

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. ($61.7 \pm 2.9/\text{mm}^2$ in normal vs. $88.7 \pm 13.3/\text{mm}^2$ in IBS, $p > 0.29$). However, the degranulation rate of MMC was significantly higher in IBS rat colon ($49.5 \pm 2.4\%$ in normal vs. $68.8 \pm 3.4\%$ 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 $22 \pm 1^\circ\text{C}$ 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

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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 Finetechnical 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 awoken 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-(1*H*-tetrazol-5-yl)-9*H*-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,

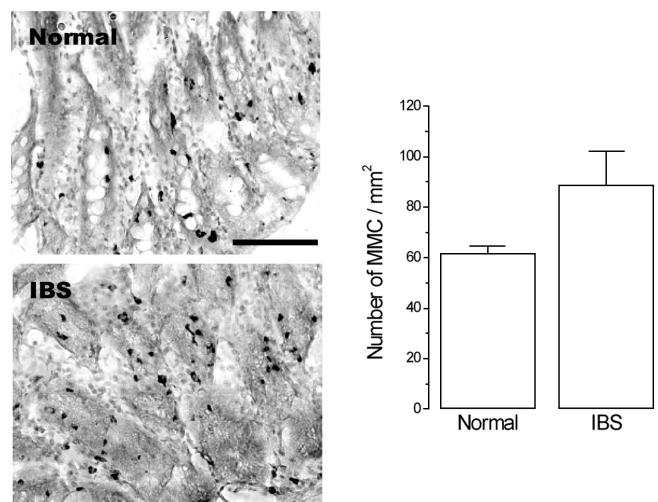


Fig. 1. The number of mucosal mast cells (MMC) in colonic mucosa. MMC was stained by 0.5% acidic toluidine blue. Bar indicates 100 µm in the upper left photograph. No statistically significant difference was detected between normal and IBS group (*P* = 0.29 by MWU test, *n* = 5 in each group).

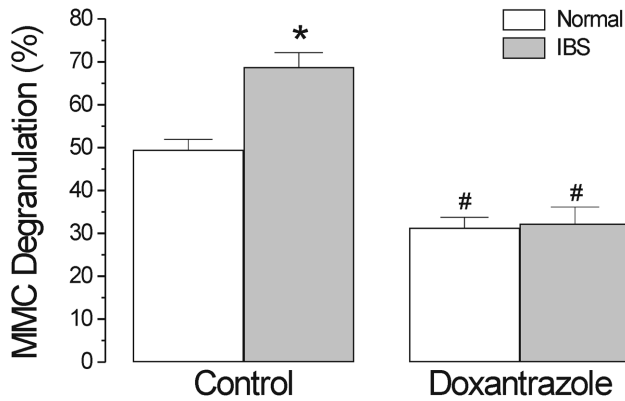


Fig. 2. The degranulation rate of MMC in colonic mucosa. Doxantrazole (5 mg/kg, i.p.) was administered 30 min prior to the sacrifice. Control animals received DMSO as a vehicle. * $p < 0.05$ vs. normal, # $p < 0.05$ vs. corresponding control group by KW test followed by Dunn's test ($n = 5$ in each group).

68.8 ± 3.4% MMC ($n = 5$) was identified to be degranulated, whereas in normal colon, 49.5 ± 2.4% MMC ($n = 5$) was degranulated ($p < 0.05$) (Fig. 2). In the groups that received a mast cell stabilizer, doxantrazole (5 mg/kg, i.p.), the degranulation rate of MMC was reduced to 31.3 ± 2.4% ($n = 5$) in normal rat colon ($p < 0.05$ vs. normal control) and to 32.2 ± 3.9% ($n = 5$) in IBS rat colon ($p < 0.05$ vs. IBS control). The degranulation rate of MMC in the doxantrazole-treated groups was not different between normal and IBS rat colon ($p > 0.8$).

Effect of a mast cell stabilizer on the responses to rectal distension

Upon phasic rectal distension, rats showed the characteristic AWR behavior and the tachycardiac pseudo-affective response. These visceral sensory responses increased as the intensity of distending stimulus was raised. As shown in Fig. 3, the visceral sensory responses to rectal distension were exaggerated in the IBS rat, resulting in the significant increase in the AUC of the stimulus-response plots (1.7 ± 0.2 vs. 2.5 ± 0.1 in the AWR score, $p < 0.01$; 38.5 ± 1.9 vs. 57.2 ± 7.7 in the Δ pulse rate, $P = 0.03$, $n = 7$ in both groups). These results indicate that the IBS rat has visceral hypersensitivity. We examined whether the visceral hypersensitivity in the IBS rat is related to degranulation of MMC. In normal rats, doxantrazole had no effect on either the visceral sensory AWR behavior or the pseudo-affective tachycardiac responses to rectal distension (Fig. 4). However, the same dose of mast cell stabilizer effectively alleviated the visceral hypersensitivity in IBS rats. By the pretreatment of doxantrazole, the AUC of the stimulus-response plots was significantly reduced from 2.5 ± 0.1 to 1.9 ± 0.2 ($P = 0.022$) in the AWR score and from 57.2 ± 7.7 to 29.5 ± 4.6 ($p < 0.01$) in the Δ pulse rate (Fig. 5).

