Amino Acids (2008) 35: 217–224
DOI 10.1007/s00726-007-0562-8
Printed in The Netherlands

Amino Acids

Attenuation by dietary taurine of dextran sulfate sodium-induced colitis in mice and of THP-1-induced damage to intestinal Caco-2 cell monolayers

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Received February 28, 2007
Accepted April 27, 2007
Published online July 6, 2007;

Summary. The effects of dietary taurine on the experimental colitis induced by dextran sulfate sodium (DSS) in mice were evaluated. C57BL/6 female mice were given 3% DSS in drinking water for 5 d to induce acute colitis. Taurine at 2% was added to the drinking water 5 d before and during the DSS-treatment to investigate its preventive effect. Taurine supplementation significantly attenuated the weight decrease, diarrhea severity, colon shortening, and the increase in the colonic tissue myeloperoxidase activity induced by DSS. Taurine also significantly inhibited the increase in the expression of a pro-inflammatory chemokine, macrophage inflammatory protein 2 (MIP-2), but not of interleukin (IL)-1β or tumor necrosis factor (TNF)-α mRNA. Furthermore, taurine significantly protected the intestinal Caco-2 cell monolayers from the damage by macrophage-like THP-1 cells in an in vitro coculture system. These results suggest that taurine prevented DSS-induced colitis partly in association with (1) its inhibitory effects on the secretion of MIP-2 from the intestinal epithelial cells and on the infiltration of such inflammatory cells as neutrophils and (2) its cytoprotective functions on the epithelial barrier from the direct toxicity of DSS and from the inflammatory cell-induced injury.

Keywords: Taurine – IBD – DSS – Caco-2 – Colitis

Abbreviations: CD, Crohn's disease; DSS, dextran sulfate sodium; IBDs, inflammatory bowel diseases; IL, interleukine; LDH, lactate dehydrogenase; MIP-2, macrophage inflammatory protein 2; MPO, myeloperoxidase; NF-κB, nuclear factor-κB; TNBS, trinitrobenzene sulfonic acid; TNF-α, tumor necrosis factor; UC, ulcerative colitis

Introduction

Inflammatory bowel diseases (IBDs), principally including Crohn's disease (CD) and ulcerative colitis (UC), are chronic recurrent inflammatory disorders of the gastrointestinal tract (Podolsky, 1991a, b). They are characterized by prominent infiltration of neutrophils and T cells into colonic lesions accompanied by epithelial cell necrosis and ulceration (Fiocchi, 1998). IBDs are thought to result from inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora (Podolsky, 2002). The epithelium of the mucosa has an essential role in the physical separation of potentially stimulating microflora and the reactive cells of the mucosal immune system (Bouma and Strober, 2003). There is no specific medicine or treatment for these diseases at present. Various drugs, including 5-aminosalicylate-based compounds, corticosteroids, and immunosuppressive and immunoregulatory agents, are commonly used alone or adjunctively to control the active disease in most patients. However, they display limited beneficial action or/and various side-effects (Podolsky, 2002; Shanahan, 2001; Sands, 2000). On the other hand, the role of nutrition has received increasing attention (O'Sullivan and O'Morain, 2004; Tsune et al., 2003). Recently, such dietary factors as glycine, a nonessential amino acid, and rutin, a flavonoid, have been shown to have preventive effects on chemical-induced experimental colitis (Tsune et al., 2003; Kwon et al., 2005).

Taurine, a sulfur-containing β-amino acid, is the most abundant free amino acid in many tissues and organs in mammals (Brosnan and Brosnan, 2006; Satsu et al., 2002). For example, the taurine concentration is thought to be in the range of 25–50 mmol/l in leukocytes and skeletal cells (Schuller-Levis and Park, 2004). Taurine is neither metabolized nor incorporated into the cellular proteins of mammals, suggesting an important requirement for the

free amino acid (Huxtable, 1992; Oudit et al., 2004). In fact, studies have demonstrated that a high level of extracellular taurine can protect cells against damaging stimuli such as ischemia-reperfusion, reactive oxygen species, toxic xenobiotics, cellular excitotoxicity, and osmotic derangements (Schaffer et al., 2003). Moreover, studies have also demonstrated that taurine and its cellular metabolite, taurine chlorine, can suppress TNF-α-induced inflammatory response in vitro (Kanayama et al., 2002), and LPS- or oxidant-induced inflammation/injury in vivo to the lung (Egan et al., 2001; Schuller-Levis and Park, 2004).

