Mini Review

Chemical Properties and Nutritional Value of Plant-Origin Glucosylceramide

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Summary Glucosylceramide (GlcCer), a representative sphingolipid in cell membranes of plants and fungi, is known to have certain benefits, such as prevention of intestinal impairment and improved skin moisturizing, when consumed. Recently, incidence rates of intestinal impairments have increased in East Asian countries due to changes of people’s diet and lifestyle. Therefore, the occurrence of these impairments needs to be prevented through dietary improvement and supplements containing GlcCer. The in vitro and in vivo effects of GlcCer on colon impairment were explored in our previous studies, with focus on sphingolipid structure. Conversely, plant cell membrane contents such as GlcCer are known to be difficult to extract due to the thick cell wall. Therefore, human and other mammals may not be able to utilize GlcCer when digesting food of plant origin. To confirm this hypothesis, we investigated the effects of polished rice and the extract on intestinal impairment. In addition, we discuss the intestinal function of GlcCer contained in polished rice and the relationship between GlcCer and other lipophilic functional components.

Key Words glucosylceramide, colon cancer, colon inflammation, polished rice, extract

Recently, incidence rates of intestinal impairments, such as colon cancer and inflammatory bowel disease (IBD), have increased in East Asian countries, including Japan, while incidence rates in Western countries remain high (1, 2). It is difficult to recover fully from IBD, and patients have an increased risk of developing colon cancer (3). Our previous research suggested that the production of inflammation-related cytokines has a connection with the formation of aberrant crypt foci (ACF), precursors of colon cancer (4). Epidemiological studies indicate that colon cancer is strongly associated with diet; thus, these inflammation-related diseases may be prevented through dietary changes and nutritional supplements (5).

Glucosylceramide (GlcCer), a representative sphingolipid in plants and fungi, consists of a sphingoid base with an amide-linked fatty acid (i.e., ceramide) attached to a glucose molecule. GlcCer is known to have positive effects on intestinal impairment, atopic dermatitis, and skin moisture (4, 6–15). Based on our previous studies that focused on sphingolipid structure, in this review we improve our understanding of sphingolipids and the in vitro and in vivo effects of GlcCer on colon impairments.

Differences between Plant and Animal Sphingolipid Structures

It is well known that there are diverse compositions of the sphingolipid classes and sphingoid bases in nature. Mammalian sphingolipid classes consist of sphingomyelin, galactocylceramide (GalCer), ceramide, and ganglioside. On the other hand, plant sphingolipid classes are mainly composed of GlcCer, ceramide, and glycosyl inositol phosphoceramide (Fig. 1). On mammalian sphingolipid bases, trans-4-sphinganine (sphin-gosine, d18:1 trans 4) is the most prevalent, and sphinganine (d18:0) and 4-hydroxyphosphogamine (t18:0) also occur frequently in small amounts. Plant sphingolipids show diverse sphingoid base structures such as trans-8-sphinganine (d18:1 cis), cis-8-sphinganine (d18:1 cis), trans-4, trans-8-sphingadienine (d18:1 cis), trans-4, cis-8-sphingadienine (d18:1 cis), 4-hydroxy-trans-8-sphing-genine (t18:1 cis), and 4-hydroxy-cis-8-sphingogamine (t18:1 cis). 9-methyl-trans-4, trans-8-sphingadienine (9-Me d18:2 cis) is a unique base found in fungi.

Digestion and Absorption of Plant Sphingolipids

Dietary complex sphingolipids with polar heads (GlcCer and sphingomyelin) are hydrolyzed to sphingoid bases through ceramides by intestinal enzymes and enteric bacteria, and then taken up by mucosal cells. A large portion of the sphingoid bases absorbed is metabolized to fatty acids, while a small number is regenerated into ceramide and complex sphingolipids. In our previous studies, the digestibility of plant and mammalian GlcCer is similar (16, 17). However, absorption rates of other sphingoid bases, when compared to that of sphingosine composed mainly of mammalian sphingolipids, are much lower. One hypothesis is that sphingoid bases
except for sphingosine appear to be transported out of cells across the apical membranes of enterocytes by P-glycoprotein after absorption (17).

**Apoptotic Effects of Plant Sphingolipids on Colon Cancer Cells**

Schmelz et al. reported that dietary sphingomyelin, a mammalian sphingolipid, suppresses colon cancer in mice (18, 19), and Selzner et al. reported that ceramide levels in human colon cancer are lower when compared to those in healthy tissue. Furthermore, the administration of ceramide analogues induces apoptosis and prevents tumor growth in human colon cancer (20). Therefore, when complex sphingolipids are ingested, hydrolysates such as ceramides and sphingoid bases, are expected to have anti-cancer effects.

Although bovine brain has been the primary resource of complex sphingolipids, outbreaks of bovine spongiform encephalopathy have made its use difficult. Effects of plant or fungus origin sphingolipids might be weaker than animal-origin due to lower absorption rates, as mentioned above.

Accordingly, we investigated the in vitro effects of various sphingoid bases on colon cancer (6). All sphingoid bases examined induced apoptosis in Caco-2 cells, a human colon cancer cell line, and the intensity of apoptotic induction was essentially the same among all sphingoid bases studied (Fig. 2). In differentiated Caco-2 cells as human normal colon cells, no apoptosis was observed. All sphingoid bases examined induced a decrease in intracellular β-catenin content, suggesting a possible mechanism.

