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Dietary glucosylceramide improves skin barrier function in hairless mice

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KEYWORDS
Glucosylceramide;
Diet;
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TEWL

Summary

Background: Sphingolipids are known to play an important role in both water retention and epidermal permeability barrier function in mammalian stratum corneum. However, little is known about the effects on epidermal function of orally administered sphingolipids.
Objective: We examined the effect of dietary glucosylceramide (GluCer) on the maintenance and recovery of epidermal barrier function.
Methods: Hairless mice were fed a particular diet (HR-AD) for 4 weeks to induce chronic skin perturbation. Subsequently, a normal diet supplemented with GluCer (from rice bran and germ) was provided for the next 4 weeks. Transepidermal water loss (TEWL) and stratum corneum flexibility were measured throughout this recovery phase. Additional hairless mice were fed a diet with or without a maize-extracted GluCer supplement for 5 weeks, then their skin was acutely perturbed with repeated tape-stripping, and the TEWL was measured.
Results: Although skin functions were generally lower following chronic perturbation, in GluCer-fed mice the TEWL was significantly reduced at 2 weeks and the stratum corneum flexibility was increased at 3 weeks compared to controls. Following acute barrier perturbation by tape-stripping, mice an HR-AD fed a GluCer diet exhibited enhanced recovery compared with the control diet group.

Abbreviations: GluCer, glucosylceramide; TEWL, transepidermal water loss

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Conclusion: These results demonstrate that in hairless mice skin barrier functions impaired by chronic or acute perturbations were improved by dietary GluCer. The oral administration of GluCer may be useful for the preservation and recovery of epidermal barrier functions an HR-AD.

1. Introduction

The stratum corneum is the extreme outer layer of the skin and is responsible for protection against external stimuli. This tissue prevents excessive transepidermal water loss (TEWL) from the body to the external environment, which is indispensable for the survival of mammals. The lamellar structure of the stratum corneum is composed of mainly lipids, including cholesterol, fatty acids, and ceramide, a fundamental constituent of sphingolipids that plays a critical role in water-retention and other epidermal permeability barrier functions [1]. In skin diseases such as atopic dermatitis, the lesional skin tends to be overly sensitive and dry, exhibiting defective permeability barrier functions. There is also a markedly altered types of ceramides in the lesional skin of atopic dermatitis patients compared with normal tissue from healthy persons [1—4].

Hairless mice (HR-1) are a known animal model for observing skin abnormalities such as atopic dermatitis. Atopic dermatitis-like symptoms can be induced in these animals by feeding them a particular diet, the HR-AD [5,6]. In addition, acute perturbations in human or hairless mice skin have been induced using a typical application of an organic solvent such as acetone or tape-stripping [7,8], both of which reportedly reduce the TEWL.

GluCer is a major sphingolipid of plant tissue, including those used as food sources [9—11]. Reportedly, the application of the sphingolipid GluCer to dorsal skin of mice significantly reduced the TEWL and barrier disruption induced by ultraviolet radiation [12]. However, it is not clear what effect oral administration of GluCer would have on skin barrier function. In the present report, we examine the efficacy of dietary GluCer in restoring epidermal barrier function using a model of chronic or acute perturbation in hairless mice (HR-1 mice). Our results indicate that in these animals orally administered GluCer improves epidermal function previously impaired by an HR-AD diet or tape-stripping. Thus, our findings suggest the possibility that dietary GluCer could be therapeutic in treating skin disorders.

2. Materials and methods

2.1. Animals

Four-week-old male hairless mice (HR-1) and 8-week-old female hairless mice (Hr/Hr) were purchased from Hoshino Experimental Animal Center (Yashio, Japan) and Charles River Laboratories Japan, Inc. (Yokohama, Japan). Animal procedures were approved by the Ethical Committee at Hokkaido University Graduate School of Medicine and were performed in accordance with their guidelines. The mice were housed in plastic cages in a barrier facility at a temperature of 22 $\pm$ 3 °C, with 50 $\pm$ 20% relative humidity, and a 12:12 h light:dark cycle.

