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Original Research Communication

A Novel Water-Soluble Vitamin E Derivative Protects Against Experimental Colitis in Rats

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ABSTRACT

This study was designed to investigate the effects of water-soluble vitamin E derivative, 2-(α-D-glucopyranosyl)methyl-2,5,7,8-tetramethylchroman-6-ol (TMG), on experimental colitis in rats. Colitis was induced in male Wistar rats weighing 200 grams using an enema of trinitrobenzene sulfonic acid (TNBS) dissolved in 50% ethanol; 1 ml of TMG dissolved in physiological saline (0.2 mg/ml, 2 mg/ml, 20 mg/ml) was injected intraperitoneally every day for 1 week after the enema. The damage score, wet weight of the colon, and increase in body weight were estimated 1 week after the enema of TNBS. Thiobarbituric acid-reactive substances (TBA-RS), an index of lipid peroxidation, and the level of α-tocopherol or TMG in the colonic mucosa were measured 1 week after the induction of colitis. As a result, increase in body weight was inhibited by the induction of colitis, although the inhibition was reduced in the group treated with TMG. The damage score, wet weight and TBA-RS were increased significantly in the colitis group; however, they were inhibited by the administration of TMG. The α-tocopherol level in the colonic mucosa was reduced by the induction of colitis, wheres TMG could not be detected in the colonic mucosa of rats treated with TMG. These results suggest that TMG is effective for the treatment of colitis in rats induced by TNBS. Antiox. Redox Signal. 1, 555–562.

INTRODUCTION

INFLAMMATORY BOWEL DISEASE (IBD), such as Crohn's disease and ulcerative colitis, is characterized by marked infiltration to the mucosa with macrophages, lymphocytes, and neutrophils. Its etiology remains unclear and a satisfying therapy has not yet been established. In recent years, it has been proposed that oxygen-derived free radicals produced by neutrophils and macrophages are implicated in the pathogenesis of IBD (Allgayer, 1991; Babbs, 1992; Simmonds *et al.*, 1992). Some studies have demonstrated that the antioxidative defense system is depleted in human IBD or an animal model of IBD, and the possibilities of prevention or treatment of IBD by enhancing the antioxidant capacity of the host are emphasized (Yoshikawa *et al.*, 1992; Buffinton and Doe, 1995; Lih-Brody *et al.*, 1996).

In rats, the administration by enema of 2,4,6-trinitrobenzene sulfonic acid (TNBS) dissolved in ethanol produces severe, transmural, granulomatous inflammation of the distal colon, which persists for more than 8 weeks (Morris *et al.*, 1989). This animal model shares many of the histopathological and clinical features of IBD in humans, particularly of Crohn's disease, and is thus useful for studying the pathophysiology of IBD (Yamada *et al.*, 1992).

Vitamin E is one of the lipid-soluble antioxidants and is generally considered to protect against lipid peroxidation of the cell membrane (Burton *et al.*, 1982; Niki *et al.* 1984) and to scav-

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enge singlet oxygen (Yamauchi and Matsushita, 1977; Mukai *et al.*, 1991) and superoxide anion radical (Nishikimi *et al.*, 1980; Fukuzawa and Gebicki, 1983). Therefore, vitamin E or its derivatives are expected to have particular application to patients suffering from IBD. 2-(α-D-glucopyranosyl)methyl-2,5,7,8-tetramethylchroman-6-ol (TMG; Fig. 1) is a novel synthesized vitamin E derivative that is responsible for the peroxyl radical scavenging activity on lipid peroxidation, in common with α-tocopherol, a major constituent of lipid-soluble vitamin E (Murase *et al.*, 1997, 1998). TMG has its excellent water solubility (>1 g/ml) by the replacement of long phytyl side chain of α-tocopherol to the glucosyl group (Murase *et al.*, 1997). Our objective was to investigate the effect of TMG in TNBS-induced colitis in rats.

MATERIALS AND METHODS

Experimental animals

Male Wistar rats weighing 190–210 grams were obtained from Keari Co. Ltd. (Osaka, Japan), and were housed at 22°C with a 12-hr lighting time and rat chow *ad libitum*. They fasted for 48 h prior to the induction of colitis, but were allowed free access to water. Care of the animals and the experimental procedures were carried out in accordance with the guidelines of the Japan Council on Animal Care.

