

Involvement of 5HT₃ Receptors in Anti-Inflammatory Effects of Tropisetron on Experimental TNBS-Induced Colitis in Rat

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ABSTRACT

Introduction: There is a pressing need for research leading to the development of new effective drugs with lower side effects and more efficacy for treating inflammatory bowel disease (IBD). The analgesic and anti-inflammatory properties of 5-Hydroxytryptamine (5-HT)-3 receptor antagonists have been shown in *in vivo* and *in vitro* studies. The present study was designed to investigate the effects of tropisetron, a 5-HT₃ receptor antagonist, on an immune-based animal model of IBD. **Methods:** In the present study, the trinitrobenzenesulfonic acid (TNBS) model of colitis in the rat was used. Two hours after induction of colitis in rats, tropisetron (2 mg/kg), dexamethasone (1 mg/kg), meta-chlorophenylbiguanide (mCPBG, 5 mg/kg), a 5-HT₃ receptor agonist, or tropisetron + mCPBG were intraperitoneally (i.p.) administered for 6 days. Animals were then sacrificed; macroscopic, histological, biochemical (myeloperoxidase [MPO]) assessments and ELISA test (tumor necrosis factor-alpha, interleukin-6 and interleukin-1 beta) were performed on distal colon samples. **Results:** Tropisetron or dexamethasone treatment significantly reduced macroscopic and microscopic colonic damages. In addition, a significant reduction in MPO activity and colonic levels of inflammatory cytokines was seen. The beneficial effects of tropisetron were antagonized by concurrent administration of mCPBG. **Conclusion:** The present study indicates that the protective effects of tropisetron on TNBS-induced colitis can be mediated by 5-HT₃ receptors.

Introduction

Inflammatory bowel disease (IBD), comprising ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder of the gastrointestinal (GI) tract characterized by a relapsing course. The etiology of both diseases remains ambiguous; however, they are likely to result from complex interactions of susceptibility genes, environment, and immune system.^{1,2} Both entities have a broad spectrum of clinical presentations. IBD refers to massive cellular infiltrates and pertains to immunological abnormalities indicating increasing number of CD4⁺ T lymphocytes, mast cells, neutrophils, and eosinophils.³ Patients with IBD frequently suffer from fatigue, inflammation, ulceration, edema, diarrhea along with blood and/or mucus, fever and gastric dysmotility.⁴ Many medical therapies have been proposed for IBD such as 5-aminosalicylate derivatives, glucocorticoids, and immunosuppressives;⁵ nonetheless, available medicines

are not universally effective and result in marked deleterious effects. Therefore, the medical management of IBD remains challenging and investigations on novel treatments are required.⁶

5-hydroxytryptamine (serotonin) is an important gastrointestinal (GI) signaling molecule involving in motor, secretory and sensory functions.⁷ It is also found in the immune-inflammatory axis and modulates the immune response in several autoimmune conditions.^{8,9} Intestinal inflammation may arise from a change in 5-HT-producing enterochromaffin (EC) cells and an increase in 5-HT content associated with the pattern of IBD.¹⁰ These actions are mediated by a large family of serotonin receptors located within the neural circuitry and on other cell types in the gut. Of the 5-HT receptors expressed in intestines, the 5-HT₃ receptor has been one of the most widely studied receptor in GI function.¹¹ To illustrate, 5-HT₃ receptors are widely expressed in cells of the immune system including primary

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human monocytes and T cells.¹² Serotonin can modulate T-cell activation and proliferation through activation of their 5-HT₃ receptors. In addition, experimental studies revealed that 5-HT₃ receptor antagonists including tropisetron and ondansetron possess both analgesic and anti-inflammatory effects.¹³ Nevertheless, the mechanisms for these impacts have not been understood yet. Fiebich *et al* reported that lipopolysaccharide-stimulated secretion of tumor necrosis factor- α (TNF- α) and interleukin-1b (IL-1b) was dose-dependently inhibited by tropisetron in human monocytes.¹³ Moreover, tropisetron inhibits both IL-2 gene transcription and IL-2 synthesis in stimulated T cell.¹⁴ Preliminary data have shown that the intra-articular administration of tropisetron exerts a beneficial effect upon patients with osteoarthritis, rheumatoid arthritis and scleroderma.¹⁵ Furthermore, Musavizadeh *et al*¹⁶ demonstrated the anti-inflammatory effect of tropisetron on acid acetic induced colitis, although the authors reported that this beneficial effect is likely to be independent of 5-HT₃ receptors.

