NOTE

Effects of Dietary Plant-origin Glucosylceramide on Colon Cytokine Contents in DMH-treated Mice

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Abstract: In this study, the effects of dietary plant-origin glucosylceramide (GlcCer) on colon cytokine contents were investigated in 1,2-dimethylhydrazine (DMH)-treated mice, a model of colon cancer. DMH treatment induced the formation of aberrant crypt foci (ACF) and the production of inflammatory cytokines and chemokines. Dietary GlcCer suppressed ACF formation and cytokine production in these mice. In particular, chemokine production was suppressed by dietary GlcCer. These GlcCer-related trends of suppression were similar to those observed in our previous study on dextran sulfate sodium salt (DSS)-treated mice. These results provide further evidence for the suppression of DMH-induced inflammation by dietary GlcCer.

Key words: sphingolipid, DMH, plant lipid, cytokine

1 INTRODUCTION

Plant sphingolipids, especially glucosylceramide (GlcCer), consist of various sphingoid bases such as trans-8-sphingenine, cis-8-sphingenine, trans-4, trans-8-sphingadienine, trans-4, cis-8-sphingadienine, 4-hydroxy-trans-8-sphingenine, and 4-hydroxy-cis-8-sphingenine^{1, 2)}. The functionality of sphingolipids has attracted a great deal of attention in food science, and several groups have reported research about colon cancer prevention by sphingolipids³⁾.

In our previous study, we investigated the effects of dietary GlcCer on aberrant crypt foci (ACF) formation in the large intestines of mice treated with 1,2-dimethylhydrazine (DMH)⁴⁾. We found that DMH administration caused injury to the crypts; however, concurrent administration of dietary GlcCer was associated with maintenance of the colon in the normal state. Therefore, we speculated that GlcCer suppresses the onset of ACF by inhibiting inflammation.

Moreover, there have been a number of studies on the association between inflammation and cellular sphingolipids. Some reported the induction of colitis by dietary sphingomyelin⁵⁾, while others reported the suppression of colitis by dietary sphingomyelin⁶⁾. In a previous study, we investigated the effects of dietary GlcCer on bowel inflam-

mation in dextran sulfate sodium salt (DSS)-treated mice, a commonly used animal model of inflammatory bowel disease (IBD). Dietary GlcCer suppressed DSS-induced body weight loss. Moreover, dietary GlcCer significantly suppressed the production of cytokines by the intestinal mucosa⁷⁾.

We therefore postulated that the suppression of DMH-induced ACF formation by dietary GlcCer was dependent on the anti-inflammatory effect of dietary GlcCer. In the present study, we report the effects of dietary plant GlcCer on cytokine production in DMH-treated mice.

2 EXPERIMENTAL PROCEDURES

2.1 Preparation of maize GlcCer

The commercial GlcCer-rich preparation from Nippon Flour Mills (Atsugi, Japan) was used for the separation of maize GlcCer. To isolate GlcCer, the preparation was dissolved in chloroform and applied to a silica gel column followed by preparative thin-layer chromatography, as described previously⁸⁾. The purity of GlcCer (>80%) was determined by using high-performance liquid chromatography, as described previously⁹⁾.

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2.2 Animals and diet

Male BALB/c mice were obtained from CLEA Japan Inc. (Tokyo, Japan) at 4 weeks of age and housed in isolator cages at 22°C under a 12-h light/dark cycle. The mice were randomly divided into three groups ($n=8$ each); Blank group: control diet with i.p. saline, Control group: control diet with i.p. DMH, GlcCer group: plus maize GlcCer diet with i.p. DMH. Moreover, these groups were divided into 2 systems ($n=4$) to confirm ACF formation and cytokine levels of intestine. After acclimation to the experimental diet for 10 days, each mouse was administered i.p. 1,2-DMH-HCl once a week for 7 weeks. The experimental diets used were based on AIN-76 (i.e. control diet), not containing sphingolipids^{3, 10}, and supplemented with GlcCer (1 g/kg diet, 0.1%)⁷. All protocols involving animals were approved by the Animal Care and Use Committee and conducted in accordance with the Obihiro University Guidelines.

2.3 Identification of aberrant crypt foci

ACF on large intestinal crypts were identified and quantified as described previously⁴. The large intestine was excised from the mice under pentobarbitone anesthesia, and the portion from the cecum to the vent was separated and rinsed in cold saline. This was followed by cell fixation overnight in 4% paraformaldehyde in phosphate-buffered saline (PBS) followed by staining with 0.3% methylene blue solution in saline for 30 min at room temperature. ACF were counted throughout the large intestine under a microscope.

2.4 Quantitative analysis of cytokine levels in the large intestine mucosa

We used a cytokine array to determine the effects of dietary GlcCer on inflammation-related colonic cytokine production in DMH-treated mice. This system can simultaneously detect 40 cytokines. Briefly, the large intestine mucosa was removed using a glass slide, homogenized with 1% Triton X-100 in PBS (pH 7.4), and centrifuged at $10,000 \times g$ for 1 min. The supernatant was applied to a Cytokine Array kit (R&D Systems, Inc., Minneapolis, MN, USA). The following cytokines were detectable: B lymphocyte chemoattractant (BLC), chemokine (C-C motif) ligand 1 (I-309), complement component 5a (C5/C5a), eotaxin, granulocyte macrophage colony-stimulating factor (GM-CSF), interferon- γ (IFN- γ), interferon- γ -induced protein 10 (IP-10), interleukin-1 α (IL-1 α), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-16, IL-17, IL-23, IL-27, metalloproteinase inhibitor 1 (TIMP-1), monocyte chemoattractant protein 2 (MCP-2), macrophage inflammatory protein-1 β (MIP-1 β), macrophage inflammatory protein-1 α (MIP-1 α), monokine induced by gamma interferon (MIG), monocyte chemotactic protein 5 (MCP-5), monocyte-specific cytokine MCP-1 (JE), macrophage colony-stimulating factor (M-

