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The Relationship between Small-Intestinal Bacterial Overgrowth and Intestinal Permeability in Patients with Irritable Bowel Syndrome

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Background/Aims: Small-intestinal bacterial overgrowth (SIBO) is a frequent finding in patients with irritable bowel syndrome (IBS). Many patients with IBS also have abnormal intestinal permeability, which is probably due to low-grade inflammation in the intestinal mucosa. Our aim was to verify the relationship between SIBO and small-intestinal permeability in IBS patients. **Methods:** A cohort of 38 IBS patients (20 women and 18 men; age range 16-70 years; mean age 40.2 years) with symptoms that fulfilled Rome-II criteria, and 12 healthy controls (5 women and 7 men; age range 25-52 years; mean age: 37.8 years) were recruited. All subjects underwent lactulose breath tests (LBTs) and intestinal permeability tests using the polyethylene glycol (PEG) 3350/400 retrieval ratio. **Results:** A positive LBT was found in 18.4% (7/38) of patients with IBS and 8.3% (1/12) of control subjects. Intestinal permeability was significantly increased in patients with IBS compared with the normal controls (0.82±0.09 vs 0.41±0.05 [mean±SD], respectively; $p<0.05$). However, the intestinal permeability did not differ significantly between IBS patients with a positive LBT and those with a negative LBT (0.90±0.13 and 0.80±0.11, respectively; $p>0.05$). **Conclusions:** Intestinal permeability was increased in patients with IBS, but this finding did not correlated with the occurrence of SIBO. **(Gut and Liver 2009; 3:174-179)**

Key Words: Small intestinal bacterial overgrowth; Intestinal permeability; Irritable bowel syndrome

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common gastrointestinal diseases. However, due to the marked variability of symptoms in patients with IBS, it is not easy to develop a unifying hypothesis to explain the pathophysiology of this disease. It has been recently suggested that small intestinal bacterial overgrowth (SIBO) may provide a framework for understanding the frequent clinical observations of IBS patients.[1]

SIBO is a frequent condition in patients with IBS. In the previous study, 10-84% of the patients with IBS presented with bacterial overgrowth using a lactulose breath test (LBT).[2-5] Treatment of SIBO with antibiotic and probiotic treatment effectively improved the gas-related symptoms of IBS[2,6,7] and these results have been proposed as evidence of SIBO as a cause of the symptoms of IBS. One of the possible consequences of the host response to SIBO is immune activation.[1] SIBO has been regarded as an important factor for the development of bacterial translocation[8] and bacterial translocation is responsible for immune activation.[9,10]

Immune activation is also present in IBS patients. There is accumulating evidence of an increased number of inflammatory cells in the mucosa[11-13] and lamina propria,[14] and an altered ratio of an anti-inflammatory cytokine to a proinflammatory cytokine in IBS patients.[15,16] This inflammation may be a cause or an effect of an altered mucosal barrier in the intestine. Inflammatory conditions in the gut such as acute gastroenteritis[17] and

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Crohn's disease[18] increase gut permeability, and some IBS subgroups may also be associated with altered gut permeability.[19] However, the previous studies on intestinal permeability in IBS patients have produced conflicting results and the selection of patients varied among the studies.[20,21]

Therefore, the aim of our study was to determine whether there was any difference in intestinal permeability between normal subjects and IBS patients, and also between the IBS subgroups (IBS with diarrhea [IBS-D] and IBS with constipation [IBS-C]) and we wanted to determine if any relationship exists between SIBO and intestinal permeability.

MATERIALS AND METHODS

1. Patients

All the patients exhibited symptoms meeting the Rome-II criteria[22]; none of the study participants had a clear history of inflammatory bowel disease or post-infectious IBS, which is diagnosed when patients have at least two of the following: fever, vomiting, diarrhea or a positive stool culture at the onset of IBS symptoms, and the previous bowel habit has been within normal limits.[23] Informed written consent was obtained from all patients, and approval for the study was received from the local ethics committee at the hospital. Twelve normal control subjects were included in the study. The control subjects had met all of the following criteria: (i) macroscopically and histologically normal colonic mucosa,[12] (ii) no persistent bowel symptoms, (iii) no organic or functional bowel disease and (iv) no history of chronic medical disease. The patients with the following criteria were excluded from the two groups: (i) a history of atopy, food allergy or asthma based on a detailed medical history, (ii) use of mast cell stabilizers or steroids during the month before the study, (iii) active diverticulitis or (iv) gastrointestinal infection.[12] All the participants underwent colonoscopy in order to exclude organic diseases, and the subjects were excluded if they had used antibiotics within 2 weeks of the LBT.