Recent progress in our understanding about mucosal immunity and pathophysiology of IBD has mostly been achieved by the development in new experimental animal models of intestinal inflammation. Large intestine inflammation induced by rectal administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS) is among the most common models of colitis described in the literature.¹⁷ This model can exhibit the inflammation related to cytokines secretion,¹⁸ and it is efficiently able to mimic both acute and chronic colitis resembling the human UC.¹⁹

With regard to the probable association between inflammation and 5-HT, it can be inferred that the anti-inflammatory property of tropisetron in experimental colitis, may be at least partly mediated through its effect on 5-HT₃ receptor pathways. The aim of this investigation was to assess the anti-inflammatory property of tropisetron (through its effect on 5-HT₃ receptors) upon colonic inflammation markers, to compare the effects of tropisetron with dexamethasone as a reference drug, and to explore the probable involvement of 5-HT₃ receptors in producing anti-inflammatory effect of tropisetron against colitis in immune-based animal model of colitis.

Materials and methods

Animals

Twelve-week-old male Wistar rats weighing approximately 250 \pm 20 g bred in animal house of School of Pharmacy, Isfahan University of Medical Sciences were used. All studies were performed under approval of Animal Care Committee of the Isfahan University of Medical Sciences (Tehran Protocol no. 302386; February 20, 2005) and were in agreement with the guidelines for the proper use of animals in biomedical research. The rats were housed in groups of 6 in temperature-controlled rooms with a 12-h light/dark cycle and fed standard pelleted chow and water *ad libitum*. Rats were ultimately sacrificed by inhalation

of ether.

Chemicals

Dexamethasone was obtained from Iran Hormone Pharmaceutical Co. (Tehran, Iran). TNBS, metachlorophenylbiguanide (mCPBG), tropisetron hydrochloride, hexadecyltrimethyl-ammonium bromide (HTAB), aprotinin A, bovine serum albumin, phenylmethylsulfonyl fluoride, benzethonium chloride, ethylene diamine tetra-acetic acid (EDTA), and Tween 20 were all purchased from Sigma Chemical Company (St. Louis, MO, USA). The amount of colonic rat TNF- α , IL-1 β and IL-6 were quantified by commercially available enzyme-linked immunosorbent assay (ELISA) kits (ALPCO, USA).

Induction of colitis

All rats were fasted for 36 h prior to the induction of colitis, with free access to water. Colitis was induced by means of method of Morris *et al.*²⁰ They were slightly anaesthetized with ether and given 10 mg (50 mg/kg) of TNBS dissolved in 0.25 mL of 50% ethanol (v/v) by means of a polyethylene catheter inserted 8 cm proximal to the anus. Following instillation of TNBS, rats were maintained in a supine Trendelenburg position for 2-3 min in order to prevent anal leakage of TNBS. Thereafter, they returned to their cages with free access to food and water. Normal group received enema of 0.25 ml of normal saline.

Grouping

Animals were randomly assigned to six groups (n = 6). TNBS-control group received normal saline, 2 h subsequent to induction of colitis. In normal group, normal saline (0.25 ml/rat) was administrated intrarectally instead of TNBS. Dexamethasone (1 mg/kg)²¹ and tropisetron (2 mg/kg)¹⁶ were administered to dexamethasone and tropisetron groups, respectively. Meta chlorophenylbiguanide (mCPBG) group received mCPBG (5 mg/kg), a selective 5-HT₃ agonist²² and finally in tropisetron + mCPBG group, tropisetron and mCPBG were administrated concurrently. All treatments were carried out intraperitoneally (i.p.), 2 h after induction of colitis by TNBS, and continued daily for six days.²⁰

Assessment of body weight changes and diarrheal status

Animal body weights and occurrence of diarrhea were recorded daily over the experiments. The Percent of body weight loss was thereafter measured. Using arbitrary criteria (1. Formed stools, 2. Loosed stools, 3. Diarrhea), fecal output was assessed every day for six days.

