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Oral administration of glucosylceramide ameliorates inflammatory dry-skin condition in chronic oxazolone-induced irritant contact dermatitis in the mouse ear

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ABSTRACT

Background: Irritant contact dermatitis (ICD) is an inflammatory skin disease triggered by exposure to a chemical that is toxic or irritating to the skin. A major characteristic of chronic ICD is an inflammatory dry-skin condition with associated itching. Although glucosylceramide (GlcCer) is known to improve the skin barrier function, its mechanism of action is unknown.
Objectives: Using a mouse model of oxazolone-induced chronic ICD, this study investigated the effects of oral administration of GlcCer on inflammatory dry skin.
Methods: Chronic ICD was induced by repeated application of oxazolone in mice. GlcCer was orally administered once daily throughout the elicitation phase. The beneficial efficacy of GlcCer on cutaneous inflammation was evaluated by assessing ear thickness, lymph node weight, histological findings, and mRNA expression of pro-inflammatory cytokines such as IL-1β and IL-6. Additionally, parameters of the itch-associated response, including scratching behavior, water content of the skin, and aquaporin-3 levels in the lesional ear, were measured.
Results: Oral GlcCer administration significantly suppressed mRNA expression of the pro-inflammatory cytokines IL-1β and IL-6. GlcCer also suppressed ear swelling, lymph node weight gains, and infiltration of leukocytes and mast cells in ICD mice. In oxazolone-induced ICD mice, GlcCer significantly inhibited irritant-related scratching behavior and dehydration of the stratum corneum, and decreased aquaporin-3 expression.
Conclusions: Our results indicate that GlcCer suppressed inflammation not only by inhibiting cytokine production but also by repairing the skin barrier function, suggesting a potential beneficial role for GlcCer in the improvement of chronic ICD.

1. Introduction

Irritant contact dermatitis (ICD), a common dermatitis triggered by exposure to a skin irritant, is characterized by erythema, edema, and dryness with itching [1]. Additionally, ICD exhibits abnormalities in the permeability barrier function as well as in immunologic functions [2,3]. Chronic perturbations of the barrier function decrease elasticity and increase susceptibility to infection and water loss, resulting in dry, scaly, itchy skin and chronic inflammation [4,5]. Histologically, skin lesions of chronic ICD are characterized by marked epidermal hyperproliferation and the presence of inflammatory infiltrates consisting of mast cells and leukocytes, such as neutrophils and monocytes [6,7]. The production of cytokines by infiltrating inflammatory cells as well as epidermal cells is a major mechanism in inflammatory skin reactions [7]. Several proinflammatory cytokines, including IL-1β and IL-6, are overexpressed in the lesional skin in inflammatory dermatitis for cutaneous homeostasis [8].

Abbreviations: GlcCer, glucosylceramide; ICD, irritant contact dermatitis; IL, interleukin; AQP3, aquaporin-3; SC, stratum corneum; AU, arbitrary units.

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Permeability barrier abnormalities are an important component of the pathogenesis of contact dermatitis. Lesional skin of various skin diseases, such as atopic dermatitis or contact dermatitis, shows impaired barrier function [9]. Acute disruption of the barrier function by external stimuli such as mechanical trauma produced by tape stripping or contact with either solvents or detergents elicits a homeostatic repair response that leads to the restoration of normal barrier function [10]. Repeated perturbations of the barrier, however, induce epidermal hyperplasia and cutaneous inflammation [11] and a decrease in stratum corneum (SC) hydration. When SC hydration decreases, epidermal hyperplasia and inflammation signs emerge.

The SC, the outermost layer of the skin, is important for protection against external stimuli and prevention of excessive transcutaneous water loss [4]. The water content of the SC is important for the appearance and physiological function of the skin. Reduced hydration in the SC has been found in many skin diseases, such as atopic dermatitis, eczema, and psoriasis [12,13], and is considered to induce itching and scratching [14]. Itch-induced scratching worsens skin conditions and newly provokes and sustains skin inflammation, which in turn leads to continued intense itching sensations.

A key factor in skin hydration and integrity is the presence of aquaporins (AQP) [15], which act mainly as water-selective pores [16]. Among the AQP families, the aquaglyceroporins (AQP3, 7, 9, and 10) are permeable to water, glycerol, and other small solutes [17]. AQP3 is expressed in keratinocytes in the basal layer of the epidermis [18] and plays a role in allowing glycerol to move into more superficial layers of the epidermis and SC. Mice deficient in AQP3 have dry skin with reduced SC hydration, decreased elasticity, and impaired barrier recovery [19,20], suggesting the importance of AQP3 in skin physiology.

