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# Protective Effect of Taurine on TNBS-induced Inflammatory Bowel Disease in Rats

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We had previously reported that the protective effect of taurine against indomethacin-induced gastric mucosal injury was due to its antioxidant effects, which inhibited lipid peroxidation and neutrophil activation. In this study, we examined the effect of taurine on reducing the inflammatory parameters of trinitrobenzene sulfonic acid (TNBS)-induced inflammatory bowel disease (IBD) in rats. In order to induce IBD, ethanolic TNBS was given to rats intracolonically. Then they received 500 mg/kg/day of taurine orally and were sacrificed one week after IBD induction. While ulceration and inflammation of distal colon with formation of granuloma in the vehicle-treated IBD rats two days after administration of TNBS were observed, treatment with taurine ameliorated colonic damage and decreased the incidence of diarrhea and adhesion. Also, colon weight as an index of tissue edema, which was markedly increased in the IBD rats, became significantly lower after taurine treatment. Myeloperoxidase (MPO) activity in the vehicle-treated IBD rats was substantially increased, compared with that of normal control. The taurine-treated animals significantly reduced MPO activity (35% lower) when compared with that of the vehicle-treated animals. Taurine treatment decreased both basal and formyl-methionyl leucyl phenylalanine-stimulated reactive oxygen generation from colonic tissue in the IBD rats. These results suggest that the administration of taurine reduce the inflammatory parameters in this IBD rat model by increasing defending capacity against oxidative damage.

**Keywords :** Taurine, Inflammatory bowel disease, Trinitrobenzene sulfonic acid, Myeloperoxidase, Reactive oxygen

## INTRODUCTION

Taurine, 2-aminoethane sulfonic acid is one of the most abundant intracellular free amino acids present in various mammalian tissues including lung, retina, intestine, exposed to high concentrations of oxidant (Fukuda *et al.*, 1982; Green *et al.*, 1991; Wright *et al.*, 1985). Taurine has been shown to possess signficant anti-inflammatory properties in various *in vivo* and *in vitro* models of inflammation (Gurujeyalakashmi *et al.*, 1996; McLoughlin *et al.*, 1991; Schuller-Levis *et al.*, 1994, Son *et al.*, 1996; Stapleton *et al.*, 1996; Wang *et al.*, 1989). Although the protective mechanisms of taurine are still unclear, much attention has focused on its ability to attenuate the oxidative cellular damage through neutrophil modulation and to scavenge the reactive oxygen species (Kim *et al.*, 1996; McLoughlin *et al.*, 1991; Murakami *et al.*, 1995; Son *et al.*, 1996).

It has been suggested that the chronically inflamed intestine and/or colon may be subjected to considerable oxidative stress (McKenzie *et al.*, 1996; Pfeiffer *et al.*, 1995; Yamada and Grisham, 1991). These oxidants seem to be usually derived from the phagocytic leukocytes since these cells are known to be present in large numbers in inflamed mucosa and produce significant amounts of reactive oxygen species in response to certain inflammatory stimuli. Because the colonic mucosa contains relatively small amounts of antioxidant enzymes (e.g. superoxide dismutase (SOD), catalase, glutathione S-transferase, peroxidase) (Buffinton *et al.*, 1995), it is possible that the gut mucosa may be overwhelmed during active inflammation which could result in intestinal injury. This may suggest that potent antioxidants prove to be beneficial in the treat-

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ment or prophylaxis of inflammatory bowel disease (IBD) (Tamai *et al.*, 1991; Yue *et al.*, 1996).

In the present study, we describe the possible anti-inflammatory effect of taurine in acute colitis rat model.

## MATERIALS AND METHODS

### Animals

Mature male Sprague-Dowley rats (Charles River, Japan) were used for these experiments. They were housed throughout the study in a room with constant temperature (22°C), humidity (55%) and photoperiod (light on for 12 h a day). Tap water and standard rat pellet food were provided ad libitum. For the experiments, rats were randomly selected and divided into 4 groups of 12 animals each.

### Trinitrobenzene sulfonic acid (TNBS)-induced rat colitis model

Colitis was induced by using the technique described by Morris *et al.* (1989). Briefly, in rats lightly anesthetized with ether, a rubber cannula (OD, 2 mm) was inserted into the lumen of the colon via the anus approximately to the splenic flexure (8 cm from the anus). TNBS (30 mg/rat) was dissolved in 50% ethanol (v/v) and injected (0.5 ml) into the colon via the rubber cannula.