Macroscopic assessment

Once the animals were sacrificed by means of ether inhalation, the distal colon was removed and opened longitudinally. Afterwards, the colonic segment was cleaned of fecal content, fat and mesentery and processed for assessment by macroscopic, histological scores and biochemical markers. Each specimen (under a constant load of 8 cm from the anus) was weighed (mg), and weight/ length ratio (mg/cm) was measured. Each colon was scored for macroscopically visible damage on a 0–15 scale by an observer unaware of the treatment, according

to criteria previously defined by Ballester *et al* (some modifications have been undertaken as shown in Table 1).²³ The colon samples were subsequently sectioned in 3-4 longitudinal fragments and immediately frozen in liquid nitrogen for biochemical assessment.

After taking photos from distal colons, ulcer area and percent of necrosis were determined according to method of measurement of ulcer area and percent of necrosis mentioned in our previous study.²⁴

Table 1. Scoring criteria for assessment of macroscopic rat colonic injuries

Adhesions	0 No adhesions
	1 Difficult dissection
	2 Visible adhesions
	3 'Wrapped' intestine
Obstruction	0 No obstruction
	1 Need for gentle manual cleaning
	2 Fecal impaction
Thickening	0 Similar to uninflamed intestine
	1 Thicker than normal (~1-2mm)
	2 Much thicker than normal (>2mm)
Hyperemia	0 Similar to uninflamed intestine
	1 Mild and generalized or intense but localized hyperemia
	2 Intense and localized hyperemia
	3 Frank hemorrhage
Necrosis	0 No signs of necrosis
	1 Small areas of necrosis
	2 Patchy necrosis
	3 Focal necrosis <0.8 cm
	4 Focal necrosis >0.8 cm
	5 Extended necrotic lesion

Histological assessment

For histological examination, a sample of colonic tissue was fixed in 10% formalin, dehydrated, paraffin embedded, processed, sliced into 4 µm-thick sections and stained with haematoxylin and eosin (H&E). The histological damage was evaluated by a pathologist coworker who was blinded to the experimental groups, according to previously described criteria.^{25,26} Total colitis index was then derived by summing three sub-scores (inflammation severity, inflammation extent, crypt damage) on H&E-stained and coded sections.

Measurement of myeloperoxidase (MPO) Activity

MPO which is an enzyme found predominantly in the azurophilic granules of neutrophils was measured as a quantitative index of inflammation in intestine, using the modified method of Bradley *et al*.²⁷ Each segment was weighed and chopped in 1 ml of 50 mM potassium phosphate buffer involving 0.5% HTAB. Having chopped, we placed the tissue in a homogenizing tube. The container was then rinsed with 2×1 ml HTAB in buffer solution. Afterwards, we added more buffer in order to provide a concentration equivalent to 5 ml per 0.1 g of colon tissue

and homogenized (15000 rpm) for 4×45 s at 1 min intervals. The homogenate was placed in a sample tube, sonicated in an ice bath for 10 s, subjected to 3 cycles of freezing and thawing, and sonicated again for 10 s. The suspensions were thereafter centrifuged (15000 rpm for 15 min at 4°C) and the supernatant was decanted for assessment. The MPO activity was analyzed spectrophotometrically as follows: 0.1 ml of the supernatant was added to 2.9 ml of 50 mM K₃PO₄ buffer (pH = 6.0) involving O-dianisidine dihydrochloride (0.167 mg/ml) and 0.005 % hydrogen peroxide. The absorbance of the reaction mixture was recorded at a wave length of 450 nm by means of a UV-Vis spectrophotometer. The data were reported as the change in absorbance/min/mg colonic wet weight.

Measurement of cytokine in the rat colon

The levels of TNF-α, IL-1b and IL-6 in the colon samples were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO, USA) as described previously.²⁸ The colon tissue segments were weighed and processed in order to determine IL-1β, IL-6 and TNF-α content. Thereafter, colon samples were homogenized in phosphate buffered saline (PBS; pH = 7.4) containing 0.4 M NaCl, 0.05% Tween-20, 0.5% bovine serum albumin, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM benzethonium chloride, aprotinin A 20 KI, and 10 mM EDTA. They were then centrifuged at 12000×g for 30 min at 4°C, and ELISA was performed in order to assess the levels of IL-1β and TNF-α in the supernatants.