Induction of colitis

TNBS-induced colitis was elicited by the method of Morris *et al.* (1989). In brief, rats were lightly anesthetized with ether following a 48-hr fast; then a rubber catheter (OD 2 mm) was inserted via the anus so that the tip was 8 cm proximal to the anus. TNBS (Wako Pure Chemical Industry, Osaka, Japan) dissolved in 50% ethanol (120 mg/ml) was instilled into the lumen of the colon through the catheter (0.25 ml in volume). Following instillation of TNBS at 30 mg per rat, the anus was occluded with a clip for 60 min.

α-Tocopherol

TMG

FIG. 1. Structures of α-tocopherol and 2-(α-D-glucopyranosyl)methyl-2,5,7,8-tetramethylchroman-6-ol (TMG). Both structures share a chromanol ring. TMG has glucosyl group, which gives it water solubility, instead of phytyl side chain of α-tocopherol.

Administration of TMG

All rats were divided into four groups on the day following the induction of colitis, besides the normal group, in which rats received physiological saline by enema instead of TNBS. One milliliter of each test drug was administered by intraperitoneal (i.p.) injection once a day for 1 week as follows: normal group and control group, physiological saline; TMG 0.2 group, 0.2 mg/ml of TMG (a gift from CCI Pharmacy, Gifu, Japan); TMG 2 group, 2 mg/ml of TMG; TMG 20 group, 20 mg/ml of TMG. TMG was dissolved in physiological saline.

Assessment of colitis

After the induction of colitis, the rats were weighed daily for 1 week to evaluate the effect on general nutrition. One week following the induction of colitis, all rats were killed by exsanguination via the abdominal aorta. The distal colon was removed, opened by a longitudinal incision and the degree of colitis was evaluated. For microscopic study, specimens of the distal colon stained with hematoxylin and eosin (H.E.) were prepared. As indices of inflammation, colonic damage was estimated macroscopically as the sum of the mucosal score and the serosal score. The mucosal score was rated on a 6-point scale (0–5) according to the criteria established by Morris *et al.* (Table 1a; Morris *et al.*, 1989). The serosal score was rated on a 4-point scale (0–3) according to the severity of serosal adhesions (Table 1b). The wet weight of the colon was determined, and thiobarbituric acid-reactive substances (TBA-RS), an index of lipid peroxidation, of the colonic mucosa were measured using the method of Ohkawa *et al.* (1979). The level of α-tocopherol in the colonic mucosa was also measured with high-performance liquid chro-

TABLE 1. CRITERIA FOR SCORING GROSS MORPHOLOGIC DAMAGE OF THE COLON

Score	*Gross morphology*
a. Mucosal score	
0	No damage.
1	Localized hyperemia, but no ulcers.
2	Linear ulcers with no significant inflammation.
3	Linear ulcer with inflammation at one site.
4	Two or more sites of ulceration and/or inflammation.
5	Two or more major sites of inflammation and ulceration or one major site of inflammation and ulceration extending >1 cm along the length of the colon.
b. Serosal score	
0	No adhesions.
1	Adhesion less than 5 mm along the length of the colon at one site.
2	Two or more major sites of adhesion less than 5 mm and/or one site of adhesion extending 5–10 mm along the length of the colon.
3	Two or more major sites of adhesion extending 5–10 mm or one site of adhesion extending >10 mm along the length of the colon.

The mucosal score is based on the criteria of Morris *et al.* (1989), and the serosal score was estimated according to the severity of serosal adhesions.

matography (HPLC) using the method of Abe *et al.* (1975).

Determination of TMG in colonic mucosa

Homogenate was mixed with 120 μl of methanol and stirred vigorously. After centrifugation at 10,000 × *g* for 5 min at 4°C, an aliquot of supernatant was filtered through a 0.45-μm pore filter and injected into a TSK-gel ODS-80Ts column (Tosoh) that eluted with methanol/water (45:55, vol/vol) containing 50 m*M* sodium perchlorate at 1 ml/min. An amperometric detector (ICA-5212, TOA Electronics Ltd.) was used to monitor the oxidation potential at 600 mV for detection of TMG and TMG metabolites.

Statistics

Colonic damage scores are presented as scatter plots. Differences between groups were compared by analysis of variance (Kruskal-Wallis test) followed by the Mann-Whitney U-test, a nonparametric test. Data on body weight, colonic wet weight, TBA-RS, α-tocopherol, and TMG are presented as the mean ± SEM, and were compared using analysis of variance (ANOVA) followed by Fisher's protected least significant difference test (Fisher's PLSD). A level of $p < 0.05$ was considered statistically significant.