2.2. Reagents

Dietary glucosylceramide (GluCer), extracted from rice germ and bran (Nippn ceramide RPG) or dietary GluCe from maize (Nippn ceramide CP-K6) were purchased from Nippon Flour Mills Co., Ltd. (Tokyo, Japan). HPLC analysis indicated a purity of greater than 6%.

2.3. Diets

The Labo MR stock (the normal rodent diet), and the HR-AD (the special diet, skin-damage is induced) were purchased from Nosan Corp. (Yokohama, Japan) [5]. The experimental treatment diet included the Labo MR stock used in the normal diet supplemented with 1000 ppm GluCer (from rice germ and bran) (Nosan Corp., Yokohama, Japan). The AIN-76A rodent diet were purchased from Research Diets, Inc. (New Brunswick, NJ, USA), which contains carbohydrate (66.0%), protein (20.3%), fat (5.0%), and very little sphingolipids (<0.005%), supplemented with 1000 ppm GluCer (from maize).

2.4. TEWL and stratum corneum flexibility assessments

All measurements were carried out at 22 $\pm$ 3 °C and a humidity level of 50 $\pm$ 20%, and were performed in triplicate for each skin spot. TEWL was measured using an Electrolytic water analyzer (Meeco,

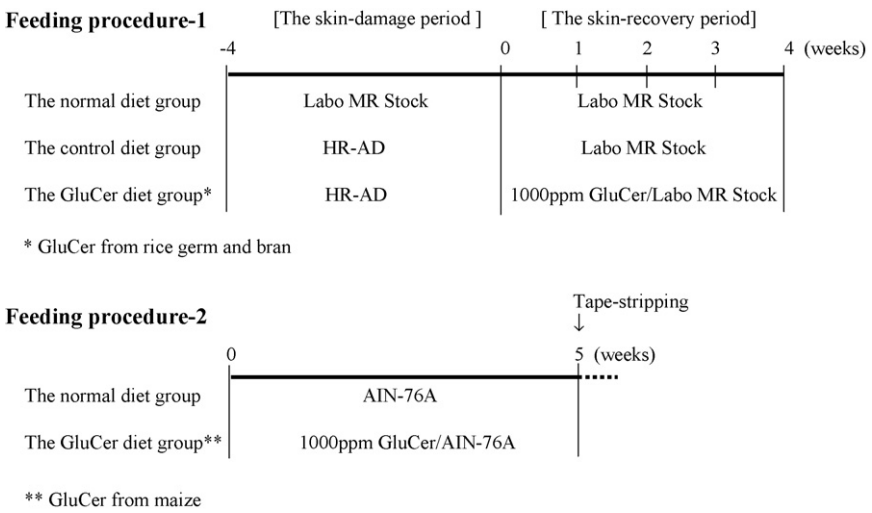

Fig. 1 Perturbation process. Feeding procedure-1: three groups (the normal diet group, the control diet group and the GluCe diet group) of hairless mice were fed a normal diet (Labo MR stock) or a special diet (HR-AD), which is known to induce skin damage, during the skin-damage period of 4 weeks. Subsequently, in the 4-week recovery period mice were fed Labo MR stock with or without GluCer (from rice). Feeding procedure-2: two groups (the normal diet group, the GluCe diet group) of hairless mice were fed a normal diet (AIN-76A) with or without GluCer (from maize) during 5 weeks. At 5 weeks, a patch of stratum corneum was removed from the dorsal skin of each mouse by stripping with adhesive tape repeatedly for five times.

Warrington, PA, USA), or an Evaporimeter AS-TW1 (Asahi Biomed, Yokohama, Japan), in accordance with the ventilated chamber method [13]. Stratum corneum flexibility was calculated by dividing the hydration measurement by the thickness of the stratum corneum, these hydration and thickness were measured by using a Corneometer ASA-M2 (Asahi Biomed, Yokohama, Japan) [13].

