Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 82 (2008) 419–423
Life Sciences
www.elsevier.com/locate/lifescie

Abolishment of TNBS-induced visceral hypersensitivity in mast cell deficient rats

Katsuyo Ohashi, Yasushi Sato, Mitsuhisa Kawai, Yoichi Kurebayashi *

Discovery Research, Pfizer Global Research and Development, Nagoya Laboratories, 5-2, Taketoyo, Chita-gun, Aichi 470-2393, Japan

Received 26 June 2007; accepted 29 November 2007

Abstract

Mucosal mast cells are implicated in visceral hypersensitivity associated with irritable bowel syndrome (IBS). In this study, we investigated the role of mast cells in the development of visceral hypersensitivity by using mast cell deficient (Ws/Ws) rats and their control (W+/W+). In W+/W+ rats, an injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS) into the proximal colon produced a significant decrease in pain threshold of the distal colon. Severe mucosal necrosis and inflammatory cell infiltration with concomitant increase in tissue myeloperoxidase activity were observed in the proximal colon that was directly insulted by TNBS, whereas neither necrosis nor increased myeloperoxidase activity occurred in the distal colon, indicating that TNBS-induced hypersensitivity is not caused by the local tissue damage or inflammation in the region of the gut where distention stimuli were applied. On the other hand, TNBS failed to elicit visceral hypersensitivity in Ws/Ws rats. This finding indicates that mast cells are essential for development of TNBS-induced visceral hypersensitivity in rats. Since the severity of TNBS-induced proximal colon injury and MPO activity was not affected by mast cell deficiency, it is unlikely that abolishment of visceral hypersensitivity in mast cell deficient rats was a result of altered development of the primary injury in the proximal colon. There was no difference between sham-operated Ws/Ws and W+/W+ rats in colonic pain threshold to distention stimuli, indicating that mast cells play no modulatory roles in normal colonic nociception. The present results support the view that mucosal mast cells play key roles in the pathogenesis of IBS.

Keywords: IBS; Mast cell deficient rats; Myeloperoxidase; TNBS; Visceral hypersensitivity

Introduction

The irritable bowel syndrome (IBS) is the most common functional gut disorder characterized by abdominal pain or discomfort (Foxx-Orenstein and Clarida 2001; Olden, 2002). A growing number of studies have demonstrated that colorectal sensory threshold to mechanical distention stimuli is markedly decreased in IBS patients, indicating a phenomenon referred to as visceral hypersensitivity (Thompson et al., 1999; Camilleri and Prather, 1992). Although the mechanisms underlying the phenomenon remained to be fully investigated, recent studies have suggested that mucosal mast cells play a key role in the pathogenesis of visceral hypersensitivity associated with IBS (Wood, 2002; Barbara et al., 2007) Mucosal mast cells locate in close proximity to the enteric sensory nerves throughout the gut, and degranulate in response to various mechanical and chemical stimuli, releasing histamine, tryptase, eicosanoids and cytokines that can excite the sensory nerves (Stead et al., 1989; Bueno et al., 1997; Galli et al., 1999; Stevens and Austen, 1989). In addition, it has been demonstrated that some IBS patients show a prominent colonic mucosal mast cell infiltration associated with increased degranulation rate and spontaneous release of histamine and tryptase (Barbara et al., 2004, 2007), suggesting that mediators released from activated mast cells are responsible for the increased sensitivity of enteric sensory nerves in patients with IBS.

The role of mucosal mast cells in the pathogenesis of visceral pain has also been investigated in rats. Coelho et al. reported that mast cell degranulation induced by lipopolysaccharide and BrX-537A resulted in a significant increase in the rectal sensitivity to mechanical distention in rats, and that the hypersensitivity was significantly suppressed by pretreatment with doxantrazole, an inhibitor of mast cell mediator release

* Corresponding author.
E-mail address: kurebayashiy@port.kobe-u.ac.jp (Y. Kurebayashi).

0024-3205/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2007.11.027

(Coelho et al., 2000). More recently, it was demonstrated that visceral hypersensitivity occurred in the rat acetic acid colitis model in association with the higher degranulation rate of colonic mucosal mast cells (La et al., 2004). These findings point to the key role played by intestinal mast cells in development of visceral hypersensitivity in rats.

