Dietary Supplementation of Catechins and α-Tocopherol Accelerates the Healing of Trinitrobenzene Sulfonic Acid-Induced Ulcerative Colitis in Rats

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Summary The effects of dietary catechins and α-tocopherol on inflammatory bowel disease in rats were examined. Male 9-week-old rats were intracolonically administrated trinitrobenzene sulfonic acid (TNBS) and fed the experimental diets containing 0.05% catechin and 0.025% α-tocopherol for 1 week, then dissected. The extent of colitis-induced TNBS was assessed macroscopically. The supplementation of catechins and α-tocopherol significantly decreased colonic damage compared with the group fed the basal diet (the disease control). In particular, catechin feeding completely inhibited the development of colon adhesions. Colonic myeloperoxidase (MPO) activity, which is a marker of neutrophil infiltration into the colonic mucosa, was lower in the groups that had been given catechins and α-tocopherol. The levels of thiobarbituric acid reactive substances in colon was highest in the disease control group; however, the differences among the groups were not significant. Plasma alkaline phosphatase activity was maintained at normal levels in the rats supplemented with catechins and α-tocopherol. These results suggest that catechins and α-tocopherol have anti-inflammatory effects on TNBS-induced rat colitis.

Key Words catechins, α-tocopherol, inflammatory bowel disease, rats, trinitrobenzene sulfonic acid

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Ulcereative colitis and Crohn’s disease are inflammatory bowel diseases (IBD) characterized by cycles of acute inflammation, ulceration, and bleeding of the colonic mucosa. The causative factors of IBD have not been defined, but mucosal lesions in IBD, dense infiltration of inflammatory cells that mainly comprise neutrophils (1) and macrophages (2), have been observed. A variety of chemical mediators in initiating and amplifying inflammatory responses, such as leukotrienes (3), cytokines (4), reactive oxygen species, and oxidant derivatives (5, 6), mediate inflammatory responses. In corticosteroid-resistant Crohn’s disease, a combination of superoxide dismutase and desferrioxamine has been found to be effective (7). In an experimental model of intestinal inflammation, antioxidants were therapeutically effective (8, 9). These findings indicate that antioxidants have anti-inflammatory effects. Antioxidants should therefore be studied as a potential treatment for IBD.

Polyphenolic compounds with low molecular weight in green tea extract are known as catechins in which the major compound is (-)-epigallocatechin 3-gallate (EGCg). Catechins extracted from green tea have been found to have distinct antioxidant effects in vitro (10) and anti-inflammatory and anticarcinogenic effects in vivo (11-13). α-Tocopherol is known to be a representative antioxidant in the cell membrane lipid phase, and it also has anti-inflammatory properties (14, 15).

The trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats is a representative model of experimental colitis (16). Acute colonic inflammation and ulceration that had developed into chronic inflammation in TNBS-administrated rats was histopathologically similar to Crohn’s disease in humans (17). In this study, we used this model to assess the protective effects of catechins and α-tocopherol on inflammation and ulceration.

MATERIALS AND METHODS

Materials. The catechin mixture called ‘Sunphenon’ was obtained from Taiyo Kagaku (Yokkaiti, Japan) and consisted of 65.1% catechins. The composition of catechins is as follows: 46.1% (-)-epigallocatechin 3-gallate (EGCg), 22.4% (+)-gallocatechin 3-gallate, 12.0% (+)-gallocatechin, 7.2% (-)-epicatechin, 6.3% (-)-epicatechin 3-gallate, and 6% other catechins. DL-α-tocopherol was provided by Eisai (Tokyo, Japan). TNBS was purchased from Nacalai Tesque (Kyoto, Japan). Other reagents of analytical grade were purchased from Wako Pure Chemicals (Osaka, Japan).

Animals. Male Sprague-Dawley rats (210–230 g) were purchased from Clea Japan (Tokyo, Japan). The rats were housed individually in wire cages and kept in a temperature-controlled room (25°C) with a 12 h light-dark cycle. They were provided free access to commercial pellets and tap water for 2 weeks before the experiments began. The protocol in this study complied with our institute’s rules for the care and use of laboratory animals.