RESULTS

Macroscopic findings of colitis

Macroscopic findings of the colon demonstrated severe colitis with edema, thickening, hyperemia, ulceration, and necrosis of the mucosa in the control group (Fig. 2b). These changes were markedly reduced in the rats of

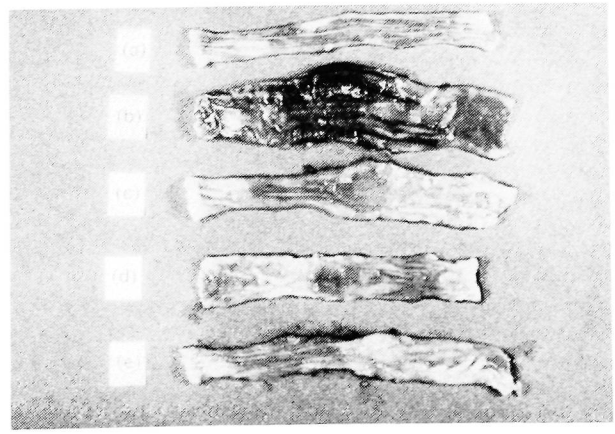

FIG. 2. Macroscopic findings of representative colons at 8 cm distal to the anus 7 days after the enema of 2,4,6-trinitrobenzene sulfonic acid (TNBS). a. Normal group. **b.** Control group. **c.** TMG (0.2 mg/body) group. **d.** TMG (2 mg/body) group. **e.** TMG (20 mg/body) group. Severe colitis was produced with edema, thickening, hyperemia, ulceration, and necrosis of the mucosa in the control group. These changes were reduced in the TMG-treated rats.

