Assessment of Intestinal Permeability and Absorption in Cirrhotic Patients with Ascites Using Combined Sugar Probes

MARC J. ZUCKERMAN, MD,* IAN S. MENZIES, FRC PATH,† HOI HO, MD,* GAVIN G. GREGORY, PhD,‡ NANCY A. CASNER, MS,* ROGER S. CRANE, FIBMS,† and JESUS A. HERNANDEZ, MD*

Gastrointestinal dysfunction in patients with cirrhosis may contribute to complications such as malnutrition and spontaneous bacterial peritonitis. To determine whether cirrhotic patients with ascites have altered intestinal function, we compared intestinal permeability and absorption in patients with liver disease and normal subjects. Intestinal permeability and absorption were investigated in 66 cirrhotic patients (48 with ascites, 18 without ascites) and 74 healthy control subjects. Timed recovery of 3-O-methyl-D-glucose, D-xylose, L-rhamnose, and lactulose in urine following oral administration was measured in order to assess active and passive carrier-mediated, and nonmediated, absorptive capacity, as well as intestinal large-pore/small-pore (lactulose/rhamnose) permeability. Test sugars were measured by quantitative thin-layer chromatography and results are expressed as a percentage of test dose recovered in a 5-h urine collection. Sugar excretion ratios relating to small intestinal permeability (lactulose/rhamnose) and absorption (rhamnose/3-O-methyl-D-glucose) were calculated to avoid the effects of nonmucosal factors such as renal clearance, portal hypertension, and ascites on the recovery of sugar probes in urine. Compared with normal subjects, the mean lactulose/rhamnose permeability ratio in cirrhotic patients with ascites was significantly higher (0.058 vs. 0.037, \( P < 0.001 \)) but not in cirrhotic patients without ascites (0.041 vs. 0.037). Cirrhotic patients with ascites had significantly lower mean recoveries of 3-O-methyl-D-glucose (23.0 vs. 49.1%; \( P < 0.001 \)), D-xylose (18.8 vs. 34.5%; \( P < 0.001 \)), L-rhamnose (4.0 vs. 9.1%; \( P < 0.001 \)), and lactulose (0.202 vs. 0.337%; \( P < 0.001 \)) than normal subjects. However, the mean rhamnose/3-O-methyl-D-glucose ratio was the same in cirrhotic patients with ascites as normal subjects (0.189 vs. 0.189), indicating that the reduction in probe recovery was due to nonmucosal factors. Compared with normal subjects, cirrhotic patients with ascites have abnormal intestinal permeability, measured by urinary lactulose/rhamnose excretion, and normal small intestinal absorption, assessed by the urinary rhamnose/3-O-methyl-D-glucose ratio. Low urine recovery of sugar probes found in cirrhotic patients appears to be the result of nonintestinal factors affecting clearance rather than reduced intestinal absorption.

KEY WORDS: cirrhosis; intestinal absorption; intestinal permeability; lactulose/rhamnose ratio; spontaneous bacterial peritonitis.
in the pathogenesis of spontaneous infections in cirrhotics (1–3, 4).

Noninvasive methods have been used to assess the barrier function of the intestine by measuring the urinary excretion of orally administered test substances (5–7). Recovery in urine of single probes such as $^{51}$Cr-EDTA and D-xylene, which have been used to determine intestinal permeability and absorptive capacity in patients with cirrhosis, may also be influenced by gastric emptying, small intestinal bacterial overgrowth and, especially, by portal hypertension and the effect of increased extracellular volume (ascites, edema) upon renal clearance (8–15). Errors introduced by such unwanted “nonintestinal” factors can be minimized by using tests employing the principle of “differential absorption.” Such tests depend upon simultaneous urinary excretion of a combination of ingested sugar probes absorbed by different pathways of mucosal transport and nonmediated permeation, results being expressed as “excretion ratios” which are unaffected by the nonintestinal factors mentioned above (5–7).

