Chapter 27
Dietary Taurine Attenuates Dextran Sulfate Sodium (DSS)-induced Experimental Colitis in Mice

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Abstract Effects of dietary taurine on the experimental colitis induced by dextran sulfate sodium (DSS) were studied. C57BL/6 mice administrated taurine or placebo for 5 days were given 3% DSS to induce acute. The colitis was as-sessed using indices such as diarrhea/bleeding scores, colon length change, histological score and tissue myeloperoxidase (MPO) activity. Further, tis-sue mRNA levels of interleukin (IL)-1β, tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)-2, were determined by real-time PCR. Taurine supplementation significantly attenuated the severity of diarrhea, colon shortening, histological score, MPO activity elevation and abnormal MIP-2 gene expression, indicating that taurine prevents DSS-induced colitis. Taurine also inhibited the TNF-α-induced secretion of IL-8 (a hu-man homologue of MIP-2) from human intestinal epithelial Caco-2 cells. Inhibition of chemokine secretion from intestinal cells may be involved in the mechanisms underlying the cytoprotective function of taurine in the intestinal epithelium.

Abbreviations *DSS*, dextran sulfate sodium; *MPO*, myeloperoxidase; *IL*, interleukin, *TNF-α*, tumor necrosis factor*α*; *MIP-2*, macrophage inflammatory protein 2

27.1 Introduction

In previous studies using human intestinal epithelial cell lines, we ob-served that intestinal taurine transporter (TAUT) activity was regulated by a variety of factors, including extracellular taurine concentration (Satsu et al. 1997), osmotic pressure (Satsu et al. 1999), lysophosphatidylcholine (Ishizuka et al. 2000; Ishizuka et al. 2002), and inflammatory cytokines, such as TNF-α and IL-1β (Mochizuki et al. 2002, Mochizuki et al. 2005). Up-regulation of TAUT by the inflammatory cytokines, followed by an increase in the intracellular taurine concentration, was particularly interesting because it indicated that the intestinal epithelial cells could

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adaptively respond to the inflammatory cytokines by increasing the amount of TAUT in the cell membrane to raise the intracellular concentration of taurine. In other words, taurine can play a cytoprotective role under inflammatory conditions.

The cytoprotective and anti-inflammatory properties of taurine have been reported in immune cells. Taurine reacts with hypochlorous acid to produce taurine chloramine, which has shown to be a powerful anti-inflammatory agent by suppressing nuclear factor-κB (NF-κB) (Kanayama et al. 2002). However, little is known about the anti-inflammatory and cy-toprotective function of taurine in intestinal epithelial cells. Son et al. (1998) reported that taurine prevents trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats (Son et al. 1998), and Satsu et al. reported that taurine protects epithelial cell damage caused by co-culturing human intestinal epithelial Caco-2 cells with human macrophage-like THP-1 cells (Satsu et al. 2006). The present study was undertaken to investigate whet-her taurine suppresses gut inflammation in an in vivo inflammatory bowel disease (IBD) model induced by dextran sulfate sodium (DSS).

27.2 Anti-Inflammatory Effects of Taurine on the IBD Model

27.2.1 Effect of Taurine on the Disease Activity Index of DSS-Induced Colitis, Histopathological Changes and Tissue Mye-loperoxidase (MPO) Activity

Female C57BL/6 mice (68 weeks old) were divided into 4 groups (6 mice in each group), and were given 2% taurine in water with or without 3% DSS (Fig. 27.1). The weight of each mouse and water intake were measured daily. Diarrhea scoring and bleeding scoring were performed on day 10 according to the method previously described (Cooper et al. 1993). Thereafter, the mice were sacrificed by cervical dislocation. The colon was removed from each mouse, and the length of the colon was measured. Two sections (1cm in length for each) were dissected from the proximal and distal colon. These were fixed in 10% formalin neutral buffer for histological assessment. Three segments from each specimen were sectioned, stained and scored. Inflammation and crypt damage were assessed for the H&E-stained sections by using a modification of the validated scoring scheme (Cooper et al. 1993; Matsuura et al. 2005). Tissue MPO activity was determined by standard enzymatic procedures (Krawisz et al. 1984) with minor changes (Zhao et al. 2006).