Statistical analysis

Data analysis was performed using the SPSS statistical package (Version 17.0). Comparison between groups was made using one-way analysis of variance (ANOVA) with Tukey post hoc test. Clinical activity score of colitis and macroscopic and histological scores were statistically analyzed using the Mann-Whitney U test. Results were reported as mean ± standard error of mean (SEM). A P-value < 0.05 was considered significant.

Results

Change in animals' body weight and diarrheal status

TNBS-treated rats experienced a body weight loss after 6 days (P < 0.001). Percentage of body weight loss in tropisetron and dexamethasone-treated groups was significantly lower than TNBS-control group after 6 days (Table 2); however, these groups showed a significant body weight loss in comparison with normal group.

As can be noted in Fig. 1, diarrheal status of TNBS-control group was significantly higher than that of normal group, over the experiment (P < 0.01). During the first four days after induction of colitis, dexamethasone and tropisetron-treated rats showed a significant increase in the diarrhea index, in comparison with normal group (P values are shown in Fig. 1). However, diarrhea index reduced gradually in these groups during this time period, and after fourth day, there was no significant difference in diarrheal status of aforementioned groups compared to

that of normal group. In addition, no significant difference was observed in the daily diarrheal status and percentage of body weight loss between the tropisetron-treated and dexamethasone-treated groups during the experiment. The mCPBG, a serotonin agonist, could not change the diarrheal status of TNBS-treated rat; however, it antagonized the beneficial effects of tropisetron.

Effect of tropisetron on macroscopic features

Six days after induction of colitis, the distal colon of the TNBS-control group showed massive injuries. The mucosa was hyperemic, inflamed, ulcerated and hemorrhagic. Necrosis and grossly visible thickness of the colon wall were observed, while the normal macroscopic features were evident in the colons of the normal group (Table 2).

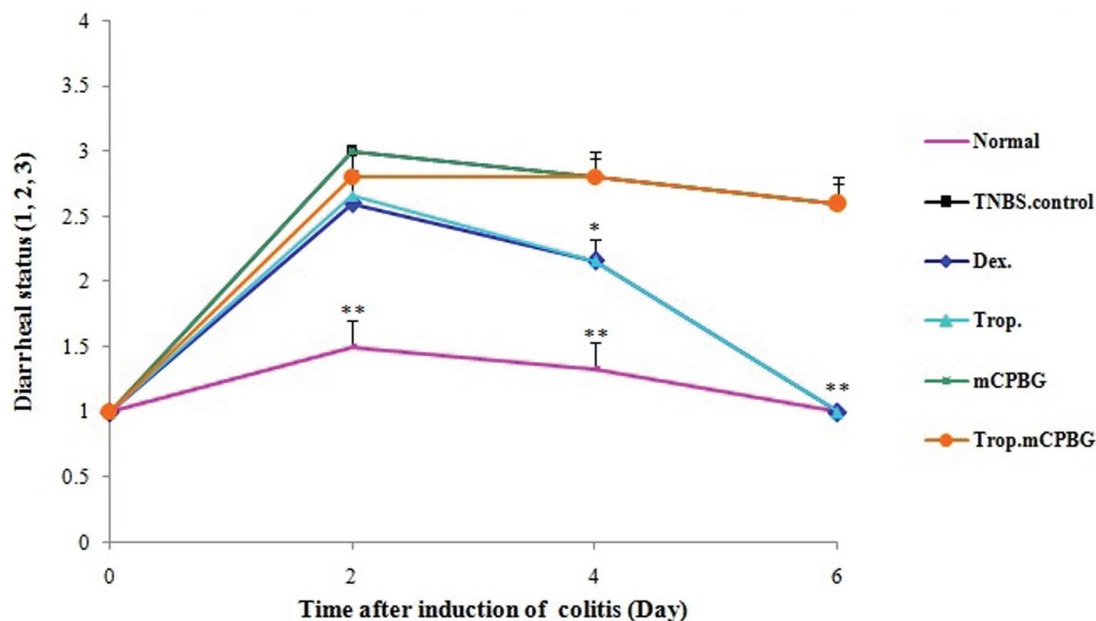


Fig. 1. Changes in diarrheal status before (Day 0) and during 6 days of treatment after induction of colitis (TNBS, 50 mg/kg) in rats. Values are means \pm SEM (n=6). **P < 0.01 and *P < 0.05 compared with TNBS-control group. TNBS, 2,4,6-trinitrobenzenesulfonic acid. Trop., tropisetron; mCPG, meta-chlorophenylbiguanide; Dex., dexamethasone. Stool consistency daily were checked in rats (1. Formed stools, 2. Loosed stools and 3. Diarrhea).