2. Questionnaire

The participants were asked to complete a questionnaire about their bowel symptoms and the psychological distress they had experienced over the last 2 weeks before the interview. The questionnaire included the duration of IBS symptoms, and an assessment of the following supportive symptoms of IBS[22] on a six-point severity scale: fewer than three bowel movements a week, more than three bowel movements a day, hard or lumpy stools, loose (mushy) or watery stools, straining during a bowel movement, urgency (having to rush to have a bowel movement), the feeling of incomplete bowel movement, passing mucus (white material) during a bowel movement and abdominal fullness, bloating or swelling. To assess the psychological well-being and disability of the subjects, all the participants were asked to complete the Beck Depression Inventory (BDI)[24] and the State-Trait Anxiety Inventory (STAI).[25]

3. Lactulose breath test

A lactulose breath test was performed 2 weeks after colonoscopy. After the ingestion of a 100-mL water solution containing 10 g of lactulose, breath samples were taken at 15-min intervals for 3 h. During the test, the subjects were forbidden to eat, smoke or exercise. Alveolar air samples were obtained after a normal inspiration by having the subjects exhale through a mouthpiece into the bags connected by a three-way valve. When the first 500 mL of expiratory air filled one plastic bag, then the end alveolar air was collected in a second bag (a 1-1 rubber anesthesia bag adapted with a one-way valve). The end alveolar air was then immediately transferred into 50 mL plastic syringes fitted with two-way stopcocks and the air was analyzed within a 2-h period. The H_2 and CH_4 concentrations in the breath samples were determined simultaneously with a Micro Lyser DP gas chromatograph (Quintron Instrument Company, Milwaukee, WI, USA). Dry air was used as the carrier gas at a flow rate of 30 mL/min. The chromatograph was calibrated with using H_2 and CH_4 reference mixtures in compressed air. The presence of small bowel bacterial overgrowth was defined by the evidence of a peak $>$20 ppm that occurred 15 min before the colonic peak. Also, the patients with an elevated fasting H_2 and/or CH_4 level ($>$12-15 ppm) were considered positive for bacterial overgrowth.

4. Measurement of intestinal permeability

Intestinal permeability was also performed 2 weeks after colonoscopy. Polyethylene glycol (PEG) 3350 was employed for this study as the permeability probe. PEG 400, which acts as an internal control for possible changes in intestinal motility and absorption, was added. The two components of PEG were administered orally; urine was collected for the following 8 hours, and the concentration of each PEG molecule was measured using high-performance liquid chromatography (Waters HPLC system). The ratio of urinary excretion of each molecule was then determined. The differential urinary excretion of the two PEG molecules (the PEG 3350/PEG 400 ratio), which corrects for possible changes in intestinal motility and ab-

sorption, was calculated to provide the "intestinal permeability index".

5. Statistical analysis

Quantitative data were compared using Student's *t* test with the results expressed as means±SEMs. All the other qualitative data comparisons used the χ^2 test. Correlation between intestinal permeability, the lactulose breath test and bowel symptoms was estimated using Pearson's correlation coefficient. The alpha level of significance was set at $p<0.05$. All the analyses were conducted using SPSS (version 11.5.0, SPSS Inc., Chicago, IL, USA).

RESULTS

1. Patients characteristics

Thirty eight IBS patients (age range: 16-70 years, mean age: 40.2 years, 20 women and 18 men) with symptoms that fulfilled the Rome-II criteria, and 12 healthy controls (age range: 25-58 years, mean age: 37.8 years, 5 women and 7 men) were recruited. Concerning the prevalence of bowel habit subtypes, 27 (71%) patients with IBS were classified as IBS-D patients, 8 (21%) were classified as IBS-C patients and 3 (8%) were classified as IBS-A patients. The mean symptom duration of IBS was 78 months (range: 6-360 months). There was no statistical difference of their demographics between two groups ($p>0.05$). In contrast, the depression scores (BDI) and state of anxiety scores (STAI-S/T) were significantly higher for the patients with IBS than those for the normal controls ($p<0.05$) (Table 1).