2.5. Chronic barrier perturbation studies

Four-week-old male hairless mice (HR-1) were randomly allocated to three groups: the normal diet group, the control diet group, and the GluCer diet group (Fig. 1, Feeding procedure-1). During the skin-damage period of 4 weeks mice were fed HR-AD or Labo MR stock diet. Subsequently, in the 4-week recovery period mice were fed Labo MR stock with or without GluCer (from rice). Food and water were provided ad libitum. TEWL or stratum corneum flexibility were measured using an Evaporimeter AS-TW1 or a Corneometer ASA-M2 (Asahi Biomed, Yokohama, Japan).

2.6. Acute barrier perturbation studies

Eight-week-old female hairless mice (Hr/Hr) were fed the AIN-76A rodent diet with or without 1000 ppm GluCer (from maize). After 5 weeks of feeding (Fig. 1, Feeding procedure-2), a patch of stratum corneum was removed from the dorsal skin of each mouse by stripping with adhesive tape (PPS Nichiban: 2.5 cm $\times$ 3.0 cm) repeatedly for five times. Before tape-stripping, TEWL was measured by using an Electrolytic water analyzer (Meeco, Warrington, PA, USA). Subsequently, immediately and 2 h after tape-stripping, TEWL was measured on the surface of tape-stripped and non-perturbed skin (as basal TEWL).

2.7. Statistics

Results were expressed as the mean $\pm$ S.D. for each group. Statistical analysis were performed using a unpaired Student's *t*-test. Statistical significance was defined as $p < 0.05$, 0.01, and 0.001.

3. Results

3.1. Effect of dietary GluCer on TEWL in hairless mice with chronic barrier perturbation

A feeding study was performed to determine the influence of GluCer (from rice) on epidermal barrier function in vivo. To induce a model of chronic barrier perturbation in skin, hairless mice were fed an HR-AD for 4 weeks (Fig. 1, Feeding procedure-1, skin-damage period); normal controls were fed a normal diet (Labo MR stock). After the skin-damage period, the mice were fed an experimental diet containing 1000 ppm GluCer (from rice) or a normal diet for 4 weeks (Fig. 1,

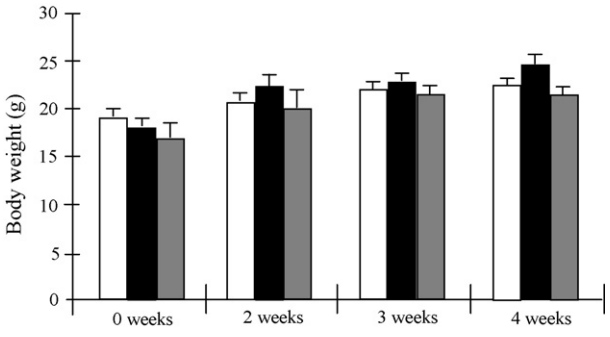

Fig. 2 Body weights of mice in the skin-recovery period. Hairless mice were fed a normal diet (Labo MR stock) or a special diet (HR-AD) for 4 weeks. Subsequently, mice were fed Labo MR stock with GluCer (the GluCer diet group) or without (the normal diet group and the control diet group), as illustrated in Fig. 1 (Feeding procedure-1). At weeks 0, 2, 3, and 4 of the skin-recovery period, the body weight of each mouse in the normal (open column), the control (black column), and HR-AD GluCer (grey column) diet groups was measured. Each column represents the mean ± S.D. of four or five animals.