Induction of colitis. Rats were randomized into 4 groups. The number of rats in the groups were as follows: catechin, 12; tocopherol, 12; disease control, 9; disease control, 9;
normal, 9. After being deprived of food for 24 h, they were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and intracolonically administrated with 0.5 mL of TNBS (60 mg/mL) in 50% (v/v) ethanol to induce colitis, as previously discussed (17). TNBS was administrated through a balloon catheter (OD, 3 mm) with a tip 4 cm from the anus. The installation procedure required 30 s, and the catheter was removed from the colon exactly 30 min after TNBS injection. The rats in the normal group were not administered TNBS/ethanol solution.

**Diet administration.** After the induction of colitis, the 4 groups of rats were fed a semisynthetic diet for 1 week. The composition of the basal diet (wt%) was as follows: casein, 20.0; corn starch, 53.0; sucrose, 10.0; soybean oil, 7.0; cellulose powder, 5.0; mineral mix. (18), 3.5; vitamin mix. (18), 1.0; choline bitartrate, 0.2; DL-methionine, 0.3. In the catechin group, the catechin mixture was added to the basal diet at a level of 0.5% (wt/wt). This level was almost comparable to those reported by Tanaka et al (19) and Kimura et al (20), who respectively demonstrated anticarcinogenic and antioxidant activities in vivo. In the tocopherol group, DL-α-tocopherol was added at a level of 0.025% (wt/wt). This level was an excess level that expected a maximum effect as an antioxidant. One of the 3 groups in which colitis had been induced (the disease control), besides the normal group, was fed the basal diet, which contained 0.005% (wt/wt) of DL-α-tocopherol. During the 1-week feeding period, the animals were provided with free access to the diet and water. Food was prepared each day.

**Assessment of the severity of colitis.** After 1 week of the experimental feeding, the rats were not fed overnight and were killed by collecting arterial blood. The distal 10 cm of the colon was removed, opened by a longitudinal incision, and rinsed with saline. The extent of the damage was assessed macroscopically as described previously (21). The presence of colonic ulceration and inflammation was scored by using the criteria outlined in Table 1. The damage score was estimated by two independent observers who were unaware of the dietary treatment. Subsequently, the colonic mucosa was scraped with a razor on an ice-cold glass plate for biochemical determinations and stored immediately at −80°C until use. Plasma obtained after centrifugation was stored at −80°C until use.

**Myeloperoxidase assay.** The myeloperoxidase (MPO) activity in the colonic mucosa was measured after Grisham et al (22). One unit was defined as the amount of enzyme present that produces a change in absorbancy per min of 1.0 at 37°C.

**Thiobarbituric acid-reactive substances (TBARS) assay.** The concentration of thiobarbituric acid reactive substances (TBARS) was measured according to Kikugawa et al (23). The amount of red pigment was determined by absorbance at 532 nm and the molecular extinction coefficient of 1:2 malonaldehyde:TBA adduct, 156 × 10³.

**α-Tocopherol assay.** The concentrations of α-tocopherol in the colonic mucosa and plasma were measured as described by Abe et al (24). α-Tocopherol was separated by HPLC and was determined fluorometrically (Ex. 298 nm, Em. 325 nm).

**Alkaline phosphatase assay.** Plasma alkaline phosphatase activity was
Table 1. Criteria for assessment of colonic damage.

| Criteria                  | Score |
|---------------------------|-------|
| Adhesions                 |       |
| None                      | 0     |
| Minimal                   | 1     |
| Involving several bowel loops | 2   |
| Strictures                |       |
| None                      | 0     |
| Mild                      | 2     |
| Severe proximal dilatation | 3   |
| Ulcers                    |       |
| None                      | 0     |
| Linear ulceration < 1 cm  | 1     |
| Two linear ulcers < 1 cm  | 2     |
| More sites of ulceration or one | 3 |
| large ulcer > 1 cm        |       |
| Wall thickness            |       |
| Less than 1 mm            | 0     |
| 1–3 mm                    | 1     |
| More than 3 mm            | 2     |

Maximum score 10

measured by a Hitachi 7170 automatic analyzer (Hitachi, Tokyo, Japan) according to Bessey et al (25).

Protein assay. The protein contents in colonic mucosa and in plasma were measured according to Lowry et al (26) and Gornall et al (27), respectively. A Hitachi 7170 automatic analyzer was used for the assay of the plasma protein.