Table 2. Effect of tropisetron (2 mg/kg, daily) on macroscopic and histopathological parameters of the colon 6 days after induction of colitis with TNBS (50 mg/kg).

Group	Colonic weight/length ratio (mg/cm)	Body weight loss after 6 days (%)	Ulcer severity (0-15)	Ulcer area (cm ²)	Necrosis (%)	Total colitis index (0-10)
Normal	64.8 \pm 2.3	-1.9 \pm 0.6	0.0	0.0	0.0	0.0
TNBS-control	256.3 \pm 9.5	7.7 \pm 0.7	12.2 \pm 0.6	6.2 \pm 0.1	51.5 \pm 2.8	9.9 \pm 0.1
Trop. (2mg/kg, i.p.)	158.3 \pm 23.6 **	3.3 \pm 0.8 **	5.6 \pm 1.1 **	4.1 \pm 0.5 *	28.1 \pm 6.8**	5.5 \pm 0.7 **
mCPBG (5mg/kg, i.p.)	252.1 \pm 8.2	8.1 \pm 0.8	11.5 \pm 0.6	6.1 \pm 0.2	51.3 \pm 3.2	9.7 \pm 0.1
mCPBG+ Trop.	245.2 \pm 27.6	7.7 \pm 0.9	11.7 \pm 0.5	5.7 \pm 0.2	51.3 \pm 4.5	9.3 \pm 0.3
Dex. (1mg/kg, i.p.)	161.9 \pm 25.9 **	3.9 \pm 0.3 *	5.33 \pm 1.4 **	4.0 \pm 0.7 *	27.0 \pm 7.9**	4.8 \pm 0.8 **

¹ TNBS,2,4,6-trinitrobenzenesulfonic acid; Trop., tropisetron; mCPG, meta-chlorophenylbiguanide; Dex., dexamethasone; i.p., intraperitoneal

² Values are means \pm SEM; n =6.

³**P < 0.01 and *P < 0.05: Significant difference compared to TNBS-control group.

The animals treated with tropisetron or dexamethasone experienced a significant decrease in ulcer severity and weight/length ratio, compared with the TNBS-control group ($P < 0.01$). No significant difference was observed in these variables between the tropisetron and dexamethasone-treated rats. Moreover, ulcer severity and weight/length ratio in the tropisetron + mCPBG-treated rats were significantly higher than in the tropisetron-treated group ($P < 0.01$).

Distal colon of the animals treated with dexamethasone and tropisetron showed a significant decrease in ulcer area and percent of necrosis compared with that of the TNBS-control group (Table 2). These macroscopic features were found more worsened in tropisetron + mCPBG-treated group, compared with the tropisetron-treated rats ($P < 0.05$ for ulcer area and $P < 0.01$ for percent of necrosis). The mCPBG, itself, did not influence macroscopic features of colitis.

Effect of tropisetron on histopathological features

The histological features of tissues were examined 6 days (24 h after the last treatment) after the administration of TNBS (50 mg/kg) showed tissue damage characterized by extensive transmural inflammation and/or diffuse necrosis, inflammatory granulomas and submucosal neutrophils' infiltration. Normal group showed a normal architecture with intact epithelium in colonic mucosa. Treatment with dexamethasone and tropisetron for six days significantly decreased total colitis index (inflammation severity, inflammation extent and crypt damage) in injurious colons, compared with that of the TNBS-control group (Table 2). Furthermore, these groups experienced re-epithelization of the mucosal layer and reduced inflammatory cell infiltration in lamina propria. Concomitant administration of mCPBG and tropisetron significantly worsened histopathological aspects, in comparison with tropisetron administration alone ($p < 0.01$). In mCPBG-treated animals, microscopic features did not differ from those of TNBS-control group (Fig. 2).