In conclusion, plant- and fungus-derived sphingoid bases induce apoptosis in colon cancer cells to the same degree as mammalian compounds, and the effects were specific to cancer cells.

**Suppression of ACF Formation by Dietary Plant GlcCer**

The in vivo effects of dietary complex sphingolipids were investigated using ACF formation in the colons of mice treated with 1,2-dimethylhydrazine (DMH), as a model for colon cancer (4, 7, 8). Administration of GlcCer from plant or fungus source suppressed ACF formation, and the suppressive effects were essentially equal between the two groups (7). Plant GlcCer was found in the feces and the intestinal mucosa of mice fed plant GlcCer. Based on results from DNA microarray analysis, dietary plant GlcCer is thought to regulate Wnt signaling and MAP-kinase pathways, and prevent the development of ACF in the colon (8). Moreover, by antibody array analysis of inflammation-related cytokines, DMH treatment increased the production of inflammatory cytokines and chemokines in the colon, while dietary plant GlcCer suppressed the production (4).

These results indicate that dietary GlcCer suppresses...
colon cancer regardless of its origin. Both GlcCer and its metabolism show suppressive effects, and the mechanism may be related to suppression of colon inflammation.

**Suppression of Colon Inflammation by Dietary Plant GlcCer**

Because experiments with DMH treated mice suggest that dietary GlcCer suppresses colon inflammation (4), we investigated these effects with mice treated by dextran sulfate sodium (DSS) as a model (9). Dietary plant GlcCer abrogated weight loss and preserved the integrity of the colon epithelium. Dietary plant GlcCer suppressed the colon expression of myeloperoxidase by neutrophils and the production of inflammatory cytokines and chemokines in the colon. These effects were also confirmed using dietary sphingomyelin (unpublished data). These results suggest that dietary complex sphingolipids play a beneficial role in the prevention of IBD.

**Effects of Plant GlcCer on Inflammatory Stresses in Normal Human Colon Cells**

Because dietary plant GlcCer suppressed colon inflammation in multiple models, namely both DMH and DSS (4, 9), we investigated the effects of complex sphingolipids on inflammation stresses in detail by using differentiated Caco-2 cells as normal human colon cells (10). Addition of tumor necrosis factor-alpha (TNF-α) or lipopolysaccharide (LPS) as an inflammatory stress decreased the viability of colon cells through the induction of apoptosis. Addition of wheat GlcCer (mainly d18:2\(^{8c,4t}\)), maize GlcCer (mainly d18:2\(^{4t,8c}\)), or bovine brain GalCer (mainly d18:1\(^{8c,4t}\)) suppressed cell injury due to inflammatory stresses and there was no difference in the suppressive effects among all complex sphingolipids studied. The production of inflammatory cytokines and chemokines induced by LPS was suppressed by plant GlcCer. In addition, exogenous plant GlcCer localized not in the cytoplasm, but on cell surface. These results can be summarized as follows: 1) complex sphingolipids have potent anti-inflammatory effects in spite of the sphingoid base composition and polar head; 2) plant GlcCer suppresses LPS-induced production of cytokines and apoptosis; 3) complex sphingolipids may act on the surface of cells to prevent the interaction between LPS and its receptor.

**Prevention of ACF Formation by GlcCer in Plant Food**

In vitro and in vivo experiments have indicated beneficial effects of plant GlcCer on intestinal impairment (4, 6–10). These experiments were performed using purified GlcCer. According to previous studies, we ingest 26–77 mg/d of plant GlcCer from our diet (21, 22). In contrast, some studies report that an intake of 0.6–1.8 mg/d from supplements, such as extracts from rice or konjac, improves human skin moisture (11, 12). There is much discussion about why supplementation with such a small amount of GlcCer, compared to that obtained from diet shows this benefit. Plant cell mem-
totic effects of T3 on cancer cells. Therefore, extrinsic GlcCer may act together with other components contained in RE to produce additional and/or a synergistic anti-cancer effects in the colon. Further studies in an in vitro intestine model are required to determine the molecular mechanisms underlying these effects.

The present study suggests that the lipophilic fraction containing GlcCer in polished rice has protective effects against intestinal impairment, but it requires extraction since digestion alone is not enough to display its full protective action.

Conclusion

Our in vitro and in vivo studies indicate that dietary purified GlcCer protects the colon epithelium from a variety of stresses (DSS, DMH, LPS, and TNF-α) (4, 6–10). These effects are produced by GlcCer as well as downstream metabolites. Although GlcCer in plant foods is difficult to digest, its effects are significantly improved by extraction, and GlcCer extracted from plant foods is expected to have additional and/or synergistic effects with other lipophilic compositions (13). Previous studies on improving atopic dermatitis and skin moisturizing suggest that GlcCer intake activates enteric canal immunity and ceramide metabolism in the skin, rather than the direct reutilization of dietary sphingolipids (14, 15). Thus, these mechanisms may be indirectly related to the maintenance of intestinal homeostasis by dietary GlcCer (Fig. 4).

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