Mucosal Mast Cell Count Is Associated With Intestinal Permeability in Patients With Diarrhea Predominant Irritable Bowel Syndrome

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Background/Aims
Although mucosal mast cell tryptase is known to significantly increase intestinal permeability, the relationship between mucosal mast cells and intestinal permeability remains unclear. The objective of this study was to evaluate the correlation among intestinal permeability, tryptase activity and mucosal mast cell count.

Methods
Rectal biopsies from 16 patients with diarrhea-predominant irritable bowel syndrome (IBS-D) and 7 normal subjects were assessed for tryptase activity and macromolecular permeability using horseradish peroxidase in Ussing chambers. In addition, mucosal mast cell levels were immunohistochemically quantified via image analysis.

Results
Rectal biopsy of tissues from IBS-D patients showed significantly increased permeability compared with those from normal controls (0.644 ± 0.08 and 0.06 ± 0.00 ng/2 hr/mm², \( P < 0.01 \)). Tryptase activity was also substantially higher in rectal biopsy samples from IBS-D patients than those from normal controls (0.86 ± 0.18 and 0.28 ± 0.04 mU/mg protein, \( P < 0.05 \)). Mucosal mast cell counts were not significantly different between the 2 groups (\( P > 0.05 \)). However, correlation analysis revealed that only mucosal mast cell count was significantly correlated with intestinal permeability in IBS-D patients (\( r = 0.558, P < 0.05 \)).

Conclusions
This study demonstrated a positive correlation between the number of mucosal mast cells and intestinal permeability, suggesting that mucosal mast cells play an important role for increased intestinal permeability in patients with IBS-D. (J Neurogastroenterol Motil 2013;19:244-250)

Key Words
Irritable bowel syndrome; Mast cell, Permeability; Tryptase
Introduction

Abnormal intestinal permeability has been implicated in the pathogenesis of many intestinal diseases, including irritable bowel syndrome (IBS). Symptoms of post-infectious IBS are associated with a subtle increase in intestinal permeability. Patients with diarrhea predominant IBS (IBS-D) have been shown to have increased small intestinal permeability compared to post-infectious IBS patients and controls. These findings suggest that an abnormal intestinal barrier facilitates enhanced antigen exposure that may activate the intestinal immune system and induce IBS symptoms such as abdominal pain.

Although the mechanism for increased intestinal permeability in IBS is still unclear, mast cells seem to play an important role. Recent studies have demonstrated that chronic stress increased the number and activity of mucosal mast cells and induced mass cell-mediated alterations in epithelial function. Furthermore, increased numbers of CD3, CD25 lymphocytes and mast cells have been detected in the colonic mucosa of IBS patients. Finally, it has been shown that IBS-D patients have increased rectal permeability responsive to mast cell tryptase.

In our previous study, we demonstrated that mucosal mast cell tryptase plays an important role in the increased rectal permeability in IBS. However, the relationship between mucosal mast cells and intestinal permeability remains unclear. Therefore, the goal of the present study is to evaluate the correlation among intestinal permeability, tryptase activity and mucosal mast cell count.

Materials and Methods

Subjects

Biopsy specimens of the rectum were obtained during routine colonoscopies of 16 patients with IBS-D and 7 healthy controls at the Kangbuk Samsung Hospital. All the IBS-D patients exhibited symptoms that fulfilled the Rome-II criteria. Participants in the control group had macroscopically and histologically normal colonic mucosa, no persistent bowel symptoms, no organic or functional bowel disease, and no history of chronic medical disease. None of the IBS-D patients had known histories of abdominal surgery, inflammatory bowel disease, or post-infectious IBS. Informed written consent was provided by all patients, and this study was approved by the hospital’s local ethics committee.

Biopsy forceps with an opening diameter of 6 mm (FB-25K-1; Olympus, Tokyo, Japan) were used for the procedures. In most cases, 2 biopsies were taken from the rectum of IBS-D patients and controls.

Staining and Quantification of Mucosal Mast Cells

Staining for mucosal mast cells

Rectal mucosal mast cells were stained using a monoclonal antibody against the human mast cell protease tryptase. Biopsy specimens were fixed in 10% neutral buffered formalin for 24 hours. For the immunohistochemistry, paraffin-embedded specimens were cut with a microtome at 4 μm thickness. Just before staining, slides were deparaffinized in xylene and rehydrated in graded alcohol solutions. After dewaxing, tissue sections were incubated with 0.5% hydrogen peroxide in methanol at 22°C for 10 minutes and then washed with running tap water for 15 minutes. Sections were treated with 0.1% trypsin (Sigma, Poole, UK) mixed in 0.1% calcium chloride (pH 7.8) for 10 minutes at 37°C. Nonspecific binding of protein was blocked by incubation in normal rabbit serum diluted 1:5 in Tris-buffered saline (TBS; pH 7.6) for 15 minutes.

The slides were then incubated for 18 hours at 4°C with the monoclonal antibody AA1 (anti-human mast cell protease tryptase; DAKO M7052, Dako Ltd., Cambridge, UK), washed in TBS for 7 minutes, and reincubated for 30 minutes with biotinylated rabbit anti-mouse IgG (Amersham, Buckinghamshire, UK). After the slides were washed again in TBS for 7 minutes, finally they were incubated with streptavidin-biotin complex conjugated with horseradish peroxidase (HRP; DAKO k0377; Dako) for 30 minutes.

The slides were developed in diaminobenzidine-hydrogen peroxide substrate (Sigma) for 10 minutes, and washed under running tap water for 5 minutes. Sections were then counterstained with hematoxylin followed by dehydration and clearance. And then they were mounted in DPX mountant (BDH Prolabo; VWR International Ltd., Leicestershire, UK).

Quantification of mucosal mast cells

Mucosal mast cells were counted under light microscopy at ×400 magnification by an expert pathologist. Immunohistochemically positive-stained mast cells were counted in five consecutive non-overlapping microscopic fields (3 sections × 2 biopsy samples) with areas of 0.24 mm². The pathologist did not know histological section’s group of origin.