Although the cytoprotective and anti-inflammatory properties of taurine may also be responsible for preventing IBDs, few studies have been done on the effects of taurine on inflammation in the gut (Kim et al., 2006; Son et al., 1998). Therefore, the aim of this present study was to evaluate the effects of dietary taurine on the gut inflammation in an in vivo IBD model induced by dextran sulfate sodium (DSS). A protective effect of taurine was also investigated using an experimental coculture system with human intestinal epithelial Caco-2 cells and human monocyte/macrophage-like THP-1 cells, which has recently been developed in our laboratory (Satsu et al., 2006).

The DSS-induced colitis animal model presents many symptoms similar to those seen in human UC, i.e., bloody diarrhea, weight loss, mucosal ulceration and neutrophilic infiltration (Elson et al., 1995; Okayasu et al., 1990). This model can induce reproducible and well-characterized mucosal colonic inflammation by an easily administered oral agent, making it widely used for studying the pathogenesis of UC and for drug screening (Egger et al., 2000; Elson et al., 1995). Although the exact mechanism for DSS colitis has not yet been established, studies have shown that the direct toxic action of DSS on the epithelium and crypts led to destruction of the mucosal barrier function, and thus caused the infiltration of bacterial products and DSS itself (Cooper et al., 1993; Dieleman et al., 1994; Ni et al., 1996). These infiltrated xenobiotics may prompt an initial response from the macrophage phagocytic system (Okayasu et al., 1990). The inflammatory mediators secreted from the macrophages and epithelial cells, and the increased infiltration of xenobiotics may subsequently activate the acquired immune system, leading to chronic inflammation (Elson et al., 1995).

Since the inflammatory mediators are important for the induction of DSS colitis and for human IBDs, we selected three of them in this study, i.e., TNF-α, interleukine (IL)-1β, and macrophage inflammatory protein 2 (MIP-2), as biomarkers to determine the anti-inflammatory effects of taurine on the induction of DSS colitis. TNF-α, a key inflammatory cytokine produced mainly from macrophages, has been shown to play a central role in the inflammatory cascade (Garside, 1999). IL-1β has been well characterized as a pivotal inflammatory cytokine produced from both inflammatory cells and mucosal epithelial cells during colonic inflammation (Tsune et al., 2003). MIP-2 (a mouse homologue of IL-8), which is produced by both intestinal epithelial cells and macrophages, plays a central role in inducing the infiltration of neutrophils into the intestinal mucosa (MacDemott et al., 1998; Ohtsuka et al., 2001). The infiltration of neutrophils is one of the important biomarkers of acute and chronic IBDs (Krawisz et al., 1984; MacDemott et al., 1998) and the MPO assay is a valid approach for evaluating inflammation, especially for estimating acute inflammation involving the infiltration of neutrophils (Krawisz et al., 1984). We also investigated the effects of taurine on the infiltration of neutrophils into the intestinal mucosa of mice by determining the tissue MPO activity. Finally, we verified whether or not taurine could protect the epithelial cell monolayers from injury by the pro-inflammatory mediators from THP-1 cells in the coculture system.

Materials and methods

Animals and in vivo experimental design

Female C57BL/6 mice (6–8 weeks old; Japan SLC, Japan) were housed in an air-conditioned room (23 ± 2 °C) with a 12-h light/dark cycle. They were allowed free access to food and tap water. After being acclimatized to the environment for 3–4 days, the mice were divided into 4 groups (6 mice in each group). They were given autoclaved tap water (normal water) only, the tap water containing 2% taurine (taurine water) only, 5-days of normal water and then another 5-days of 3% DSS water, or 5-days taurine water and then another 5-days of DSS plus taurine water. The weight of each mouse and the water intake were measured daily. Diarrhea scoring and bleeding scoring were performed on d 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 (from the ileocecal junction to the anal verge) was measured. Two sections (1 cm in length for each) were dissected from the proximal (1–2 cm from the cecum) and distal (1–2 cm from the anal verge) colon. These were fixed in a 10% formalin neutral buffer for histological assessment. All animals received humane care, and the study protocols were approved by the Committee for Care of Laboratory Animals in the Graduate School of Agricultural and Life Sciences at the University of Tokyo. The dose and duration of taurine treatment were determined by the results of the preliminary experiments.