Compared with the control mice maintained on normal water, mice maintained on water supplemented with 2% taurine exhibited normal food and water intake (∼3.7 mL/day, mouse), as well as weight gain. Five days following the addition of 3% DSS to the drinking water body weight fell 5%. Taurine supplementation significantly retarded DSS-induced weight loss (data not shown). It also significantly attenuated the diarrhea symptom, and appeared to diminish the severity of fecal bleeding induced by DSS (Fig. 27.2A and 27.2.B, respectively). Furthermore,

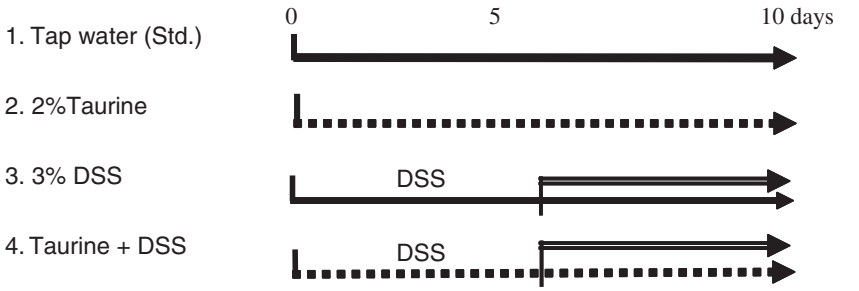

Fig. 27.1 Experimental design for DSS-induced IBD in mice. Six mice/group

taurine supplementation significantly inhibited DSS-mediated shortening of colon length (Fig. 27.2.C).

The severity of colonic mucosal damage and inflammation evaluated by histopathology is shown in Fig. 27.3. DSS induced a mild to moderate inflammatory infiltrate and crypt damage in the proximal colon. Taurine appeared to attenuate the inflammatory response, in particular DSS-mediated crypt damage. The total colitis scores for the DSS and DSS-Tau groups were 11.8 ± 2.0 and 8.8 ± 1.5, respectively (Fig. 27.3A). Although these scores were not statistically different, they revealed a trend implying a beneficial effect of taurine against DSS-induced colitis. To quantitatively evaluate the inflammatory changes in the colon, MPO activity of whole colonic tissue was determined (Fig. 27.3B). MPO activity of DSS-treated colonic

