

Enteral diets enriched with medium-chain triglycerides and N-3 fatty acids prevent chemically induced experimental colitis in rats

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The specific purpose of this study was to evaluate the significant effects of medium-chain triglycerides (MCTs) and N-3 fatty acids on chemically induced experimental colitis induced by 2,4,6-trinitrobenzene sulphonic acid (TNBS) in rats. Male Wistar rats were fed liquid diets enriched with N-6 fatty acid (control diets), N-3 fatty acid (MCT– diets), and N-3 fatty acid and MCT (MCT+ diets) for 2 weeks and then were given an intracolonic injection of TNBS. Serum and tissue samples were collected 5 days after ethanol or TNBS enema. The severity of colitis was evaluated pathologically, and tissue myeloperoxidase activity was measured in colonic tissues. Furthermore, protein levels for inflammatory cytokines and a chemokine were assessed by an enzyme-linked immunosorbent assay in colonic tissues. Induction of proinflammatory cytokines tumor necrosis factor- α and interleukin- 1β in the colon by TNBS enema was markedly attenuated by the MCT+ diet among the 3 diets studied. Furthermore, the induction of chemokines macrophage inflammatory protein-2 and monocyte chemoattractant protein-1 also was blunted significantly in animals fed the MCT+ diets. As a result, MPO activities in the colonic tissue also were blunted significantly in animals fed the MCT+ diets compared with those fed the control diets or the MCT– diets. Furthermore, the MCT+ diet improved chemically induced colitis significantly among the 3 diets studied. Diets enriched with both MCTs and N-3 fatty acids may be effective for the therapy of inflammatory bowel disease as antiinflammatory immunomodulating nutrients. (Translational Research 2010;156:282–291)

Abbreviations: ANOVA = analysis of variance; ELISA = enzyme-linked immunosorbent assay; IBD = inflammatory bowel disease; IL = interleukin; LPS = lipopolysaccharide; MCT = medium-chain triglyceride; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; MPO = myeloperoxidase; SEM = standard error of the mean; TNBS = 2,4,6-trinitrobenzene sulphonic acid; TNF = tumor necrosis factor

Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders of the gastrointestinal tract that are of unknown origin.¹ IBDs are characterized by an infiltration of neutrophils into the colon accompanied by necrosis of epithelial cells and ulceration.

Although the exact pathogenesis of IBDs is poorly understood, evidence indicates that it involves an interaction between the innate and the acquired immune system, genetic susceptibility, and bacterial flora.² Historically, therapy for IBD commonly has included

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AT A GLANCE COMMENTARY

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Background

In the present study, medium-chain triglycerides (MCTs) inhibited the expression of inflammatory cytokines/chemokines in the colonic tissue, production of those mediators by activated macrophages, and accumulation of activated neutrophils into the colon, which ameliorated colonic injury.

Translational Significance

These findings clearly indicate that dietary supplementation of MCTs protect against 2,4,6-trinitrobenzene sulphonic acid-induced colitis, an animal model of Crohn's disease. Because an MCT is a general nutrient in a variety of types of enteral nutrition, the feasibility of a therapeutic approach for Crohn's disease using an MCT-rich enteral diet is promising. In conclusion, MCTs or N-3 fatty acids as immunomodulating nutrients most likely are useful for the treatment and maintenance of the remission of Crohn's disease.

glucocorticoids and mesalamine products. More recently, immunomodulators and antitumor necrosis factor (TNF)-alpha based therapies have been used increasingly. However, each of these agents have their limitations including the predisposition to potentially serious infections.³ Immunosuppressive drugs also have been applied to control severe illness, although they have more serious complications and toxic side effects.⁴ Alternatively, nutrition is also the major factor affecting the intestinal environment, and therefore, diets have been implicated in the pathogenesis of IBDs.^{5,6} The role of nutrition in the management of IBDs has received increasing attention. Consequently, various dietary measures have been recommended as adjuvant, if not primary, therapies.^{7,8}

A daily supplement of medium-chain triglycerides (MCTs) prevented increases in intestinal and hepatic injury after the administration of endotoxin.^{9,10} In those studies, MCTs inhibited the activation of the hepatic macrophage by inhibiting the expression of CD14.⁹ Intestinal macrophages activated by internal endotoxin are involved in the mechanism of Crohn's disease.¹¹ These findings support the hypothesis that it is very possible that MCTs also may be effective against Crohn's disease. Indeed, it was reported from this laboratory that MCT-enriched diets prevented chemical-induced IBD in

rats.¹² Furthermore, clinical data from patients with Crohn's disease suggested that replacing part of the dietary fat with MCT may help in inducing clinical remission¹³; however, the effect of MCTs on Crohn's disease has not yet been elucidated clearly. Alternatively, N-3 fatty acids also have protective effects on mucosal injury resulting from a 2,4,6-trinitrobenzene sulphonic acid (TNBS) enema in rats, suggesting that an N-3 fatty acid-rich diet may be applicable for enteral nutrition in the treatment of IBD patients.¹⁴ However, it also was reported that colonic inflammation was prevented in both patients treated with diets enriched with MCT and patients treated with diets enriched with both MCT and N-3 fatty in Crohn's disease.¹⁵ Thus, significant effects of MCT and N-3 fatty acids on Crohn's disease are still unclear. Therefore, the specific purpose of this study was to evaluate the significant effects of MCTs and N-3 fatty acids on chemically induced experimental colitis by TNBS in rats as a model of human Crohn's disease.

