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Oral glucosylceraminde reduces 2,4-dinitrofluorobenzene induced inflammatory response in mice by reducing TNF-alpha levels and leukocyte infiltration

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Running title: Anti-inflammatory Property of Orally Administered Glucosylceramide

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Abstract

Sphingolipids are constituents of cellular membranes and play important roles as second messengers mediating cell functions. As significant components in foods, sphingolipids have been proven to be critical for human health. Moreover, diverse metabolic intermediates of sphingolipids are known to play key roles both in proinflammatory and in anti-inflammatory effects. However, the effect of dietary sphingolipids on inflammation is a complicated field that needs to be further assessed. Our study evaluated the effects of orally administered maize glucosylceramide (GluCer), one of the most conventional dietary sphingolipids, on inflammation using the 2, 4-dinitro-1-fluorobenzene (DNFB)-treated BALB/c murine model. Oral administration of GluCer inhibited ear swelling and leukocyte infiltration to the inflammatory site, suggesting that dietary GluCer has anti-inflammatory properties. ELISA analyses revealed that oral administration of GluCer for 6 days was not modified the Th1/Th2 balance, but significantly down-regulated the activation of TNF-α at the inflammatory site. Based on these results, the down-regulation of TNF-α by dietary GluCer may suppress vascular permeability and reduce the migration of inflammatory cells. Our findings increase understanding of the actions dietary sphingolipids on the balance of the immune response.

Keywords

Sphingolipids, Dietary supplements, glucosylceramide, Anti-inflammatory agents, DNFB, BALB/c mice, TNF-α, immune response

Abbreviations

DNFB 2, 4-dinitro-1-fluorobenzene

GluCer glucosylceramide

IFN-γ interferon-gamma

IgE immunoglobulin E

IL-1β interleukin-1beta

IL-4 interleukin-4

IL-6 interleukin-6

NF-κB receptor activator of nuclear factor-kappa B

Th1 T-helper 1

Th2 T-helper 2

TNF-α tumor necrosis factor-alpha

Introduction

Sphingolipids are commonly believed to protect the cell surface against harmful environmental factors by forming the mechanically stable and chemically resistant outer leaflet of the plasma membrane lipid bilayer [1-4]. Sphingolipids generate diverse metabolic intermediates, notably ceramide, sphingosine, sphingosine-1-phosphate and ceramide-1-phosphate, which serve as important mediators in the signaling cascades involved in apoptosis, proliferation, and stress responses [5-8]. Although we have already demonstrated that dietary sphingolipids are poorly absorbed by the intestine [9], sphingolipids that are significant components of foods have gained considerable attention for their potential and essential roles in human health [10-14]. It has been reported that dietary supplementation with sphingolipids has diverse physiological effects, such as lowering plasma lipids [15], improving skin barrier function [16], preventing melanin formation [17], contributing to central nervous system myelination [18] as well as protecting the colon against inflammation [19-25]. However, the functional, regulatory, and physiological significance of the immune regulating effects of dietary sphingolipids is an appreciably complicated field that is not well understood.

One hypothesis of immune regulation involves the balance between T-helper 1 (Th1) and T-helper 2 (Th2) cells, which direct different immune response pathways. Th1 cells drive the "cellular immunity" pathway to fight viruses and other intracellular pathogens, eliminate cancer cells, and stimulate delayed-type hypersensitivity skin reactions. Th2 cells are involved in "humoral immunity" and up-regulate antibody production to fight extracellular organisms. Either pathway can down-regulate the other. Disruption of the Th1/Th2 balance can cause immunological diseases [26, 27]. Via the actions of sphingolipid degrading enzymes, such as sphingomyelinase, glycoceramidases and ceramidase, dietary sphingolipids are hydrolyzed to various kinds of metabolic intermediates which are critical for the activation and mediation of various types of immune cells. Metabolites of sphingolipids initiate and maintain diverse aspects of immune cell balance and functional responses by regulating cell migration and inflammatory pathways [8, 20, 28-31]. For instance, sphingolipid hydrolysis products regulate cyclooxygenase-2, interleukin 1β (IL-1β), interleukin 6 (IL-6), tumor necrosis factor α (TNF-α) and nuclear factor kappa B (NF-κB) via the sphingosine kinase 1/ sphingosine-1-phosphate and ceramide kinase 1/ceramide-1-phosphate pathways, and thus cause the activation of mast cells, control thymocyte maturation and regulate the balance of lymphocyte subpopulations [32-37].