Recently, it has been reported that an injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS) into the rat proximal colon resulted in a significant decrease in the sensory threshold of the non-inflamed distal colon to mechanical distention stimuli (Diop et al., 2002). This experimental visceral hypersensitivity is unique compared to other animal models in that pain threshold to mechanical distention can be determined at the intact region of the gut, and that the hypersensitivity lasts for over 2 weeks, providing a useful tool for investigation of the pathophysiology and therapy of IBS (Diop et al., 2002; Ohashi et al., 2007a,b). Earlier workers have demonstrated a significant suppression of TNBS-induced visceral hypersensitivity by pretreatment with antibodies against nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) that are known to be released from mast cells, and hypothesized that mast cells are playing an important role as a source of the neurotrophins in the development of the experimental visceral hypersensitivity in rats (Delafoy et al., 2003, 2006). In a previous study, we have found that a prominent mucosal mast cell infiltration with a concomitant increase in spontaneous mediator release occurred in the sensitized colon of the TNBS-treated rats, and that the induced visceral hypersensitivity was significantly suppressed by a mast cell stabilizer doxantrazole, indicating a possible involvement of mucosal mast cells (Ohashi et al., 2007a).

In the present study, in order to confirm and further extend the roles of mucosal mast cells in the pathogenesis of IBS, we investigated the impact of mast cell deficiency on development of TNBS-induced visceral hypersensitivity using mast cell deficient Ws/Ws rats.

Materials and methods

Animals

Male Ws/Ws and W+/W+ rats were purchased from SLC Co. (Shizuoka, Japan). The origin of Ws/Ws and W+/W+ rats has been described in the previous paper (Niwa et al., 1991). They were kept under conditions of constant temperature (23±2 °C) and humidity (55±15%) with a 12-h light/dark conditions with free access to normal laboratory chow and tap water. All procedures employed in this study were approved by the institutional Animal Ethics Committees according to the Laboratory Animal Welfare guidelines.

TNBS-induced hypersensitivity

After 16–18 h fasting, the animals were anesthetized by combined intramuscular administration of ketamine (40 mg/kg) and xylazine (6 mg/kg), abdominal laparotomy was made for an injection of TNBS (50 mg/1.5 ml/kg) into the proximal colon (1 cm distal from the cecum). The sham-operated rats were prepared with the same surgical procedure, but received vehicle alone instead of TNBS. Measurement of visceral pain threshold was carried out on day 7 post-surgery as described previously (Diop et al., 2002). In short, a 5-cm latex balloon (Okamoto, Japan) was inserted through the anus and placed in the distal colon at 5 cm from the anus. After 30-min acclimation, the balloon was progressively inflated from 0 to 70 mmHg, by 5 mmHg increments every 30 s using the electronic barostat (G&J, Canada). The distention procedure was repeated twice with 10 min interval, i.e. the first one for preliminary conditioning and the second one to determine the pain threshold. The pain threshold was defined as the pressure that was required to elicit any behavioral signs of pain, corresponding to the repeated waves of contraction of oblique musculature with inward turning of the hind limb, or to hump-backed position, or to squashing of the lower abdomen against the floor (Wesselmann et al., 1998).

Histological study

On day 7 post-TNBS injection, a segment was taken from the proximal colon (5 cm proximally from the cecum) and distal colon (5 to 10 cm from the anus), sectioned transversely in their entirety, and fixed overnight in 4% paraformaldehyde. The fixed tissues were processed in paraffin, cut into 5 μm sections, stained with hematoxylin–eosin (H&E) or toluidine blue, and examined with light microscopy. All sections were masked to avoid any biases in histological examinations.

Colonic mucosal myeloperoxidase activity

Colon samples were taken on day 7 post-TNBS, cut into ~5 mm^2 pieces, and homogenized in 1 ml of 0.5% hexadecyltrimethylammonium bromide per 100 mg of colon tissue. The homogenate was stored at −80 °C in a deep freezer. Myeloperoxidase (MPO) activity was determined according to the method described in a previous report (Diop et al., 2002). The results were expressed as MPO units per milligram of tissue.

Compounds

2,4,6-Trinitrobenzene sulfonic acid was purchased from Fluka (Buchs, Switzerland) and dissolved in 30% ethanol at 33 mg/ml for intra-colonic injection. Ketamine/xylazine solution was purchased from Sigma Aldrich Co. (St. Louis. MO). All other reagents used in this study are of reagent grade.

Statistical analysis

The pain threshold data are represented as medians and the 1st and 3rd quartiles that indicate the range of median values calculated by Prism Software (GraphPad, CA, USA). The statistical analysis was carried out using Kruskal–Wallis testing followed by individual Mann–Whitney *U*-test and *p*-values less than 0.05 were considered as statistical significance. The