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) fluorescent 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 fluorescent 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. 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.

RESULTS

Indomethacin causes pan-small bowel transmural and histologic injury proportional to dosing (Fig 2). The median MB-402:MB-301 ratios followed smooth curvilinear trajectories over 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).

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 impede gut damage and inflammation. 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, 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.

REFERENCES


MATERIALS AND METHODS

245 rats were randomized into 6 groups: sham, MB-402 (20 mg/kg), MB-301 (20 mg/kg), MB-402 (25 mg/kg), MB-301 (25 mg/kg), MB-402 (30 mg/kg), MB-301 (30 mg/kg). Animals were euthanized and necropsied. Tissues were fixed and stained.

REFERENCES