Intestinal permeability and absorptive capacity can be assessed together using a quadruple test solution containing lactulose (Lac), L-rhamnose (Rham), 3-O-methyl-D-glucosamine (3mGlc), and D-xylene (Xyl). Lac and Xyl have affinity for specific intestinal transport systems, 3mGlc being very efficiently absorbed and fully excreted in the urine, while absorption of Xyl is less efficient and excretion incomplete, being subject to partial metabolism in the liver. Absorption of the monosaccharide Rham is mainly by permeation through small aqueous pores of high incidence, and uptake of the disaccharide Lac is restricted to aqueous pores of larger (“macromolecular”) dimension but much lower incidence in the intestinal mucosa. Neither Lac nor Rham has any affinity for carrier-mediated transport, and both are fully excreted in the urine following absorption, without significant metabolic loss (7). Disease states (e.g., Crohn’s disease and celiac disease) associated with injury of the small intestinal mucosa have abnormal mucosal permeability characterized by increased disaccharide/monosaccharide (Lac/Rham) permeation (6), and the urinary Rham/3mGlc excretion ratio relates to small intestinal absorption (5, 7, 16, 17).

Reports of simultaneous investigation of intestinal permeability and absorption in cirrhotics are few (18, 19), especially in cirrhotics with ascites, who are of particular interest due to their predisposition to spontaneous bacterial peritonitis (SBP).

**METHODS**

**Study Population.** The test solution was administered to 66 successive patients with cirrhosis admitted to Thomas General Hospital or seen at Texas Tech Medical Clinic in El Paso. All patients had either biopsy-proven cirrhosis or a clinical picture, with abnormal liver–spleen scan with colloid shift, consistent with cirrhosis. There were 48 cirrhotic patients with ascites and 18 cirrhotic patients without ascites. Severity of liver disease was determined according to Child–Pugh criteria. Diagnosis of spontaneous bacterial peritonitis was made on the basis of a polymorphonuclear cell count in ascitic fluid >250 cells/mm$^3$ with no apparent source of infection (20). Testing was done concurrently in a comparison group of 74 normal volunteers (21). Subjects with known gastrointestinal or renal disease or diabetes mellitus were excluded. Also excluded were patients receiving substances known to affect intestinal permeability test results (6) such as lactulose, nonsteroidal antiinflammatory drugs, or alcohol, in the previous 2 weeks.

The study was approved by the Texas Tech University Health Sciences Center Institutional Review Board in El Paso. All subjects in the study gave informed consent.

**Intestinal Permeability and Absorption Test Procedure.** The test sugars were prepared as a concentrated syrup (>2000 mosmol/kg) which resists bacterial degradation at room temperature. Immediately before use, 10 ml of this syrup, containing a single dose, viz., 0.2 g 3mGlc, 0.5 g Xyl, 1.0 g Rham, (Sigma Chemical Co.), Poole, Dorset, UK, and Lac, 5.0 g, (>7.5 ml lactulose syrup, 679, w/v, Duphar Laboratories Ltd., West End, Southhampton, UK), was dispensed with a syringe and diluted to 100 ml with drinking water to give an approximately iso-osmolar solution (300 mosmol/kg). Medications were excluded from 12 h before until the end of each test, food and nutritious drinks being excluded from 4 h before until 2 h after taking the test solution. After an overnight fast and bladder emptying, the patient ingested the test solution and then made a complete 5-h urine collection into a bottle containing preservative. The volume of each 5-h urine collection was recorded and a 10-ml aliquot, preserved with thiomersal (>10 mg/100 ml) (22) was sent for sugar analysis to St. Thomas Hospital, London, where they were processed within 4 months using the same technique undertaken or supervised by the same person (Dr. I. S. Menzies).

Analytical Methods. Test sugars in urine were measured by quantitative thin-layer chromatography using a modification of a previously described method (7, 25). Following development, and a 4-amino salicylic acid/orthophosphoric acid color reaction, scanning densitometry was undertaken employing either a Chromscan (Joyce-Loebl & Co., Durham) or a Bio Rad Model G57670 Molecular Analytix (Bio Rad Systems, USA). Sugar concentrations were derived by comparing the optic densities of test zones with a standard concentration curve plotted from standard zones run in the same chromatogram. Results are expressed as percentages of test dose recovered in each 5-h urine collection.

**Statistics.** Results in each group are expressed as means ± standard error. Analyses of mean excretion of sugars and mean Lac/Rham ratios between groups were done using the Kruskal–Wallis nonparametric one-way ANOVA, with subsequent multiple comparisons using the Bonferonni method. Pairwise correlations were assessed by the Kendall rank correlation statistic. Analyses were performed using the BMDP software (BMDP Statistical Software, Inc., Los Angeles, CA). Statistical significance was set at $P < 0.05$ (two-tailed tests).