The goal of this study was to evaluate the effects of orally administered glucosylceramide (GluCer), one of the most important dietary sphingolipids, against DNFB-induced ear swelling in the BALB/c murine model, to provide further understanding of how dietary sphingolipids act on the balance between proinflammatory and anti-inflammatory responses.

Materials and Methods

Maize GluCer preparation

GluCer from maize was kindly donated by Nippon Flour Mills Co. Ltd. (Atsugi, Japan). The purity of this GluCer was 96%, which was determined by HPLC equipped with an evaporative light-scattering detector, as described previously [12].

Animals

Female BALB/c mice (6 weeks old, 15–20 g body weight) were purchased from Japan SLC Inc. (Shizuoka, Japan). Animals were group-housed at 6 mice per cage, and were bred at the Institute's animal facilities at 25 °C with a 12-hour light/dark cycle. Pure water and AIN-93G diet (Oriental Yeast Co., LTD., Tokyo, Japan) were available *ad libitum*. All experiments were performed according to the guidelines of Kyoto University for the use and care of laboratory animals.

Contact hypersensitivity induced by DNFB

After a 2-week acclimatization period, allergic contact dermatitis was induced by DNFB in BALB/c mice according to a previously published method with minor modifications [38]. Briefly, mice were sensitized on day 0 by application of 100 µl 0.5% DNFB in acetone-soybean oil (4:1, v/v) on their shaved dorsal skin. The mice were divided into control, low dose (5 mg) and high dose (50 mg) groups (n=12 in each group). An identifying mark was made on the tail of each mouse.

The maize GluCer was suspended in 0.5% carboxymethyl cellulose (CMC) (Nacalai Tesque Co. Ltd., Kyoto, Japan) solution and was orally administered at 5 or 50 mg to each mouse daily for six days. One hour after the final treatment, mice were challenged with 20 µL 0.5% DNFB in acetone–soybean oil (4:1) on both ears. The thickness of the right ear of each mouse was measured with a Dial Thickness Gauge (Mitutoyo Co., Kanagawa, Japan) at 0, 6 and 24 hours after the DNFB challenge. Ear swelling was calculated as the difference in thickness before and after challenge [39].

Six hours (n=6) and 24 hours (n=6) after DNFB treatment, blood was collected and mice were sacrificed under anesthesia. The right ear and spleen of each mouse was immediately excised and frozen in liquid nitrogen, then stored at -80°C until use.

Morphological analysis

The left ear of each mouse was fixed in 10% neutral buffered formalin solution and was then processed routinely into paraffin wax. Formalin fixed paraffin sections were stained with hematoxylin and eosin (H&E) to observe morphological changes using a microscope (Keyence Co., Osaka, Japan).

Measurement of cytokine production and serum immunoglobulin E (IgE)

Amounts of IFN-γ, interleukin-4 (IL-4), TNF-α and interleukin-10 (IL-10) in homogenates of tissues were quantified using Murine IL-4 (Diaclone Research, Besancon, France), Murine IFN-γ (Diaclone Research), Mouse TNF-α (Pierce Biotechnology Inc., Rockford, IL, USA), and Murine IL-10 (Diaclone Research) ELISA kits, respectively, according to the manufacturer's instructions. Levels of those cytokines in each supernatant were normalized to total protein content, which was determined using a DC Protein assay kit (Bio-Rad Laboratories, Hercules, CA, USA). Total serum IgE levels of DNFB-challenged mice were quantified using a Mouse IgE ELISA kit (Immunology Consultants Laboratory, Newberg, Oregon, USA) according to the manufacturer's instructions.

Statistical analysis

Data are reported as means ± SD. Statistical analyses were performed using one-way analysis of variance (ANOVA) with Fisher's PLSD method to identify levels of significance between the groups.

