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Research Paper

Anti-inflammatory effect of enzymatic hydrolysate of corn gluten in an experimental model of colitis

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Abstract

Objectives Intestinal bacteria are thought to be involved in the initiation and perpetuation of inflammatory bowel diseases. Prebiotics (non-digestable dietary carbohydrate) have beneficial properties that alter the intestinal flora and contain glutamine-rich protein. Glutamine significantly decreases indices of inflammation. In this study, an enzymatic hydrolysate of corn gluten (EHCG) was administered by gavage to Sprague-Dawley rats fed an elemental diet to determine whether EHCG can ameliorate experimental colitis.

Methods Colitis was induced by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid after 10 days' daily oral administration of EHCG at 100 and 300 mg/kg. Macroscopic damage was assessed using a scoring system. The mucosa homogenate was sonicated and myeloperoxidase activity and histamine levels measured.

Key findings Treatment with EHCG significantly decreased the severity of injury and reduced myeloperoxidase activity and histamine levels in the distal colon mucosa.

Conclusions EHCG may have therapeutic benefit as a supplement in enteral nutrition for patients with inflammatory bowel diseases.

Keywords enzymatic hydrolysate of corn gluten; histamine; inflammatory bowel diseases; myeloperoxidase activity

Introduction

Ulcerative colitis and Crohn's disease are inflammatory bowel diseases (IBD) characterised by cycles of acute inflammation and ulcerative bleeding of the colonic mucosa. However, little is known about the aetiology of these diseases. The intestinal bacteria are thought be involved in the initiation and perpetuation of IBD.[1,2]

The use of dietary fibre and germinated barley foodstuff (GBF), a glutamine-rich protein, has been shown to reduce inflammatory scores in rats with colitis induced by dextran sulfate sodium (DSS).[3,4] The dietary fibre component, goats milk oligosaccharide or lactulose, has a beneficial effect on rats with colitis induced by DSS[5] or 2,4,6-trinitrobenzene sulfonic acid (TNBS).[6] However, the effect of the protein component of GBF is not clear.

IBD are characterised by a marked increase in the number of mast cells, resulting in infiltration of inflammatory cells into mucosal lesions.[7] Histamine has been recognised as a major mediator in allergic disease, and highly elevated histamine levels have been detected in the mucosa of patients with IBD.[8]

In this study, we administered an enzymatic hydrolysate of corn gluten (EHCG) by gavage to Sprague-Dawley rats, to assess whether EHCG can ameliorate experimental colitis, in order to determine its efficacy as a supplement for enteral nutrition.

Materials and Methods

Chemicals

TNBS was purchased from Wako Pure Chemicals (Osaka, Japan). EHCG was purchased from Sigma (St Louis, MO, USA).

Animals

Twenty-four male Sprague-Dawley rats weighing 180 ± 20 g were obtained from Japan SLC Inc. (Shizuoka, Japan). They were housed in standard cages and fed a commercial elemental

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diet (Elental, Ajinomoto Pharma Co., Ltd, Tokyo, Japan) and tap water ad libitum. All procedures for handling animals were approved by the Animal Experimentation Committee of Gifu Women's University Graduate School of Human Life Science.

Experimental procedure

The rats were divided into four groups of six rats. EHCG was administered at either 300 mg/kg (high dose) or 100 mg/kg (low dose) daily by gavage for 10 days in two groups. A third group, the disease control group, were given saline (EHCG vehicle). Colitis was induced after 10 days' treatment in these three groups of rats by intrarectal administration of TNBS, 120 mg/kg, dissolved in 1 ml 50% (v/v) ethanol through a 2 mm silicone rubber tube approximately 5 cm proximal to the anus.[9] The fourth group of rats were the untreated control group, given intrarectal ethanol. Rats were sacrificed 12 h after administration of TNBS and the descending colon was cut at the pubic symphysis.