MATERIALS AND METHODS

TNBS-induced colitis model. Male Wistar rats weighing 200 g were obtained from Japan SLC Inc. (Shizuoka, Japan). All animals received humane care, and the study protocols were approved by the Committee of Laboratory Animals at University of Yamanashi according to institutional guidelines. Rats were fed liquid diets enriched with N-6 fatty acid (control diets), N-3 fatty acid (MCT- diets), and N-3 fatty acid and MCT (MCT+ diets) for 2 weeks (Table I). After 2 weeks, rats under light ether anesthesia either were given a single enema of TNBS solution (50 mg in 50% of ethanol/rat; Wako Pure Chemical Industries, Ltd, Osaka, Japan) or 50% of ethanol via a catheter inserted 8-cm lengths from the anus,¹⁶ and animals were fed the same diets for an additional week. The diets were given freely throughout the observation periods, and body weight was recorded.

Animals were sacrificed 5 days after the TNBS enema to obtain blood and tissue specimens. Tissue specimens were kept at -80°C until assayed. The colonic tissue specimens then were fixed in buffered formalin and embedded in paraffin, and tissue sections were stained by hematoxylin and eosin. Colonic inflammation in the histology specimen was assessed using a modification of the pathologic grading system of Macpherson and Pfeiffer¹⁷ as follows: grade 0 = normal findings; grade 1 = mild mucosal and/or submucosal inflammatory infiltrate (admixture of neutrophils) and edema, punctate mucosal erosions often associated with capillary proliferation, and muscularis mucosae intact; grade 2 = grade 1 changes involving 50% of the specimen; grade 3 = prominent inflammatory infiltrate and edema

Table I. Composition of the experimental diets (per 100 mL)

Composition and ingredients	Liquid diets enriched with N-6 fatty acid* (Control diet)	Liquid diets enriched with N-3 fatty acid† (MCT diets)	Liquid diets enriched with n-3 fatty acid and MCT (MCT+ diets)
Diet composition			
Protein (g)	3.52	4.38	4.38
Fat (g)	3.52	2.23	2.23
Carbohydrate (kcal)	13.72	15.62	15.62
Energy (kcal)	100	100	100
(protein/fat/carbohydrate)	(14.1/31.5/54/5)	(18/20/62)	(18/20/62)
Fat ingredients			
MCT (Trucaprylin, g)	—	—	0.750
Soybean oil (g)	0.160	0.699	0.699
Perilla oil (g)	—	0.180	0.180
Palm oil (g)	—	1.084	0.334
Corn oil (g)	3.320	—	—
n-6/n-3	44	3	3

*Liquid diets enriched with N-6 fatty acid contain the following vitamins and minerals: VA 250 IU, VD 20 IU, VE 3 mg, VK 7 μ g, VB₁ 15 μ g, VB₂ 172 μ g, VB₆ 200 μ g, VC 15.2 mg, nicotinamide 2 mg, pantothenic acid 0.5 mg, folic acid 20 μ g, biotin 15.2 μ g, Na 80 mg, K 148 mg, Ca 52 mg, Mg 20 mg, P 52 mg, Cl 136 mg, Fe 0.9 mg, Zn 1.5 mg, Mn 200 μ g, and Cu 100 μ g.

†Liquid diets enriched with N-3 fatty acid contain the following vitamins and minerals: VA 207 IU, VD 13.6 IU, VE 0.65 mg, VK 62.5 μ g, VB₁ 380 μ g, VB₂ 245 μ g, VB₆ 0.32 μ g, VC 28.1 mg, nicotinamide 2.5 mg, pantothenic acid 0.96 mg, folic acid 37.5 μ g, biotin 3.8 μ g, Na 73.8 mg, K 138 mg, Ca 44 mg, Mg 19.3 mg, P 44 mg, Cl 117 mg, Fe 0.63 mg, Zn 0.64 mg, Mn 133 μ g, and Cu 125 μ g.

(neutrophils usually predominating), frequently with deeper areas of ulceration extending through the muscularis mucosae into the submucosa, and rare inflammatory cells invading the muscularis propriae but without muscle necrosis; grade 4 = grade 3 changes involving 50% of the specimen; grade 5 = extensive ulceration with coagulative necrosis bordered inferiorly by numerous neutrophils and lesser numbers of mononuclear cells and necrosis extending deeply into the muscularis propria; grade 6 = grade 5 changes involving 50% of the specimen. All scoring was performed by the same individual under blind conditions to prevent observer's bias.