Table 1. Clinical Characteristics and Psychopathology of IBS Patients and Controls

	IBS patients	Controls	p-value
No. of patients	38	12	
Age (years)*	40.2±2.6	37.8±2.6	NS
Women (%)	52.7	41.7	NS
Description	27 diarrhea 8 alternating 3 constipation		
Psychopathology*			
STAI-S	43.6±1.8	37.5±2.1	0.04
STAI-T	44.3±1.7	38.6±1.1	0.01
BDI	12.6±1.5	7.0±41.4	0.01

IBS, irritable bowel syndrome; STAI, State-Trait Anxiety Inventory; BDI, Beck Depression Inventory; NS, not significant.
*Values are expressed as means±standard error of means (SEMs).

2. Positivity of the lactulose breath test in the patients with IBS and the controls

Of the 38 patients with IBS, 7 were positive on the LBT (18.4%), as compared with 1 of 12 control subjects (8.3%). The difference between the groups was statistically insignificant ($p>0.05$). The percentage of patients with positivity on the LBT was higher for the patients with IBS-D (6/27) than those with IBS-C (0/8) and IBS-A (1/3); however, this did not reach statistical significance ($p>0.05$) (Table 2).

3. Intestinal permeability results for the patients with IBS and the controls

The intestinal permeability was significantly increased in the patients with IBS compared with the normal controls (0.82±0.09 vs 0.41±0.05, respectively; $p<0.05$) (Fig. 1). However, no significant difference for intestinal permeability was observed among the patients with IBS-D, IBS-C and IBS-A (0.85±0.12, 0.74±0.13 and 0.77±0.30, respectively; $p>0.05$) (Fig. 2).

Table 2. Prevalence of a Positive LBT among Patients with IBS and Controls

	LBT positivity (%)	p-value
IBS total	7/38 (18)	>0.05
Control	1/12 (8)	
IBS-D	6/27 (22)	>0.05
IBS-C	0/8 (0)	
IBS-A	1/3 (33)	

LBT, lactulose breath test; IBS, irritable bowel syndrome.

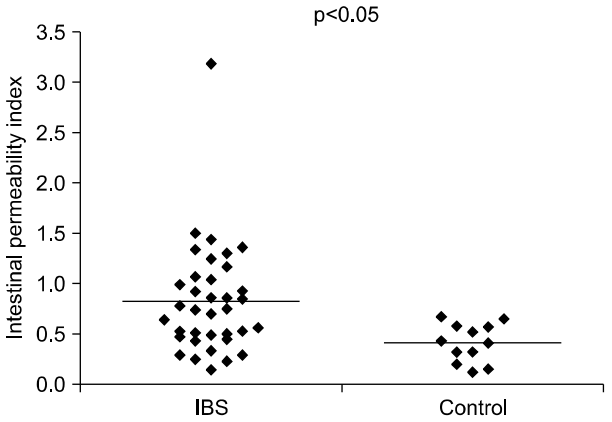

Fig. 1. Comparison of intestinal permeability between patients with IBS and controls. Intestinal permeability was significantly higher in the IBS patients than in the normal controls ($p<0.05$).
IBS, irritable bowel syndrome.

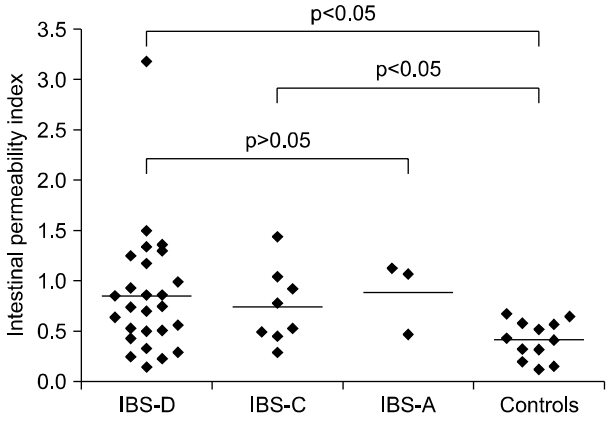

Fig. 2. Comparison of intestinal permeability between IBS subgroups and controls. Intestinal permeability did not differ significantly among the IBS-D, IBS-C, and IBS-A patients, but it was significantly higher in the IBS-D and IBS-C patients than in the controls ($p<0.05$).
IBS, irritable bowel syndrome.