Histological analysis

After the colonic specimens had been fixed by formalin, they were embedded in paraffin, sectioned, and the sections were stained with H&E. Three segments from each specimen were sectioned, stained and scored, the presented score being the average value for those three segments. Inflammation and crypt damage were assessed for the H&E-stained sec-

tions by using a modification of the validated scoring scheme (Cooper et al., 1993; Matsuura et al., 2005). Briefly, the sections were graded as to inflammation severity (4 grades), inflammation extent (4 grades), and crypt damage (5 grades). Each of these grades was also scored as to the percentage involvement. Each subscore (inflammation severity score, inflammation extent score, and crypt damage score) was the product of the grade multiplied by the percent involvement. The total colitis score is the sum of the three subscores, with the highest total colitis score being 40. All scoring was assessed by two histologists and the scores are presented as the average from the two histologists (standard deviations were 0.03–0.6).

Measurement of the tissue MPO activity

The tissue MPO activity was determined by the standard enzymatic procedure (Krawisz et al., 1984) with minor changes (Zhao et al., 2006).

Quantitative analysis of the effect of taurine on the TNF-α-induced IL-8 secretion and mRNA expression in Caco-2 cells

To verify the anti-inflammatory effects of taurine in vitro, we subsequently determined whether or not taurine could suppress the TNF-α-induced IL-8 (human homologue of MIP-2) secretion from the Caco-2 cells. Caco-2 cells were pre-cultured on 24-wells plates for 14 d as previously described (Satsu et al., 2006), before being treated with taurine for 3 h and then with 50 ng/ml of TNF-α for a further 1 h (mRNA determination) or 24 h (protein determination). The dose range of taurine is 0–50 mol/l. The protocol including pretreatment time was determined according to the results of preliminary experiments. mRNA of IL-8 in cells was extracted and quantified by real-time PCR. IL-8 concentration in supernatants was determined by ELISA.

Coculture system

To verify the cytoprotective effects of taurine on the intestinal epithelial cells in vitro, we determined the effects of taurine on the cell damage to Caco-2 monolayers in a coculture system using human intestinal epithelial Caco-2 cells and human monocytic leukemia THP-1 cells (Fig. 1). The coculture system was constructed by culturing two cell lines in a Transwell with bicameral chambers as described previously (Satu et al., 2006). Briefly, the semipermeable support membrane on which the Caco-2 cell monolayers had been cultured for 14 days was placed on the macrophage-like THP-1 cells that had been cultured on the 12-well plates (Fig. 1). The Caco-2 cells were collected for a lactate dehydrogenase (LDH) assay 48 h after starting the coculture in the presence or absence of taurine in the medium for Caco-2.

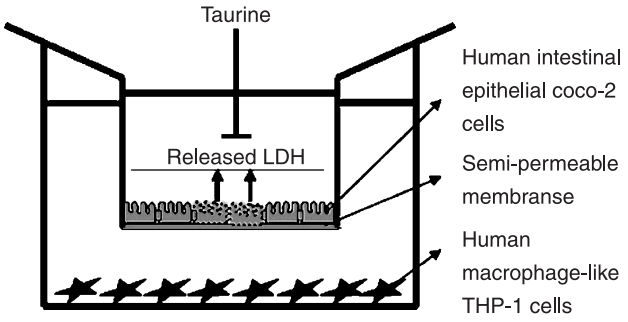

Fig. 1. Experimental design of the study with the in vitro coculture system. *LDH* Lactate dehydrogenase

Quantitative analysis of the gene expression of pro-inflammatory cytokines by real-time PCR

Total RNA was extracted from a tissue homogenate or cell lysate by the guanidium thiocyanate-phenol-chloroform method according to the manufacturer's instructions (Isogen, Nippon Gene Co. Japan). Total poly A^+ mRNA was subsequently purified from the total RNA by using Oligotex-dT30-super (Takara, Japan).

The steady-state levels of TNF-α, IL-1β, MIP-2, IL-8, β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined by real-time PCR. Total mRNA (15 ng) was reverse-transcribed with QuantiTect Reverse Transcription kit (Qiagen, Japan). To effectively remove the possible contaminating genomic DNA. Each RNA sample was pre-incubated in gDNA Wipeout Buffer (Qiagen, Japan) before the reverse transcription. The obtained complementary DNA (1 μl) was amplified by a QuantiTect SYBR Green real-time PCR kit (Qiagen, Japan), using the Lightcycler system (Roche Diagnostics, Germany). The reaction mixture was incubated for 15 min at 95 °C, and then subjected to 50 amplification cycles consisting of denaturing at 95 °C for 15 sec, annealing at 59 °C (TNF-α and IL-β) or at 57 °C (the others) for 15 sec, and extension at 72 °C for 15 sec. The primers used in the experiment are shown in Table 1. Their specificity were verified through analysis of melting curve of each product