Measurement of myeloperoxidase activity. Tissue myeloperoxidase (MPO) activity was determined by a standard enzymatic procedure as previously described by Krawisz et al¹⁸ with slight modifications. Briefly, each tissue specimen was homogenized in buffer (0.5% hexadecyltrimethylammonium bromide in 50 mmol/L potassium phosphate buffer, pH 6.0) for 90 s on ice. Then the tissue homogenate was sonicated for 10 s before undergoing 3 cycles of freeze-thaw ($-70^{\circ}\text{C}/37^{\circ}\text{C}$). Samples were centrifuged at $20,000 \times g$ for 20 min at 4°C , and the supernatant was collected. Samples were added to 2.9 mL of 50 mmol/L phosphate buffer (pH 6.0) containing 0.167 mg/mL O-dianisidine hydrochloride and 0.0005% hydrogen peroxide, and the kinetics of absorbance at 460 nm were measured using a spectrophotometer at 25°C . Protein concentration of the supernatant was determined using a Bradford assay kit (Bio-Rad Laboratories, Hercules, CA) for calibration, and values were standardized using MPO

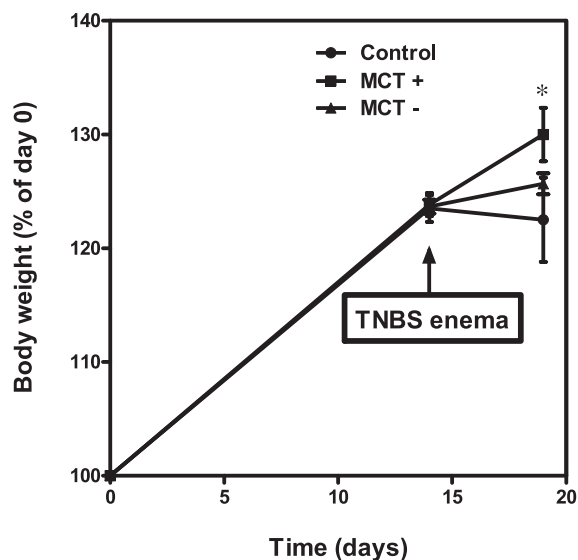


Fig 1. Body weight. Body weight was measured at 0, 14, and 19 days. Values were means \pm SEM ($n = 8$). ●, rats fed the control diets (Control); ▲, rats fed the MCT- diets (MCT-); and ■, rats fed the MCT+ diets (MCT+). * $P < 0.05$ compared with animals fed the control diets and treated with the TNBS enema.

purified from human leukocytes (Sigma Chemical Co., St. Louis, MO).

Endotoxin assay. Blood was collected via the aorta 5 days after TNBS treatment in a pyrogen-free heparinized syringe and was centrifuged at 1200 rpm for 10 min. Plasma was stored at -80°C in pyrogen-free glass tubes until assay using a limulus amoebocyte lysate test kit (Kinetic-QCL; BioWhittaker, Walkersville, MD).^{19,20}

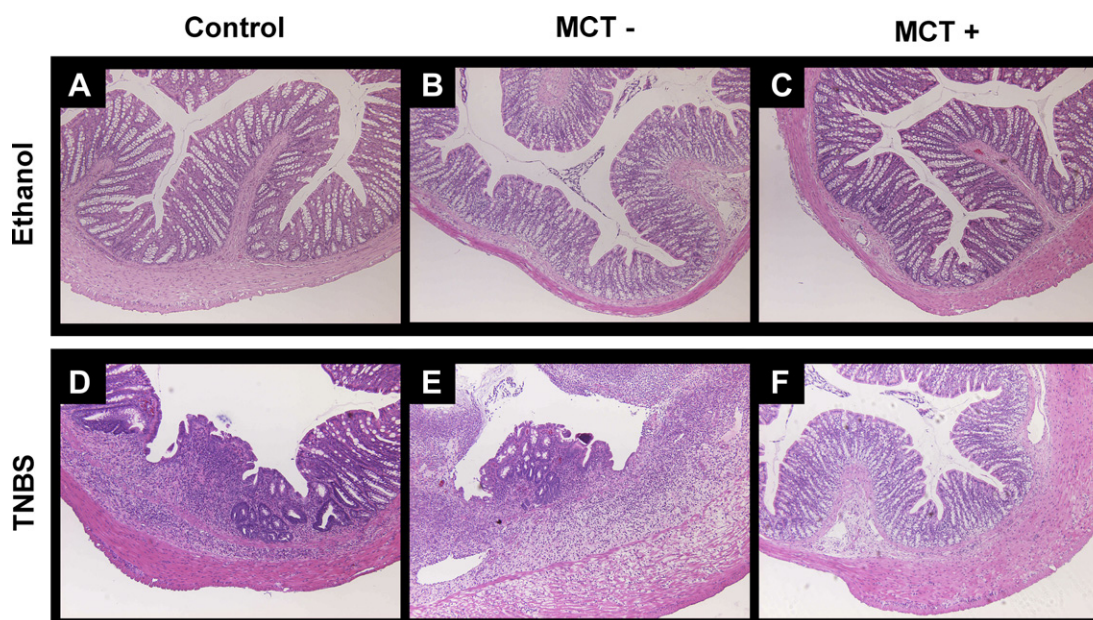


Fig 2. Pathological findings in the colon. Representative microphotographs of colonic tissues from animals fed the control diets and treated with an ethanol vehicle (A) or TNBS (D); animals fed MCT– diets and treated with an ethanol vehicle (B) or TNBS (E); and animals fed MCT+ diets and treated with an ethanol vehicle (C) or TNBS (F) are shown. Original magnification is $\times 400$.

