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Increased Intestinal Permeability in Patients with Inflammatory Bowel Disease

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Abstract: Intestinal permeability can be measured by the sugar absorption test. This test is based on determining the ratio of the urinary excretion of a large and a small carbohydrate after oral administration. The aim of this study was to determine which combination of carbohydrates used in the test gives the highest correlation with disease activity in inflammatory bowel disease. 26 patients with Crohn`s disease, 21 patients with ulcerative colitis and 27 healthy control subjects were included in the study. The patients with inflammatory bowel disease had either minimal or highly active disease or were in remission. Two disaccharides (lactulose: L, and cellobiose: C) and two smaller carbohydrates (rhamnose:R, and mannitol:M) were given orally and the urinary excretion was measured by high pressure liquid chromatography followed by pulsed amperometric electrochemical detection on a gold electrode. The ratios C/R, L/R, C/M and L/M were used as indicators for intestinal permeability.

There were no side effects of oral sugar administration. All patients tolerated the test well. Lactulose, rhamnose and cellobiose concentrations are easily be measured in the urine whereas mannitol measurement requires the use of an anion exchanger. This produced inconsistent results. Patients with Crohn`s disease or with ulcerative colitis had increased permeability indices in comparison to healthy controls, even in remission. The L/R ratio gave a better differentiation between the healthy controls and patients with active disease than the other agents. Changes in disease activity are best reflected by use of cellobiose/rhamnose excretion quotient.

Key words: Inflammatory bowel disease, intestinal permeability, Crohn´s disease, ulcerative colitis

Introduction

The epithelium of the small and large intestine is a selective barrier to the absorption of potentially harmful bowel contents. However, this barrier is incomplete and it enables small molecules to diffuse into the intercellular space. In patients with Crohn's Disease (CD), this barrier function is disturbed resulting in increased permeability. This increase in permeability is found in patients with active inflammatory bowel disease (IBD) and there is evidence that it does not return to normal when the disease is in remission. A number of studies have found increased permeability in healthy relatives of patients with IBD and this may indicate a role of disturbed permeability in the pathogenesis of this disease.

Increased intestinal permeability is not a specific finding in CD. It is also found in other conditions and following intake of non-steroidal anti-inflammatory drugs [21]. Meddings et al. (2000) [20] found that environmental stress increases the gastrointestinal permeability.

Intestinal permeability is defined as the ability of compounds to cross the intestinal mucosa through the paracellular tight junction areas. Bjarnason et al. (1995) [19] proposed that the size of the paracellular pores decrease along the crypt-villus axis. Therefore large molecules are absorbed mainly between the cells and near the crypts. Smaller molecules are absorbed along the whole crypt-villus axis. The surface for absorption of smaller molecules is therefore much greater than for larger molecules.

The intestinal permeability can be assessed non-invasively by measurement of urinary excretion after oral intake of carbohydrates which are not metabolised [1, 2, 3]. A higher recovery of the administered carbohydrate in the urine corresponds to a higher gastrointestinal absorption which in turn reflects increased permeability. However several factors such as gastrointestinal motility, absorptive capacity, urinary volume and renal function can influence the absorption and thus the urinary excretion. Therefore, it is standard practice to administer two carbohydrates simultaneously (a small and a large molecule). The assumption is that the small molecule is absorbed to a high extent even in healthy subjects. The absorption of the larger molecule is limited and this is dependent on the gastrointestinal permeability. By determining the ratio of two different carbohydrates, the influence of gastric emptying, intestinal transit time, dilution by intestinal secretions and renal function could be reduced. An increase in the ratio of urinary excretion of the large and the small carbohydrate is assumed to reflect an increase in paracellular permeability of the tight junctions. Other factors such as shortening of villus height may also influence this ratio [1, 4].

The study of small intestine permeability by measuring absorption of two non-metabolised sugars, one of lower and one of higher molecular weight, is a well established method. The use of the ratio of excretion of the two absorbed sugars allows a more definitive

measurement of permeability changes than the use of only one single carbohydrate. Several carbohydrates such as lactulose, cellobiose, rhamnose and mannitol are used to assess the intestinal permeability. Rhamnose and mannitol reflect the absorption of small molecules, whereas lactulose and cellobiose reflect permeability to larger molecules.

The aim of this study was to evaluate various carbohydrates for assessment of intestinal permeability in patients with inflammatory bowel diseases.

Materials and Methods

Patients

Twenty-six patients with Crohn´s disease (CD) (mean age: 34.6 years, range 14 to 58 years, 10 male, 16 female) and 21 patients with ulcerative colitis (UC) (mean age: 37.4 years; range 18 to 67 years; 11 male, 10 female) and 27 healthy volunteers (mean age: 31.0; range 20 to 58 years, 13 male, 14female) gave informed consent and were included. The volunteers had no history of digestive, allergic or dermatological diseases and did not take any medication at the time they were included in the control group. The diagnosis of CD or UC was based on clinical grounds and on typical endoscopic, radiological and histological criteria. Two patients with CD (2 males, mean age 26 years) and 5 patients with UC (3 males, 2 females, mean age 43 years) were analysed twice with different disease activity each (Table 1).