CSF), neutrophil-activating protein 3 (KC), interferon-inducible T cell alpha chemoattractant (I-TAC), regulated on activation, normal T cell expressed and secreted (RANTES), soluble intercellular cell adhesion molecule-1 (sICAM-1), stromal cell-derived factor 1 (SDF-1), thymus and activation regulated chemokine (TARC), triggering receptor expressed on myeloid cells 1 (TREM-1), and tumor necrosis factor- α (TNF- α).

2.5 Statistical analysis

The results were analyzed by using analysis of variance and Scheffe's test. In all analyses, P values < 0.05 were considered statistically significant.

3 RESULTS and DISCUSSION

The results showed that body weight gain and liver weight for the GlcCer group were essentially the same as those of the Control group throughout the period of experimental feeding. The microscopic images of ACF are shown in Fig. 1. ACF stained darker than the normal intestinal tissue and had a three-dimensional structure. At 7 weeks of DMH treatment, ACF were formed in the large intestines of the mice in the Control group. In contrast, ACF were not formed in mice of the GlcCer group. DMH administration caused injury to the crypts, but treatment with dietary GlcCer maintained the colon in the normal state. The results obtained were essentially the same as those of our previous studies⁴.

Fig. 1 Aberrant crypt foci formation induced by DMH in large intestinal crypts.

(A) Large intestine from an untreated BALB/c mouse (Blank group). (B) Large intestine of DMH-treated mouse (Control group). (C) The large intestine of DMH-treated mouse fed with GlcCer (GlcCer group). A, B, and C were stained by methylene blue ($\times 100$).

A cytokine array was used to determine the effects of dietary GlcCer on inflammation-related cytokine production in DMH-treated mice for 7 weeks. This array can simultaneously detect 40 cytokines. DMH treatment induced cytokine production compared to the Blank group (Fig. 2). The densities of positive and negative control spots in this kit were almost equal, indicating that this method is suitable for the analysis of cytokine content. Inflammatory cytokine levels increased with DMH administration, but no marked increases were observed. The levels of anti-inflammatory cytokines and other cytokines did not change significantly. However, some chemokines were markedly induced upon DMH treatment. Dietary GlcCer suppressed

the production of most inflammatory cytokines and chemokines induced by DMH administration. Specifically, IP-10, MIG, and RANTES were strongly suppressed by dietary GlcCer.

In our previous study in a model of DSS-induced colitis, dietary GlcCer suppressed chemokine production, especially IP-10 and MIG, and chorionic crypt injury⁷. DSS, a mucopolysaccharide, induced damage to the colon epidermal cells after oral administration. Damage to the barrier system of the mucosa allowed DSS to reach the lower layer of the mucosa where it was phagocytosed by antigen-presenting cells¹¹. DSS administration as well as DMH induced inflammatory cytokines. In particular, chemokines were strongly induced. Treatment with dietary GlcCer reduced the levels of inflammatory cytokines and chemokines, with particularly marked reductions in chemokine levels. Patients with IBDs, such as ulcerative colitis and Crohn's disease, are at an increased risk of developing colon cancer, confirming that chronic inflammation leads to a predisposition to tumor development¹². Moreover, chemokines are upregulated in both IBD and colon cancer¹³. These results suggested that chemokine modulation by dietary GlcCer may suppress the progression of both IBD and cancer.

In this study, DMH administration induced chemokine expression, particularly IP-10¹⁴, MIG¹⁵, and RANTES¹⁵. These chemokines are involved in the migration of lymphocytes under inflammatory conditions¹⁶. The administration of GlcCer markedly reduced the levels of these cytokines. DMH is a potent carcinogen and has been reported to induce colon cancer in experimental animals. Humans are exposed to DMH and other hydrazines through both the environment and diet¹⁷. DMH is metabolized in the liver resulting in the production of electrophilic diazonium ions, which are known to induce oxidative stress^{18, 19}. Dietary GlcCer was shown to suppress the production of inflammatory cytokines and chemokines in a previous study that used a different mechanism to induce colon crypt injury⁷, and the pattern of suppression was similar to that observed in the present study. The mechanism underlying the suppressive effect of dietary GlcCer on inflammatory cytokine and chemokine levels induced by these agents remains unknown. However, dietary GlcCer probably exerts its anti-inflammatory/protective effects by inhibiting the intestinal absorption of various drugs because the effects on the intestinal cytokine amounts induced by various chemical agents are similar.

This results of this study showed that dietary GlcCer could inhibit the production of DMH-induced cytokines, especially the production of chemokines. The effects on intestinal inflammatory cytokines and chemokines were similar to those reported previously in a DSS inflammation model. Thus, dietary GlcCer may protect the colon surface from the harmful effects of various drugs. Further studies in model cell systems are required to determine the molec-

Fig. 2 Effects of dietary GlcCer on cytokine levels in colon mucosa of DMH-treated mice (fold change). ■: Control group; □: GlcCer group. (A) Inflammatory cytokine. (B) Chemokine. (C) Anti-inflammatory cytokine and others. a and b: Values with different superscript letters in the same column differ significantly ($p < 0.05$). Error bars indicate standard deviation.

ular mechanism underlying these effects.

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