Measurements of protein levels of cytokines and chemokines by enzyme-linked immunosorbent assay (ELISA). The colonic tissue was homogenized in cold phosphate-buffered saline using a Polytron-type homogenizer. Tissue homogenate then was centrifuged at $20,000 \times g$ for 20 min at $4^{\circ}C$ to obtain the supernatant. The total protein concentrations of the tissue supernatant and the whole-cell lysate were measured using a Bio-Rad protein assay kit (Bio-Rad Laboratories) for calibration, and protein concentrations of interleukin (IL)- 1β , TNF- α , IL-6, IL-12, macrophage inflammatory protein (MIP)-2, and monocyte chemoattractant protein (MCP)-1 in the tissue homogenate, and culture media were determined using enzyme-linked immunosorbent assay (ELISA) kits (IL- 1β , TNF- α , IL-12, and IL-6; R&D Systems Inc, MIP-2 and MCP-1; Immuno-Biological Laboratories Inc, Gumma, Japan) according to the manufacturer's instructions.

Statistical analysis. Data are expressed as mean \pm standard error of the mean (SEM). Analysis of variance (ANOVA) with the Bonferroni *post hoc* test or the student *t* test was used to determine significance when appropriate. $P < 0.05$ was considered significant.

RESULTS

Effects of MCTs on pathophysiological changes after TNBS enema. No significant differences were noted in food intake in each group (the control diet group, 60 ± 15 mL/day; the MCT– diet group, 62 ± 13 mL/day;

and MCT+ diet group, 61 ± 11 mL/day), and steady weight gain was observed in each treatment group (Fig 1). No significant differences were noted in body weight gain among the 3 groups before TNBS or ethanol enema.

First, the symptomatic parameters including body weight loss and diarrhea caused by colitis were monitored after a single intracolonic injection of TNBS. Rats fed the control diets, the MCT–, diets or the MCT+ diets gained body weight after the ethanol enema, the average increment reaching 3.0 ± 0.3 g, 2.9 ± 0.3 g, and 3.0 ± 0.2 g, respectively, in 1 day ($n = 8$) (data not shown). However, after receiving a single intracolonic TNBS injection, rats fed the control diets lost body weight almost 1.9 ± 0.7 g in 1 day ($n = 8$) (Fig 1). In contrast, the MCT– diets prevented the loss of body weight caused by TNBS, the increment being 2.1 ± 0.4 g in 1 day ($n = 8$); however no significant differences were observed between the control group and the MCT– group. Importantly, the MCT+ diets prevented the loss of body weight caused by TNBS almost completely, with the increment being 2.8 ± 0.4 g in 1 day ($n = 8$). Significant differences were observed between the control group and the MCT+ group.

Histopathologic changes in the colon. The severity of colonic inflammation and ulceration was evaluated further by histopathologic observations (Fig 2). As shown in Fig 2, no pathological changes occurred in animals treated with ethanol enema in all groups. In contrast,

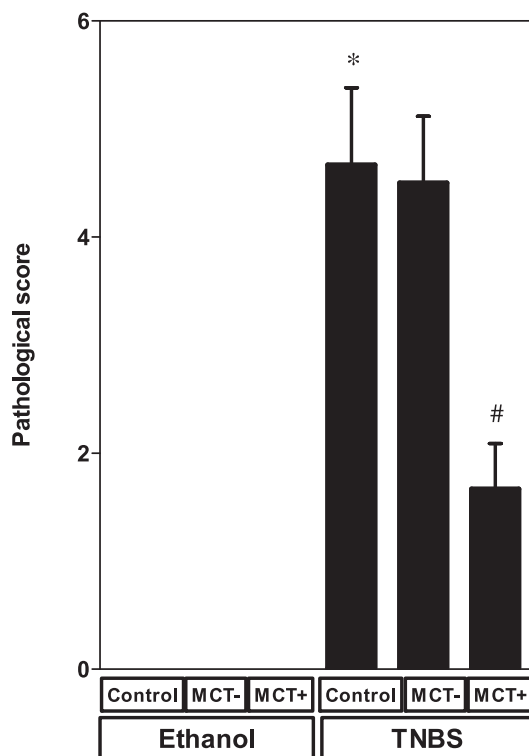


Fig 3. Pathological score. Pathological changes were scored in the colonic tissue 5 days after treatment with an ethanol vehicle or TNBS as described in the Materials and Methods section. Data represent means \pm SEM ($n = 8$). * $P < 0.05$ compared with animals fed the control diets and treated with the ethanol vehicle; and # $P < 0.05$ compared with animals fed the control diets and treated with TNBS by the Mann-Whitney rank sum test.

ulcer formation and massive transmural infiltration of inflammatory cells, predominantly polymorphonuclear neutrophils, were observed 5 days after intracolonic injection of TNBS in animals fed the control diets or the MCT- diets. In contrast, ulceration was mild, and inflammatory cells were localized in the mucosa and the submucosal area in animals fed the MCT+ diets.