Results

GluCer suppresses DNFB-induced ear swelling of BALB/c mice

After challenge with DNFB, typical allergic contact dermatitis was provoked in ears of BALB/c mice, which was characterized by an initial increase of ear thickness and visible congestion of blood vessels. Oral treatment with maize GluCer suppressed DNFB-induced inflammatory symptom (redness and thickness) of ears. As shown in Fig. 1, a significant depression of ear thickness was observed at 6 h in both low (5mg/day) and high dose (50mg/day) GluCer treated groups ($p<0.05$). At 24 h, the average value of ear thickness was also reduced by GluCer, but there were no statistical differences among the three groups, which may due to large individual differences. The reduction of DNFB-induced ear swelling implies dietary GluCer has anti-inflammatory property.

GluCer inhibits inflammatory infiltrates in the ears of BALB/c mice

Histological specimens of ears were prepared at 6 h and 24 h after topical application of DNFB in BALB/c mice. In the control group, typical allergic contact dermatitis with congested blood vessels and apparent edema could be observed by H&E staining. As shown in Fig. 2A and Fig. 2D, microvascular dilations and dense leukocytes infiltrating the connective tissue, which are characteristics of inflammatory reactions, were clearly observed in the control group. At higher magnification, various kinds of migrated inflammatory cells could also be observed, including fibrocytes, mononuclear cells, degranulated mast cells and other leukocytes. These results confirm that DNFB induces severe inflammation in the ears of BALB/c mice and that a variety of lymphocytes migrated out from blood vessels during this contact sensitivity procedure. In both the low (5 mg/day) and the high (50 mg/day) dose GluCer-treated groups, microvascular dilation and leukocytes in inflammatory infiltrates were inhibited at 6 h (Fig. 2 B&C) and 24 h groups (Fig. 2 E&F). These results show that dietary GluCer inhibits microvascular dilation and inflammatory infiltration of DNFB-induced BALB/c mice.

GluCer inhibits inflammation by reducing TNF-α production in the ear

To clarify the effect of GluCer on DNFB-induced inflammation, especially on Th1/Th2 balance, levels of IFN-γ as an indicator of Th1 cells and IL-4 as an indicator of Th2 cells were measured by ELISA assay. IFN-γ and IL-4 levels of GluCer-treated group were not significantly altered (Table 1). In other words, Th1/Th2 balance was not modified by oral administration of GluCer for 6 days.

For further evaluating the effect of GluCer on DNFB-induced inflammation, IL-10 was determined. In ear, IL-10 was significantly decreased at 6 h both low and high dose groups, whereas this effect did not prolong to 24 h (Table 1). However, in spleen, IL-10 was increased by GluCer treatment at 24 h, but not reached a statistical significance in 6 h because of the relatively large individual differences (Table 1).

TNF-α as the most important proinflammatory cytokine and IgE as the most important antibody in the serum were also measured. As shown in Fig. 3A, the TNF-α level in the ear was significantly suppressed both in the low and high dose GluCer groups ($p<0.05$). Moreover, TNF-α level was also significantly down-regulated at 24 h by the effect of high dose GluCer (Fig. 3B). In contrast, IgE in the serum was almost at the same level among the control, low and high GluCer dose groups (Fig. 3 C&D). The anti-inflammatory effect of dietary GluCer on contact dermatitis in DNFB-induced BALB/c mice is via regulation of the level of TNF-α secreted by inflammatory cells in the ear.

Discussion

The findings presented here indicate that dietary plant GluCer, one of the most important and abundant sphingolipids in food [12], suppresses the DNFB-induced ear swelling of BALB/c mice, and inhibits the microvascular dilation and inflammatory infiltration response via down-regulating levels of TNF-α, but not modifying the balance of Th1/Th2. Meanwhile, over-expressed IL-10 in inflammatory ear skin was suppressed by dietary GluCer. This anti-inflammatory effect of dietary GluCer increases our understanding of biofunctional sphingolipids.