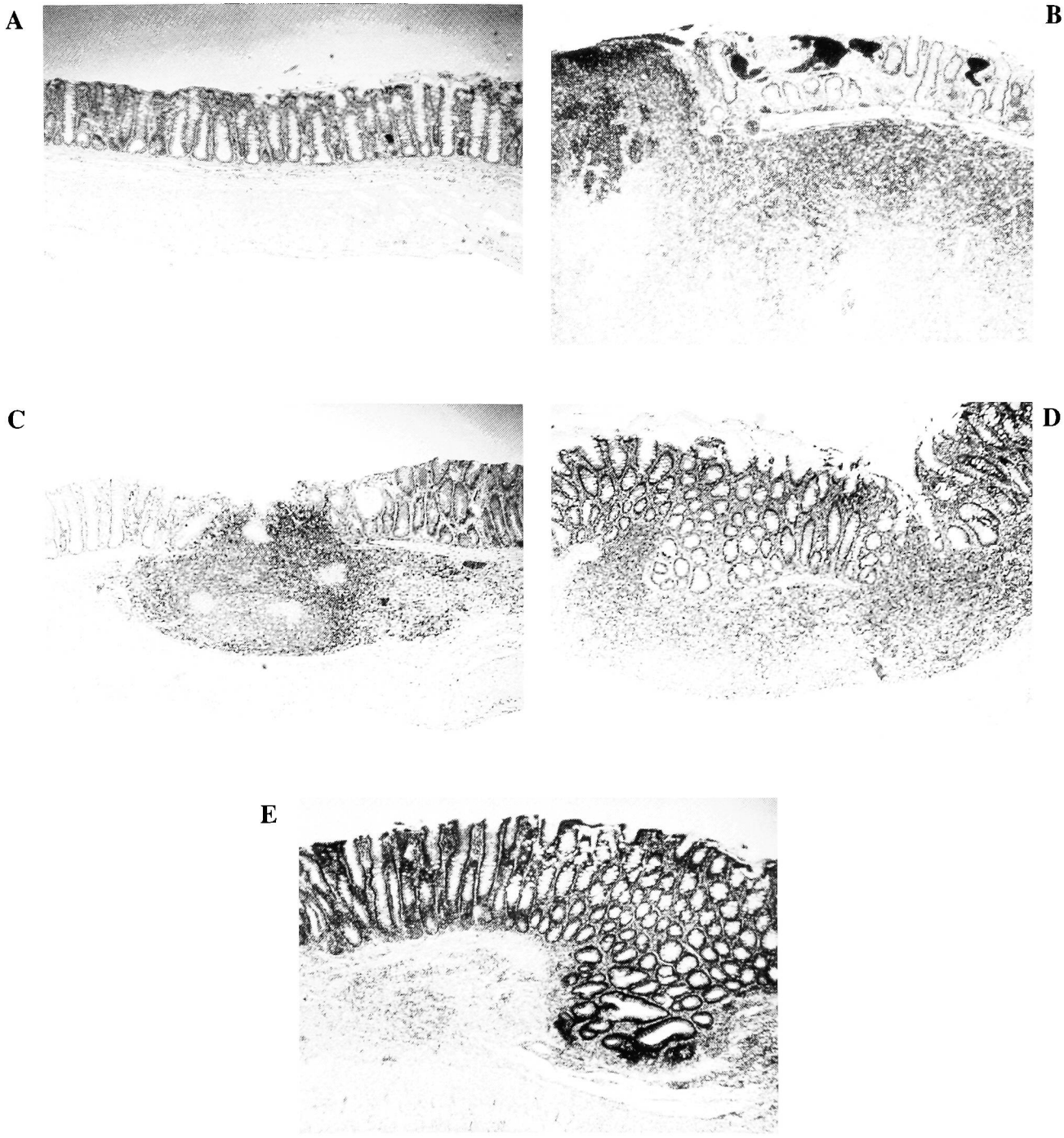

FIG. 3. Microscopic findings of representative colons at 8 cm distal to the anus 7 days after the enema of TNBS. **A.** Normal group. **B.** Control group. **C.** TMG (0.2 mg/body) group. **D.** (2 mg/body) group. **E.** TMG (20 mg/body) group. In the control group, marked thickening of the colonic wall was observed with the infiltration and aggregation of numerous inflammatory cells with prominent lymphocytes, which are also found in smaller numbers in the groups treated with TMG. Magnification, 40× (hematoxylin & eosin).

the TMG 0.2 group, the TMG 2 group, and the TMG 20 group, without remarkable differences among them (Fig. 2c,d,e).

Microscopic findings

Light microscopic study of H.E.-stained colonic specimens of control rats showed marked thickening of the colonic wall, with the infiltration and aggregation of numerous inflammatory cells having prominent lymphocytes in clusters forming a lymph follicle. These changes extended transmurally with small noncaseating granulomas distributed throughout (Fig. 3B). These findings were not found in the other groups, particularly in the TMG 0.2, TMG 2, and TMG 20 groups, in which specimens showed only mild or moderate degrees of infiltration of inflammatory cells and slight mural thickening (Fig. 3c,d,e).

Changes in body weight of rats

The increase of body weight for 1 week was significantly smaller in the control, TMG 0.2, TMG 2, and TMG 20 groups with experimental colitis than in the normal group (Fig. 4). All of the TMG-treated groups showed significantly less inhibition of weight gain compared with the control group, but no significant differences among the three groups.

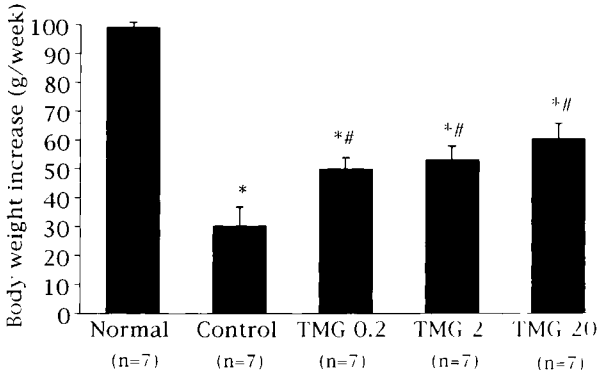

FIG. 4. Effect of TMG on increase in body weight for 1 week after the enema of TNBS. One milliliter of TMG solution (0.2 mg/ml, 2 mg/ml, or 20 mg/ml) was injected i.p. daily for 1 week after the enema of TNBS. Rats in the normal group and the control group received i.p. injection of saline instead of TMG. Rats in the normal group received an enema of saline instead of TNBS. Data are expressed as the mean ± SEM. $^{*}p < 0.05$ vs. normal; $^{\#}p < 0.05$ vs. control.

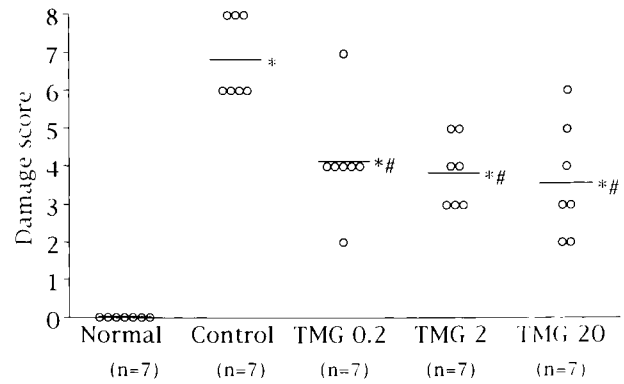

FIG. 5. Effect of TMG on the damage score 1 week after the enema of TNBS. TMG was administered to rats in the same manner as described in Fig. 4. Data are expressed as a scatter plot. $^{*}p < 0.05$ vs. normal; $^{\#}p < 0.05$ vs. control.