Table 1. Primer sets used for real-time PCR

Gene	GenBank reference	Primer sequences	Major product size (base pairs)
Mouse TNF-α	NM-013693	Forward: 5′ CCA CGC TCT TCT GTC TAC TGA 3′ Reverse: 5′ GCT ACA GGC TTG TCA CTC G 3′	145
Mouse IL-β	NM-008361	Forward: 5′ TCG GAC CCA TAT GAG CTG A 3′ Reverse: 5′ CCA CAG GTA TTT TGT CGT TGC 3′	115
Mouse MIP-2	NM-009140	Forward: 5′ CCT GGT TCA GAA AAT CAT CC 3′ Reverse: 5′ GCT ACA GGC TTG TCA CTC G 3′	102
Mouse β-actin	X-03672	Forward: 5′ GGC CAG GTC ATC ACT ATT G 3′ Reverse: 5′ TCT TTG GTT CTT CCG TTG AG 3′	147
Mouse GAPDH	NM-008084	Forward: 5′ TAC ACT GAG GAC CAG GTT GT 3′ Reverse: 5′ CTG TAG CCG TAT TCA TTG TC 3′	137
Human IL-8	NM000584	Forward: 5′ AGA GTG ATT GAG AGT GGA CC 3′ Reverse: 5′ ACT TCT CCA CAA CCC TCT G 3′	118
Human β-actin	BC002409	Forward: 5′ CCA CGA AAC TAC CTT CAA C 3′ Reverse: 5′ GAT CTT CAT TGT GTG CTG GG 3′	160

and agarose gel electrophoresis. Quantification of mRNA was performed using a comparative method, $\Delta\Delta C_T$ method (Pfaffl et al., 2002). Gene expression levels of the target molecules were finally normalized by using two housekeeping genes, β-actin and GAPDH. To detect the contamination from genomic DNA and/or the preparation of real-time PCR, RT minus control and non-template control were included in all the quantitative analysis.

ELISA

The protein concentration of IL-8 in each collected supernatant was determined by using an ELISA kit (Pierce Biotechnology, IL, USA) according to the manufacturer's instructions. Standard Human IL-8/CXCL8 (R&D Systems, UK) was diluted with the same medium as the one used in the cell culture. All standards and samples were assayed in duplicate in each test. The quantification was accomplished using calibration of the standards.

LDH assay

The cell damage to the Caco-2 monolayers was evaluated by the LDH release from the cells as measured by an LDH assay. First, the Caco-2 monolayers that had been cocultured were rinsed twice with a Hank's balanced salt solution (HBSS) and then incubated with 0.5 ml/well of HBSS at 37 °C for 1 h. Second, the supernatant was collected and 0.5 ml of 0.1% Triton X-100 was added to each well to solubilize the cells. Last, LDH activity was measured in both the supernatants and the cell lysate fractions using an LDH-cytotoxic test kit (Wako, Osaka, Japan). The percent of LDH release from the cells was determined using the following formula: LDH release = (the amount of LDH in the supernatant)/(the total amount of LDH in both the supernatant and cell lysate) × 100.

Statistical analysis

Each data value is presented as mean ± SEM, the sample size being 6, unless otherwise specified in the text. For the data of in vitro studies, Tukey's multiple-range test was used when significant differences ($P < 0.05$) were obtained by one-way ANOVA. For the data of in vivo studies, because the variances were unequal, a non-parametric test (Mann–Whitney U test) was used and significance was set at $P < 0.05$.

