GASTROENTEROLOGY 1989;97:927–31

Intestinal Permeability in Patients With Crohn's Disease and Their Healthy Relatives

K. D. KATZ, D. HOLLANDER, C. M. VADHEIM, C. McELREE, T. DELAHUNTY, V. D. DADUFALZA, P. KRUGLIAK, and J. I. ROTTER

Division of Gastroenterology, University of California at Irvine, and Division of Medical Genetics, Departments of Medicine and Pediatrics, Cedars-Sinai Medical Center and University of California at Los Angeles, California

The healthy relatives of patients with Crohn's disease were previously found to have increased intestinal permeability to polyethylene glycol 400. To determine whether the abnormal permeability is uniquely detectable by polyethylene glycol 400, we studied the intestinal permeability of three new probes (lactulose, rhamnose, and mannitol) in 25 patients with Crohn's disease, 41 of their healthy relatives, and 29 normal controls without a family history of inflammatory bowel disease. Patients with Crohn's disease had increased lactulose permeability when compared with relatives or controls. Lactulose absorption by patients with Crohn's disease was 0.41% ± 0.07% (mean ± SE), whereas that of their relatives and unrelated controls was 0.28% ± 0.03% and 0.26% ± 0.03%, respectively. There was no significant difference between the relatives and controls, but both groups differed from the patients ($p < 0.05$ and $p < 0.025$, respectively). The patients' lactulose/rhamnose ratio was 70.5% ± 9.2% vs. 37.2% ± 3.3% in relatives and 40.6% ± 5.7% in unrelated controls ($p < 0.0005$ and $p < 0.0025$, respectively). The two intermediate-sized probes, rhamnose and mannitol, did not detect permeability differences among the three groups. The inability of lactulose, rhamnose, or mannitol to detect permeability abnormalities in healthy relatives of patients with Crohn's disease suggests that these probes penetrate the intestinal barrier by routes or mechanisms that are different from those of polyethylene glycol 400. Lactulose, in particular, detects permeability changes in patients with intestinal inflammation, and polyethylene glycol 400 is able to detect permeability changes in the healthy relatives of our patients. These data indicate that permeability may be abnormal as a secondary result of inflammation, or as a result of a primary genetic abnormality.

The etiology of Crohn's disease (CD) remains unknown despite intensive research efforts. Increased intestinal permeability to luminal antigens could be one etiologic factor in CD (1,2). If this hypothesis is correct, material excluded by the normal bowel could penetrate through the mucosa, and instigate an inflammatory or immune reaction. Indeed, studies with the probes polyethylene glycol 400 (PEG 400) (2,3), ^{51}Cr-ethylenediaminetetraacetic acid (4,5), and lactulose (6–8) have shown increased intestinal permeability in patients with CD. Whether the abnormal permeability is a genetically determined, primary etiologic factor, or perhaps a result of intestinal inflammation was not answered by the previous studies. To answer these questions we performed permeability studies in a unique fashion by studying not only patients with the disease but also their healthy relatives. As it is known that the relatives of patients with CD have a 17–70-fold increased risk of developing inflammatory bowel disease, we reasoned that if a permeability abnormality is of etiologic importance then it would be found in some of the healthy relatives of patients with CD (9).

In our previous study we found that intestinal permeability to PEG 400 is significantly increased in the healthy relatives of patients with CD. However, it is not known whether different permeability probes

Abbreviations used in this paper: CD, Crohn's disease; IBD, inflammatory bowel disease.

0016-5085/89/$3.50

use different absorptive "pathways" (10,11), whether differing physical structure, shape, size, solubility, and other properties of the different probes affect their intestinal penetration (12–14), or whether the abnormality seen in patients with CD and their relatives is specific to one pathway and a specific probe. Therefore, in this study we used several different probes to determine if the abnormal permeability in patients with CD and their healthy relatives is specific to some probes or whether it is shared by other permeability probes as well. We used three additional probes: lactulose (a globular sugar with a cross-sectional diameter of 9.5 Å), mannitol (a linear sugar with a diameter of 6.7 Å), and rhamnose (a globular sugar with a diameter of 8.3 Å) (12). In contrast to these sugars, PEG 400 is a linear polymer with a cross-sectional diameter of 5.3 Å (12). By using these sugars as probes we tested whether the abnormal permeability seen in both healthy relatives and patients with CD is specific to PEG and its pathway or whether it is a more generalized abnormality detectable by other probes and their absorptive pathways.

