Measurement of gut permeability using fluorescent tracer agent technology

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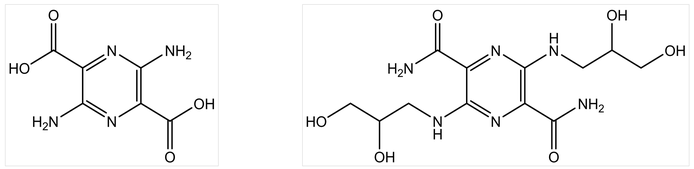
ABSTRACT

Background: The healthy gut restricts molecular and bacterial movement across tight junctions. Dual sugar absorption tests are commonly used to measure permeability, but have technical challenges. We evaluated orally-administered fluorescent tracers to measure gut mucosal integrity, because these agents are amenable to specimen-free evaluation of mucosal integrity. **Methods:** After challenging rats with increasing doses of indomethacin, we measured urinary ratios of orally-administered fluorescent tracers MB-402 and MB-301, or of lactulose (L) and rhamnose (R). We also tested intravenous (IV) fluorophore clearance, and transcutaneous readouts. **Results:** Urinary MB-402:MB-301 and L:R ratios reflect gut injury proportional the challenge dose. The fluorophores generated smooth curvilinear trajectories with wide dynamic ranges. Urinary L:R ratios were chaotic, and had narrower dynamic ranges. The urinary clearance of IV-administered fluorescent tracers was similar in challenged and control rats. Transcutaneously measured fluorophore ratios distinguished challenged and control rats.
Conclusion: Orally-administered fluorescent tracers detect small bowel injury and generate a dose-response dynamic range suitable for intervention studies. They can also be measured transcutaneously, obviating drawbacks of dual sugar absorption tests.

INTRODUCTION

A cardinal function of the gut is to maintain tight junction integrity between epithelial cells. Increased gut permeability accompanies the many disorders with morphologic injury to cells (celiac disease [1], Crohn's Disease, and childhood enteropathy). In humans, dual sugar absorption tests (DSATs) are most commonly used to assess gut permeability. DSATs compare urinary excretion of orally ingested sugars, generally a disaccharide (L (MW = 342)), and a monosaccharide, usually mannitol (M) (MW=182) or R (MW=164).

DSATs have many technical limitations, including those related to timing of collection, bacterial and fecal contamination, and assay availability[2]. Fluorophores MB-402 (MW=422) and MB-301 (MW=198) are pyrazine analogs that have excretion properties similar to those of L, M, and R, and differentiating incident and emission wavelengths, so circulating ratios can be measured through intact skin (Fig 1). We sought to determine if, in a rat model of small bowel injury, fluorescent and sugar tracers perform equivalently.



MB-301, MW 198, light abs max 405λ MB-402, MW422, light abs max 500λ

Figure 1. Properties of fluorescent tracers.

MATERIALS AND METHODS

245 g female S-D rats are challenged with high (15 mg/kg), intermediate (10 mg/kg) or low (5 mg/kg) dose indomethacin in 2% methylcellulose, vehicle alone. The rats were anesthetized 18-20 hr later, and their bladders catheterized. MB-402 (10.67 mg/kg) and MB-301 (2.67 mg/kg), or L (50 mg/kg) and R (12.5 mg/kg) were gavage-administered, and urines collected over 8hr. Indomethacin-challenged and control rats were also tested with IV MB-402 (4 mg/kg) and MB-301 (1 mg/kg). We also transcutaneously measured the circulating fluorophore ratios. Urinary tracer concentrations are determined by normal phase isocratic HPLC-tandem ESI mass spectrometry (L, R)[3], or a Waters Alliance 2695 HPLC system or Acquity H Class UPLC system (MB-301, MB-402)[4]. At experiment end, animals are euthanized and necropsied. Tissues are fixed and stained.

REFERENCES

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RESULTS

Indomethacin causes pan-small bowel transmural and histologic injury proportional to dosing (Fig 2). The median MB-402:MB301 ratios followed smooth curvilinear upward trajectories during the 8 hr of sampling, across all dosing levels, and in proportion to the dose of the indomethacin administered. L:R trajectories were more chaotic. For L:R, the 8 hr median ratios were 1.6-, 2.5-, and 6.7-fold greater than in controls after low, intermediate- and high-dose indomethacin, respectively; the corresponding ratios for the fluorescent tracers were 4.1-, 9.0-, and 30.0–fold (Fig 3). Indomethacin-challenged and control rats cleared IV-administered fluorophores similarly (Fig 4), suggesting the differential ratios in Fig 1 are related to intestinal factors. Finally, the ratios of the peak fluorescence emissions determined by transcutaneous sensors categorically distinguished challenge and control rats (Fig 5).