The first and the second groups were treated with taurine (500 mg/kg, once a day) or sulfasalazine (50 mg/kg, once a day) respectively, by oral gavage from 24 h after the induction of colitis until the animals were sacrificed. The third group for normal control received an equal volume of the vehicle.

Animals were observed for clinical symptoms of colitis such as weight change and diarrhea including loose stools, daily. All scoring of damage and excision of tissue samples were performed by an observer unaware of the treatment group. The rats in the various treatment groups were randomized before being killed. The rats were weighed and killed by cervical dislocation and the distal 10 cm of colon was removed. The colon was opened by a longitudinal incision, rinsed with tap water and pinned out on a wax block. Macroscopically visible damage was scored on a 0~10 scale using the scoring system described in Table I, which was based on the area of involvement and the presence of ulcers. The presence or absence of adhesions between the colon and other organs was also noted. Tissue samples were excised for subsequent measurement of myeloperoxidase (MPO) activity and eicosanoids synthesis.

**Table I.** Grading criteria for macroscopic damage score of large intestine

| Grade | Gross lesion |
|---|---|
| 0 | No damage |
| 1 | Hyperemia |
| 2 | Hyperemia with thickening of the bowel wall |
| 3 | Linear ulceration without hyperemia or thickening |
| 4 | Two or more sites of ulceration and/or inflammation |
| 5 | Two or more major site of inflammation and ulceration or inflammation extending > 1 cm along the length of the colon |
| 6~10 | If ulceration is more than 2 cm along the legth of the colon, the score is increased by 1 for each additional cm of involvement (i.e., if ulceration is 3 cm along the length of the colon, the score would be 7). |

### MPO activity

The MPO activity of colonic tissue was determined as an index of inflammation (Krawisz *et al.*, 1984). Finely minced segments of the colon (200~300 mg) were taken from ulcerated and/or inflamed areas. After weighing, segments of the colon were suspended in 0.5% hexadecyl trimethyl ammonium bromide (pH 6.5, 50 mg of tissue per ml) and were then homogenized. After freezing and thawing the homogenate three times, the tissue levels of MPO were determined by the technique utilizing 0.0005% hydrogen peroxide as a substrate for the enzyme. One unit of MPO activity was defined as a degradation of 1 μl peroxide per minute at 25°C and the activity was expressed in units per gram weight (U/g) of wet tissue.

### Luminol-dependent chemiluminescence (CL)

The mucosa scraped from the rectum and colon were washed immediately in PBS. The tissue suspension was then divided equally into the well of microplate containing 100 μl of minimal essential medium (MEM; Gibco, N.Y., USA). After addition of 0.02 mg luminol (Sigma, Louis, USA) and 0.14 mg/ml formyl-methionyl leucyl phenylalanine (FMLP) to the well of microplate, LitePlate (PACKARD, Meriden, USA), CL was determined using a TopCount (PACKARD, Meriden, USA). CL values were expressed as counts/s/mg wet weight of the colon after subtraction of background (Nielsen *et al.*, 1994; Simmonds *et al.*, 1992).

### Colonic eicosanoids formation

Segments of the colon (200~300 mg) taken from ulcerated and/or inflamed areas minced finely. After the addition 500 μl of Tris buffer (50 mM, pH 7.5 at 0°C), they were centrifuged for 10 s at 9000×g as described by Whittle (1981). The supernatant was transferred to microcentrifuge tubes containing indomethacin (final concentration 10 μg/ml) and frozen to -20 °C. The levels of two major eicosanoids, leukotriene $B_4$ ($LTB_4$) and prostaglandin $E_2$ ($PGE_2$), were measured by radioimmunoassay kits (Amersham, Buckinghum-

shire, UK).

### Statistical analysis

All data were expressed as means±standard error (SE). Comparisons between groups of nonparametric data such as the damage scores were made with the Wilcoxon rank-sum test. Comparisons between groups of parametric data were made with student's t-test for unpaired data. With all statistical analyses, an associated probability (P value) less than 5% was considered significant.