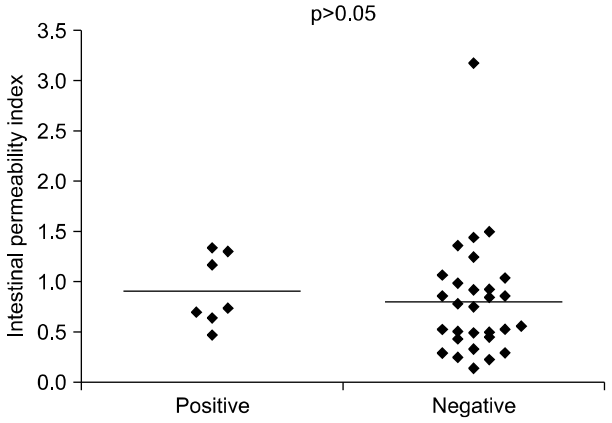

Fig. 3. Comparison of intestinal permeability between IBS patients with a positive LBT and those with a negative LBT. There was no evidence that intestinal permeability was higher in patients with a positive LBT than in those with a negative LBT ($p>0.05$).
IBS, irritable bowel syndrome; LBT, lactulose breath test.

4. Relation between intestinal permeability, the lactulose breath test and bowel symptoms

The various symptoms of IBS failed to show any significant correlation with intestinal permeability or LBT. However, the symptom score of hard stool significantly correlated with positivity on the LBT ($r=-0.32$, $p=0.04$).

5. Comparison of intestinal permeability between patients with positive and negative breath tests

When the intestinal permeability between the IBS patients having a positive LBT and those with a negative LBT was compared, there was no evidence that the patients with a positive LBT had increased intestinal permeability compared with those patients with a negative LBT (0.90 ± 0.13 and 0.80 ± 0.11, respectively; $p>0.05$) (Fig. 3).

DISCUSSION

This study revealed that intestinal permeability was significantly increased in the patients with IBS compared with the normal controls. However, no significant difference in intestinal permeability was observed among the patients with IBS-D, IBS-C and IBS-A; moreover, contrary to our expectation, there was no significant difference in intestinal permeability between the IBS patients with a positive LBT and those IBS patients with a negative LBT.

The explanation for why the patients with IBS in this study had a lower rate of a positive LBT compared with previous study remains unclear. Rigorous selection criteria for the patients with IBS and differences in race and geographic area may have played some role in this discrepancy.[2,6] Above all, LBT after two weeks of colonoscopy may be most responsible for our paucity of abnormal LBTs. Bowel cleansing with polyethylene glycol (PEG, Colyte, 4 L) removes the bulk of bacterial flora including hydrogen producing ones[26,27] and the higher rates of positive LBTs for the IBS patients correlated with a longer time limit (2 to 3 months) after colonoscopy than did our results.[2,4] However, most patients with IBS continued complaining of their symptoms when they re-visited 2 weeks after colonoscopy. Therefore, a proper time limit for administering the LBT after colonoscopy should be evaluated in the future.

Our finding of a higher positive rate on the LBT for the patients with IBS-D was consistent with a previous report[4] and it also coincided with the result of this study that the IBS patients with a positive LBT have less hard stool than those with a negative LBT. Therefore, even though it was not statistically significant, it might be interpreted that SIBO may be an underlying cause for IBS-D.