We found major differences in the probes' ability to detect permeability abnormalities in patients with CD and their asymptomatic, clinically healthy relatives. Therefore, this study provides important new clinical information that will serve as a basis for the design of laboratory studies to investigate mechanisms and routes of permeation of different probes by both normal and diseased intestinal epithelium.

Materials and Methods

We studied the intestinal permeability of 25 patients with CD and 41 of their clinically unaffected relatives. Patients were recruited from University of California at Irvine- and University of California at Los Angeles-affiliated Crohn's disease centers and from community physicians. Patients who participated in the study had minimal disease activity and did not require corticosteroids for control of their disease at the time of the study. One or several of the authors visited the patients' homes and obtained detailed medical and family histories before the permeability studies were performed. Written informed consent and release-of-medical-information forms were obtained. The patients' medical information was reviewed by a gastroenterologist who documented the presence of CD as proven by surgical exploration, colonoscopy, or classic radiologic findings.

After a 3-h fast, the subjects ingested 7.5 g of lactulose, 1 g of rhamnose, and 1 g of mannitol (Sigma Chemical Co., St. Louis, Mo.) in 6–8 oz of water. Water was allowed during the fast, which continued for the full 6 h of urine collection. The total volume of urine was measured, and several aliquots were frozen at −20°C for subsequent analysis. The urinary concentrations of lactulose, mannitol, and rhamnose were determined by high-performance liquid chromatography analysis (15). The samples were thawed and 50 μl of saturated NaOH was added to 5 ml of urine to precipitate the urinary proteins. The samples were centrifuged and the supernate was removed. We added 1.23 g of MB-3 resin (Sigma Chemical Co.) to the supernate and whirled it for 10 s and repeated the process once. A 20-μl portion of this supernate was then injected into a Rezex Ca^{2+} (Phenomenax, Rancho Palos Verdes, Calif.) high-performance liquid chromatography column. This technique allows rapid reproducible determinations with a sensitivity of 1 mg/ml. Injections of known probe concentrations, and "spiked urine" samples served as standards and controls. Total urinary excretion was calculated for each of the sugars, and results were expressed as percent recovery of the ingested probe. The data for each group were compared by analysis of variance, and when significance was found a Fisher PLSD (Protected Least Significant Differences) (16) was used to determine which differences were indeed significant. The study was approved by the University of California at Irvine and Cedars-Sinai Human Studies review committees.

Results

Description of Study Subjects

Eleven patients had undergone both small bowel and colonic resections, 5 had undergone small bowel resections only, and 9 had had no bowel resections. Eleven of the patients were men (44%) and 14 were women. The mean age of the patients was 40.8 ± 17.3 yr. Thirty-four of the patients' relatives were first-degree, 3 were second-degree, and 4 were third-degree relatives. Of the relatives, 17 (41%) were men (16 first degree) and 24 were women (19 first degree). Their mean age was 37.6 ± 18.4 yr. None of the relatives had clinical signs or symptoms of CD and none had sought medical attention for a CD evaluation. Those relatives taking aspirin or nonsteroidal antiinflammatory drugs were excluded (the final number of relatives studied was 35). No subjects had ingested alcohol for at least 24 h before the study. Twenty-eight unrelated healthy controls without a family history of inflammatory bowel disease were also studied. None had clinical signs or symptoms of CD, and none had sought medical attention for a CD evaluation. None had ingested alcohol for at least 24 h before the study, and none were taking medications regularly. Eighteen (64%) were men and 10 were women. Mean age was 36.4 ± 10.1 yr.

Permeability

Rates of intestinal permeability were based on the amounts of probe recovered in the urine. The absorption (mean ± SE) in patients, relatives, and controls, respectively, was as follows: rhamnose,

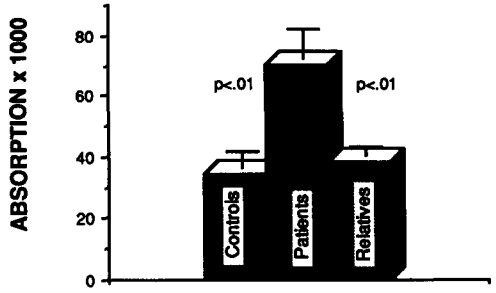

Figure 1. Comparison of lactulose/rhamnose permeability values ($\times 10^3$) in patients with CD, their healthy family members, and normal controls. Statistical differences above are by Fisher PLSD.