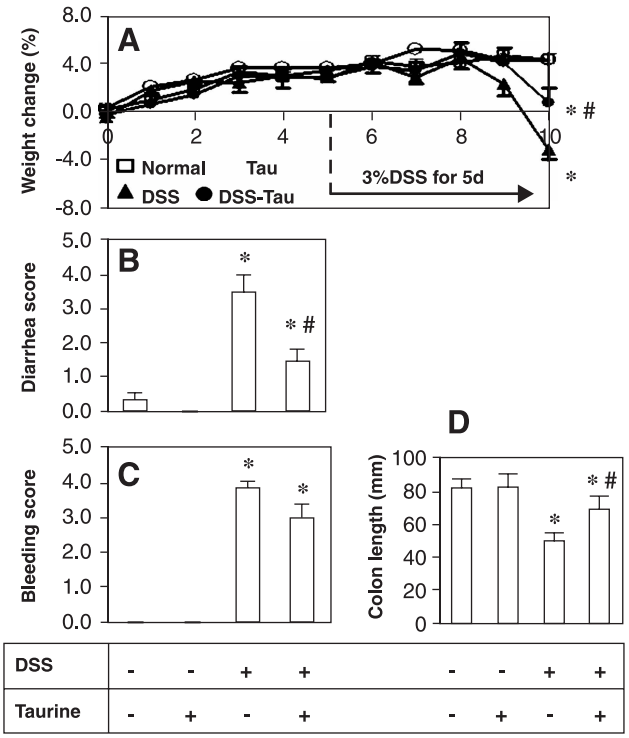

Fig. 2. Protective effects of taurine on the body weight change (**A**), diarrhea score (**B**), bleeding score (**C**), and colonic length (**D**) in dextran sulfate sodium (DSS)-treated mice. The mice were given *ad libitum* autoclaved tap water only (Normal group) or the tap water containing 2% taurine (Tau group), 3% DSS (DSS group), or 3% DSS plus 2% taurine (DSS-Tau group). Data for weight change are expressed as the mean percentage change from the starting body weight. The diarrhea and bleeding scores were evaluated on d 10 (5 days after starting the DSS treatment). Each value is the mean ± SEM, $n = 6$. $^{*}P < 0.05$ compared with the Normal group, $^{\#}P < 0.05$ compared with the DSS group. Mann–Whitney test

Results

Effects of taurine on the disease activity index for DSS-induced colitis in mice

Compared with the effect from normal water, water supplemented with 2% taurine affected neither the food nor water intake of the mice (~3.7 ml/day, mouse), nor their weight change (Fig. 2A). Adding 3% DSS to the drinking water induced about a 5% body weight loss by the fifth day after starting the DSS treatment. Taurine supplementation significantly retarded this weight loss induced by DSS (Fig. 2A). Taurine supplementation also significantly attenuated the diarrhea symptom ($p < 0.01$, Mann–Whitney test), and appeared to attenuate the fecal bleeding symptom ($p = 0.08$, t-test: two-sample assuming unequal variances) induced by DSS (Fig. 2B and C, respectively). Furthermore, taurine supplementation significantly inhibited the colon length shortening induced by DSS (Fig. 2D).

Effects of taurine on the histopathological changes and tissue MPO activities in the DSS-treated colon of mice

The severity of colonic mucosal damage and inflammation was evaluated by histopathological observations (Fig. 3). In general, DSS induced more severe mucosal damage and inflammation in the distal colon than in the proximal colon, which showed the region-specific susceptibility of colon to DSS. This consisted with the results of Okayasu et al. (1990). In this study, we focused on the proximal colon because there appeared to be no difference in mucosal damage or inflammation in the distal colon between the DSS and DSS-Tau groups. DSS induced a mild to moderate inflammatory infiltrate and crypt damage in the proximal colon (Fig. 3C). The sub-scores for

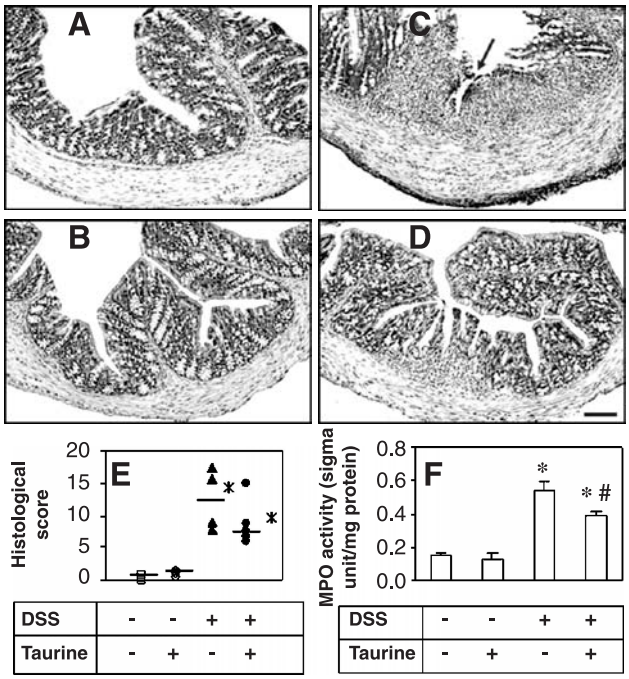

Fig. 3. Histopathological changes and myeloperoxidase (MPO) activity in the colon after the dextran sulfate sodium (DSS) treatment. Representative microphotographs of the proximal colonic tissue (H&E staining, Bar = 100 μm) are from the mice that were given autoclaved tap water only (**A**), or the tap water containing 2% taurine (**B**), 3% DSS (**C**), or 3% DSS plus 2% taurine (**D**). The histological scores were evaluated on d 10 (5 days after starting the DSS treatment). The total colitis score, (**E**, $n = 5$) is the sum of the 3 subscores for inflammation severity, inflammation extent, and crypt damage. MPO activity (**F**) in the whole colonic tissue was determined on d 10 by the standard method described in the Materials and methods section. Each value in **F** is the mean ± SEM, $n = 6$. $^{*}P < 0.05$ compared with the Normal group, $^{\#}P < 0.05$ compared with the DSS group. Mann–Whitney test