Effects of tropisetron on MPO activity in inflamed colonic tissues

As shown in Table 3, MPO activity significantly increased in the TNBS-control group compared with normal group. This finding was in agreement with the histological assessment, which showed increased leucocyte infiltration in the TNBS control group. In the groups treated with dexamethasone and tropisetron, the MPO activity of colonic tissues was lower than that in the TNBS-control group ($P < 0.05$). A concurrent treatment with mCPBG and tropisetron significantly resulted in a high MPO activity, in contrast to tropisetron administration alone ($P < 0.05$). Data from mCPBG group did not differ from TNBS-control group.

Effect of tropisetron on cytokines production

As can be noted in Table 3, TNF- α , IL-6 and IL-1 β contents increased in the TNBS-control group, compared with those of normal rats. These parameters reduced

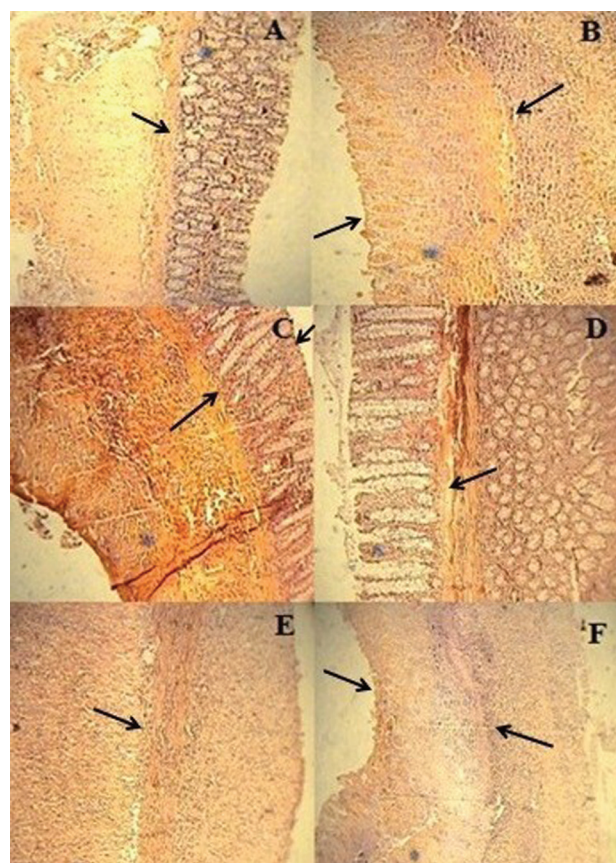


Fig. 2. Microscopic presentation of TNBS-induced colitis in rats (hematoxylin and eosin staining; original magnification 10 \times). (A) Normal group: mucus layer and crypts are normal; (B) TNBS-control group: epithelial distortion, crypt damage and inflammatory cell infiltrates; (C & D) Dexamethasone and tropisetron groups respectively: mild to moderate mucosal and submucosal inflammation and mucosal inflammatory cell infiltrates; (E & F): mCPBG and mCPBG + tropisetron, respectively: infiltration of neutrophils and destruction of mucosal architecture.

significantly in rats treated with either tropisetron or dexamethasone. No significant difference was observed in the content of TNF- α , IL-1 β and IL-6 between tropisetron and dexamethasone-treated rats. In the group concurrently treated with tropisetron and mCPBG, the levels of aforementioned cytokines were significantly higher than those treated with tropisetron alone ($P < 0.05$ for TNF- α and IL-6, $P < 0.01$ for IL-1 β). There was no significant difference in inflammatory cytokines content between mCPBG-treated and TNBS-control groups.

Discussion

In the present study, the administration of tropisetron markedly attenuated the inflammatory response to TNBS-induced colitis in rat. This is evidenced by improved signs, decreased percentage of body weight loss and colonic weight/length ratio, reduced colonic macroscopic and microscopic damage scores, inhibited MPO activity and abated inflammatory cytokine levels. As these effects were antagonized by concurrent administration of mCPBG (a

Table 3. Biochemical and inflammatory parameters of the rat colon 6 days after induction of colitis with TNBS (50 mg/kg).