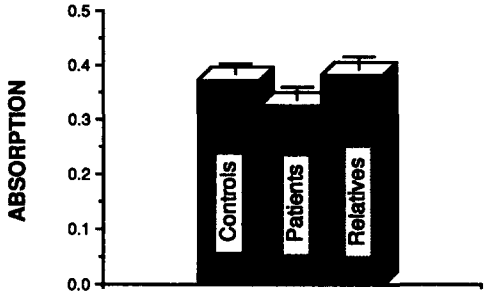

Figure 3. Comparison of rhamnose/mannitol permeability values in patients with CD, their healthy family members, and normal controls. No statistical differences by analysis of variance were seen.

5.9% ± 0.7%, 7.4% ± 0.5%, and 7.4% ± 0.7%; mannitol, 17.9% ± 2.6%, 20.6% ± 1.7%, and 20.5% ± 1.7%; lactulose, 0.41% ± 0.07%, 0.28% ± 0.03%, and 0.26% ± 0.03%. Urinary excretion of rhamnose and mannitol was similar in the three groups ($p = 0.16$, and $p = 0.58$, respectively, by analysis of variance). A significant difference between the three groups was found in the absorption of lactulose ($p = 0.04$ by analysis of variance); the relatives and controls differed from the patients ($p < 0.05$ and $p < 0.025$, respectively, by Fisher PLSD) but no significant differences were seen between the controls and the relatives ($p = 0.75$, Fisher PLSD).

To correct for potential variability in parameters such as urinary excretion between subjects, we calculated the ratios of the sugars as lactulose/rhamnose, lactulose/mannitol, and rhamnose/mannitol. These results are shown in Figures 1–3. In comparison to their clinically healthy relatives and the unrelated healthy controls, patients with CD had significantly higher lactulose/mannitol ratios ($p < 0.01$, Fisher PLSD) and lactulose/rhamnose values. No significant difference between groups was observed in the rhamnose/mannitol determination ($p = 0.13$ by analysis of variance). The lactulose/rhamnose ratios in the three groups showed the greatest differences between patients and controls ($p < 0.0025$) and between patients and relatives ($p < 0.0005$) with numerical differences of 30 and 33, respectively.

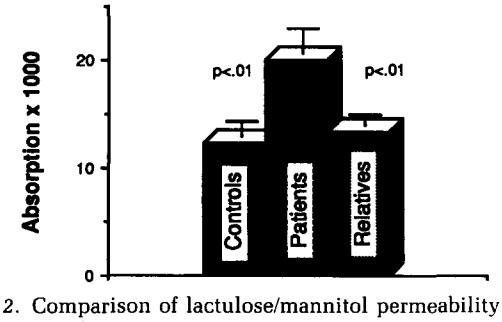

Figure 2. Comparison of lactulose/mannitol permeability values ($\times 10^3$) in patients with CD, their healthy family members, and normal controls. Statistical differences above are by Fisher PLSD.

The effects of ileal resection, colonic resection, or both ileal and colonic resections on the permeability values were not statistically significant (by analysis of variance: lactulose, $p = 0.95$; mannitol, $p = 0.33$; and rhamnose, $p = 0.43$). Likewise, there was no correlation between the site of bowel involvement and the magnitude of intestinal permeability ($p = 0.69$).

Intestinal transit was assessed in the three groups of subjects by measuring breath hydrogen following a standard test meal (17). Oral-to-cecal mean transit times of patients, relatives, and controls were 3.0 ± 0.36, 2.9 ± 0.60, and 3.2 ± 0.39 h, respectively. These differences were not statistically different ($p > 0.05$).

Discussion

The intestinal epithelium forms a vital barrier against the penetration of antigenic, carcinogenic, or inflammation-producing compounds into the intestinal tissue and the systemic circulation. The penetration of the intestinal barrier by medium- or large-sized molecules is often referred to as selective intestinal permeability (4,10,11). To quantitate the degree of penetration of the intestinal barrier, intestinal permeability is often assessed by the absorption rate of nonnutritive, nonmetabolized large molecules, such as lactulose, mannitol, rhamnose, cellobiose, ^{51}Cr-ethylenediaminetetraacetic acid, and PEG 400 (11). The normal intestine appears to discriminate between permeability probes according to their cross-sectional diameters (12). Normally, small molecules, such as water and sodium, readily permeate the intestine, whereas larger molecules, such as dextran (18) and inulin (19), are excluded.