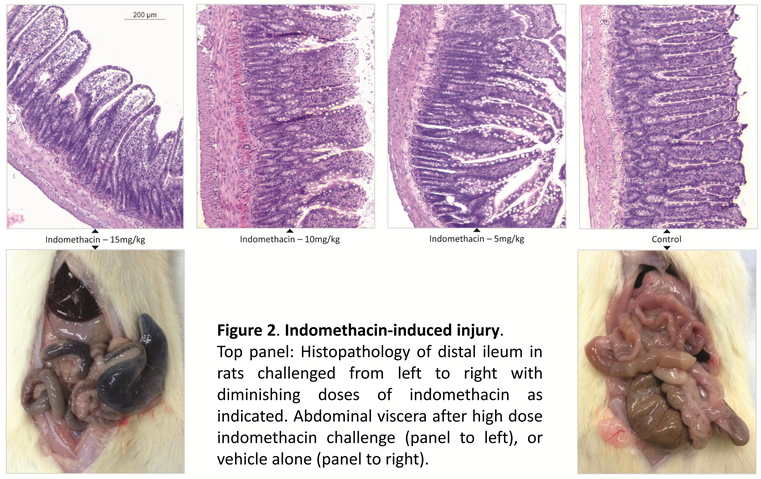

Figure 2. Indomethacin-induced injury. Top panel: Histopathology of distal ileum in rats challenged from left to right with diminishing doses of indomethacin as indicated. Abdominal viscera after high dose indomethacin challenge (panel to left), or vehicle alone (panel to right).

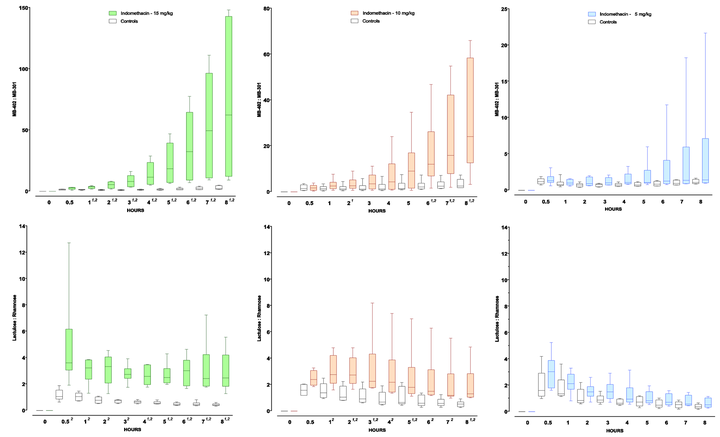

Figure 3. Urinary ratios of gavaged tracers. Ratios of tracers (Y-axes, as indicated) in urines are plotted against time after gavage (X-axes). Box plots portray medians and IQRs for each point, for controls (open boxes) and rats challenged with increasing doses of indomethacin (colored boxes). [a]=p<0.05 challenged rats vs. rats challenged with low-dose indomethacin; [b]=p<0.05 challenged rats vs. controls.

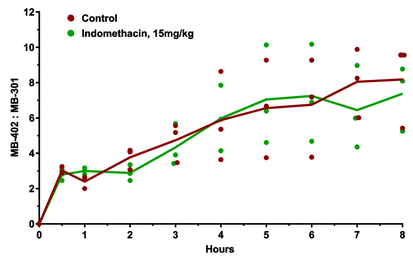

Figure 4. Urinary ratios of IV-administered tracers. Ratios of urinary tracers following IV injection of M-402 and MB-301in challenged (●) and control (●) rats (3 rats each). Lines = means, dots = individual measurements.

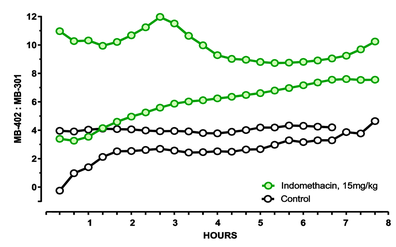

Figure 5. Continuous transcutaneous tracing of orally administered fluorescent tracers. MB-402:MB-301 ratios (Y-axis) were determined transcutaneously over time after fluorophore gavage in two rats challenged with indomethacin (15 mg/kg) (o) and two rats challenged with vehicle alone (o).

DISCUSSION

Pyrazine-based fluorophores reflect intestinal injury better than DSATs. Most notably, 8 hrs after tracer administration, the MB-402:MB-301 ratios present a more robust dynamic range than do the L:R ratios. These greater relative differences, and the smoother curves generated, endorse fluorescent tracers for testing interventions to improve tight junction integrity. Moreover, transcutaneous monitoring provides real-time data and obviates taking possession of, preserving, transporting, and analyzing urines. Finally, fluorophores with MWs > than that of MB-402 could be adapted to measure gut permeability across a gradient of injury severity. IV fluorophore MB-102 is under study in humans to measure glomerular filtration [21], so using this technology should be feasible for testing gut permeability.

In summary, enterally-administered fluorophores reflect gut injury in a dose-dependent manner in a rat model. Tracer ratios in blood can be determined transcutaneously. If the wide dynamic dose-response range reflects graded severity of human gut disorders, these fluorophores could enable broader clinical use of permeability assessments, to detect, monitor, and repair barrier defects. We are now adapting this specimen-free test to measure intestinal permeability in additional models, and in humans with disorders of gut function and inflammation.

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