Under normal conditions, PEG with a molecular weight of 400 or less is absorbed by the intestinal tract and then excreted in urine. In contrast, PEGs with a molecular mass exceeding 3,350 are poorly absorbed in intestinal tract.[28]; thus, this has been used to assess intestinal permeability under acute conditions such as inflammation, shock and burn.[29] The safety of PEG 400 and 4,000 have already been established in humans,[30] and PEG as permeability probes can offer three major advantages over ^{51}Cr-EDTA and small sugars. First, PEG is safe and easy to handle. Second, these polymers cover a wide range of

molecular sizes, including those of macromolecules, and last, their chromatographic determination is unaffected by the presence of sugars found in food and urine, and subjects are not required to adapt their diet prior to taking the test.[31] PEG has been criticized for not being sufficiently sensitive to assess intestinal permeability in some of the more subtle disorders of barrier function due to the low urine excretion levels of PEG 400.[32] However, our results of intestinal permeability for the normal subjects are in agreement with those of a previous study,[31] and when we checked the intestinal permeability twice for the same subject, we found that this method gave very similar results. Thus, we could verify the reproducibility of this method.

In the current study, we could not find any difference in intestinal permeability between the subgroups of IBS. This finding was not consistent with an earlier report that the intestinal permeability profiles differ among the IBS subtypes with higher small bowel permeability in the patients with PI-IBS and IBS-D when compared with IBS-C patients.[19] The possible causes for this discrepancy are the bias from the small number of IBS-C and IBS-A patients and the different time periods for urine collection. The urine samples for this study were collected during 8 hours, which represents absorption from the small intestine to the proximal colon. The mixed intestinal permeability of the small and large bowel may lead to different result. However, we could not exclude the possibility of increased intestinal permeability in the patients with IBS-C, Since earlier study have reported that the number of mast cells was greater in the patients with IBS-C than that in the patients with IBS-D and IBS-A,[33] and mediators from mast cell plays an important role in the increase of intestinal permeability.[34] Therefore, the exact intestinal permeability in the patients with IBS-C remains to be established.

This study could not provide any evidence that a causal relationship exists between SIBO and intestinal permeability. Although studies showing the symptom improvement after antibiotic treatment in patients with IBS have been used to further support the conclusion that SIBO is a pathophysiological factor in IBS,[2,6] this pathophysiology may be explained by alterations of colonic bacterial flora that result in symptom improvement, rather than by removal of the bacterial overgrowth.[3] Furthermore, there is a possibility that lactulose breath test was not sensitive enough to detect the SIBO properly in the small intestine and that SIBO does not cause immune activation in the GI tract, or that other factors such as stress and alcohol may play more crucial roles in the development of enhanced intestinal permeability. Therefore, further study is needed in the future to verify the cause of increased intestinal permeability in the intestine of patients with IBS and the relationship between SIBO, immune activation and intestinal permeability.

REFERENCES

1. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. JAMA 2004; 292:852-858.
2. Pimentel M, Chow EJ, Lin HC. Normalization of lactulose breath testing correlates with symptom improvement in irritable bowel syndrome: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol 2003;98:412-419.
3. Posserud I, Stotzer PO, Bjornsson ES, Abrahamsson H, Simren M. Small intestinal bacterial overgrowth in patients with irritable bowel syndrome. Gut 2007;56:802-808.
4. Lupascu A, Gabrielli M, Lauritano EC, et al. Hydrogen glucose breath test to detect small intestinal bacterial overgrowth: a prevalence case-control study in irritable bowel syndrome. Aliment Pharmacol Ther 2005;22:1157-1160.
5. Walters B, Vanner SJ. Detection of bacterial overgrowth in IBS using the lactulose H2 breath test: comparison with 14C-D-xylose and healthy controls. Am J Gastroenterol 2005;100:1566-1570.
6. Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol 2000;95:3503-3506.
7. Hong KS, Kang HW, Im JP, et al. Effect of probiotics on symptoms in Korean adults with irritable bowel syndrome. Gut Liver 2009;3:101-107.
8. Berg RD, Garlington AW. Translocation of certain indigenous bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs in a gnotobiotic mouse model. Infect Immun 1979;23:403-411.
9. Woodcock NP, Robertson J, Morgan DR, Gregg KL, Mitchell CJ, MacFie J. Bacterial translocation and immunohistochemical measurement of gut immune function. J Clin Pathol 2001;54:619-623.
10. Nieuwenhuijs VB, Verheem A, van Duijvenbode-Beumer H, et al. The role of interdigestive small bowel motility in the regulation of gut microflora, bacterial overgrowth, and bacterial translocation in rats. Ann Surg 1998;228:188-193.
11. Weston AP, Biddle WL, Bhatia PS, Miner PB Jr. Terminal ileal mucosal mast cells in irritable bowel syndrome. Dig Dis Sci 1993;38:1590-1595.
12. O'Sullivan M, Clayton N, Breslin NP, et al. Increased mast cells in the irritable bowel syndrome. Neurogastroenterol Motil 2000;12:449-457.
13. Gwee KA, Leong YL, Graham C, et al. The role of psychological and biological factors in postinfective gut dysfunction. Gut 1999;44:400-406.
14. Salzmann JL, Peltier-Koch F, Bloch F, Petite JP, Camilleri JP. Morphometric study of colonic biopsies: a new method of estimating inflammatory diseases. Lab Invest 1989;60: 847-551.
15. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenter-