In this study, using a large globular permeability probe (lactulose) and two smaller probes (rhamnose and mannitol), we assessed the intestinal barrier of patients with CD and their healthy relatives. We

found that lactulose had an abnormally high level of absorption in patients with CD but not in their healthy relatives or healthy unrelated controls. Neither rhamnose nor mannitol, the intermediate-sized probe molecules, showed any differences in the intestinal permeability of patients with CD, their healthy relatives, and unrelated healthy controls. In an earlier study PEG 400, a linear probe molecule with a cross-sectional diameter of 5.3 Å, was shown to penetrate the intestine of both the healthy relatives and of patients with CD at an abnormally high level (2).

It is not known whether lactulose penetrates the intestinal barrier through the paracellular tight junctions or directly through the cell membranes, or by a combination of the two routes. Furthermore, it is not known whether the transport of lactulose is by passive diffusion along its concentration gradient or whether other mechanisms such as endocytosis or carrier mediation are involved in its transepithelial transfer (10,19–21). What can be concluded from the present study is that lactulose permeation does not detect the genetically determined abnormality of CD (22,23) because its intestinal permeation in the healthy relatives of our patients was normal. In contrast, PEG 400 permeability, as shown in our previous study of relatives of patients with CD, was abnormal in both the patients with CD and their healthy relatives (2). We conclude from these experiments that PEG 400 permeates the intestine by a pathway that detects a genetically determined abnormality, whereas lactulose permeates the intestine by a route that is sensitive to inflammation (7,8).

These findings are not surprising. Lactulose differs in its shape and size from PEG 400. We know that PEG 400 is passively transported across the tight junctions by a process that is modulated by solvent drag (24). We do not know the precise mechanisms of transport or the route of lactulose permeability (10). We do know, however, that lactulose is closely related in size, shape, and permeability characteristics to another common permeability probe, ^{51}Cr-ethylenediaminetetraacetic acid. Experiments both in patients and in animal models have clearly established that both ^{51}Cr-ethylenediaminetetraacetic acid and lactulose permeation increase as the degree of tissue inflammation increases (5,7,8). As lactulose is similar in structure and size to ^{51}Cr-ethylenediaminetetraacetic acid, we propose as a working hypothesis that lactulose is also primarily sensitive to tissue inflammation and does not detect underlying genetic abnormalities in permeability. Therefore, our present findings of increased lactulose permeability in patients with CD, but not in their healthy relatives, are not surprising, as the relatives would not be expected to have intestinal inflammation.

In contrast to lactulose, neither rhamnose nor mannitol showed any differences in permeability between patients with CD, their healthy relatives, and healthy controls. Both probes are much smaller than lactulose. Although the intestinal routes and mechanisms of permeation of these two probes are not clearly understood, our data suggest that these two probes are sensitive to neither intestinal inflammation, as are lactulose and ^{51}Cr-ethylenediaminetetraacetic acid (5,7,8), nor the underlying genetically determined permeability defect in CD, as is PEG 400 (2).

These clinical findings of probe specificity for detecting underlying genetic- versus inflammation-induced abnormalities provide directions for future laboratory investigation. Using intestinal loops, everted sacs, membrane vesicles, and cell cultures, investigators must seek explanations for the permeability patterns of different probes in CD. By learning how various probes permeate the intestinal epithelium and what factors influence their permeation rates we will be closer to understanding the clinical data of probe specificity and to designing new therapeutic approaches to CD (25).