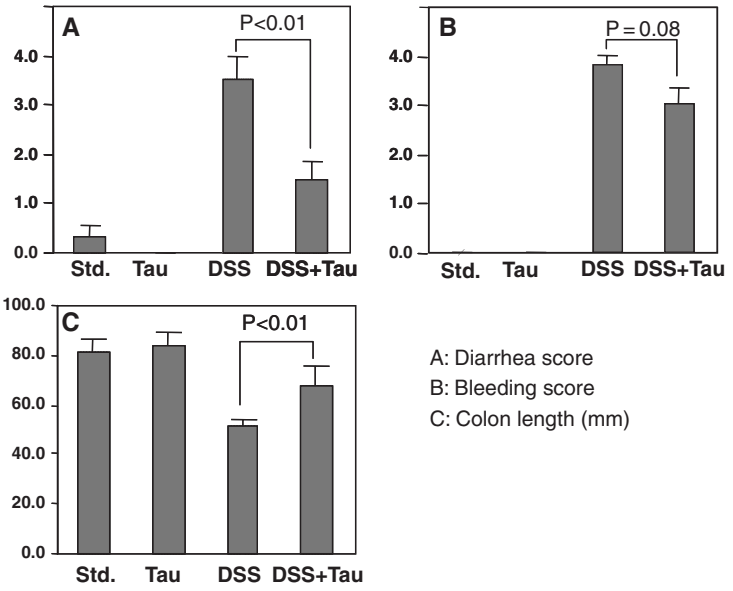

Fig. 27.2 Effect of taurine on the disease activity index of DSS-induced colitis

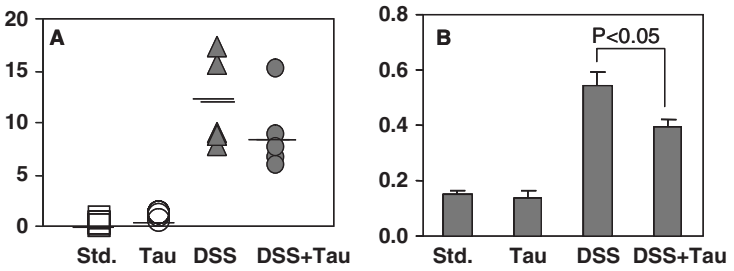

Fig. 27.3 Histological score (**A**) and MPO activity (**B**) of colonic tissue

tissue was 3.6-fold greater than that of normal tissue. Taurine treatment led to a 27% decrease in MPO activity compared to that of the DSS plus taurine-treated colonic tissue. TNBS- and DSS-induced colitis animal models are the most widely used for studying Chron's disease (CD) and ulcerative colitis (UC), respectively (Elson et al. 1995; Okayasu et al. 1990). Previous studies (Kim et al. 2006, Son et al. 1998) have demonstrated a therapeutic effect of taurine on TNBS-induced colitis in rats. Our present study further showed the beneficial effect of taurine against DSS-induced colitis in mice, suggesting that taurine would be useful for the treatment of both CD and UC.

27.2.2 Effect of Taurine on Expression of Pro-inflammatory Cytokines in the DSS-Treated Colon

RNA was extracted from tissue homogenate using the guanidium thiocyanate-phenol-chloroform method. Total poly A+ mRNA was subsequently purified from total RNA using Oligotex-dT30-super (Takara, Japan). The mRNA levels for TNF-α, IL-1β, MIP-2 were determined by real-time PCR. Total mRNA was then subjected to reverse transcription with the QuantiTect Reverse Transcription kit (Qiagen, Japan). The resulting complementary DNA was amplified using a QuantiTect SYBR Green real-time PCR kit (Qiagen, Japan). The reaction mixture was incubated for 15 min at 95°C, and then subjected to 50 amplification cycles, which included denaturation at 95°C for 15 s, an annealing step at 59°C (TNF-α and IL-1β) or 57°C (the others) for 15 s, and extension at 72°C for 15 s. Quantification of mRNA was performed using a comparative method, ΔΔCT method (Pfaffl et al. 2002 2002). Gene expression levels of the target molecules were finally normalized by using two housekeeping genes, β–actin and GAPDH.

The expression of MIP-2, TNF-α, and IL-1β was significantly increased in DSS-treated mice. The increase in MIP-2 mRNA levels was significantly reduced by taurine supplementation (Fig. 27.4A), whereas IL-1β and TNF-α mRNA levels were not significantly affected (Fig. 27.4.B, C). MIP-2, a mouse homologue of human IL-8, is a chemokine that plays a crucial role in mediating the infiltration of neutrophils into the intestinal mucosa. Taurine reduced MPO activity in DSS-treated

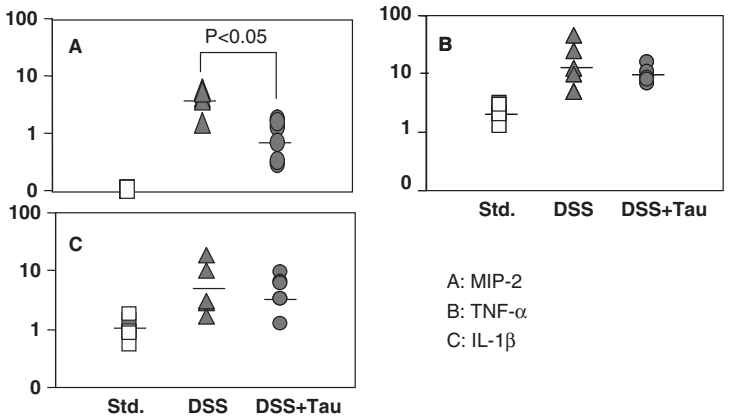

Fig. 27.4 Effect of taurine on the mRNA levels of pro-inflammatory cytokines in the DSS-treated colon

