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Dietary sericin enhances epidermal levels of glucosylceramides and ceramides with up-regulating protein expressions of glucosylceramide synthase, ß-glucocerebrosidase and acidic sphingomyelinase in NC/Nga mice

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ABSTRACT

We have previously reported that dietary sericin improves epidermal dryness with the increased total Ceramide (Cer) in NC/Nga mice, an animal model of atopic dermatitis (AD). In this study, we hypothesized that the increased level of total Cer induced by dietary sericin would be related to the altered metabolism of glucosylceramide (GlcCer) and sphingomyelin (SM), major precursors of Cer generation. NC/Nga mice were fed a control diet (group CA: atopic control) or diets with 1% silk protein, either sericin (group S) or fibroin (group F) for 10 weeks. In the epidermis of group CA, total Cer (including Cer1, 2, 3/4 and 6) and all GlcCer species were reduced; these levels in group S were increased to levels similar to or higher than in the normal control group of BALB/c mice (group C). In addition, the protein expressions, but not mRNA expressions, of GlcCer synthase, β-glucocerebrosidase, and acidic sphingomyelinase, enzymes for GlcCer synthesis, GlcCer and SM hydrolysis, respectively, were highly increased in group S. The epidermal levels of total Cer (including Cer2, 3/4, and 6) and all GlcCer species and of these enzyme proteins in group F were lower than in group S. Notably, alterations in total SM, SM1, SM3, and SM synthase 1, which were increased in group CA, were not significant between groups S and F. Cer5 and SM2 were not altered among groups. Dietary sericin enhanced the epidermal levels of all GlcCer and most Cer species with up-regulating protein expressions of GlcCer synthase, β-glucocerebrosidase, and acidic sphingomyelinase.

1. Introduction

Ceramide (Cer), combined with cholesterol and free fatty acids, forms the extracellular lamellar membrane structure of the epidermal barrier. Cer bears the structural moieties of amide-linked non-hydroxy acids, α-hydroxy acids, ω-hydroxy acid and ester-linked fatty acids on sphingoid bases, which are thought to play a role in maintaining the lamellar integrity

Abbreviations: AD, atopic dermatitis; aSMase, acidic sphingomyelinase; β-GlcCer'ase, β- glucocerebrosidase; Cer, ceramide; CDase, ceramidase; GlcCer, glucosylceramide; GlcCer synthase, glucosylceramide synthase; SM, sphingomyelin; SMase, sphingomyelinase; SM synthase, sphingomyelin synthase; SPT, serine palmitoyl transferase.

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of the epidermal barrier. Furthermore, the distinct compositions of these structural moieties are composed of 9 heterogeneous Cer species (Cer1-9) in the epidermis [1].

During the differentiation processes of the epidermis, Cer is synthesized de novo with the enzymatic condensation of serine and palmitoyl-Co A by serine palmitoyl transferase (SPT) [1]. The newly synthesized Cer is promptly modified at the 1-hydroxy position to either glucosylceramide (GlcCer) by GlcCer synthase or to sphingomyelin (SM) by SM synthase. Although various species of Cer are generated from these 2 precursors during the final stages of epidermal differentiation [1-3], all 9 Cer species (Cer1-9) are generated mainly from GlcCer hydrolysis by β-glucocerebrosidase (β-GlcCer'ase) [1,2]. Cer2 or Cer5 are generated in part from SM hydrolysis by sphingomyelinase (SMase) [1,3]. Cer ultimately undergoes degradation by ceramidase (CDase) into sphingosine and fatty acids [1]. Depletion of Cer has been reported in skin condition that involve barrier defects, such as AD [4-6]. Moreover, the marked decrease of GlcCer and SM, coupled with alterations of Cer metabolizing enzymes and of the degradative enzyme, CDase, has been frequently reported in the epidermis of AD [4-7].

In our search for a dietary source that enhances the epidermal level of Cer, our attention has been drawn to silk protein. Silk consists of 2 types of proteins, fibroin and sericin. In silk textile processing, sericin, which envelops fibroin with successive sticky layers, is mostly removed, and the fibrous protein fibroin is purified. Although fibroin is reported to be a useful biomaterial for skin health [8,9], our previous studies demonstrated that dietary sericin improves epidermal dryness [10], a major symptom of AD [11], in parallel with increased epidermal levels of total Cer [12] in NC/Nga mice, an animal model of AD [7,13]. However, dietary sericin and fibroin both inhibit mRNA and protein expressions of epidermal SPT, and not of CDase [12], indicating that dietary sericin does not enhance de novo Cer synthesis or inhibit Cer degradation. Based on our previous studies, we hypothesized that the increased level of total Cer induced with dietary sericin would be related to the altered metabolism of GlcCer and SM. In this study, we examined the dietary effect of silk proteins on epidermal levels of individual species of Cer, GlcCer and SM and of GlcCer synthase, SM synthase, β-GlcCer'ase and SMase. NC/Nga mice were fed 1% silk protein, either sericin or fibroin, and the epidermal levels of individual Cer, GlcCer and SM species were determined. mRNA and/or protein expressions of GlcCer synthase, SM synthase, β-GlcCer'ase, and SMase were also determined.

