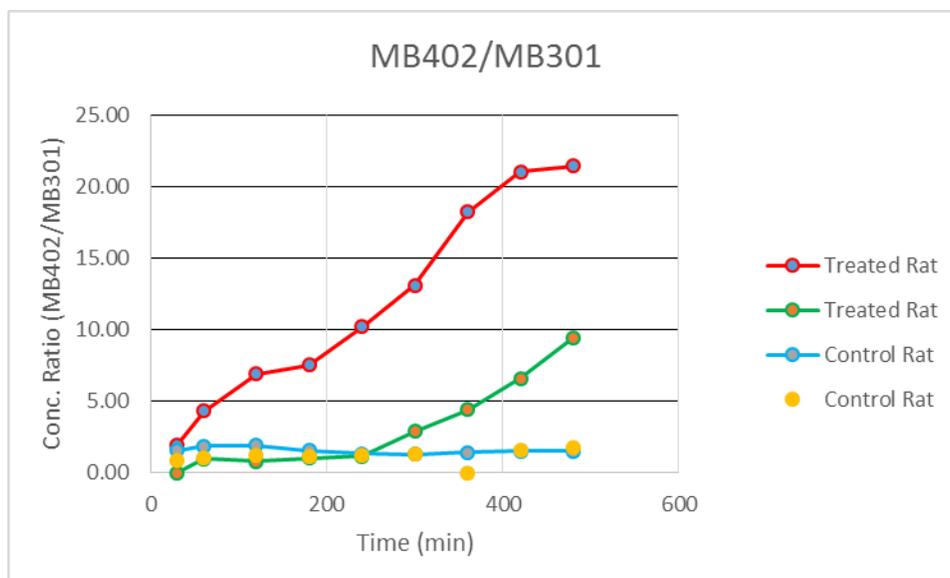


Figure 3. Ratio of the measured concentrations in urine of MB-402 to MB-301. The circles are the measured concentration ratio of MB-402 to MB-302 at each time point and the line is a guide for the eye.

4. DISCUSSION

Oral administration of our renally clearing fluorophores results in their appearance and quantitative detectability in the urine. Results shown herein mimic the standard, but not clinically amenable, dual sugar test for gut permeability determination. Our next step will be to use our transdermal fluorescence detection instrumentation to simultaneously measure both fluorophores in vivo and thus have a true point-of-care gut permeability measurement without the collection of bodily fluid samples and subsequent laboratory analysis.

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