Dietary GluCer is known to be hydrolyzed to ceramide, sphingosine and free fatty acids in the intestinal lumen. In mucosal cells, exogenous free sphingosine and dihydrosphingosine are rapidly absorbed and metabolized to palmitic acid [40]. A smaller portion of the sphingoid bases is reincorporated into ceramide and more complex sphingolipids. Our recent findings revealed that dietary GluCer originating from higher plants can be hydrolyzed in the intestine and that the intact plant form of sphingoid bases is barely absorbed by the tissues [9,41]. Ono *et al.* reported that dietary maize and yeast GluCer did not alter the sphingoid base composition in the skin of NC mice [42]. We speculate that dietary maize GluCer accomplishes its anti-inflammatory effect, not only by producing bio-active metabolic intermediates through the sphingolipid metabolic pathways, but also via the activation of sphingolipid metabolic enzymes that affect endogenous sphingolipids at the inflammatory site, because diverse metabolic intermediates of sphingolipids, including ceramide, sphingosine, sphingosine-1-phosphate and ceramide-1-phosphate are well known important and highly bioactive endogenous regulators, which are involved in a complex metabolism network and play critical roles in inflammation [43-46].

In the case of our study, dietary maize GluCer accomplishes its inhibition of inflammation via the down-regulation of TNF-α level at the inflammatory site. TNF-α, produced by mononuclear phagocytes and other inflammatory cells (neutrophils, lymphocytes, natural killer cells and mast cells) or non-inflammatory cells (endothelial cells), is known to be one of the most important inflammatory mediators [47,48]. TNF-α facilitates the formation of adhesion molecules, vascular permeability and migration of leukocytes to sites of inflammation by affecting endothelial cells [49-51]. Our results reveal that dietary maize GluCer down-regulates levels of TNF-α in inflammatory ears. This down-regulation of TNF-α may affect endothelial cells and inflammatory cells, and also can inhibit vascular permeability at the site of inflammation. As a result, leukocyte migration is reduced. In the inflammatory cells, DNFB activates NF-κB by depredating the inhibitor of NF-κB (IκB) [52]. In addition, it has been reported that sphingolipids down regulated TNF-α via inactivating of NF-κB in histamine-induced mouse skin tissues [53]. Thus, dietary GluCer seems to inhibit the DNFB activated NF-κB and down-modulate TNF-α expression in this study.

Moreover, IL-10 levels were increased in spleens but suppressed in ears by dietary GluCer. This well known anti-inflammatory cytokine, IL-10, has been reported over-expressed during the antigen-specific type of skin inflammation [54] and DNFB-challenged ear [55, 56]. IL-10 is released by CD4+ T helper 2 (Th2) cell clones and a variety of other cells, including keratinocytes, macrophages, B lymphocytes, and mast cells [56]. The suppressive effect of dietary GluCer on IL-10 expression in DNFB-challenged ears might be caused by the inhibition of leukocytes infiltrating to inflammatory site.

Ono *et al.* demonstrate that supplementation of 0.1% GluCer diet for 7-week prevented atopic dermatitis-like symptoms in a mouse model by regulating the Th1/Th2 balance [42]. However, IFN-γ, IL-4 and IgE levels, the markers of Th1/Th2 balance, were not notably affected by GluCer administration for 6 days in the present study. It appears that the period of treatment is important for immunological response of dietary GluCer.

Furthermore, allergic inflammatory skin disease is associated with a loss of ceramide in the extracellular lamellar membranes which causes an abnormal barrier function of the stratum corneum [57]. Application of ceramide on diseased skin could significantly reduce allergic inflammatory reactions by improving the severity score, stratum corneum cohesion and hydration [58-60]. Dietary GluCer might also improve the stratum corneum cohesion and hydration of ear skin to reduce the skin inflammation.