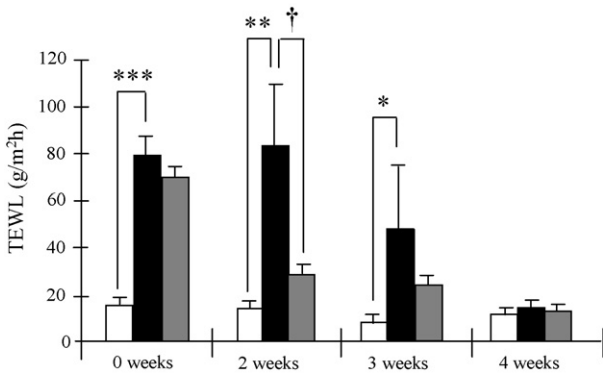

Fig. 3 Effect of dietary GluCer on transepidermal water loss (TEWL) in hairless mice with chronic barrier perturbation. Hairless mice were fed a normal diet (Labo MR stock) or a special diet (HR-AD) for 4 weeks. Subsequently, mice were fed a diet of Labo MR stock with GluCer (the GluCer diet group) or without (the normal diet group and the control diet group), as illustrated in Fig. 1 (Feeding procedure-1). At 0, 2, 3, and 4 weeks of the skin-recovery period, the TEWL was measured in each mouse of the normal (open column), the control (black column), the GluCer (grey column) diet groups using an Evaporimeter AS-TW1. Each column represents the mean ± S.D. of four or five animals. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. $^{\dagger}p < 0.05$.

Feeding procedure-1, the skin-recovery period). During the skin-recovery period, there were no significant differences in body weights among the three groups (Fig. 2).

We first determined the TEWL as a marker of epidermal barrier function, measured using an Evaporimeter AS-TW1 (Asahi Biomed, Yokohama, Japan), at 0, 2, 3, and 4 weeks of the skin-recovery period. In the normal diet group, the TEWL was approximately 12 g/(m^2 h) throughout the skin-recovery period (Fig. 3). In mice fed the HR-AD, the TEWL markedly increased to approximately 74.0 g/(m^2 h) by the end of the skin-damage period (0 weeks). Two weeks into the skin-recovery period, the TEWL of the control HR-AD group had not changed, but the TEWL of the GluCer diet group was significantly decreased (Fig. 3). By 3 weeks the TEWL in the GluCer group still had a tendency to be reduced compared to the control diet. These results demonstrate that the oral administration of GluCer can significantly improve the barrier function of skin affected by chronic perturbation.

3.2. Effect of dietary GluCer on stratum corneum flexibility in hairless mice

Next, we examined the effect of the diet on the stratum corneum flexibility, measured using a Corneometer ASA-M2 (Asahi Biomed, Yokohama, Japan). At the end of the skin-damage period (0 weeks) (Fig. 1, Feeding procedure-1), the flexibility was significantly reduced in the groups fed the HR-AD compared with those consuming the normal diet group (Fig. 4). Three weeks into the skin-recovery period, the GluCer diet group showed significantly increased stratum corneum flexibility compared with that of the control diet group, and nearly equal to that of the normal diet group. These results indicate that the oral administration

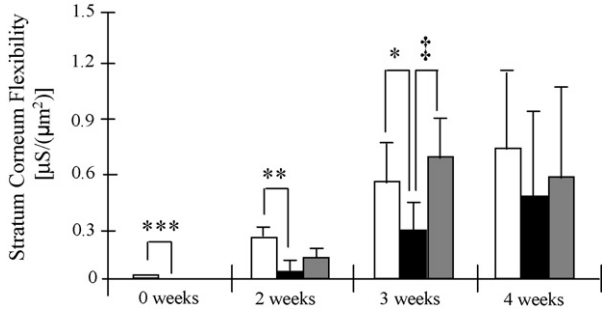

Fig. 4 Effect of dietary GluCer on stratum corneum flexibility in hairless mice with chronic barrier perturbation. Hairless mice were fed a normal diet (Labo MR stock) or a special diet (HR-AD) for 4 weeks. Subsequently, mice were fed Labo MR stock with GluCer (the GluCer diet group) or without (the normal diet group and the control diet group), as illustrated in Fig. 1 (Feeding procedure-1). At 0, 2, 3, and 4 weeks of the skin-recovery period, the stratum corneum flexibility was measured for each mouse in the normal (open column), the control (black column), and the GluCer (grey column) diet groups. Each column represents the mean ± S.D. of four or five animals. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$. $^{\ddagger}p < 0.01$.