ology 2005;128:541-551.
16. Al-Khatib K, Lin HC. Immune activation and gut microbes in irritable bowel syndrome. Gut Liver 2009;3:14-19.
17. Forget P, Sodoyez-Goffaux F, Zappitelli A. Permeability of the small intestine to [51Cr]EDTA in children with acute gastroenteritis or eczema. J Pediatr Gastroenterol Nutr 1985;4:393-396.
18. Bjarnson I, O'Morain C, Levi AJ, Peters TJ. Absorption of 51chromium-labeled ethylenediaminetetraacetate in inflammatory bowel disease. Gastroenterology 1983;85:318-322.
19. Dunlop SP, Hebden J, Campbell E, et al. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am J Gastroenterol 2006;101: 1288-1294.
20. Tibble JA, Sigthorsson G, Foster R, Forgacs I, Bjarnason I. Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. Gastroenterology 2002;123:450-460.
21. Dainese R, Galliani EA, De Lazzari F, Di Leo V, Naccarato R. Discrepancies between reported food intolerance and sensitization test findings in irritable bowel syndrome patients. Am J Gastroenterol 1999;94:1892-1897.
22. American Gastroenterology Association. American Gastroenterological Association medical position statement: irritable bowel syndrome. Gastroenterology 2002;123:2105-2107.
23. Spiller RC. Postinfectious irritable bowel syndrome. Gastroenterology 2003;124:1662-1671.
24. Beck A, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561-571.
25. Spielberger C. Manual for the state-trait anxiety inventory. Palo Alto: Consulting Psychologists Press, 1983.
26. Peled Y, Weinberg D, Hallak A, Gilat T. Factors affecting methane production in humans: gastrointestinal diseases and alterations of colonic flora. Dig Dis Sci 1987;32:267-271.
27. DiPalma JA, Brady CE 3rd, Stewart DL, et al. Comparison of colon cleansing methods in preparation for colonoscopy. Gastroenterology 1984;86:856-860.
28. Schiller LR, Santa Ana CA, Porter J, Fordtran JS. Validation of polyethylene glycol 3350 as a poorly absorbable marker for intestinal perfusion studies. Dig Dis Sci 1997; 42:1-5.
29. Winne D, Gorig H. Appearance of 14C-polyethylene glycol 4000 in intestinal venous blood: influence of osmolarity and laxatives, effect on net water flux determination. Naunyn Schmiedebergs Arch Pharmacol 1982;321:149-156.
30. Ameno H, Tani T, Hanasawa K, Kodama M. New method for the detection of bacterial translocation using intestinal permeability with polyethylene glycol 4000. Eur Surg Res 2000;32:23-29.
31. Loret S, Nollevaux G, Remacle R, et al. Analysis of PEG 400 and 4000 in urine for gut permeability assessment using solid phase extraction and gel permeation chromatography with refractometric detection. J Chromatogr B Analyt Technol Biomed Life Sci 2004;805:195-202.
32. Bjarnason I, MacPherson A, Hollander D. Intestinal permeability: an overview. Gastroenterology 1995;108:1566-1581.
33. Chadwick VS, Chen W, Shu D, et al. Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology 2002;122:1778-1783.
34. Jacob C, Yang PC, Darmoul D, et al. Mast cell tryptase controls paracellular permeability of the intestine: role of protease-activated receptor 2 and beta-arrestins. J Biol Chem 2005;280:31936-31948.