In summary, our data provide evidence that maize GluCer has anti-inflammatory effects on the DNFB-induced inflammation of BALB/c mice. We confirmed that dietary GluCer inhibits ear swelling and leukocyte infiltration. Furthermore, our results indicate that this effect is accomplished mainly by down-regulating TNF-α, but does not significantly affect Th1/Th2 balance or IgE levels in the serum. We hypothesize that dietary GluCer accomplishes its anti-inflammatory effect by down-regulating TNF-α to suppress vascular permeability. This reduces the migration of inflammatory cells, affects endogenous sphingolipids through metabolic pathways by activating sphingolipid-related enzymes, and moreover, by hydrolysis to ceramide to improve skin barrier function at the dermatitis site. Our findings increase the comprehensive understanding of the actions of dietary sphingolipids on the balance of immune responses.

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References

1. Lingwood D, Simons K (2010) Lipid rafts as a membrane-organizing principle. Science 327: 46 – 50
2. Bartke N, Hannun YA (2009) Bioactive sphingolipids: metabolism and function. J Lipid Res 50: 91 – 96
3. Prabuddha S, Barbara B, David H (2007) Lipid rafts, fluid/fluid phase separation, and their relevance to plasma membrane structure and function. Semin Cell Dev Biol 18: 583-590
4. Merrill AH Jr, Schmelz EM, Dillehay DL, Spiegel S, Shayman JA, Schroeder JJ, Riley RT, Voss KA, Wang E (1997) Sphingolipids--the enigmatic lipid class: biochemistry, physiology, and pathophysiology. Toxicol Appl Pharmacol 142: 208-225
5. Spiegel S, Merrill AH Jr (1996) Sphingolipid metabolism and cell growth regulation. FASEB J 10: 1388-1397
6. Zheng W, Kollmeyer J, Symolon H, Momin A, Munter E, Wang E, Kelly S, Allegood JC, Liu Y, Peng Q, Ramaraju H, Sullards MC, Cabot M, Merrill AH Jr (2006) Ceramides and other bioactive sphingolipid backbones in health and disease: Lipidomic analysis, metabolism and roles in membrane structure, dynamics, signaling and autophagy. Biochim Biophys Acta 1758: 1864-1884
7. Nixon GF (2009) Sphingolipids in inflammation: pathological implications and potential therapeutic targets. Br J Pharmacol 158: 982-993
8. El Alwani M, Wu BX, Obeid LM, Hannun YA (2006) Bioactive sphingolipids in the modulation of the inflammatory response. Pharmacol Ther 112: 171-183
9. Sugawara T, Tsuduki T, Yano S, Hirose M, Duan J, Aida K, Ikeda I, Hirata T (2010) Intestinal absorption of dietary maize glucosylceramide in lymphatic duct cannulated rats. J Lipid Res 51: 1761-1769
10. Yunoki K, Ogawa T, Ono J, Miyashita R, Aida K, Oda Y, Ohnishi M (2008) Analysis of sphingolipid classes and their contents in meals. Biosci Biotechnol Biochem 72: 222-225
11. Duan J, Sugawara T, Hirata T (2010) Sphingolipids in seafood using HPLC with evaporative

light-scattering detection: Its Application in tissue distribution of Sphingolipids in fish. J Oleo Sci 59: 509-513

12. Sugawara T, Miyazawa T (1999) Separation and determination of glycolipids from edible plant sources by high performance liquid chromatography and evaporative light scattering detection. Lipids 34: 1231–1237
13. Nyberg L, Duan RD, Nilsson Å (1998) Sphingomyelin- a dietary component with structural and biological function. Prog Colloid Polym Sci 108: 119-128
14. Vesper H, Schmelz EM, Nikolova-Karakashian MN, Dillehay DL, Lynch DV, Merrill AH Jr (1999) Sphingolipids in food and the emerging importance of sphingolipids to nutrition. J Nutr 129: 1239–1250
15. Duivenvoorden I, Voshol PJ, Rensen PC, van Duyvenvoorde W, Romijn JA, Emeis JJ, Havekes LM, Nieuwenhuizen WF (2006) Dietary sphingolipids lower plasma cholesterol and triacylglycerol and prevent liver steatosis in APOE*3Leiden mice. Am J Clin Nutr 84:312-321
16. Tsuji K, Mitsutake S, Ishikawa J, Takagi Y, Akiyama M, Shimizu H, Tomiyama T, Igarashi Y (2006) Dietary glucosylceramide improves skin barrier function in hairless mice. J Dermatol Sci 44: 101—107
17. Kinoshita M, Hori N, Aida K, Sugawara T, Ohnishi M (2007) Prevention of melanin formation by yeast cerebroside in B16 mouse melanoma cells. J Oleo Sci 56: 645-648
18. Oshida K, Shimizu T, Takase M, Tamura Y, Shimizu T, Yamashiro Y (2003) Effects of dietary sphingomyelin on central nervous system myelination in developing rats. Pediatr Res 53: 589-593
19. Sugawara T, Kinoshita M, Ohnishi M, Miyazawa T (2002) Apoptosis induction by wheat-flour sphingoid bases in DLD-1 human colon cancer cells. Biosci Biotechnol Biochem 66: 2228–2231
20. Duan RD, Nilsson Å (2009) Metabolism of sphingolipids in the gut and its relation to inflammation and cancer development. Prog Lipid Res 48: 62-72
21. Merrill AH Jr, Schmelz EM, Wang E, Schroeder JJ, Dillehay DL, Riley RT (1995) Role of