These histopathological findings were graded using criteria described in detail in the Materials and Methods section. No pathological changes were observed in animals treated with an ethanol vehicle (Fig 3). In the control group, pathological scores were 4.7 after TNBS enema. In the MCT- diet group, the pathological scores were about 4.5, and no significant differences were observed between the control group and the MCT- group. In contrast, the MCT+ diet blunted these scores significantly by 64%, supporting the histopathological findings.

Plasma endotoxin levels. Plasma endotoxin levels were minimal 5 days after the ethanol enema in all 3 groups (Fig 4, A). In contrast, endotoxin levels were elevated significantly to about 200 pg/mL after the TNBS enema in the control group. Although endotoxin levels were

lower in animals fed the MCT+ diet compared with those fed the control diet or the MCT- diets, no significant differences were noted among the 3 groups.

MPO activity in the colon. To evaluate inflammation in the colon, MPO activities in the colonic tissue were measured by a standard enzymatic assay (Fig 4, B). MPO activities were 5 ng/mL/mg protein 5 days after the ethanol enema. In contrast, this activity increased significantly by nearly 6-fold after the TNBS enema in the control diet group. Furthermore, in animals fed the MCT- diets, values also were elevated to the same levels as those of the control group. However, MPO activity did not increase in animals fed the MCT+ diets after the TNBS enema.

Protein levels of inflammatory cytokines in the colon. Tissue protein levels of TNF- α , IL-1 β , IL-6, and IL-12 were measured by ELISA as shown in Fig 5. After the ethanol enema, protein expression of TNF- α was about 4 pg/mL/mg proteins in all 3 groups (Fig 5, A). In contrast, values increased to about 28 pg/mL/mg protein 5 days after the TNBS enema in the control group. Furthermore, in the MCT- group, levels were about 15 pg/mL/mg proteins after the TNBS enema. Significant differences were observed between the control group and the MCT- group. Moreover, in the MCT+ group, values were blunted significantly to 6 pg/mL/mg proteins after the TNBS enema.

Protein levels of IL-1 β were about 100 pg/mL/mg protein after the ethanol enema in all 3 groups (Fig 5, B). In contrast, after the TNBS enema, values increased significantly to about 900 pg/mL/mg proteins in the control group. The MCT- diets decreased tissue IL-1 β levels significantly by about 70% after the TNBS enema. Furthermore, the MCT+ diet also blunted these levels, and values were significantly lower in animals fed the MCT+ diets than those fed the MCT- diets. Collectively, these findings indicated that MCTs prevented colonic inflammation caused by TNBS by preventing the expression of the proinflammatory cytokines TNF- α and IL-1 β .

After the ethanol enema, protein expression of IL-6 was about 7 AU/mL/mg proteins in the control group (Fig 5, C). This level was blunted by about 50% after the TNBS enema. In the MCT- group, the levels were about 5 AU/mL/mg proteins after the ethanol enema, and the values did not change after the TNBS enema. In the MCT+ group, values were about 7 AU/mL/mg proteins after ethanol enema. These values increased significantly 1.4-fold after the TNBS enema.

In the control group, IL-12 levels were about 190 pg/mL/mg protein 5 days after the ethanol enema (Fig 5, D). After the TNBS enema, the values did not change and no significant differences were noted among the groups studied.

