Role of mucosal mast cells in visceral hypersensitivity in a rat model of irritable bowel syndrome

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The involvement of mucosal mast cells (MMC) in the pathophysiology of irritable bowel syndrome (IBS) is still controversial. We aimed to re-evaluate the role of MMC in visceral hypersensitivity associated with IBS using a rat IBS model that develops the IBS symptom after a subsidence of acetic acid-induced colitis. No significant difference in the number of MMC was observed between normal rat colon and IBS rat colon. (61.7 ± 2.9/mm² in normal vs. 88.7 ± 13.3/mm² in IBS, p > 0.29). However, the degranulation rate of MMC was significantly higher in IBS rat colon (49.5 ± 2.4% in normal vs. 68.8 ± 3.4% in IBS, p < 0.05). Pretreatment of a mast cell stabilizer, doxantrazole (5 mg/kg, i.p.), reduced the degranulation rate of MMC and significantly attenuated visceral hypersensitivity to rectal distension in IBS rat, whereas it had no effect on the visceral sensory responses in normal rat. These results suggest that, although the number of MMC is not significantly changed in IBS rat colon, the higher degranulation rate of MMC is responsible for visceral hypersensitivity in this model IBS.

Key words: Irritable bowel syndrome, visceral hypersensitivity, mucosal mast cell.

Introduction

Irritable bowel syndrome (IBS), a chronic disorder in the absence of objective abnormalities in structures, is the most common disorder encountered by gastroenterologist [6]. Investigators have demonstrated that one of the important pathophysiological features of IBS is a lowered threshold for visceral pain elicited by luminal mechanical and chemical stimuli, a phenomenon referred to as visceral hypersensitivity [19,26]. However, the etiology and the pathophysiological mechanism of IBS remain poorly understood.

Recently, researchers have suggested the role of inflammatory cells in the pathogenesis of IBS [2]. Patients with ulcerative colitis in remission often express IBS-like symptoms [16], and IBS patients have a low but significant increase in immune cells in colonic mucosa and in jejunal myenteric plexus [14,27]. Among the inflammatory cells, mucosal mast cells (MMC) have gained researcher’s interest in relation to IBS. MMC locates throughout the gut in close proximity to enteric nerves [24], and secretes numerous inflammatory substances including histamine, cytokines, proteases, and eicosanoids [4] that are known to sensitize visceral sensory nerve fibers [12].

Despite these findings, it is still controversial whether MMC plays a key role in IBS. Several investigators have noted an increased number of MMC in terminal ileum [28] and in caecum of IBS patients [21], but others found neither changes in the number of MMC in IBS patients [15,23,25] nor correlation between the number of MMC and the extent of IBS symptoms [7]. Thus, we aimed to re-evaluate the pathophysiological role of MMC in IBS using an animal model of IBS. In our previous study, we reported that rats develop IBS symptoms after subsidence of acetic acid-induced colitis, showing visceral hypersensitivity to rectal distension [17]. In the present study, we focused to investigate (1) whether the number of MMC in colon is changed in this rat IBS model, and (2) whether the IBS symptom (visceral hypersensitivity) can be alleviated by the inhibition of mast cell degranulation.

Materials and Methods

Experimental animals and induction of IBS

Male Sprague-Dawley rats (270–310 g) were housed in stainless steel hanging cages in colony room maintained under a 12 h light/dark cycle with a room temperature of 22 ± 1°C and humidity of 65-70%. Water and food were available ad libitum. IBS symptoms were produced as described previously [17]. Briefly, colitis was induced by intracolonic instillation of 1 ml 4% acetic acid. Control
animals received saline instead of acetic acid. Rats were left to recover from colitis for 6 days, and used for experiments at 7 days after induction of colitis.

Mucosal mast cells counting
On the day of experiments, rats were sacrificed by cervical dislocation and the distal colon was dissected. The colonic samples were fixed in Carnoy’s fixative for 2 hr at room temperature, and then transferred to 30% sucrose in phosphate buffered solution at 4°C overnight. Sample blocks embedded in OCT compound (Sakura Finetechical Co., Japan) were cut into 10 µm thick transverse sections in a cryostat-microtome. The sections were reacted with 0.5% toluidine blue in 0.5 N HCl for 30 min. Specimen was examined under a light-microscope (Axioskop, Carl Zeiss, Germany) and digitally photographed at ×400 (Micromax cooled-CCD, Princeton Instrument, USA). Three sections per animal were examined and the number of MMC was counted in at least 10 randomly selected fields using an image analyzing software. Loss of intracellular granules, with stained material dispersed diffusely within the lamina propria, was taken as an evidence of MMC degranulation [5]. The number of MMC was expressed as mean number of cells per mm², and the degranulation rate of MMC was expressed as a percentile proportion of the degranulated MMC to the total MMC in the photographed fields.

Visceral sensory responses to rectal distension
Overnight fasted rats were lightly anesthetized with either, and disposable silicon balloon-urethral catheter for pediatric use (6 Fr, Sewoon Medical, Korea) was inserted intra-anally until the end of the balloon was 2 cm inside the rectum. Rats were placed in a transparent cubicle (20 × 8 × 8 cm) on a mirror-based elevated platform and were allowed to recover and acclimate for a minimum of 30 min before testing. After animals were fully awaken and acclimate, ascending-limits phasic distension (0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and 1 ml) with pre-warmed (37°C) water was applied for 30 sec every 4 min. The visceral sensory responses to rectal distension was quantified by scoring the abdominal withdrawal reflex (AWR), as described previously [17], and simultaneously measuring the concomitant increase in arterial pulse rate (tachycardia) from caudal artery using a non-invasive pulse transducer (MLT125R, AD Instruments, Australia). The AWR score and the pseudo-affective tachycardiac responses are reported to be the reliable indicators of the visceral sensory response to luminal distension [1,17,20].

The overall difference in the visceral sensory responses between groups was determined by taking the area under the curve (AUC) that was calculated as the sum of responses plotted against the distension volume using the trapezoidal rule.

Effect of a mast cell stabilizer
The effect of doxantrazole ((3-(1H-tetrazol-5-yl)-9H-thioxanthen-9-one 10,10-dioxide monohydrate, Aldrich, Milwaukee, WI, USA), a mast cell stabilizer, was investigated in normal and IBS rats. Doxantrazole (5 mg/kg, i.p.) was dissolved in dimethylsulfoxide (DMSO) and injected 30 min before experimentation. Control animals received an equal amount of DMSO as a vehicle.

Statistical analyses
Data were expressed as mean ± SEM, with n, the number of animals. The difference in the values was statistically analyzed using Mann-Whitney U (MWU)-test at the p < 0.05 significance level. Comparisons between three or more groups were performed with Kruskal-Wallis test (KW test) followed by nonparametric Dunn’s test.

Results
The number of MMC in distal colon
In the toluidine blue-stained colonic specimens, MMC was easily identified in the lamina propria. The mean number of MMC was 61.7 ± 2.9/mm² (n = 5) in normal rat colon and 88.7 ± 13.3/mm² (n = 5) in IBS rat colon (Fig. 1). There was no statistically significant difference between these values (p > 0.29).

The degranulation rate of MMC in distal colon
The degranulation rate of MMC was significantly higher in IBS rat colon than in normal colon. In IBS rat colon, the number of MMC and the degranulation rate were compared to normal rats.

Fig. 1. The number of mucosal mast cells (MMC) in colonic mucosa. MMC was stained by 0.5% acidic toluidine blue. Bar indicates 100 µm in the upper left photograph. No statistically significant difference was detected between normal and IBS group (P = 0.29 by MWU test, n = 5 in each group).