Macroscopic assessment of colon damage

Macroscopic damage was assessed using a scoring system that quantifies the area of inflammation and the presence or absence of ulcers using four standard parameters:[10] adhesions (0 = none, 1 = minimum, 2 = involving several bowel loops); wall thickness (0 = less than 1 mm, 1 = 1–3 mm, 2 = more than 3 mm); strictures (0 = none, 2 = mild, 3 = severe proximal dilatation); and ulcers (0 = none, 1 = linear ulceration < 1 cm, 2 = two linear ulcers < 1 cm, 3 = more sites of ulceration or one large ulcer > 1 cm).

Measurement of myeloperoxidase activity

Samples were collected according to previously reported methods.[9,11] The mucosa was scraped from 5-cm long samples and homogenised in hexadecyltrimethylammonium bromide buffer (pH 6.0). The homogenate was sonicated and subjected to three cycles of freezing and centrifugation at 20 000*g* for 30 min at 4°C. An aliquot of the supernatant was assayed for myeloperoxidase (MPO) activity according to a method described previously.[12] The rate of change in absorbance in the mixture of supernatant and guaiacol–H_2O_2 buffer was measured at 460 nm. One MPO activity unit corresponds to 1 μmol H_2O_2 degraded in 1 min. Protein was quantified using a commercial protein assay kit (Coomassie protein assay reagent; Pierce, Rockford, IL, USA).

Measurement of histamine levels

Levels of histamine in the mucosa were determined using a commercial histamine enzyme immunoassay kit (SPI-BIO, Montigny-Le-Bretonneux, France).

Statistical analysis

Results are given as means ± SE. Differences between groups were tested using the Kruskal–Wallis test and Nemenyi test; $P < 0.05$ was considered significant.

Results

Severity of colitis

The effects of EHCG on strictures and ulcers are shown in Table 1. Pretreatment with high-dose EHCG significantly decreased the extent and severity of the injury as evidenced by macroscopic damage.

Myeloperoxidase activity

Acute injury by TNBS administration was characterized by an increase in MPO activity in the inflamed mucosa. Pretreatment with high-dose EHCG significantly reduced MPO activity (Figure 1).

Histamine levels

Acute injury by TNBS administration was characterized by an increase in the histamine in the inflamed mucosa.

Table 1 Effects of enzymatic hydrolysate of corn gluten (EHCG) on the severity of colitis

	Damage score	
	Mean ± SD	**Median (range)**
Untreated	0.20 ± 0.20	0 (0–1)
Disease control	6.50 ± 0.41	7 (6–7)
EHCG, 100 mg/kg	5.33 ± 0.33	5 (5–6)
EHCG, 300 mg/kg	1.66 ± 0.67*	1 (1–3)

Values are from six experiments. *$P < 0.05$ vs disease control group.

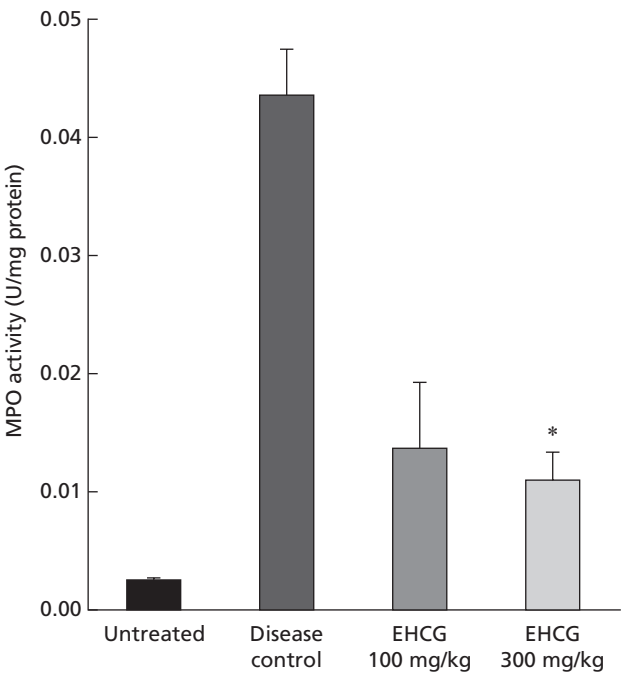

Figure 1 Effects of pretreatment with an enzymatic hydrolysate of corn gluten (EHCG) on myeloperoxidase (MPO) activity in the distal colon mucosa of rats with experimental colitis. Bars show means ± SE ($n = 6$). *$P < 0.05$ vs disease control group

