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Dietary gangliosides increase the content and molecular percentage of ether phospholipids containing 20:4n-6 and 22:6n-3 in weanling rat intestine

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Abstract

This study was conducted to determine whether dietary ganglioside (GG) increases the content of ether phospholipids (EPL) in intestinal mucosa. Weanling Sprague-Dawley rats were fed a semipurified diet consisting of 20% fat as a control diet. Two experimental diets were formulated by adding either 0.1% (w/w fat) GGs (GG diet) or 1.0% (w/w fat) sphingomyelin (SM diet) to the control diet. Fatty acid methyl esters from the alkenylacyl, alkylacyl and diacyl subclasses of phospholipids were measured to determine total and molecular percentage of EPL comprising the choline phosphoglyceride (CPG) and ethanolamine phosphoglyceride (EPG) fraction. Animals fed the GG diet significantly increased total EPL content both in CPG (by 36%) and in EPG (by 66%), and the molecular percentage of EPL in CPG (by 76%) and in EPG (by 59%) compared to animals fed the control diet. Dietary GG-induced increase in EPL resulted in a higher level of polyunsaturated fatty acids (PUFA) specifically in 20:4n-6 and 22:6n-3 compared to control animals, leading to a decrease in the ratio of saturated fatty acids (SFA) to PUFA both in CPG and in EPG. Feeding animals the SM diet showed a higher level of EPL than control animals with a concomitant increase in 22:6n-3 in EPL. The present data demonstrate that dietary GG increases the content and composition of EPL containing PUFA in the weanling rat intestine.

Keywords: Ganglioside; Ether phospholipid; Polyunsaturated fatty acid; Intestine; Diet

1. Introduction

Gangliosides (GG), sialic acid-containing glycosphingolipids, act as a receptor for *E. coli* and *Cholera* toxins [1] and a stimulator of the immune system in the intestine [2]. Rat intestine has 20–30% of the total lipids as glycosphingolipids, one third of which are GGs [3]. Change occurs in the composition and molecular structure of GGs during intestinal development [4]. Monosialoganglioside GM3 (GM3) is the major GG in rat intestine [5] and is localized at the brush border membrane while disialoganglioside GD3 (GD3) is present at the basolateral membrane [6]. These findings imply that intestinal function may be influenced by the presence and composition of constituent sphingolipids (SPL).

Exogenous GG and sphingomyelin (SM) are hydrolyzed by enterocyte membrane-bound enzymes such as sialidase, sphingomyelinase and/or ceramidase [7–10]. When rats were fed radiolabelled sphingosine, ceramide or SM, about 10–55% of radioactivity was found in the rat intestine, and

Abbreviations: CPG, choline phosphoglycerides; EPG, ethanolamine phosphoglycerides; EPL, ether phospholipids; GG, gangliosides; GLC, gas liquid chromatography; GD3, disialoganglioside GD3; GM1, monosialoganglioside GM1; GM3, monosialoganglioside GM3; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelin; SPL, sphingolipids; TLC, thin layer chromatography.

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30–60% was found in lymph lipids after 24 h [9]. Metabolites transported into enterocytes are reutilized in the synthesis of GGs or SM or both [7,8]. These studies indicate that dietary SPL are digested, metabolised and transported into other tissues.

Several studies suggest a possible interaction between SPL and phospholipids [7–9,11]. Sphingosine-1-phosphate, a metabolic derivative of SPL, is metabolized into phosphoethanolamine and hexadecanal, both prerequisite materials for phospholipid synthesis [7,8]. In animal studies, dietary [3-^{3}H] sphingosine–SM is absorbed, of which 70% is fatty acid and glyceride [9]. An appreciable amount of sphingosine is incorporated into hepatocyte phospholipids in the ether phospholipid (EPL) form when radiolabelled [^{3}H] sphingosine-GM1[2] was intraperitoneally injected into mice [11].