The patients with IBD were divided into three groups (high activity, low activity and remission). To be included in the remission group, patients had to be free of symptoms and have no laboratory evidence of inflammation. Patients with low activitiy Crohn`s disease had a CDAI value (Crohn`s disease activity index) between 150 and 250. Patients with highly active Crohn`s disease had a CDAI value above 250. Patients with low activity ulcerative colitis suffered from mild diarrhoea (maximum bowel movements per day of five) without further clinical symptoms. Patients with more frequent bowel movements or with additional clinical symptoms were included in the group with high disease activity.

Three patients with ulcerative colitis and 15 patients suffering from Crohn´s disease had undergone surgery in the past, including eleven patients with who had an ileocoecal resection.

18 of the patients with Crohn´s disease had involvement of the terminal ileum and the other 8 patients had involvement of the colon. Nine patients with ulcerative colitis had localized disease of the left colon, whilst the remainder suffered from pan colitis The diagnosis of IBD had been established between 1 and 20 years before inclusion in the study (mean 5 years).

Sixteen patients with UC and 19 patients with CD were treated with systemic steroids while being studied. No surgical procedure had been performed at least 6 months prior to the study.

Laboratory Tests

The following laboratory tests were performed at the time of the sugar absorption test: White blood cell (WBC) and platelet counts; erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and plasma albumin level. (Table2)

Measurement of Intestinal Permeability

The test was performed on an outpatient basis. After an overnight fast, the subjects were asked to completely empty the urinary bladder. The subjects then took a solution of 10 g lactulose (L), 10 g cellobiose (C), 3 g l-rhamnose (R) and 2,5 g d-mannitol (M) in 250 ml water orally. Their urine was collected for the next 5 hours in a plastic container containing 0.5 g chlorhexidine and refrigerated to avoid bacterial degradation of the sugars. The subjects were not allowed to eat during the 5-hour collection period but were allowed to drink 750 ml of water two hours after the initial ingestion. The urine collection containers were returned to our outpatient clinic within 24 hours. The volume of the 5-hour urine was measured and aliquots were stored at -20 °C until analysis.

Protein was removed from the urine with 0.5ml of periodic acid (0.03 mol/L in 0.25 mol/L sulfuric acid. The sample was then vortexed for 10 sec and centrifuged for 10 min at 2500 rpm. The supernatant was treated with Amberlite MB-3 resign (Polysciences Europe, 69214 Eppelheim, Germany) in the acetate form, swirled for 10 minutes and centrifuged. The concentrations of the four carbohydrates were measured by high performance liquid chromatography using two anion exchange columns in series (Carbonpack PA1, Dionex, Breda, The Netherlands) with a 0.1 mol/l NaOH solution as the isocratic mobile phase. This was followed by pulsed amperometric electrochemical detection on a gold electrode (Beckmann, System Gold, Autosampler 507). The urinary recovery of lactulose, mannitol, cellobiose and rhamnose was expressed as a percentage of the orally administered dose recovered in the 5-hour urine. From these data, the permeability indices C/R, L/ R, C/M and L/M were derived.

Statistics

The data were expressed as mean and standard error of the mean (SEM) unless otherwise mentioned.

Results:

The sugar solution was well tolerated and no patient reported diarrhoea after oral administration. There was no significant difference in relation to gender or age. The demographic data and disease activity according to the clinical findings are summarised in Table 1. There was no correlation between permeability, age and gender. The laboratory findings are summarized in Table 2.

In comparison to the intestinal permeability of healthy volunteers, all patients suffering from inflammatory bowel disease showed increased ratios of C/R and L/R, C/M and L/M ratios (see Tables 3a and 3b). In patients with active disease, these ratios were higher than in patients in remission. The ratios of L/R and L/M for those patients in remission were higher than

Table 1. Demographical data.

	Controls	**Crohn´s disease**			**ulcerative colitis**		
		remission	**low activity**	**high activity**	**remission**	**low activity**	**high activity**
male	13	5	5	0	4	6	1
female	14	4	9	3	3	6	1
subgroup		9	14	3	7	12	2
Total	27	26			21		

Table 2. Laboratory tests (mean ± SEM).

	WBC (10^3/*l)		**C-reactive protein** (mg/dl)		**ESR** (mm/h)	
Disease activity	**Crohn´s disease**	**ulcerative colitis**	**Crohn´s disease**	**ulcerative colitis**	**Crohn´s disease**	**ulcerative colitis**
all	10.8 ± 3.8	10.5 ± 3.9	1.4 ± 1.7	1.5 ± 3.4	20 ± 14	19 ± 17
remission	9.0 ± 2.6	8.4 ± 2.7	0.5 ± 0.3	0.5 ± 0.5	11 ±8	10 ± 6
low activity	10.6 ± 4.3	11.5 ± 3.9	1.5 ± 1.1	0.8 ± 0.7	22 ±7	14 ± 11
high activity	12 ± 1.6	12.9 ± 4.4	4.9 ± 0.4	8.1 ± 7.4	26 ± 2.5	47 ±17

Table 3a. Permeability indices (mean ± SEM) (Factor of mean increase in comparison to remission).