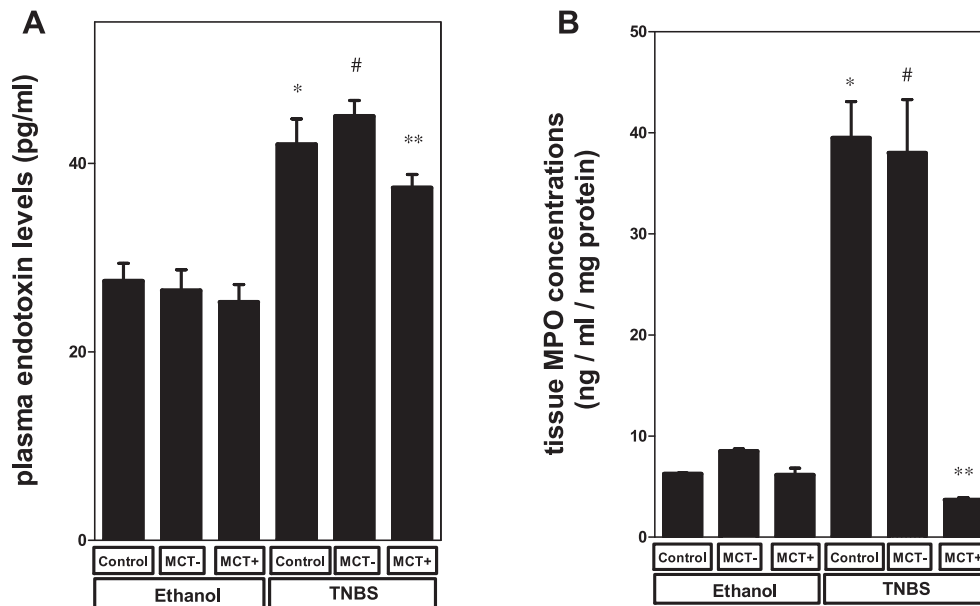


Fig 4. Plasma endotoxin concentrations and MPO activity in the colon. Plasma endotoxin concentrations (**A**) and MPO activity in the colon (**B**) after treatment with ethanol or TNBS were determined as described in the Materials and Methods section. Data represent means \pm SEM (n = 8). * $P < 0.05$ compared with animals fed the control diets and treated with the ethanol enema; # $P < 0.05$ compared with animals fed the MCT- diets and treated with the ethanol enema; and ** $P < 0.05$ compared with animals fed the MCT+ diets and treated with the ethanol enema by ANOVA with the Bonferroni *post hoc* test.

Protein levels of inflammatory chemokines in the colon. Protein levels of MIP-2 were minimal after the ethanol enema in all groups, as shown in Fig 6, A. In contrast, protein levels of MIP-2 increased significantly to about 60 pg/mL/mg protein after the TNBS enema in the control group. Although values also increased to about 25 pg/mL/mg protein in the MCT- group, these values were significantly lower compared with the control group. Importantly, in the MCT+ group, values did not increase after the TNBS enema.

Protein levels of MCP-1 were about 400 pg/mL/mg protein after the ethanol enema in all groups (Fig 6, B). In contrast, protein levels of MCP-1 increased significantly to about 700 pg/mL/mg protein after the TNBS enema in the control group. In the MCT- group, values were not different compared with the control group after the TNBS enema. In contrast, values were blunted by about 20% in the MCT+ group after the TNBS enema.

DISCUSSION

Effects of dietary fatty acids on IBD. Dietary fatty acids are an important factor involved in the pathogenesis of IBD. Indeed, it was reported that a significant correlation exists between occurrences of IBD and increases in the intake of dietary N-6 fatty acid.²¹ Alternatively, an N-3 fatty acid-rich diet effectively reduced early mucosal

inflammation in TNBS-induced enteritis in rats.¹⁴ Furthermore, a fish-oil-containing diet also prevented TNBS-induced colitis in rats,²² suggesting that N-3 fatty acid has an antiinflammatory effect. Indeed, in the present study, an N-3 fatty-acid-enriched diet improved the expression of inflammatory mediators in the colon and colitis compared with the control group (N-6 enriched diets without MCTs) (Figs 2, 3, 5, and 6). Thus, N-3 fatty acids have antiinflammatory effects in IBD.

Alternatively, MCTs have been shown to be protective against various types of organ injuries including endotoxin-induced hepatic and intestinal injuries⁹ and alcohol-induced liver damage.²³ In the present study, treatment with the MCT-enriched diet also prevented colitis induced by TNBS. These results demonstrate the protective effect of MCTs against inflammation in the colon, as previously reported.²⁴ Thus, therapy for IBD using diets containing MCTs and/or N-3 fatty acids is promising.

Among the inflammatory mediators expressed by proinflammatory cytokines are chemoattractant factors such as MIP-2 and MCP-1. They control the nature and magnitude of inflammatory cell infiltration to the site of inflammation, and they also are involved in organ injury.²⁵ MCTs and N-3 fatty acids also inhibited the expression of MIP-2 and/or MCP-1 after the TNBS enema (Fig 6). Thus, MCTs and N-3 fatty acids may inhibit the