2. Methods and materials

2.1. Animals and diets

Five-week-old male BALB/c mice (n = 10) and 5-week-old male NC/Nga mice (n = 30) were purchased from SLC Japan (Shizuoka, Japan). After a 1-week adaptation period, the NC/Nga mice were assigned to three groups of 10 mice each: an atopic control group (group CA) with a control diet, and groups S and F with diets supplemented with 1.0% powdered extracts of sericin (S) or fibroin (F), respectively. The mice were fed the experimental diets for 10 weeks. A normal control group of BALB/c mice was fed a control diet for 10 weeks (group C). The ingredient composition of the experimental diets is shown in Table 1. The diets of groups C and CA included no silk protein. The diet of group S included 10 g/kg sericin. The diet of group F included 10 g/kg fibroin. The preparation and the molecular weights of the sericin and fibroin powders and the amino acid (AA) compositions of casein, sericin and fibroin have been described previously [10,12].

During the 10-week feeding period, all mice were maintained under conventional laboratory conditions without air filtration to induce AD as described previously [7,13]. The mice were housed under conditions of controlled temperature (22°C-24°C), humidity (55%-60%) and light (lights on from 07:00 to 19:00). Food intakes and body weights of all groups were monitored weekly over the 10-week feeding period. Animal care and handling conformed to the guidelines provided by the Animal Care and Use Review Committee of Kyung Hee University. At the end of week 10, all mice were euthanized by pentobarbital sodium (at a dose of 185 mg/kg IV). Epidermal strips were removed after overnight incubation of whole skin in an ice-cold 1:1 mixture of dispase II (2.4 U/mL,

Table 1 – Ingredient composition of the experimental diets fed to mice (gram per kilogram diet)

Ingredient	Experimental diets [1]			
	C	CA	S	F
Casein	230	230	220	220
Sericin	-	-	10	-
Fibroin	-	-	-	10
L-cystine	3	3	3	3
Corn oil	100	100	100	100
Cellulose	50	50	50	50
Vitamin mix [2]	10	10	10	10
Mineral mix [3]	35	35	35	35
Sucrose	200	200	200	200
Corn starch	372	372	372	372

[1] Group C, BALB/c mice fed a control diet; Groups CA, S and F, NC/Nga mice fed a control diet (group CA) or diets supplemented with either 1% sericin (group S) or fibroin (group F).

[2] Vitamin mix composition, AIN-93 vitamin mix #310025 (Dytes Inc, Bethlehem, PA, USA): niacin 3 g/kg, calcium pantothenate 1.6 g/kg, pyridoxine HCl 0.06 g/kg, thiamine HCl 0.6 g/kg, riboflavin 0.6 g/kg, folic acid 0.2 g/kg, biotin 0.2 g/kg, vitamin E acetate (500 IU/g) 15 g/kg, vitamin B_{12} (0.1 %) 2.5 g/kg, vitamin A palmitate (500000 U/g) 0.8 g/kg, vitamin D_3 (400000 IU/g) 0.25 g/kg, vitamin K1/dextrose mix (10 mg/g) 7.5 g/kg and sucrose 967.23 g/kg.

[3] Salt mix composition : AIN-93G salt mix #210025 (Dytes Inc, Bethlehem, PA, USA): calcium carbonate 357 g/kg, potassium phosphate (monobasic)196 g/kg, potassium citrate H_2O 70.78 g/kg, sodium chloride 74 g/kg, potassium sulfate 46.6 g/kg, magnesium oxide 24 g/kg, ferric citrate U.S.P 6.06 g/kg, zinc carbonate 1.65 g/kg, manganous carbonate 0.63 g/kg, cupric carbonate 0.3 g/kg, potassium iodate 0.01 g/kg, sodium selenate 0.01025 g/kg, ammonium paramolybdate $4H_2O$ 0.00795 g/kg, sodium metasilicate $9H_2O$ 1.45 g/kg, chromium potassium sulfate $12H_2O$ 0.275 g/kg, lithium chloride 0.0714 g/kg, boric acid 0.0815 g/kg, sodium fluoride 0.0635 g/kg, nickel carbonate 0.0318 g/kg, ammonium vanadate 0.066 g/kg, and sucrose finely powdered sucrose 221.026 g/kg.