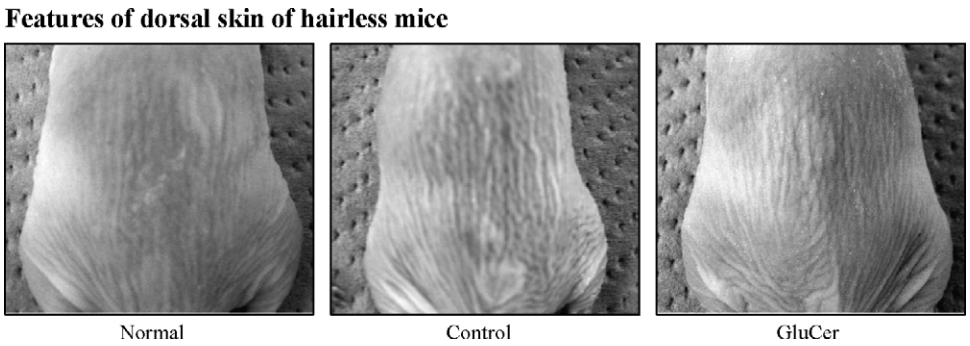

Fig. 5 Effect of dietary GluCer on skin appearance in hairless mice with chronic barrier perturbation. Hairless mice were fed a normal diet (Labo MR stock) or a special diet (HR-AD) for 4 weeks. Subsequently, mice were fed Labo MR stock with GluCer (the GluCer diet group) or without (the normal diet group and the control diet group), as illustrated in Fig. 1 (Feeding procedure-1). At 3 weeks of the skin-recovery period, the dorsal skins in a mouse of the normal diet group (left panel), the control diet group (middle panel), or the GluCer diet group (right panel) were photographed.

of GluCer can significantly elevate stratum corneum flexibility in skin with induced chronic perturbation.

3.3. Effect of dietary GluCer on skin appearance in hairless mice

At 3 weeks of the skin-recovery period (Fig. 1, Feeding procedure-1), the dorsal skin of mice from each of the three diet groups was examined (Fig. 5). The dorsal skin of the GluCer diet group showed reduced skin creases compared to creases in the control diet group, similar to skin of the normal diet group. The appearance of the dorsal skin revealed that the oral administration of GluCer reduced the number of skin creases.

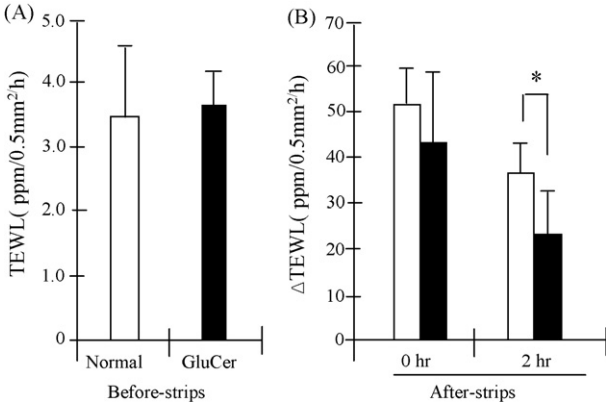

Fig. 6 Effect of dietary GluCer on the protection and recovery from acute barrier perturbation. (A) At the end of oral administration of GluCer (from maize) for 5 weeks, the TEWL were measured using a Meeco electrolytic water analyzer. (B) Following the feeding procedure in (A), the dorsal skin of each mouse was tape-stripped five times to perturb the skin barrier function. Immediately after tape-stripping (0 h) and 2 h later, the TEWL was measured in areas of perturbed and non-perturbed skin and the difference (ΔTEWL) was calculated for each mouse of the normal (open column) and GluCer (solid column) diet groups. Each column represents the mean ± S.D. of eight animals. $^{*}p < 0.05$.