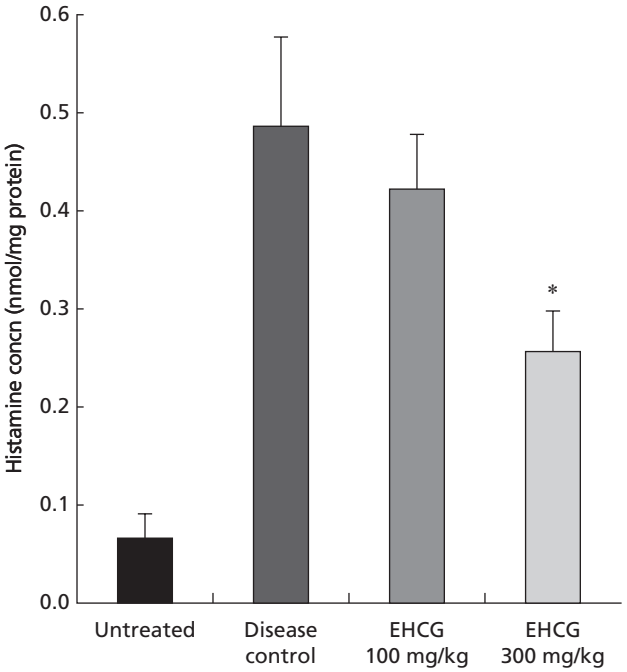

Figure 2 Effects of pretreatment with an enzymatic hydrolysate of corn gluten (EHCG) on histamine levels in the distal colon mucosa in rats with experimental colitis. Bars show means ± SE ($n = 6$). $^{*}P < 0.05$ vs disease control group

Pretreatment with high-dose EHCG significantly reduced this increase in histamine (Figure 2).

Discussion

IBD are complex autoimmune diseases whose aetiology and pathogenesis have not been fully elucidated. Multiple aetiological theories have been proposed. TNBS-induced colitis shares many of the histopathological and clinical features of human IBD and is used as an animal model of immunological colon injury. Macroscopic damage is commonly assessed by a scoring system[10] and inflammation is accompanied by a drastic elevation of MPO activity.

In the present study, administration of high-dose EHCG significantly attenuated the severity of colitis, MPO activity and histamine levels. However, low-dose EHCG did not attenuate the severity of colitis and histamine levels. These results suggest that the severity of colitis depends on histamine levels as a chemoattractant.

Mast cells are major participants of allergy reactions and are known as a source of inflammatory chemicals, including histamine, heparin, neutral proteases and cytokines. Marked increases in the number of mast cells and highly elevated histamine levels are seen in the mucosa of patients with IBD.[7,8] Histamine also activates neutrophils as a chemoattractant. This study showed that histamine levels and MPO activities were increased in rats with TNBS-induced colitis.

Intestinal bacteria are thought to be involved in the initiation and perpetuation of IBD.[2] Probiotic bacteria and prebiotics (non-digestable dietary carbohydrate) have beneficial effects on the intestinal flora. GBF is a prebiotic product made from malt which contains glutamine-rich protein and hemicellulose-rich fibre. GBF significantly attenuated mucosal damage and reduced levels of interferon-γ and interleukin-6 in the colonic mucosa in mice.[13] Glutamine significantly decreased indices of inflammation when administered before induction of colitis by TNBS in rats.[14]

Other indicators of inflammation such as nitrates/nitrites can also be evaluated. Nitric oxide (NO) production is a sign of granulocyte and mast cell activation in coeliac disease.[15] The relative NO production was lower after corn gluten challenge than after wheat gluten challenge in patients with coeliac disease. Corn gluten is a non-toxic ingredient that is included in the diets of patients with coeliac disease. In this study, administration of EHCG significantly attenuated the increase in mast cell degranulation in rats with TNBS-induced colitis. We now plan to examine the effects of EHCG on mucosal histology.

Conclusions

These results indicate that EHCG attenuates inflammatory histamine levels and neutrophil migration, and may therefore have therapeutic efficacy as a supplement in enteral nutrition for patients with IBD.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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