Group	MPO activity (Unit/100 mg wet tissue)	TNF- α (pg/g wet tissue)	IL-6 (pg/g wet tissue)	IL-1 β (pg/g wet tissue)
Normal	0.6 \pm 0.1	141.0 \pm 13.8	3663.3 \pm 240.2	1647.5 \pm 452.4
TNBS-control	3.4 \pm 0.4	249.9 \pm 26.6	5210.3 \pm 457.9	15296.0 \pm 1579.7
Trop. (2mg/kg, i.p.)	1.9 \pm 0.2 *	163.7 \pm 8.6 *	3800.5 \pm 164.8 *	8770.8 \pm 1047.3 **
mCPBG (5mg/kg, i.p.)	3.5 \pm 0.3	250.0 \pm 23.1	5152.0 \pm 385.3	15317.9 \pm 1315.7
mCPBG+ Trop.	3.4 \pm 0.3	251.8 \pm 18.5	5249.0 \pm 312.5	15580.9 \pm 1486.3
Dex. (1mg/kg, i.p.)	1.9 \pm 0.4 *	160.6 \pm 12.6 *	3821.7 \pm 270.7 *	8641.2 \pm 985.2 **

¹TNBS, 2,4,6-trinitrobenzenesulfonic acid; Trop., tropisetron; mCPG, meta-chlorophenylbiguanide; Dex., dexamethasone; i.p., intraperitoneal.

² Values are means \pm SEM; n = 6.

³ *P < 0.01 and [†]P < 0.05: Significant difference compared to TNBS-control group.

selective 5-HT₃ agonist), a possible explanation is that the protection conferred by tropisetron is at least partly mediated by 5-HT₃ receptors.

Tropisetron as a 5-HT₃ receptor antagonist is a potent antiemetic;²⁹ besides, recent studies have also revealed new potential applications for this drug.³⁰ The anti-inflammatory property of tropisetron has been demonstrated by *in vivo* and *in vitro* studies. Fiebich *et al.*¹³ reported that lipopolysaccharide-stimulated secretion of TNF- α and IL-1 β was dose-dependently inhibited by tropisetron in human monocytes. Additionally, preliminary studies showed clinical efficacy of tropisetron as an analgesic and anti-inflammatory agent in patients with chronic inflammatory joint diseases and soft tissue rheumatism.³¹ Musavizadeh *et al.*¹⁶ reported the salutary effects of single dose of tropisetron administration in experimental colitis. In spite of the fact that the authors did not investigate probable involvement of 5-HT₃ receptors in protection provided by tropisetron against acetic acid-induced colitis in rats, they ultimately suggested that the anti-inflammatory effect of tropisetron may be mediated by pathways other than 5-HT₃ receptors. The present study set out to determine whether the 5-HT₃ receptors are involved in producing anti-inflammatory effect of tropisetron on an immune-based animal model of IBD (TNBS model) in a 6-day treatment. Our study produced results which corroborate the findings of Mousavizadeh *et al.* showing protective role of tropisetron against TNBS-induced colitis. Nevertheless, we demonstrated that the anti-inflammatory effect of tropisetron is at least partly mediated through its effect on 5-HT₃ receptor pathways. One of the most widely used models for human IBD is colitis induced by haptinizing agent. TNBS is a simple and reproducible process and can mimic efficiently the pattern of inflammation similar to human UC.³² As increasing 5-HT availability has been demonstrated in TNBS-induced colitis, this model is likely to be ideal for assessment of drugs which influence serotonin pathway

in intestinal inflammation.³³ In addition, TNBS model is associated with activated T helper (h)₁ and Th₂ cells.³⁴ As mentioned above, TNBS model can mimic both acute and chronic phases of colitis predicated upon experiment period. Present study was designed as a six-day experiment to evaluate the effect of administration of tropisetron on TNBS-induced colitis. This period is corresponding with the previous studies carried out on models of TNBS-induced chronic inflammation in the rat.^{20, 24}