Statistics. The results are expressed as mean ± SE. The results from scoring the colonic damage were analyzed by the Kruskal-Wallis test and the Mann-Whitney test. All data except for the damage score was analyzed with the one-way ANOVA and Newman-Keuls test. These statistical analyses were performed with the statistics program Statmate (Nankodo, Tokyo, Japan) on a Macintosh.

RESULTS

Effects of the TNBS administration on criteria of rats

A single intracolonic administration of TNBS induced a syndrome that was characterized by diarrhea, anorexia, loss of body weight, and severe colonic damage. Just after the administration of TNBS, severe diarrhea and bleeding from the anus were observed in all rats, and food intake decreased; however, this stopped within 3 d, and their food intake increased to normal. No significant difference in the changes of body weight during 3 d after TNBS-treatment suggested that the severity of colonic damages were similar among rats of the three groups.
Fig. 1. Effects of catechins and α-tocopherol on the severity of colitis. Colonic damage was scored on a scale of 0 (normal) to 10 (severe) by two independent observers.

Table 2. Effects of catechins and α-tocopherol treatment on body weight gain, colonic weight, and incidence of adhesions.

| Groups                  | Body weight gain during the experimental feeding period (g) | Colonic weight (g/100 g body weight) | Incidence of adhesions of colon (%) |
|-------------------------|------------------------------------------------------------|--------------------------------------|-------------------------------------|
| Catechin (12)           | 38.9 ± 5.1<sup>a</sup>                                      | 0.68 ± 0.03<sup>a</sup>              | 0                                   |
| Tocopherol (12)         | 28.3 ± 6.2<sup>b</sup>                                      | 0.83 ± 0.11<sup>a</sup>              | 25                                  |
| Disease control (9)     | 19.4 ± 4.3<sup>b</sup>                                      | 1.83 ± 0.42<sup>b</sup>              | 78                                  |
| Normal (9)              | 43.2 ± 2.8<sup>a</sup>                                      | 0.41 ± 0.01<sup>a</sup>              | —                                   |

Values (mean ± SE) without a common superscript letter (a and b) are significantly different (p < 0.05). The number of rats in the assay is in parentheses.

Macroscopic colonic damage scores and body weight gain

Figure 1 shows the severity of the colitis, which was macroscopically assessed 8 d after TNBS administration. In the rats fed catechins and α-tocopherol, a significant decrease in the macroscopic damage was observed, compared with the disease control group fed the basal diet. In the disease control group, perforation of the distal colon and massive adhesions between the colon and other abdominal tissue were frequently observed (Table 2). However, perforation of the distal colon and adhesions were not observed in the catechins group. In the α-tocopherol group, the incidence of adhesions were a third of that in the disease control group.

Body weight gain during the experimental period is shown in Table 2. A reduction in body weight during the first 3 d after TNBS administration was ob-
Table 3. Markers of inflammation and peroxidation in rat colitis.

| Groups        | Colonic MPO (U/mg protein) | Colonic TBARS (nmol/mg) | Colonic TOC (nmol/mg protein) | Plasma TOC (ng/mg protein) | Plasma AP (ng/mg protein) |
|---------------|----------------------------|-------------------------|-------------------------------|----------------------------|---------------------------|
| Catechin      | 19.4 ± 2.7<sup>a,b</sup>   | 0.5 ± 0.1               | 65.3 ± 4.5<sup>a</sup>        | 19.4 ± 1.2<sup>a</sup>     | 8.5 ± 0.4<sup>a</sup>    |
| Tocopherol    | 25.8 ± 5.7<sup>a</sup>     | 0.6 ± 0.1               | 94.8 ± 7.9<sup>b</sup>        | 36.6 ± 1.7<sup>b</sup>     | 7.2 ± 0.4<sup>a</sup>    |
| Disease control | 43.0 ± 3.2<sup>c</sup>  | 0.8 ± 0.1               | 111.3 ± 10.9<sup>b</sup>      | 17.6 ± 2.7<sup>a</sup>     | 14.9 ± 2.6<sup>b</sup>  |
| Normal        | 11.1 ± 1.7<sup>b</sup>    | 0.6 ± 0.1               | 63.2 ± 4.7<sup>a</sup>        | 22.2 ± 2.2<sup>a</sup>     | 7.8 ± 0.4<sup>a</sup>   |

Values (mean ± SE) without a common superscript letter (a, b, and c) are significantly different (p < 0.05).
The number of rats in the assay is in parentheses. MPO, myeloperoxidase; TBARS, thiobarbituric acid reactive substance; TOC, α-tocopherol; AP, alkaline phosphatase.
served in all three groups that were administered TNBS as described above. However, body weight gain in the catechin group after 8d was almost equal to that in the normal group and was significantly higher than in the disease control group ($p<0.05$). In the disease control group, the colonic weight was 4 times greater than the normal group and 2 times greater than the catechin and tocopherol groups.