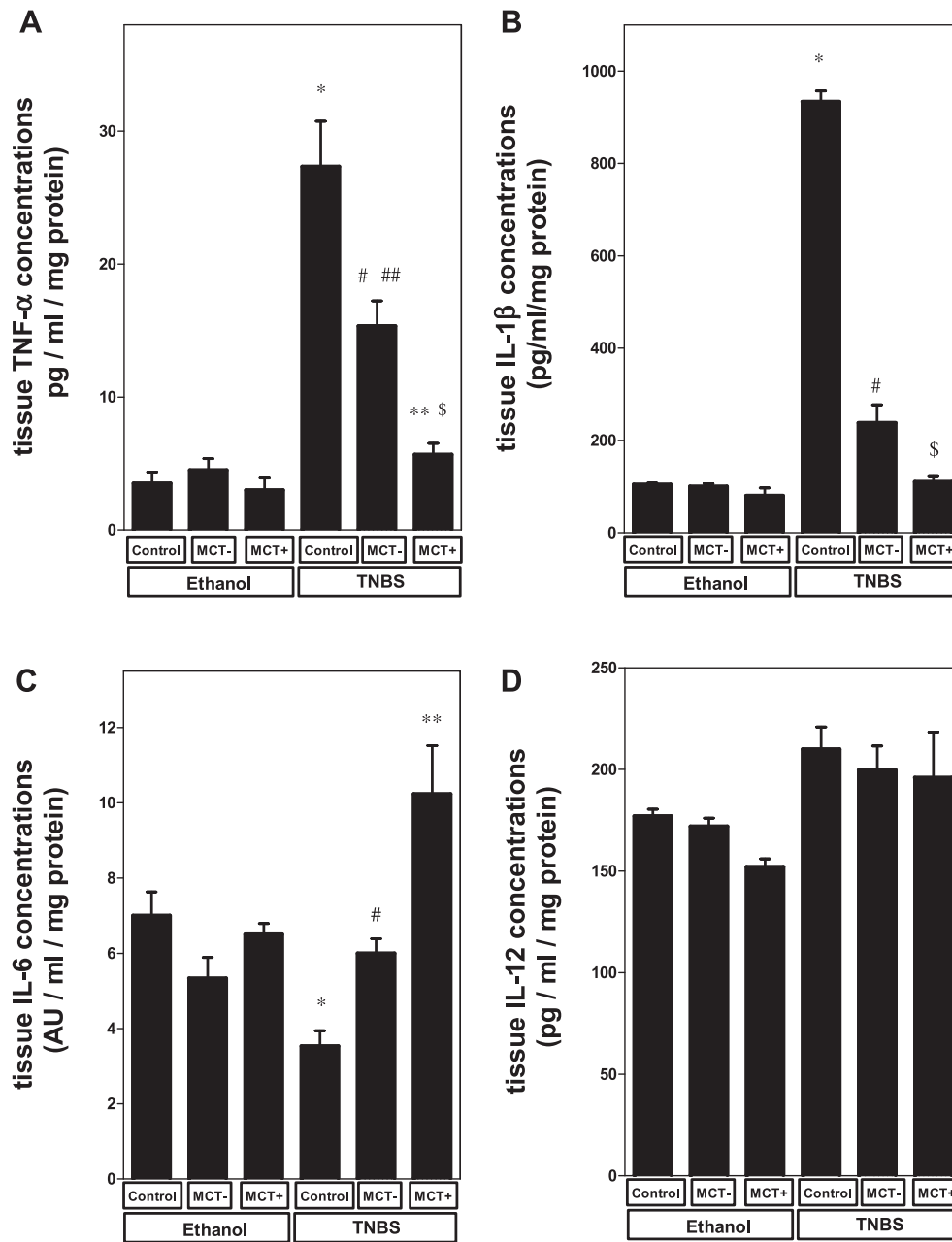


Fig 5. Protein expression of TNF- α , IL-1 β , IL-6 and IL-12 in the colon. The protein expression of TNF- α (A), IL-1 β (B), IL-6 (C), and IL-12 (D) in the colonic tissue 5 days after treatment with ethanol or TNBS was measured as described in the Materials and Methods section. Data represent mean \pm SEM (n = 8). * $P < 0.05$ compared with animals fed the control diets and treated with the ethanol enema; # $P < 0.05$ compared with animals fed the MCT- diets and treated with the ethanol enema; ** $P < 0.05$ compared with animals fed the MCT+ diets and treated with the ethanol enema; ## $P < 0.05$ compared with animals fed the control diets and treated with the TNBS enema; and \$ $P < 0.05$ compared with animals fed the MCT- diets and treated with the TNBS enema by ANOVA with the Bonferroni *post hoc* test.

infiltration of inflammatory cells such as neutrophils and macrophages into the gut, leading to decreases in inflammation. Alternatively, IL-12 plays pivotal roles in the pathogenesis of TNBS-induced and other Th1 cytokine-dominated inflammations.²⁶ In the present

study, although IL-12 levels increased after the TNBS enema, no significant differences were noted among the groups studied (Fig 5). MCTs and N-3 fatty acids have no effect on the expression of IL-12 in the experimental colitis.