3.4. Effect of dietary GluCer on recovery following acute barrier perturbation

To confirm the effect of orally administered GluCer in epidermal barrier function, we analyzed a second type of skin damage, acute perturbation. Eight-week-old female hairless mice (Hr/Hr) were fed an AIN-76A diet with or without 1000 ppm GluCer from maize (Fig. 1, Feeding procedure-2). After 5 weeks (before tape-stripping), no significant difference was observed in the TEWL of the two diet groups (Fig. 6A). To perturb the epidermal barrier function, patches of dorsal skin of each mouse were tape-stripped five times. Immediately after tape-stripping (0 h) and 2 h later, the TEWL was measured for areas of perturbed and non-perturbed skin, and the difference (ΔTEWL) was calculated for each mouse. Two hours after the damage, the difference in the ΔTEWL was significantly less in the GluCer (from maize) diet group compared with that in the normal group (Fig. 6B). This result indicated that the oral administration of GluCer accelerated recovery from acute barrier perturbation.

4. Discussion

In hairless mice with skin damage, oral administration of GluCer appeared to aid in the improvement of epidermal barrier function that had been reduced by chronic or acute perturbation. Mice fed a special skin-damaging diet (the HR-AD) exhibited increased TEWL and reduced stratum corneum flexibility. The declined skin functions improved

in mice fed a diet supplemented with GluCer. In addition, dietary GluCer aided in resistance against acute physical barrier perturbation, by tape-stripping, and enhanced the subsequent recovery. These findings suggest that dietary GluCer from plant extracts may be effective in the maintenance and improvement of epidermal barrier function.

Mice fed an HR-AD are known to exhibit atopic dermatitis-like skin symptoms, accompanied with a decline in epidermal barrier functions. Although the causative mechanism is not clear, previous reports have suggested that a dietary deficiency of some kind, perhaps in magnesium or zinc, may be involved [6]. Our study indicates that a diet supplemented with GluCer can enhance the recovery of epidermal barrier perturbation induced by an HR-AD. This finding suggests that a deficiency in GluCer might contribute to the induction of atopic dermatitis-like skin symptoms in HR-AD-fed mice. The GluCer from fungi are known to show a characteristic fungal consensus structure [11]. And then, We are investigating the effect of GluCer from tamogitake mushroom (edible fungus) on skin barrier function. In preliminary data, the application of this GluCer improved the skin-damage.

Sphingolipids, which contain ceramide and GluCer, are important constituents of the plasma membrane in mammalian cells. Sphingolipids are known to be fundamental components of the lamellar structure that mediates the epidermal permeability barrier function and skin homeostasis [14—17]. Kono et al. [18] reported that dietary sphingolipids are degraded on the brush border membrane of the small intestine, and are then taken up by enterocytes. In mice engineered with a skin-targeted conditional knockout of serine palmitoyltransferase, the enzyme responsible for the first step of sphingolipid biosynthesis, the sphingolipid composition was reportedly normal [19]. Moreover, we demonstrated that keratinocytes utilize exogenous sphingolipids to construct their own sphingolipid compositions [20]. Hence, dietary sphingolipids may be degraded and incorporated intracellularly, and used in the composition of the lamellar lipids in skin.

Upon such stimuli as exposure to ultraviolet radiation on dorsal skin or the stress of overcrowding, the TEWL of these mice is increased and the metabolism of ceramidein the epidermis is altered [21,22]. In several skin diseases a disturbance in ceramide metabolism supposedly influences skin barrier functions. In patients with atopic dermatitis or psoriasis, decreased ceramide levels and increased TEWL were observed in the lesional stratum corneum [1,23,24]. These observations suggest that a deficiency in ceramide in the epidermis might be involved in the barrier disruption observed in skin diseases such as atopic dermatitis or psoriasis.

In this study utilizing hairless mice, epidermal barrier dysfunction induced by a skin-damaging diet (HR-AD) or by tape-stripping, was shown to improve following the oral administration of GluCer extracted from plants. Therefore, dietary GluCer may be useful in preserving skin function and in recovering from epidermal barrier perturbation.

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