**Colonic MPO activity**

Table 3 shows the effects of catechins- and α-tocopherol-treatment on colonic MPO activity. The MPO activity was highest in the disease control group. In the tocopherol group, the activity was significantly higher than in the normal group ($p<0.05$), but significantly lower than in the disease control group ($p<0.05$). The activity in the catechin group was close to that in the normal group and was 45% of that in the disease control group.

**Colonic TBARS levels**

Table 3 presents the TBARS levels. TBARS was used as an index of lipid peroxidation after the induction of colitis. No significant differences were found among the groups, though the level in the disease control group was higher than in the other groups.

**Colonic and plasma α-tocopherol levels**

As shown in Table 3, the concentration of α-tocopherol in plasma was highest in rats fed the α-tocopherol-enriched diet; however, no significant differences were found among the three groups fed a basal level of α tocopherol. In the colonic mucosa, the concentration in the disease control group and in the tocopherol group was significantly greater than in the other two groups.

**Plasma alkaline phosphatase activity**

The activity of plasma alkaline phosphatase was significantly higher in the disease control group than in the other three groups, in which the levels were almost equal (Table 3).

**DISCUSSION**

Treatment with catechins and α-tocopherol significantly lessened colonic macroscopic damage. The occurrence of adhesions between the colon and other abdominal tissue, which results from transmural inflammation, is a common feature of human Crohn’s disease (28) and TNBS-induced colitis (29). Catechins completely inhibited the development of adhesions, and the α-tocopherol also decreased their incidence. This suggests that both catechins and α-tocopherol suppressed the extension of the inflammatory processing in TNBS-induced colitis. Rachmilewitz et al (30) found that the colon’s wet weight was a sensitive indicator of the severity and extent of acute inflammatory response. The dietary catechins and α-tocopherol
prevented an increase in colonic weight. In TNBS-induced colitis, the activity of colonic alkaline phosphatase increases (9). Actually, the colonic damage score showed a good correlation with plasma alkaline phosphatase activity \((r=0.68, n=38, p<0.01)\). The activity of plasma alkaline phosphatase appeared to be a reliable marker of colonic inflammation in rats; however, it was not sensitive enough to detect the mild damage in the catechin and the tocopherol groups.

MPO, which is predominantly found in the neutrophils, is a sensitive marker of neutrophil infiltration and a useful marker for inflammation in the colon (17). Catechins and \(\alpha\)-tocopherol significantly decreased colonic MPO activity. This indicates that both catechins and \(\alpha\)-tocopherol suppressed neutrophil infiltration.

The mild damage in the catechin group might be partly attributed to the inhibition of 5-lipoxygenase by catechins (31). The inhibition of \(\text{LTB}_4\) synthesis (32) or the blockade of the \(\text{LTB}_4\) receptor (33) is beneficial in the experimental colitis, whereas the administration of exogenous \(\text{LTB}_4\) exacerbates inflammation (34). Oxygen radicals are suggested to play an important role in the pathophysiology of intestinal inflammation (5), and the supplementation of \(\alpha\)-tocopherol suppresses neutrophil activation (35, 36). In hyperoxia/oxidant model in a guinea pig, hepatic \(\alpha\)-tocopherol appears to be redistributed to other organs, including the lungs and brain, via plasma carrier proteins under oxidative stress (37). Siems et al (38) found an increase in TBARS in TNBS-induced colitis. The distribution of \(\alpha\)-tocopherol from the blood or the liver might be enhanced in colons with extensive damage in the disease control group, and the mobilization of \(\alpha\)-tocopherol might have prevented an increase in the TBARS level in the colonic mucosa.

Catechins and \(\alpha\)-tocopherol have anti-inflammatory effects on TNBS-induced colitis in rats. These effects seemed to be related to the impairment of neutrophil functions; however, we were unable to define the relationship. Catechins and other antioxidants, the former in particular, may be useful in the pharmacological treatment of inflammatory bowel disease.

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