inflammation severity, extent, and crypt damage were 4.3 ± 0.7, 3.4 ± 0.5, and 4.2 ± 0.8 vs. their maxima of 12, 12, and 16, respectively. Taurine appeared to attenuate the inflammation and particularly the crypt damage induced by DSS (Fig. 3D vs. C). The total colitis scores for the DSS and DSS-Tau groups were 11.8 ± 2.0 and 8.8 ± 1.5, respectively (Fig. 3E). These scores were not statistically significant perhaps due to the sample size ($n = 5$) was not large enough. However, the limited data showed a trend of the preventive effect of taurine on the colitis (Fig. 3E).

To quantitatively evaluate the inflammatory changes in the colon, the MPO activity in the whole colonic tissue was determined by a standard enzymatic procedure (Fig. 3F). The MPO activity in the DSS-treated colonic tissue was 3.6-fold greater than in the normal tissues. Taurine treatment led to a 27% decrease in the DSS plus taurine-treated colonic tissue.

Effects of taurine on the mRNA expression of pro-inflammatory cytokines in the DSS-treated colon

The expression of MIP-2, TNF-α, and IL-1β mRNA in the DSS-treated mice was about 25, 3.5, and 2 times higher than that in the normal mice, respectively (Fig. 4A–C). The increased mRNA level of MIP-2 was significantly reduced by taurine supplementation (Fig. 4A). However,

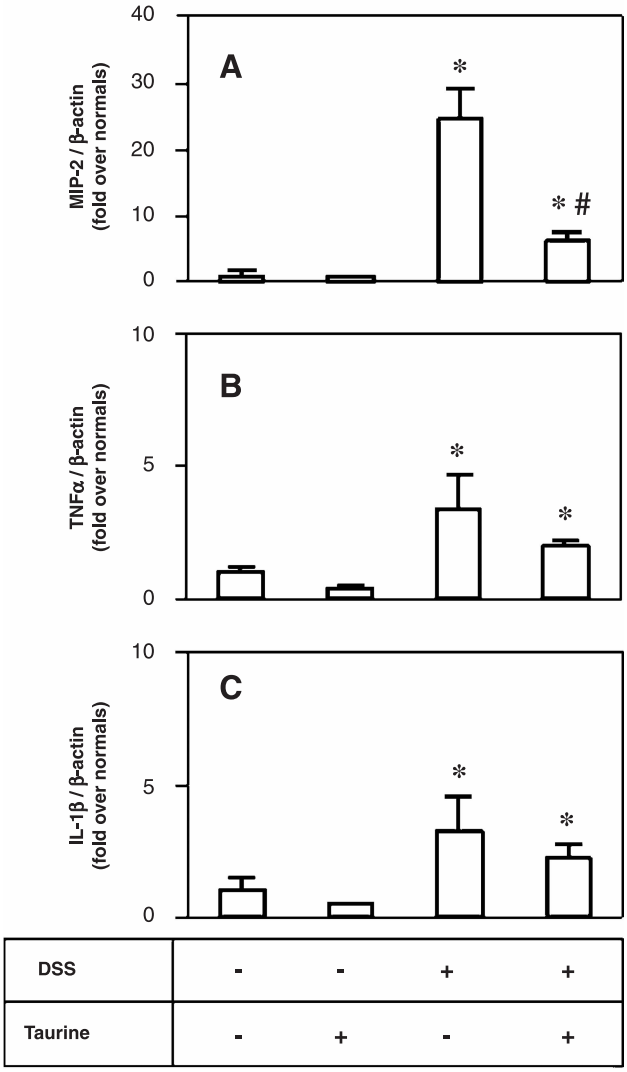

Fig. 4. Effects of taurine on the mRNA expression of pro-inflammatory cytokines MIP-2 (**A**), TNF-α (**B**), and IL-β (**C**) in the dextran sulfate sodium (DSS)-treated colon. The mice were given *ad libitum* autoclaved tap water only (Normal group) or the tap water containing 2% taurine (Tau group), 3% of DSS (DSS group), or 3% DSS plus 2% taurine (DSS-Tau group). The mRNAs of pro-inflammatory cytokines in the colonic tissue were determined on d 10 (5 days after starting the DSS treatment) by real-time PCR and are normalized against β-actin. The data normalized against GAPDH (not shown) are similar to those against β-actin (shown here), $n = 6$. $^{*}P < 0.05$ compared with the Normal group, $^{\#}P < 0.05$ compared with the DSS group. Mann–Whitney test