## RESULTS

An intracolonic administration of TNBS to rats produced a series of syndromes characterized by diarrhea, anorexia, loss of weight and severe colonic damage. Body weight gain was reduced by 50% in the TNBS-treated animals, whereas that of saline-treated normal rats was not reduced. The colon of the TNBS-treated animals showed diffuse hemorrhagic necrosis of the mucosa and bowel wall thickening, typically extending 3 to 7 cm along the length. They were assigned a median score of 8.0. Severe adhesion of colon to adjacent organs (intestinal loops, stomach, etc.) were noted in 90% of the TNBS-control animals. None of saline treated rats presented signs of diarrhea, adhesion or colonic damage. The mortality rate for the experiment was less than 1%. Oral treatment of colitic rats with 500 mg/kg/day of taurine, whose concentration was determined on the basis of our previous study (Son *et al.*, 1996), significantly recovered the decrease of body weight gain. Although there were little effect on incidence of diarrhea, taurine treatment significantly reduced the adhesion (data not shown) and colon weight as an index of edema. Colonic damage score was lower in taurine-treated group than in the TNBS-control group, representing a 27% decrease in the damaged area. However, intracolonic treatment of taurine had no significant effect on all the inflammation factors. These difference between oral and intracolonic administration of taurine is supposed to be due to the difference of tauirne uptake and should be more investigated.

Sulfasalazine which is widely used in the therapy of IBD showed significant effect on all indices of disease and inflammation.

### MPO activity

Fig. 1 shows the MPO activity in colonic mucosa. In the inflamed tissue the MPO activity, an indicator of neutrophil infiltration, showed a 3-fold increase in 1 week after TNBS treatment, as compared with noninflamed tissue of normal colon. This increase of MPO activity was reduced by treatment with taurine or sulfasalazine for 1 week.

### Luminol-dependent chemiluminescence

Superoxide level measured by luminol-dependent

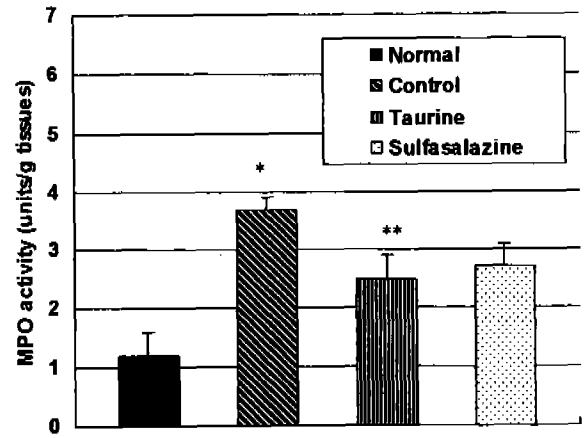


**Fig. 1.** Effect of taurine on colonic myeloperoxidase (MPO) in rat TNBS-induced colitis. Taurine was given to colitic rat induced by intracolonic administration of TNBS for 1 week as described in Materials and Methods. Data show mean± S.E.; n=6~12 animals/group. *vs normal, p<0.05; **vs control, p<0.05

**Table II.** Effect of oral treatment of taurine on the anti-inflammatory activity to TNBS-induced colitis[a] in rat

| Drug | Dose (mg/kg) | Change of weight | | Incidence of diarrhea (%) | Lesion score |
|---|---|---|---|---|---|
| | | body (g/day) | colon (g/10 cm) | | |
| Normal | - | 8.5±1.2 | 0.7±0.1 | - | - |
| Control | - | 4.2±1.5 | 2.2±0.3 | 38.1 | 7.0±0.5 |
| Taurine | 500 | 6.7±1.6 | 1.8±0.2 | 29.6 | 5.1±0.4* |
| Sulfasalazine | 50 | 7.5±1.3 | 1.6±0.1 | 14.3 | 3.9±0.7* |

[a]Colitis was induced by intracolonic administration of TNBS/ethanol (30 mg/50% ethanol, 0.5 mg/rat).
Taurine and other drugs were administrated to rats for 1 week.
*significantly different from controls at P<0.05.
Data show mean±S.E.