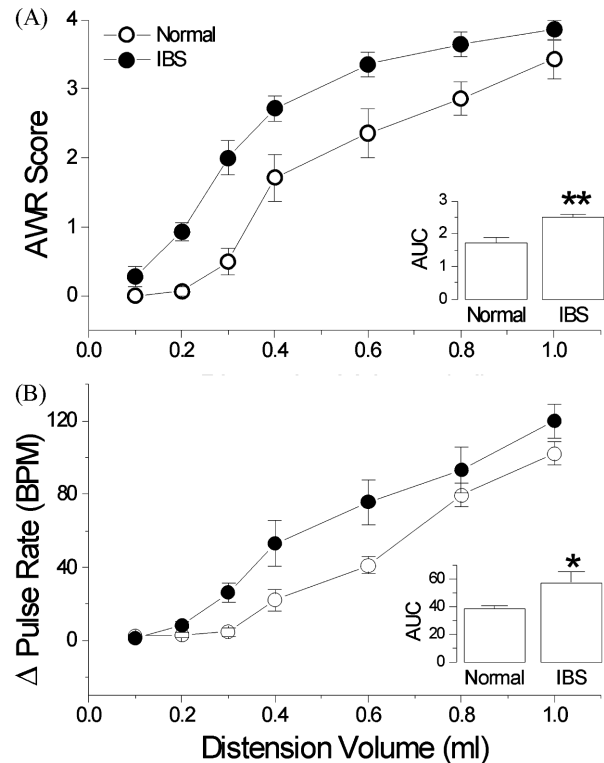


Fig. 3. Visceral sensory responses to phasic rectal distension. (A) Score of the AWR behavior and (B) the extent of tachycardiac pseudo-affective response upon rectal distension. Inset plot represents the area under the curve (AUC). * $p < 0.05$, ** $p < 0.01$ vs. normal ($n = 7$ in each group).

Discussion

This study demonstrates that the number of MMC is not changed in IBS rat (Fig. 1). Because the rat model of IBS used in this study develops IBS symptom after subsidence of colonic inflammation, our observation could reflect the condition of post-infectious IBS (PI-IBS) patients who acquire the IBS symptoms after an episode of intestinal infection and inflammation. Thus, it can be supposed that patients with PI-IBS may not show notable changes in the number of MMC in colonic mucosa. Indeed, it was recently reported that the number of MMC in PI-IBS patients is virtually identical to that in healthy subjects [11,23].

Although no change was observed in the number of MMC in IBS rat, this result does not necessarily mean that MMC does not play a role in the pathophysiology of IBS. We found that the percentage of degranulated MMC is significantly higher in IBS rat colon than in normal colon (Fig. 2). This finding implies that the state of MMC degranulation, rather than the total number of MMC, may be related to the IBS symptom development. Supporting this notion, we observed that the inhibition of MMC degranulation by doxantrazole could effectively attenuate

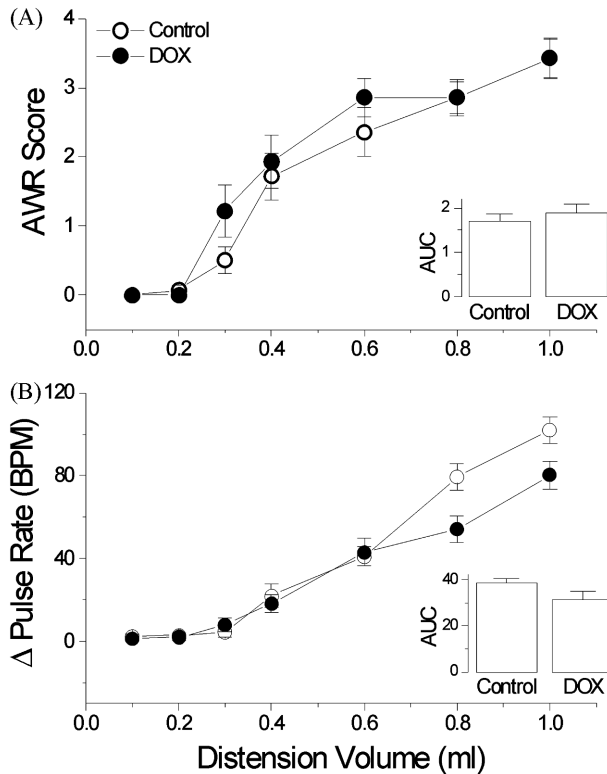


Fig. 4. Effect of doxantrazole on the visceral sensory responses in normal rats. Doxantrazole (DOX, 5 mg/kg, i.p.), administered 30 min before the rectal distension, had no effect on either (A) the AWR score or (B) the extent of tachycardiac response in normal rats ($n=7$ in each group).