IL-1β and TNF-α mRNA levels were not significantly affected (Fig. 4B, C).

Inhibition by taurine of the TNF-α-induced IL-8 secretion from human epithelial Caco-2 cells

To verify the anti-inflammatory activity of taurine toward human intestinal epithelial Caco-2 cells, we determined the suppressive effect of taurine on the TNF-α-induced IL-8 secretion from the Caco-2 cells. Adding taurine to the medium of the cells significantly inhibited the IL-8 secretion and its mRNA expression by the Caco-2 cells in a dose-dependent manner (Fig. 5A, B).

Effect of taurine on the THP-1-induced cell damage to Caco-2 monolayers

The cytoprotective effect of taurine on epithelial cells was further verified in the coculture system. Coculturing the Caco-2 monolayers with activated macrophage-like THP-1 cells for 48 h led to about a 10-fold higher LDH release from the Caco-2 cells than no-coculturing. This over-release of LDH was significantly reduced by adding taurine to the medium of the Caco-2 cells (Fig. 5). The result indicates that taurine reduced the THP-1-induced cell damage to the Caco-2 monolayers.

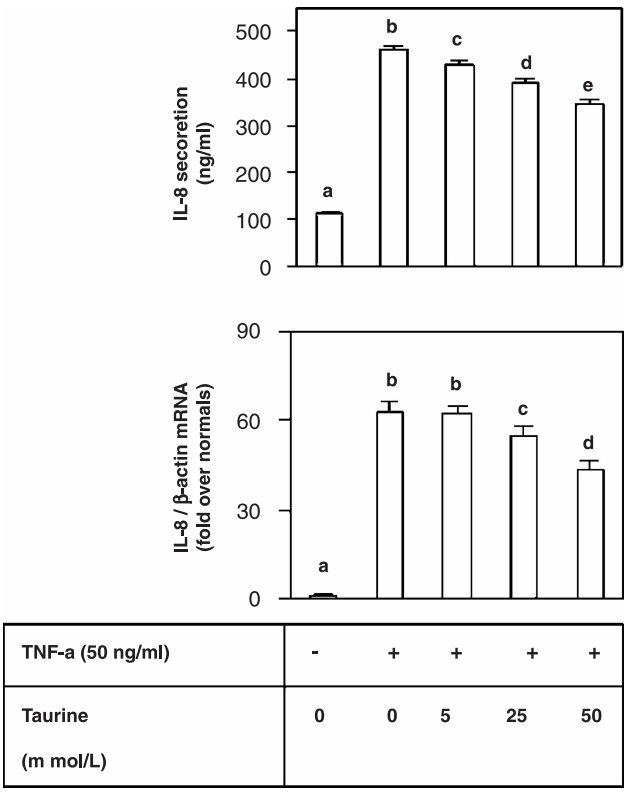

Fig. 5. Inhibition by taurine of the TNF-α-induced interleukin (IL)-8 secretion (**A**, $n = 6$) and its mRNA expression (**B**, $n = 4$) in Caco-2 cells. Caco-2 cells were treated with taurine for 3 h and then incubated with 50 ng/ml of TNF-α for a further 24 h (for protein determination) or 1 h (for mRNA determination). The supernatants were collected and assayed for its IL-8 concentration by ELISA. The mRNA expression was determined by real-time PCR. Each value is the mean ± SEM. Means without common letters differ, $P < 0.05$, Tukey's multiple-range test