**Table III.** Effect of intracolonic treatment of taurine on the anti-inflammatory activity to TNBS-induced colitis[a] in rat

| Drug | Dose (mg/kg) | Change of weight | | Incidence of diarrhea (%) | Lesion score |
|---|---|---|---|---|---|
| | | body (g/day) | colon (g/10 cm) | | |
| Normal | - | 6.0±2.4 | 0.7±0.1 | - | - |
| Control | - | 0.8±2.0 | 1.3±0.6 | 51.9 | 8.9±1.9 |
| Taurine | 500 | 1.0±1.9 | 1.2±0.4 | 47.2 | 8.3±1.4 |
| Sulfasalazine | 50 | 3.0±1.8 | 1.2±0.3 | 26.9 | 6.3±2.3 |

[a]Colitis was induced by intracolonic administration of TNBS/ethanol (30 mg/50% ethanol, 0.5 mg/rat).
Taurine and other drugs were administrated to rats for 1 week.
Data show mean±S.E.

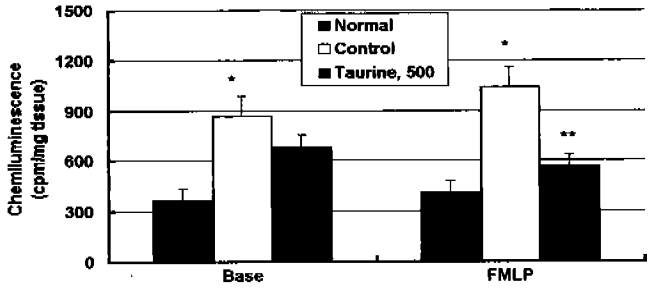


**Fig. 2.** Effect of taurine on luminol-dependent chemiluminescence in the mucosa of inflamed colon induced by TNBS. Taurine was given to colitic rat induced by intracolonic administration of TNBS for 1 week as described in Materials and Methods. Data show mean±S.E.; n=6~12 animals/group. *vs normal, p<0.05; **vs control, p<0.05

chemiluminescence showed that there was an approximately 2.5-fold increase in basal superoxide generation of colon tissue in colitic rats in 1 week after TNBS treatment. Treatment with taurine decreased the level of superoxide anion generation by 20%. When animals were stimulated with 0.14 mg/ml of FMLP, there was a substantial increase in superoxide generation measured from tissues of colitic rats 1 week after TNBS administration. Taurine treatment significantly decreased the superoxide generation by 50% (Fig. 2).

### Colonic eicosanoids formation

TNBS-induced colitis resulted in elevating the colonic levels of $LTB_4$ and $PGE_2$ when compared with that of the normal group. As shown in Fig. 3, $LTB_4$ synthesis in inflamed tissues was significantly elevated in 1 week after TNBS administration. Treatment with taurine or sulfasalazine significantly influenced colonic $LTB_4$ levels, reducing by 38% and 44%, respectively.

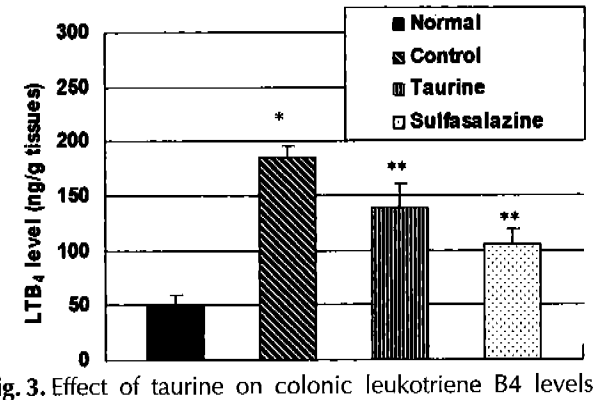


**Fig. 3.** Effect of taurine on colonic leukotriene B4 levels in rat TNBS-induced colitis. Taurine was given to colitic rat induced by intracolonic administration of TNBS for 1 week as described in Materials and Methods. Data show mean±S.E.; n=6~12 animals/group. *vs normal, p<0.05; **vs control, p <0.05