visceral hypersensitivity to rectal distension in the IBS rat. Doxantrazole significantly inhibited the AWR behavior and the tachycardiac pseudo-affective responses to rectal distension (Fig. 5). Recent studies further support the importance of MMC degranulation in the IBS symptoms. The degranulation rate of MMC in close proximity to nerves is reported to be significantly higher than that of MMC away from nerves in the colorectal specimens of diarrhea-predominant IBS patients [22], and a significant correlation is found between vicinity of MMC to nerves and the severity of abdominal pain/discomfort symptom of IBS [3]. These findings, together with our current observation, suggest that the degranulation of MMC is an important pathophysiological factor in visceral hypersensitivity associated with IBS. In experimental animals, the role of MMC degranulation in visceral hypersensitivity has been demonstrated; doxantrazole significantly inhibited the exaggerated depressor response to jejunal distension in rats at 30 days post-nematode infection [18] and the increased abdominal response to rectal distension in stressed rats [13].

It is interesting that about a half of MMC is in the degranulated state in normal rat colon, and this degranulation rate is reduced to $31.3 \pm 2.4\%$ by the pretreatment of doxantrazole (Fig. 2). In keeping with our conclusion that

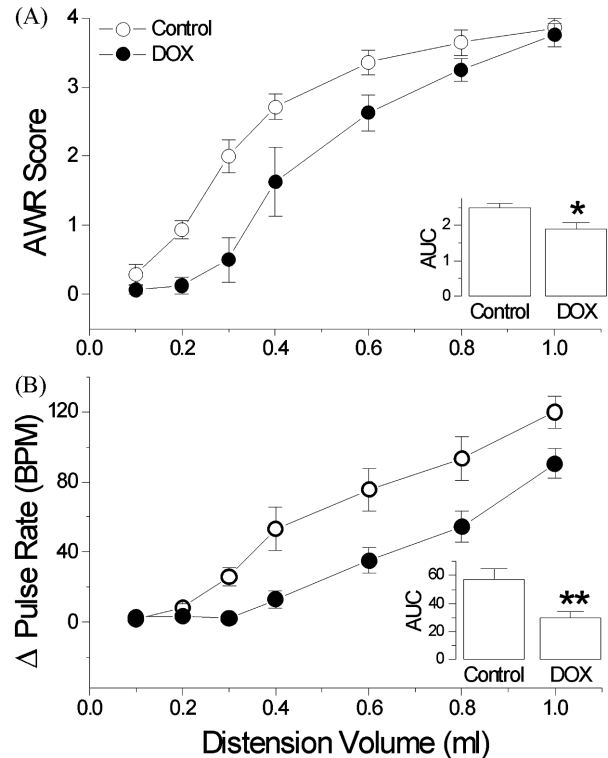


Fig. 5. Effect of doxantrazole on the visceral hypersensitivity in IBS rats. Pretreatment of doxantrazole (DOX, 5 mg/kg, i.p.) attenuated both (A) the AWR score and (B) the extent of tachycardiac response in IBS rats. * $p<0.05$, ** $p<0.01$ vs. control by MWU test ($n=7$ in each group).

degranulation of MMC causes the exaggeration of the visceral sensory responses to rectal distension, it is quite expectable that, in normal rat, doxantrazole can also reduce the visceral sensory behavior and the pseudo-affective response to some extent. However, we did not find any significant effect of doxantrazole on the visceral sensory responses to rectal distension in normal rats. Considering that the activation rate of MMC away from nerves was similar between IBS patients and control subjects [22], it could be assumed that such MMC degranulation rate as in normal rat colon represents the activation state of MMC away from nerves, and hence cannot produce any significant change in visceral sensory function. A further ultrastructural study is required to provide evidences for this assumption.

It is well known that mast cells can synthesize a variety of substances, and these substances can sensitize sensory neuron [4]. Although it is beyond the scope of the present study to identify the most important mast cell mediators that are responsible for visceral hypersensitivity in IBS, it is worthy to mention that antagonists of H_1 , H_2 , and H_3 histamine receptors did not modify but 5-HT_{1A} receptor antagonist effectively reduced the mast cell activator-induced visceral allodynia [8]. In addition, it was also reported that experimental activation of proteinase-activated

receptor-2 (PAR-2), which could be activated endogenously by mast cell tryptase [10], induced visceral hyperalgesia in rats [9]. It would be of interest in future study to examine whether serotonin acting on 5-HT_{1A} receptor and tryptase acting on PAR-2 are involved in visceral hypersensitivity in IBS rats.

In conclusion, we found that the number of MMC in colon is not changed, but the degranulation rate of MMC is higher in IBS rats. Additionally, we observed that the inhibition of the MMC degranulation could attenuate visceral hypersensitivity in IBS rats. These results suggest that the increased degranulation of MMC might be responsible for the visceral pain/discomfort symptom of IBS.

Acknowledgments

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