Damage scores

Colonic damage scores showed significant increases in the four colitis groups compared with the normal group (Fig. 5). These increases were significantly inhibited in the three groups treated with TMG.

Wet weight

Colonic wet weight increased significantly in the four colitis groups compared with the normal group (Fig. 6). These increases were significantly inhibited in all groups treated with TMG, without significant differences among the TMG-treated groups.

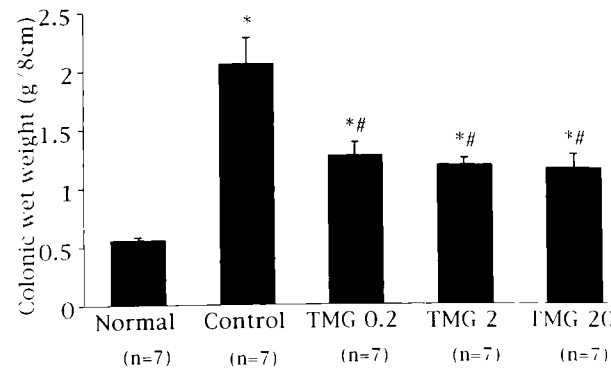

FIG. 6. Effect of TMG on colonic wet weight 1 week after the enema of TNBS. TMG was administered to rats in the same manner as described in Fig. 4. Data are expressed as the mean ± SEM. $^{*}p < 0.05$ vs. normal; $^{\#}p < 0.05$ vs. control.

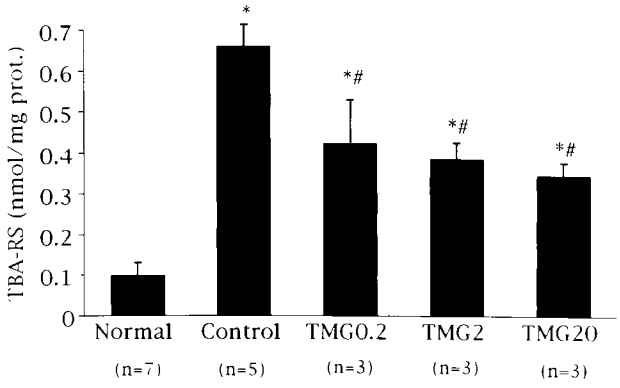

FIG. 7. Effect of TMG on thiobarbituric acid-reactive substances (TBA-RS) in colonic mucosa 1 week after the enema of TNBS. TMG was administered to rats in the same manner as described in Fig. 4. Data are expressed as the mean ± SEM. $^{*}p < 0.05$ vs. normal; $^{\#}p < 0.05$ vs. control.

TBA-RS

The control group showed a significant increase in TBA-RS compared with the normal group (Fig. 7). However, the increase was significantly reduced in all groups treated with TMG.

α-Tocopherol and TMG

The α-tocopherol level in the colonic mucosa decreased significantly in the control group compared with the normal group (Fig. 8), whereas TMG in the colonic mucosa was not detected in all groups treated with TMG.

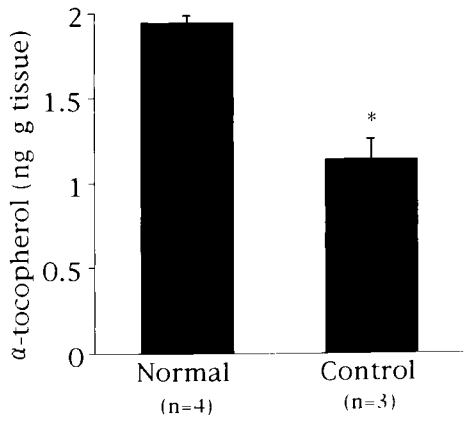

FIG. 8. The level of α-tocopherol in colonic mucosa 1 week after the enema of TNBS. Data are epxressed as the mean ± SEM. $^{*}p < 0.05$ vs. normal.