Discussion

The epithelium of the mucosa plays an essential role in the pathogenesis of IBDs (Bouma and Strober, 2003). The normal epithelium contains highly evolved tight junctions and mucin, constructing an effective barrier against luminal agents/antigens. Genetic and/or environmental factors may compromise the integrity of the barrier and lead to an increase in the epithelial permeability (Podolsky, 2002). Such increased epithelial permeability may allow sufficient luminal antigens to enter the lamina propria and trigger an over-response of the mucosal immune system, by which excessive pro-inflammatory cytokines and chemokines are secreted. These pro-inflammatory mediators further increase the epithelial permeability, setting up a vicious cycle of chronic inflammation (MacDonald and Monteleon, 2005). Maintaining the integrity of the mucosal barrier and inhibiting the over-secretion of pro-inflammatory cytokines and chemokines may therefore be the key points in preventing IBDs. In this study, the damage by DSS to the epithelium in the DSS-Tau group appeared to be slightly less than that in the DSS group (Fig. 3C vs. D), suggesting that taurine supplementation may protect the mucosal epithelium from injury by the direct toxicity

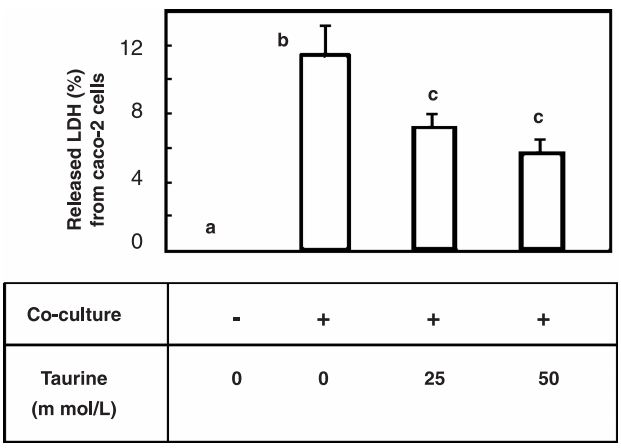

Fig. 6. Inhibition by taurine of the THP-1-induced increase in the release of lactate dehydrogenase (LDH) from Caco-2 cell monolayers. Released LDH from Caco-2 cells was measured after the cells had been cocultured with THP-1 cells for 48 h. Each value is the mean ± SEM, $n = 6$. Means without common letters differ, $P < 0.05$, Tukey's multiple-range test

of DSS itself and/or the infiltrated inflammatory cells. Such an in vivo result was complemented by the in vitro studies, that is, taurine significantly attenuated the macrophages-like THP-1-induced disruption to the human intestinal epithelial Caco-2 monolayers in the coculture system (Fig. 6).

On the other hand, taurine supplementation significantly reduced the over-expression of MIP-2 mRNA (Fig. 4A), a crucial chemokine in inducing the infiltration of neutrophils into the intestinal mucosa which is produced by both intestinal epithelial cells and macrophages (MacDemott et al., 1998; Ohtsuka and Sanderson, 2003; Ohtsuka et al., 2001). As expected, taurine correspondingly reduced the MPO activities in DSS-treated colonic mucosa, indicating its suppressive effect on the infiltration of neutrophils (Fig. 3F). Taurine in intra- and extra-leukocytes reacts with hypochlorous acid to produce taurine chloramines. The latter has shown powerful anti-inflammatory activity by depressing nuclear factor-κB (NF-κB) and down-regulating pro-inflammatory mediators such as TNF-α, PGE2, and COX-2 in both rodent and human leukocytes (Kanayama et al., 2002; Schuller-Levis and Park, 2004). The anti-inflammatory properties of taurine chloramine is considered to be the major mechanism of taurine in preventing LPS-induced acute lung injury in sheep (Egan et al., 2001) and trinitrobenzene sulfonic acid (TNBS)-induced colitis in rat (Kim et al., 2006). Interestingly, our in vitro studies showed that taurine inhibited the secretion of IL-8 induced by TNF-α in the Caco-2 cell line (Fig. 5). Because the Caco-2 cells, without MPO, do not convert taurine into taurine chloramine, our results indicate that intact taurine may also have anti-inflammatory potential and thus contribute to its preventive effect on DSS-induced colitis.

Although CD and UC show such common symptoms as diarrhea, fecal bleeding and weight loss, clinical experience has led to the generally accepted notion that they are distinct entities involving possible different pathogenesis (Bouma and Strober, 2003; Podolsky, 2002). This would account for the fact that some therapies may be very effective on one of CD or UC but not on the other (Podolsky, 2002). TNBS- and DSS-induced colitis animal models are the most widely used for studying CD and UC, respectively (Elson et al., 1995; Okayasu et al., 1990). Studies (Kim et al., 2006; Son et al., 1998) have shown the therapeutic effect of taurine on TNBS-induced colitis in rats. Our present study further showed the preventive effect of taurine on DSS-induced colitis in mice. These studies strongly suggest that taurine would be useful for the treatment of both CD and UC.

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