An alteration in EC cell numbers and in amount of 5-HT was observed in intestinal mucosal inflammation such as ulcerative colitis.¹⁰ Intestinal inflammation is associated with mucosal recruitment of macrophages which are a major source of proinflammatory cytokines IL-1 β , IL-6, and TNF- α . Serotonergic receptors were found in immune cells including macrophages.¹² Due to the strategic location of EC, it is likely that 5-HT may serve an important role in infiltration and activation of macrophages in the gut inflammation. More to the point, intestinal immunocytes can release cytokines, which bring about activation of adjacent EC cells to liberate 5-HT. Through a positive feedback mechanism, increased 5-HT secretion can activate more 5-HT₃ receptors on EC cells.^{35,36} Immune cells also liberate serotonin and this exogenously added serotonin can increase T-cell proliferation.¹² It was demonstrated that the T-cells play a crucial role in pathogenesis of IBD. Activation of Th₁ cell gives rise to liberation of proinflammatory cytokines. Furthermore, it stimulates tissue macrophages in order to release additional proinflammatory cytokines (e.g. TNF- α , IL-1 β , IL-6, IL-8, and IL-12), nitric oxide and reactive oxygen species.³⁷

The Proinflammatory cytokines (e.g., TNF- α , IL-1 β , and IL-6) are elevated in the most inflammatory states. Many studies have shown that the levels of these cytokines liberated from macrophages, neutrophils and endothelial cells are increased in TNBS-induced colitis³⁸ and in human IBD.³⁹ They can liberate other cytokines,

arachidonic acid metabolites and lytic enzymes, and thus result in edema, fibrosis and necrosis.⁴⁰ TNF- α and IL-1 β are the main mediators pertaining to neutrophil activation and mobilization, fibroblasts' proliferation and cytotoxic initiation.⁴¹ In the present study, the extent of these proinflammatory cytokines increased subsequent to TNBS instillation. Additionally, the colonic profile of these mediators was reduced by administration of tropisetron. This result is possibly due to inhibition of synthesis and/or release of these mediators.

MPO activity, a quantitative index of neutrophil influx into inflamed intestinal tissue, is reportedly augmented in both experimental and human IBD.⁴² In the present study, the increase in MPO activity in TNBS-treated animals reduced after administration of tropisetron. This finding was consistent with attenuation of macroscopic and microscopic damage scores. This suggests its protective effect against colonic injury.

Neurogenic inflammation can pertain to colitis.⁴³ Local liberation of neuropeptides, for instance, substance P (SP) and calcitonin gene-related peptide (CGRP), from enteric and sensory afferent neurons mediates this type of inflammation. In fact, these neuropeptides and their receptors influence initiation and modulation of GI inflammation. However, inhibition of proinflammatory neuropeptides' release from enteric and/or sensory afferent nerves can abate colonic inflammation. Moreover, SP-induced neurogenic inflammation was shown to be associated with 5-HT through acting at 5-HT₃ receptors on capsaicin-sensitive fibers.^{43, 44}

It is plausible that some useful influences of tropisetron on experimental rat colitis can be at least explained by its proclivity to block 5-HT-induced inflammatory neuropeptide release.

According to our finding, mCPBG, a potent and selective 5-HT₃ receptor agonist, negated the salutary effect of tropisetron on TNB-induced colitis. Animals treated with mCPBG experienced colitis comparable to that of TNBS-control group. This might result from the severity of colitis reached its climax at the day of evaluation of colon damage and thus no more severity could be achieved by mCPBG. Furthermore, it can be deduced that there is the maximum level of involvement of serotonin pathway in TNBS-induced colitis and the administration of mCPBG as a potent 5-HT₃ agonist, cannot further aggravate colitis through activation of this pathway.

Conclusion

In conclusion, the data from the present study reveal that treatment with tropisetron is able to alleviate intestinal inflammation in the TNBS model of colitis in rats. Tropisetron reduced significantly neutrophil infiltration and colonic levels of inflammatory cytokines. In addition, clinical efficacy of tropisetron as an analgesic agent in patients with chronic inflammatory joint diseases and soft tissue rheumatism has been documented. According

to our finding, it is likely that anti-inflammatory effects of tropisetron on TNBS-induced colitis, at least partly arise from the ability of this drug to block 5-HT₃ receptors. Considering the low incidence of side-effects of tropisetron, it is contemplated that tropisetron may be useful in the therapy of patients with IBD. However, further studies are required to predict the efficacy and effectiveness of treatment with tropisetron in IBD.

Competing interests

The authors declared no competing interests.

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