DISCUSSION

The results of the present study suggest that TMG, a water-soluble vitamin E derivative, is effective in TNB-induced colitis in rats. In this animal model, TBA-RS in the colonic mucosa were increased by the induction of colitis, while the α-tocopherol level in the colonic mucosa decreased. These findings suggest that α-tocopherol was consumed through acting as a radical-scavenging antioxidant in the colonic inflammation.

For induction of colitis, the dose of TNB was fixed at 30 mg per rat and the concentration of ethanol was 50%. This regimen was shown to be most effective in inducing severe colitis (Morris *et al.*, 1989; Yoshikawa *et al.*, 1992). Although TNB itself produces reactive oxygen species (ROS) in its oxidative metabolism process (Grisham *et al.*, 1991), the majority of ROS in the colonic mucosa has been proposed to be released by macrophages and neutrophils, which are markedly increased in the colon during acute and chronic inflammation (Yoshikawa *et al.*, 1992; Palmen *et al.*, 1995). As has been reported, ROS and lipid peroxidation has been implicated in the formation of rats TNB-induced colitis (Siems *et al.*, 1992; Yoshikawa *et al.*, 1991), as well as in human IBD (Suematsu *et al.*, 1987; Kitahora *et al.*, 1988). In addition, the generated ROS simultaneously attenuate antioxidative defense system in the colonic mucosa, which includes superoxide dismutase (SOD), glutathione peroxidase, vitamin E, and some kinds of trace elements (Yoshikawa *et al.*, 1992; Buffinton and Doe, 1995; Lih-Brody *et al.*, 1996; Bousvaros *et al.*, 1998). In such a pathological state, it is reasonable to supplement with antioxidant agents for prophylaxis or therapy. Emerit *et al.* reported that the administration of CuZn-SOD reduced colonic inflammation in patients with IBD (Emerit *et al.*, 1991). We also reported that agents having antioxidant activity, such as Mn-SOD (Yoshikawa *et al.*, 1992) or zinc-carnosine chelate compound (Yoshikawa *et al.*, 1997), could protect against TNB-induced colitis through scavenging ROS and inhibiting lipid peroxidation. Our present study showed that TMG inhibited lipid peroxidation in the colonic

mucosa and reduced colonic inflammation. In the TMG-treated rats, TMG could not be detected in the colonic mucosa 24 hr after the last administration. The possible effect of TMG could be associated with scavenging of hydroxyl radical, which is only ROS able to initiate lipid peroxidation. Another effect of TMG could be associated with intramembrane termination of lipid peroxidation and consequently in decrease of TBA-RS.

Vitamin E is well known as the major lipid-soluble antioxidant preventing oxidative attack of membrane lipids and other membrane compounds. Antioxidant activity of vitamin E is partially based on its ability to break the chain reaction of lipid peroxidation (Burton *et al.*, 1982; Niki *et al.*, 1984), and to scavenge singlet oxygen (Yamauchi and Matsushito, 1977; Mukai *et al.*, 1991) and superoxide anion radical (Nishikimi *et al.*, 1980; Fukuzawa and Gebicki, 1983) efficiently. However, vitamin E is lipophilic and, to be supplied to tissue, vitamin E must trace the same pathway after oral intake as does the vitamin E that is absorbed at microvilli of the small intestine independently or by the intermediating chylomicron, and then carried to the liver by way of the lymphatic system, distributed among lipoproteins, and transported to general organs (Drevon, 1993).

The high water solubility of TMG enables it to be carried to general organs and supplied to tissue rapidly. TMG also shows an excellent antioxidant activity equally to α-tocopherol or ascorbic acid (Murase *et al.*, 1997). It is reported that TMG is located within the membrane surface and can scavenge radicals generated either in the lipid or in the aqueous phase (Murase *et al.*, 1998). Therefore, TMG has many advantages over therapeutics of IBD as an antioxidant in comparison with α-tocopherol.

In summary, the results of the present study indicate that TMG inhibited lipid peroxidation and reduced development of the colonic inflammation induced by TNB in rats. Although the main drugs used for treatment of IBD are sulfasalazine and corticosteroids, they have many side effects. This investigation suggests that TMG may become a new therapeutic agent for IBD.

ABBREVIATIONS

H.E., hematoxylin and eosin; IBD, inflammatory bowel disease; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA-RS, thiobarbituric acid-reactive substances; TMG, 2-(α-D-glucopyranosyl)methyl-2,5,7,8-tetramethylchroman-6-ol; TNBS, trinitrobenzene sulfonic acid.

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