Despite powerful evidence for the importance of barrier function abnormalities, treatments for ICD largely address immunologic abnormalities related to inflammation. Although the use of topical corticosteroid for ICD is widespread, its long-term application, even with intermittent use, induces undesirable side effects. Lipid-based moisturizers are often used for the prevention and treatment of ICD, aimed at retaining moisture and restoring the skin barrier function [21]. The clinical efficacy of moisturizer, however, remains a topic of controversy. Hence, there is a strong need for alternative therapies that are safe and effective and that are aimed at improving the barrier dysfunction in inflammatory skin diseases such as ICD.

Glucosylceramide (GlcCer), the glycosylated form of ceramide, is a major sphingolipid in plants such as soybean, corn, rice, and wheat [22]. GlcCer has recently attracted intense interest because of the beneficial effects of GlcCer in improving the skin barrier function. For example, skin barrier function in hairless mice was improved by dietary GlcCer [23] and topical application to the dorsal skin [24]. Furthermore, a konjac extract containing GlcCer improved transepidermal water loss (TEWL) in healthy subjects [25]. Although a number of studies have demonstrated the efficacy of GlcCer in controlling eczematous skin diseases such as chronic contact dermatitis, the mechanism of GlcCer activity is unknown.

Using a mouse model of oxazolone-induced chronic contact dermatitis, we investigated the efficacy of GlcCer in reducing cutaneous inflammation and restoring epidermal hydration and examined the possible mechanism involved.

2. Materials and methods

2.1. Animals

Male BALB/c mice (6 weeks old, 18–22 g body weight) were purchased from Samtaco Co. (Seoul, Korea). The animals were group housed, six mice per cage, in standard cages at 23 ± 2 °C and 40–60% humidity with a 12-h light/dark cycle (08:00–20:00 h light, 20:00–08:00 h dark) and provided with mouse chow and water ad libitum. All procedures concerning animals were conducted in accordance with the guidelines of NIH and the Institutional Animal Care and Use Committee of Kyung Hee University.

2.2. Oxazolone-induced chronic contact hypersensitivity

The sensitization and challenge protocol was carried out according to the procedure described by Shin [26], with slight modifications (Fig. 1). Briefly, eight mice per group were sensitized on day 0 by a single application of 50 μL of 1.5% oxazolone (Sigma, MO, USA) in ethanol on the abdomen. Seven days later, the mice were challenged with a total of 20 μL of 1% oxazolone in a mixture of acetone and olive oil (4:1) to the inner and outer surfaces of the both ears at 3-day intervals for 2 weeks post-sensitization. Animals in the control group were treated with vehicle alone (acetone and olive oil (4:1)).

2.3. Glucosylceramide administration and tissue preparation

Soybean-derived GlcCer was manufactured and kindly provided by Doosan Co. Glonet BU (Suwon, Korea).

The applied GlcCer formula contains 61.3% GlcCer, 11.5% steryl glycoside, 7.4% phosphatidylcholine, 6.2% phosphatidylinositol, 3.5% triglyceride, 3.5% lysophosphatidylcholine, 2.4% free fatty acid, etc. The GlcCer was comprised primarily of ceramide with 4,8-sphingadiene (d18:2) and alpha-hydroxypalmitic acid (h16:0). GlcCer was orally administered once daily from day 7 to day 22, the end of experimental period. Prednisolone, a corticosteroid, was used as a positive control (30 mg/kg; Sigma, USA). All compounds were administered in a volume of 1 mL per 100 g body weight. On the day of each challenge with oxazolone, each test compound was administered 1 h after the oxazolone application. On the last day of the experiment (day 22), the animals were sacrificed by cervical dislocation 1 h after the final GlcCer administration (2 h after the final oxazolone challenge), and samples were collected.

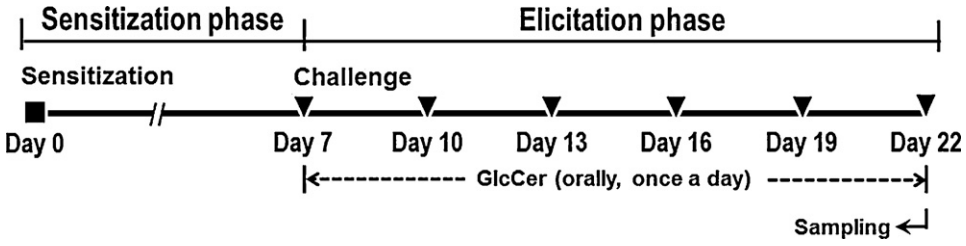

Fig. 1. Experimental schedule of oxazolone induction of chronic contact dermatitis and the oral administration of GlcCer in mice. Mice were sensitized on the abdomen with oxazolone 7 days before the first challenge and were repeatedly challenged on the ear with oxazolone every 3 days until day 22. GlcCer or prednisolone was orally administered once a day during the elicitation phase, from days 7 to 22, as described in the materials and methods. A quadrangle indicates the time of oxazolone sensitization, and the inverted triangles indicate times of oxazolone challenge.