colonic mucosa (Fig. 27.4A), an observation that would explain the suppression of neutrophil infiltration by taurine treatment (Fig. 27.3B).

27.3 In Vitro Effect of Taurine on IL-8 Secretion by Intestinal Epithelial Cells

To verify the anti-inflammatory effects of taurine in vitro, we determined whether taurine could suppress TNF-α-induced IL-8 secretion by human intestinal Caco-2 cells. Caco-2 cells were pre-cultured on 24-well plates for 14 days (Mochizuki et al. 2002), before being treated with taurine for 3 h followed by TNF-α (50 ng/mL) for either 1h (mRNA determination) or 24 h (protein determination by ELISA). IL-8 mRNA was extracted and quantified by real-time PCR. Adding taurine to the medium significantly inhibited IL-8 secretion and decreased IL-8 mRNA levels of the Caco-2 cells in a dose-dependent manner (Fig. 27.5A, B). The taurine-mediated decrease in mRNA levels of IL-8 in vitro (Fig. 27.5B) and MIP-2 in vivo (Fig. 27.4A) suggests that regulation of chemokine production is involved in the beneficial effect of taurine on IBD.

Taurine reacts with hypochlorous acid to produce taurine chloramine which exhibits anti-inflammatory activity by depressing NF-κB and down-regulating pro-inflammatory mediators, such as TNF-α, PGE2, and COX2 in both rodent and human leukocytes (SchullerLevis and Park 2004; Kanayama et al. 2002). The anti-inflammatory properties of taurine chloramine are considered to be a major mechanism underlying the beneficial effect of taurine against LPS-induced acute lung injury in sheep (Elson et al. 1995) and TNBS-induced colitis in rat (Son et al. 1998). Our in vitro study showed that taurine inhibited TNF-α-induced secretion of IL-8 from the Caco-2 cell line. Because the Ca-co-2 cells, which produce no MPO,

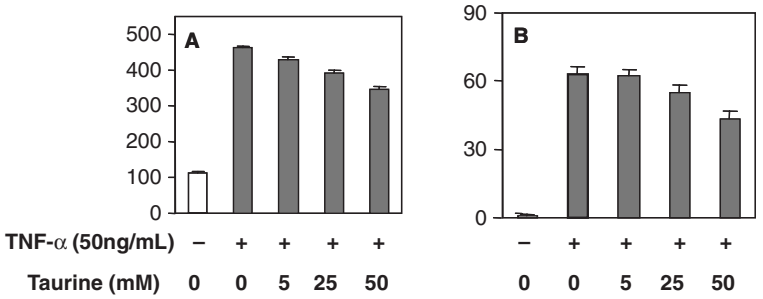

Fig. 27.5 Effect of taurine on IL-8 secretion (A; pg/ml) and mRNA levels (B; rela-tive value) in Caco-2 cells induced by TNF-α treatment

cannot convert taurine into taurine chloramine, our results indicate that intact taurine may also have anti-inflammatory potential and thus contribute to its beneficial effect against DSS-induced colitis (Zhao et al. 2007).

27.4 Conclusion

Taurine prevented DSS-induced colitis in mice. The inhibitory effect of taurine on the secretion of MIP-2 from intestinal epithelial cells would represent one of the cytoprotective mechanisms of taurine. Although taurine chloramine is considered to play a major role in preventing inflammatory diseases by down-regulating pro-inflammatory mediators, our in vitro study suggests that taurine itself also exerts some anti-inflammatory action.

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