Protective Effect of (–)-Epigallocatechin Gallate on Acute Experimental Colitis

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We studied the ameliorative effect of (–)-epigallocatechin gallate (EGCG) on inflammatory bowel disease (IBD) induced by ethanolic 2,4,6-trinitrobenzene sulfonic acid (TNBS) in male 7-week-old rats. Intestinal lesions were associated with macroscopic damage score and measured as increase in myeloperoxidase (MPO) activity in mucosa. Pretreatment with EGCG (30 mg/kg daily i.g. for 10 days) significantly reduced macroscopic damage score, inhibited MPO activity and enhanced superoxide dismutase activity. These observations confirmed that EGCG can ameliorate acute experimental colitis by suppression of superoxide generation from colonic tissue.

Key words —— 2,4,6-trinitorobenzene sulfonic acid, myeloperoxidase, superoxide dismutase, inflammatory bowel disease, (–)-epigallocatechin gallate

INTRODUCTION

Inflammatory bowel disease (IBD) is an immune-mediated disorder of the gastrointestinal tract characterized by inflammation lesions and ulceration. At present, treatments are far from perfect and natural and relatively nontoxic products are needed for improved therapies. IBD is associated with production of nitric oxide, O_2^- and neutrophil infiltration.[1–3] A large number of dietary components have been evaluated as potential chemopreventive agents, and curcumin,[2] taurine[4] and catechin mixture and alpha-tocopherol[5] have been shown to exhibit marked antioxidant effects and ameliorate acute experimental colitis induced by intracolonic instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS).

We reported previously that powdered green tea increased activity of superoxide dismutase.[6] In this report, we examine the protective effect of (–)-epigallocatechin gallate (EGCG) on TNBS-induced colitis in the rat.

MATERIALS AND METHODS

Animals and Chemicals —— Male Sprague-Dawley (SD) rats weighing 200 ± 10 g were obtained from Japan SLC Inc. (Shizuoka, Japan). The rats were housed in standard cages and fed with standard laboratory chow and tap water ad libitum. EGCG was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). TNBS was from Wako Pure Chemicals (Tokyo, Japan).

Experimental Coltis —— Colitis was induced by intrarectal administration of TNBS, 24 mg/rat, dissolved in 1 ml of 50% (v/v) ethanol[3] through a 2 mm silicone rubber tube approximately 5 cm proximal to the anus. Saline was instilled as a control (untreated healthy group). In the TNBS treated group, EGCG was administered daily (30 mg/kg) for 10 days by gavage before TNBS-induced colitis. At 12 hr after TNBS, rats were sacrificed and the descending colon was cut at the pubic symphysis.

Macroscopic Assessment —— Macroscopic damage was assessed by a scoring system taking into account the area of inflammation and the presence or absence of ulcers.[7] The following grading criteria were used to assess macroscopic damage: 0, no ulcer, no inflammation; 1, no ulcer, local hyperaemia; 2, ulceration without hyperaemia; 3, ulceration and inflammation at one site only; 4, two or more sites of ulceration and inflammation; 5, ulceration extending more than 2 cm.

Myeloperoxidase (MPO) Activity Measurement —— Samples were collected according to a method previously described.[3] Mucosa was scraped from 5-cm long samples and homogenized in hexadecyltrimethylammonium bromide buffer (pH 6.0). The homogenate was sonicated and subjected to three cycles of freezing and centrifuged for 30 min at 20000 × *g* at 4°C. The aliquot of the supernatant was assayed for myeloperoxidase (MPO) activity according to a method previously described.[8] The rate of change in absorbance was measured at 460 nm in the mixture of supernatant and guaiacol-H_2O_2 buffer. One MPO activity unit corresponds to 1 μmol of H_2O_2 degraded in 1 min. Protein was quantified us-

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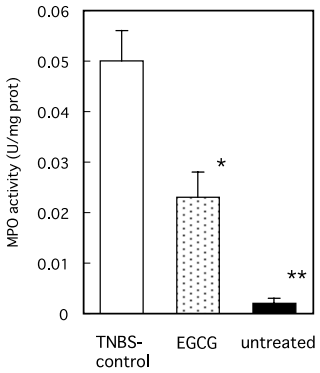

Fig. 1. Effect of EGCG Treatment on Neutrophil Granulocyte Infiltration into Distal Colon Mucosa

Thirty mg/kg of EGCG was given for 10 days prior to the administration of TNBS. Results are presented as the mean ± S.E. of six experiments: *$p < 0.05$; **$p < 0.01$.

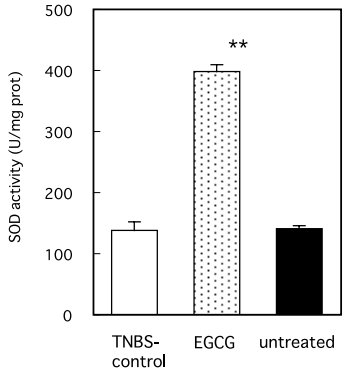

Fig. 2. Effect of EGCG on SOD Activity in Distal Colon Mucosa

Experimental procedure is as described in Fig. 1. Results are presented as the mean ± S.E. of six experiments: **$p < 0.01$.

ing a protein assay kit (Pierce, IL, U.S.A.).

Superoxide Dismutase (SOD) Activity —— Aliquots of the colon mucosa samples were assayed for superoxide dismutase (SOD) activity using a commercial kit (Wako Pure Chemicals).

Statistics —— Results are expressed as the mean ± S.E. Student's *t*-test was used for statistical comparison.

RESULTS AND DISCUSSION

TNBS induced an ulcerative inflammation of the distal colon characterized by reddish edematous mucosa. Treatment with EGCG resulted in a decrease in the extent and severity of the injury. The histological scores for the EGCG pretreated group (1.6 ± 0.4, $n = 6$) were tended to be lower than those of the TNBS control group (2.3 ± 0.4, $n = 6$). The dietary supplement of catechins significantly decreased colonic damage.[5] These results suggest that catechins have an anti-inflammatory effect against mucosal injury.

Colonic MPO activity, which is a marker of neutrophil infiltration into the mucosa, was significantly increased in the TNBS control group compared with the untreated group (Fig. 1). Treatment with EGCG significantly inhibited MPO activity by 46% ($p < 0.05$). These protective effects were due to reduced neutrophil activation as described in other food flavonoids such as curcumin[2] or morin.[9]

Treatment with EGCG increased SOD activity significantly (Fig. 2). These results suggest that superoxide was generated in TNBS-induced colitis and EGCG was effective by decreasing superoxide generation due to its radical scavenging activity.

The results of this study suggest that administration of EGCG may be therapeutically beneficial for the treatment of IBD in humans.

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