Ether phospholipid has an ester linkage at the sn-2 position, and an ether linkage, either to an alkyl or alkenyl group, at the sn-1 position [14]. Ether phospholipid tends to be enriched in mammalian intestinal cells [15]. One type of EPL known as plasmalogen, 1-*O*-alkenyl-2-acyl-glycero-phospholipids, accounts for about 6–12% of ethanolamine phosphoglyceride (EPG) in rat intestinal mucosa [15,16]. A high content of EPL may contribute to maintenance of cell integrity and function [17–23] such as permeability [18], fluidity [24] and endogenous antioxidant for membrane peroxidation [25,26]. Ether phospholipid also induces cell apoptosis [17], cytotoxicity [19,20] and antitumor activity [21–23], which could have potential in anticancer applications. These cellular functions of EPL seem to be dependent on membrane cholesterol content [27]. For example, cholesterol reduction in the membrane causes increased EPL uptake into the membrane [27] and increases activity of Δ-5 and Δ-6 desaturase enzymes [28].

Our previous work demonstrates that dietary GG increases the total content of GGs and decreases cholesterol content in developing rat intestine [6]. Thus it was logical to hypothesize that dietary GG will increase the content of polyunsaturated fatty acids (PUFA) in EPL in the intestine by increasing derivatives of SPL and by decreasing cholesterol content.

Suckling babies ingest about ~35–170 mg SPL from mothers' milk per day [29]. No clear information is available to explain the metabolic fate of dietary SPL to EPL in the developing intestine. Thus, the objective for this study was to determine whether dietary GG increases total membrane EPL content. This study also examined whether increased EPL content was accompanied with higher PUFA in EPL. Sphingomyelin and GG have a ceramide molecule anchored in the cell membrane, but attached to a different head group. Thus, SM was used as a second control to compare bioavailability with GGs. Using rats, the present study demonstrates that dietary GG increases total content and composition of EPL containing PUFA in the developing intestine.

[2] Nomenclature recommended by Svennerholm [12] and IUPAC-IUB [13].

2. Materials and methods

2.1. Animals and diets

The protocol for this study was approved by the Animal Care Committee at the University of Alberta, Canada. Male Sprague-Dawley rats (18-day-old, 40±4.5 g) were housed in polypropylene cages and maintained at a constant room temperature of 23°C and a 12-h light/dark cycle for 2 weeks. Animals had free access to water and were randomized to be fed one of three semipurified diets containing 20% (w/w) fat (Table 1). The control diet fat was a blend of triglyceride including corn oil, canola oil, coconut oil and olive oil, which reflected the fat composition of an existing infant formula. The composition of basal diet is reported elsewhere [30]. Two experimental diets were formulated by adding either SM (SM diet; 1.0% w/w fat; Sigma, MO) or GG (GG diet; 0.1% w/w fat; Fontera, Cambridge, New Zealand) to the control diet. The amount of SM and GGs added to diets was similar to that of human milk [31,32]. The GG fraction in the GG-enriched diet contained about 80% (w/w) GD3. Ganglioside GD1b, GM3 and other GGs were 9%, 5% and

Table 1
Composition of experimental diets

	Control	SM	GG
Basal diet[a] (g/100 g)	80.0	80.0	80.0
Casein	27.0	27.0	27.0
Starch	20.0	20.0	20.0
Glucose	20.765	20.765	20.765
Nonnutritive cellulose	5.0	5.0	5.0
Vitamin mix[b]	1.0	1.0	1.0
Mineral mix[c]	5.085	5.085	5.085
Choline	0.275	0.275	0.275
Inositol	0.625	0.625	0.625
L-Methionine	0.25	0.25	0.25
Oils	20.0	20.0	20.0
Triglyceride[d]	20.0 (100)[e]	19.8 (99.0)	19.9 (98.6)
Sphingomyelin	–	0.2 (1.0)	tr
Ganglioside	–	–	0.02 (0.1)
Phospholipid	–	–	0.05 (0.25)
Cholesterol	–	–	tr (0.002)

tr represents trace amount.

[a] The composition of the basal diet was described by Clandinin and Yamashiro [30].

[b] AOAC vitamin mix (Teklad Test Diets, Madison, WI): 20,000 IU vitamin A; 2000 IU vitamin D; 100 mg vitamin E; 5 mg menadione; 5 mg thiamine-HCl; 8 mg riboflavin; 40 mg pyridoxine-HCl; 40 mg niacin; 40 mg pantothenic acid; 0.4 mg biotin; 2 mg folic acid; 30 mg vitamin B_{12} per kilogram of complete diet.

[c] Bernhart-Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH): 77.5 mg Mn^{2+}; 0.06 mg Se^{2+} per kilogram of complete diet.

[d] The fatty acid composition of the triglyceride fed was similar to that of an infant formula (Table 2).

[e] Values in parenthesis represent the percentage of total fat.