References

1. Shorter RG, Huizenga KA, Spencer RJ. A working hypothesis for the etiology and pathogenesis of nonspecific inflammatory bowel disease. Am J Dig Dis 1972;17:1024–31.
2. Hollander D, Vadheim CM, Brettholz E, Petersen GM, Delahunty T, Rotter JI. Increased intestinal permeability in patients with Crohn's disease and their relatives—a possible etiological factor. Ann Intern Med 1986;105:883–5.
3. Olaison G, Leandersson P, Sjodahl R, Tagesson C. Intestinal permeability to polyethyleneglycol 600 in Crohn's disease. Peroperative determination in a defined segment of the small intestine. Gut 1988;29:196–9.
4. Bjarnason I, O'Morain C, Levi AJ, Peters TJ. Absorption of ^{51}chromium-labeled ethylenediaminetetraacetate in inflammatory bowel disease. Gastroenterology 1983;85:318–22.
5. Jenkins RT, Jones DB, Goodacre RL, et al. Reversibility of increased intestinal permeability to ^{51}Cr-EDTA in patients with gastrointestinal inflammatory disease. Am J Gastroenterol 1987;82:1159–64.
6. Ukabam SO, Clamp JR, Cooper BT. Abnormal small intestinal permeability to sugars; in patients with Crohn's disease of the terminal ileum and colon. Digestion 1983;27:70–4.
7. Andre F, Andre C, Emery Y, Forichon J, Descos L, Minaire Y. Assessment of the lactulose-mannitol test in Crohn's disease. Gut 1988;29:511–5.
8. Sanderson IR, Boulton P, Menzies I, Walker-Smith JA. Improvement of abnormal lactulose/rhamnose permeability in active Crohn's disease of the small bowel by an elemental diet. Gut 1987;28:1073–6.
9. Fielding JF. The relative risk of inflammatory bowel disease among parents and siblings of Crohn's disease patients. J Clin Gastroenterol 1986;8:655–7.
10. Maxton DG, Bjarnason I, Reynolds AP, Catt D, Peters TJ, Menzies IS. Lactulose, ^{51}Cr-labelled ethylendiaminetetra-acetate, L-rhamnose and polyethyleneglycol 500 as probe mark-

ers for assessment in vivo of human intestinal permeability. Clin Sci 1986;71:71–80.
11. Anonymous. Intestinal permeability. Lancet 1985;i:256–8.
12. Hollander D, Ricketts D, Boyd CAR. The importance of "probe" molecular geometry in determining intestinal permeability. Can J Gastroenterol 1988;2:35–8.
13. Cobden I, Dickinson RJ, Rothwell J, Axon ATR. Intestinal permeability assessed by excretion ratios of two molecules: results in coeliac disease. Br Med J [Clin Res] 1978;ii:1060.
14. Sawiers WM, Andrews DJ, Low-Beer TS. The double sugar test of intestinal permeability in the elderly. Age Ageing 1985;4:312–5.
15. Delahunty T, Hollander D. Liquid-chromatography method for estimating urinary sugars: application to studies of intestinal permeability. Clin Chem 1986;32:351.
16. O'Brien PC, Shampo MA. Statistical considerations for performing multiple tests in a single experiment. 2. Comparisons among several therapies. Mayo Clinic Proc 1988;63:816–20.
17. Read NW, Miles CA, Fisher D, et al. Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhea. Gastroenterology 1980;79:1276–82.
18. Palosuo T, Milgrom F. Appearance of dextrans and antidextran antibodies in human sera. Int Arch Allergy Appl Immunol 1981;65:153–61.
19. Loehry CA, Axon ATR, Hilton PJ, Hider RC, Creamer B. Permeability of the small intestine to substances of different molecular weight. Gut 1970;11:466–70.
20. Jenkins RT, Goodacre RL, Rooney PJ, Bienenstock J, Sivakumaran T, Walker WHC. Studies of intestinal permeability in inflammatory disease using polyethylene glycol 400. Clin Biochem 1986;9:298–302.
21. Menzies IS, Laker MF, Pounder R, et al. Abnormal intestinal permeability to sugars in villous atrophy. Lancet 1979;ii:1107–9.
22. McConnel RB. Genetic aspects of idiopathic inflammatory bowel disease. In: Kirsner JS, Shorter RG, eds. Inflammatory Bowel Disease. Philadelphia: Lea & Febiger, 1988:87–95.
23. McConnel RB, Vadheim CM. Inflammatory bowel disease. In: King RA, Rotter JI, Motulsky AG, eds. The Genetic Basis of Common Disorders. New York: Oxford University Press, 1988.
24. Krugliak P, Hollander D, Tran D, Dadufalza VD, Katz KD, Le K. Mechanisms of polyethylene glycol 400 permeability in perfused rat intestine. Gastroenterology (in press).
25. Hollander D. Crohn's disease—a permeability disorder of the tight junction? Gut 1988;29:1621–4.

Received September 29, 1988. Accepted April 19, 1989.

Address requests for reprints to: Daniel Hollander, M.D., C-340 Medical Sciences I, University of California at Irvine, Irvine, California 92717.

This work was supported in part by the Goldsmith Family Foundation, grants AG 2627 and DK35200, from the National Institutes of Health, and the Stuart Foundation.