	C/R			**L/R**		
Disease activity	**Controls**	**Crohn´s disease**	**ulcerative colitis**	**Controls**	**Crohn´s disease**	**ulcerative colitis**
remission	0.05 ± 0.06	0.07 ± 0.08	0.12 ± 0.13	0.01 ± 0.01	0.16 ± 0.11	0.29 ± 0.22
low activity		0.14 ± 0.16 (2.0)	0.21 ± 0.03 (1.75)		0.31 ± 0.19 (1.94)	0.4 ± 0.38 (1.38)
high activity		0.29± 0.13 (4.14)	1.19± 0.45 (9.9)		0.54± 0.12 (3.38)	1.11± 0.33 (3.83)

Table 3b. Permeability indices, continued (mean ± SEM) (Factor of mean increase in comparison to remission)

	C/M			**L/M**		
Disease activity	**Controls**	**Crohn´s disease**	**ulcerative colitis**	**controls**	**Crohn´s disease**	**ulcerative colitis**
remission	0.18 ± 0.22	0.01 ± 0.01	0.02 ± 0.01	0.04 ± 0.06	0.02 ± 0.01	0.03 ± 0.02
low activity		0.06 ± 0.14 (6.0)	0.05 ± 0.07 (2.5)		0.28 ± 0.72 (14.0)	0.1 ± 0.12 (3.3)
high activity		0.02 ± 0.01 (2.0)	0.33 ± 0.2 (16.5)		0.36± 0.12 (18.0)	0.31 ± 0.21 (10.3)

in the control group even in patients in remission. In contrast, the ratios C/R and C/M were lower for patients with Crohn´s disease or ulcerative colitis in remission than for healthy controls. There was no significant difference in the ratios between localised disease or pancolitis.

Changes in disease activity in IBD patients were best reflected by use of the cellobiose/rhamnose excretion quotient since this parameter shows the best correlation to disease activity both in Crohn's disease and in ulcerative colitis (Table 3a,b).

In patients with ulcerative colitis, the ratios were strongly increased only in patients with high disease activity. In patients suffering from Crohn´s disease, an increase was observed even in patients with mild disease activity, although the findings were not statistically significant.

Discussion

Several agents of different molecular size are available to assess the intestinal permeability [1, 3, 5, 6]. Lactulose (L) and cellobiose (C) are disaccharides and therefore larger than the small carbohydrates L-rhamnose (R) and D-mannitol (M). Lactulose, with a molecular weight of 342 dalton and a molecular size of 0.54 nm is the most widely used disaccharide for measuring intestinal permeability. It cannot pass through the pores in the enterocyte lipid membranes. It is absorbed paracellularly through tight junctions or extrusion zones in the villus tips. Cellobiose with a molecular weight of 342 dalton and a molecular size of less than 0.5 nm has the theoretical disadvantage that there may be cellobiase activity at the jejunal brush border in rare circumstances.

L-rhamnose (164 dalton) and D-mannitol are the most frequently used small carbohydrates for measuring gastrointestinal permeability. D-mannitol is a polyhydric alcohol of a molecular weight of 182 dalton with a size of only 0.4 nm which is passively absorbed through the small pores in the lipid membranes of the enterocytes. These pores allow the passage of small hydrophilic molecules. The absorption of these carbohydrates is influenced more by the size of the absorptive areas of the gastrointestinal mucosa than by changes in the mucosal permeability [7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18]. One disadvantage of mannitol is the possibility of endogenous mannitol production.

The major aim of this study was to evaluate four ratios of the intestinal absorption of different carbohydrates in their sensitivity to predict clinical activity in patients with Crohn`s disease or ulcerative colitis. Two small carbohydrates (R and M) and two larger disaccharides (L and C) were administered to the subjects. Therefore, four ratios as assessments for gastrointestinal permeability could be calculated: C/R, L/R, C/M and L/M.

Lactulose, rhamnose and cellobiose could be measured adequately in the urine whereas mannitol measurement required the use of an anion exchanger and can produce inconsistent results. Both Crohn`s disease and ulcerative colitis patients have higher permeability indices than healthy controls, even when they are in remission. Patients with active disease have elevated permeability indices compared to those who are in remission. In this study, changes in disease activity in IBD patients are best reflected by use of the cellobiose/rhamnose excretion quotient.

The data obtained with mannitol did not show a good correlation between clinical activity and the ratios C/M or L/M. This could in part be due to the possibility of endogenous mannitol production which could influence the findings [4]. The ratios based on L-rhamnose absorption were more consistent. The C/R ratio showed a higher relative increase in patients with high disease activity than the L/R ratio and therefore may be a more sensitive marker of disease activity.

Our results indicate that the cellobiose/rhamnose excretion quotient is the most useful combination of carbohydrates to evaluate increased intestinal permeability as a predictor of disease activity in IBD in future studies.

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