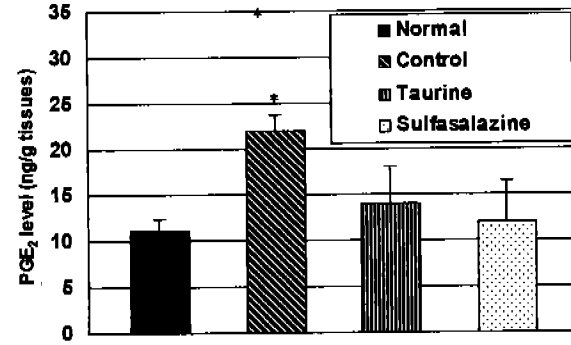


**Fig. 4.** Effect of taurine on colonic prostaglandin E2 levels in rat TNBS-induced colitis. Taurine was given to colitic rat induced by intracolonic administration of TNBS for 1 week as described in Materials and Methods. Data show mean±S.E.; n=6~12 animals/group. *vs normal, p<0.05; **vs control, p <0.05

$PGE_2$ level was increased in 1 week after TNBS administration and this increase was inhibited by treatment with taurine or sulfasalazine for 1 week (Fig. 4).

## DISCUSSION

In this study, it was observed whether there was a reduction in inflammation of taurine-treated animals with IBD, compared with non-treated animals. Thus, the inflammatory indices such as lesion score, diarrhea, adhesion, colon weight and body weight change, were measured. The results showed that the inflammatory responses in taurine-treated animals were reduced, suggesting that taurine have anti-inflammatory activity.

The mucosal lesion in IBD is characterized by dense inflammatory cell infiltrates, mainly comprising neutrophils, macrophages and lymphocytes. Although many inflammatory mediators including the cytokines and leukotrienes secreted by these cells together with luminal bacterial products, have been implicated in the mucosal injury observed in IBD (Sator, 1994), the molecules that mediate tissue damage remain poorly understood. However, a recent indirect evidence has implicated the important roles of reactive oxygen and nitrogen species (RONS) in the pathogenesis of the mucosal lesion (McKenzie *et al.*, 1996). Incresed production of RONS has been observed after *in vitro* stimulation of whole colonic mucosa of IBD patients (Simmonds *et al.*, 1992). Furthermore, reactive oxygen scavengers and inhibitors including SOD and sulfasalazine, have shown anti-inflammatory activities (Tamai *et al.*, 1991; Yue *et al.*, 1996). Sulfasalazine, a 5-aminosalicylic acid (5-ASA) derivative, is the first line of IBD therapy. Although its mode of action is still unclear and its various activities have been reported, one possibility is that 5-ASA acts as an antioxidant (Tamai *et al.*, 1991).

TNBS caused an increase in MPO activity and super-

oxide anion generation in animals. Taurine treatment was effective in reducing MPO activity and superoxide generation by TNBS administration. It has been reported that activated granulocytes secrete MPO into the extracellular medium where it catalyze the oxidation of $Cl^-$ by $H_2O_2$ to yield HOCl. HOCl react rapidly with primary amines to yield chloramines (RNHCl) (Yamada and Grisham, 1991). And taurine reacts readily with MPO-derived $HOCl/OCl^-$ to form long-lived anionic oxidant known as taurochloramine (Test *et al.*, 1984). Therefore, taurine has been proposed to modulate the inflammatory process by scavenging HOCl and preventing feed back inhibition of HOCl on MPO (Kim *et al.*, 1996).

There were considerable evidences involving several eicosanoids as key proinflammatory mediators in chronic inflammation including IBD (Boughton-Smith *et al.*, 1988; Sharon and Stenson, 1984). Arachidonic acid metabolism in experimental colitis has been shown to closely resemble that of human IBD. Administration of taurine to colitic rat reduced colonic $LTB_4$ and $PGE_2$ synthesis. Several studies have shown that taurine reduced $LTB_4$ production by manipulation of the MPO-$H_2O_2$ halide system in human leukoyte (Gurujeyalakshmi *et al.*, 1996) and $PGE_2$ production in Raw 264.7 cells (Quinn *et al.*, 1996). Colitic rats induced by TNBS showed the increase of these eicosanoids level in colonic mucosa and this increase inhibited by taurine treatment. Thus, the inhibition of eicosanoids production by taurine is likely to be due to the formation of taurochloramine in inflammatory cells.

In this study, we demonstrated that taurine had anti-inflammatory effect on IBD, which might be through the inhibition of MPO activity and the reactive oxygen generation, and modulation of eicosanoids synthesis.

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