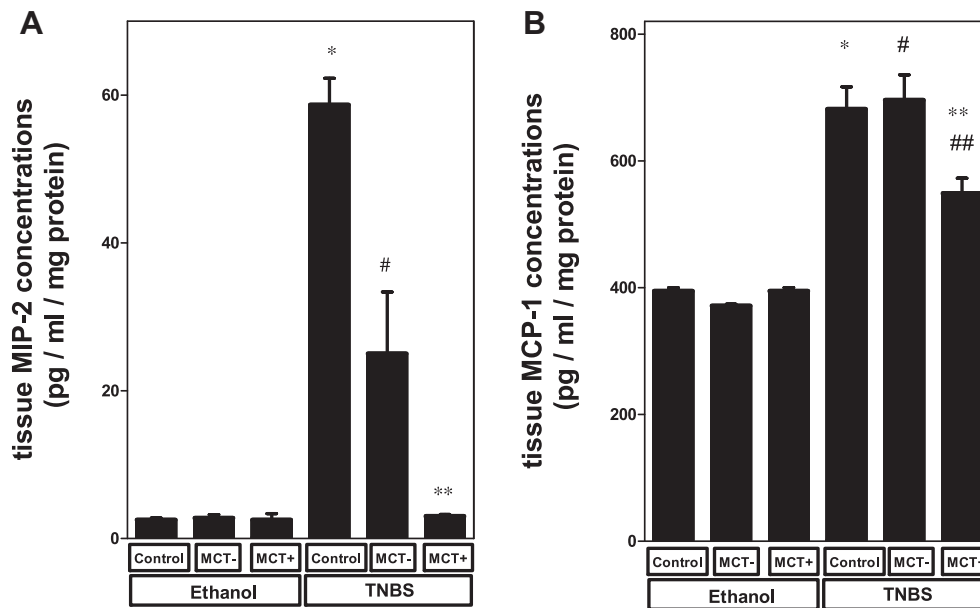


Fig 6. Protein expression of MIP-2 and MCP-1 in the colon. The protein expression of MIP-2 (A) and MCP-1 (B) in the colonic tissue 5 days after treatment with ethanol or TNBS were measured as described in the Materials and Methods section. Data represent mean \pm SEM (n = 8). * $P < 0.05$ compared with animals fed the control diets and treated with the ethanol enema; # $P < 0.05$ compared with animals fed the MCT- diets and treated with the ethanol enema; ** $P < 0.05$ compared with animals fed the MCT- diets and treated with the TNBS enema; and ## $P < 0.05$ compared with animals fed the MCT+ diets and treated with the ethanol enema by ANOVA with the Bonferroni *post hoc* test.

Significant effects of dietary MCTs on IBD. Recent studies have reported that the proinflammatory cytokine TNF- α plays a pivotal role in the inflammatory cascade²⁷ because a neutralizing antibody for TNF- α prevents TNBS-induced colitis in mice.²⁸ In the present study, MCT and N-3 fatty acid prevented the expression of proinflammatory cytokines after the TNBS enema (Fig 5), indicating that MCTs and N-3 fatty acid most likely improve colonic injury by preventing the production of proinflammatory cytokines in the colon. Importantly, those effects were more significant in animals fed liquid diets containing both MCT and N-3 fatty acids compared with those fed liquid diets containing only N-3 fatty acids, suggesting that antiinflammatory effects are greater in MCTs than in N-3 fatty acids.

Chemokines such as IL-8 play a pivotal role in the accumulation of neutrophils into inflammatory foci in TNBS-induced colitis.²⁹ TNBS-induced colitis is characterized as an inflammation of colonic tissue resulting from a severe infiltration of inflammatory cells, predominantly neutrophils.³⁰ In this study, therefore, protein levels of MIP-2, rat homologues of IL-8, were measured in the colonic tissue after TNBS treatment. Importantly, both MCT- and N-3 fatty-acid-enriched diets significantly prevented increases in protein levels of MIP-2 in the colon compared with N-3-enriched diets after the TNBS enema (Fig 6). This result is explained in

part by a down-regulation of TNF- α in animals fed the MCT diets because TNF- α is a key proinflammatory cytokine that induces MIP-2. Collectively, it also is hypothesized that MCTs prevent both accumulation and activation of neutrophils, thereby attenuating neutrophil-dominant inflammation in the colonic tissue caused by TNBS.

Among the cytokines produced in the intestinal mucosa during inflammation, IL-6 is important because of its multiple biological effects in both the intestine and other organs and tissues. IL-6 is an integral part of the inflammatory response to sepsis and endotoxemia. Under different conditions, IL-6 may exert pro- or antiinflammatory effects. In conditions of “uncontrolled” inflammation, high IL-6 levels contribute to morbidity and mortality.³¹ Systemic IL-6 levels gradually increased up to 6 h after a lethal dose of lipopolysaccharide (LPS), and this level correlated with pathophysiology and mortality.⁹ Furthermore, levels were increased significantly in rats given corn oil and correlated with organ injury and mortality.⁹ Importantly, MCTs significantly prevented these events. In addition to the biological roles of systemic levels of IL-6, IL-6 has important biological effects on the intestinal mucosa.³² Mucosal levels of IL-6 regulate enterocyte acute phase protein synthesis,³³ protein synthesis in the mucosa,³⁴ and intestinal secretory IgA production.³⁵

IL-6 is also an important regulator of secretory IgA production by B cells in the Peyer's patches. Indeed, intestinal and serum secretory IgA levels were increased significantly in rats given MCTs compared with those given corn oil after LPS administration¹⁰ because, in the present study, the expression of IL-6 was enhanced markedly by the MCT-containing diets after the TNBS enema (Fig 5). MCTs increase the expression of intestinal IL-6 after the TNBS enema, which possibly correlates with the prevention of colonic injury.

Clinical implications. In the present study, MCTs inhibited the expression of inflammatory cytokines/chemokines in the colonic tissue, production of those mediators by activated macrophages, and accumulation of activated neutrophils into the colon, which ameliorated colonic injury. These findings clearly indicate that dietary supplementation of MCTs protect against TNBS-induced colitis, an animal model of Crohn's disease. Because MCT is a general nutrient in a variety of types of enteral nutrition,³⁶ the feasibility of a therapeutic approach for Crohn's disease using an MCT-rich enteral diet is promising. In conclusion, as nutrients with immunomodulatory potential, MCTs and/or N-3 fatty acids may have use as an adjunctive therapy or in the maintenance of remission in human Crohn's disease.

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