dietary sphingolipids and inhibitors of sphingolipid metabolism in cancer and other diseases. J Nutr 125: 1677–1682

22. Aida K, Kinoshita M, Tanji M, Sugawara T, Tamura M, Ono J, Ueno N, Ohnishi M (2005) Prevention of aberrant crypt foci formation by dietary maize and yeast cerebrosides in 1,2-dimethylhydrazine-treated mice. J Oleo Sci 54: 45-49
23. Berra B, Colombo I, Sottocornola E, Giacosa A (2002) Dietary sphingolipids in colorectal cancer prevention. Eur J Cancer Prev 11: 193-197
24. Sugawara T, Kinoshita M, Ohnishi M, Miyazawa T (2002) Apoptosis induction by wheat-flour sphingoid bases in DLD-1 human colon cancer cells. Biosci Biotechnol Biochem 66: 2228–2231
25. Kinoshita M, Aida K, Tokuji Y, Sugawara T, Ohnishi M (2009) Effects of dietary plant cerebroside on gene expression in the large intestine of 1,2-dimethylhydrazine (DMH)-treated mice determined by DNA microarray analysis. J Food Lipids 16: 200-208
26. Kidd P (2003) Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. Altern Med Rev 8: 223-246
27. Hernández-Pando R, Orozcoe H, Sampieri A, Pavón L, Velasquillo C, Larriva-Sahd J, Alcocer JM, Madrid MV (1996) Correlation between the kinetics of Th1, Th2 cells and pathology in a murine model of experimental pulmonary tuberculosis. Immunology 89: 26-33
28. Dinarello CA (1997) Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. Chest 112: 321-329
29. Lahiri S, Futerman AH (2007) The metabolism and function of sphingolipids and glycosphingolipids. Cell Mol Life Sci 64: 2270–2284
30. Yopp AC, Randolph GJ, Bromberg JS (2003) Leukotrienes, sphingolipids, and leukocyte trafficking. J Immunol 171: 5-10
31. Melendez AJ, Khaw AK (2002) Dichotomy of Ca^{2+} signals triggered by different phospholipid pathways in antigen stimulation of human mast cells. J Biol Chem 277: 17255-17262
32. Jolly PS, Bektas M, Olivera A, Gonzalez-Espinosa C, Proia RL, Rivera J, Milstien S, Spiegel S

(2004) Transactivation of sphingosine-1-phosphate receptors by FcepsilonRI triggering is required for normal mast cell degranulation and chemotaxis. J Exp Med 199: 959-970

33. Snider AJ, Orr Gandy KA, Obeid LM (2010) Sphingosine kinase: Role in regulation of bioactive sphingolipid mediators in inflammation. Biochimie 92: 707-715

34. Gilfillan AM, Tkaczyk C (2006) Integrated signalling pathways for mast-cell activation. Nature Rev Immunol 6: 218–230

35. Allende ML, Dreier JL, Mandala S, Proia RL (2004) Expression of the sphingosine 1-Phosphate Receptor, S1P1, on T-cells Controls Thymic Emigration. J Biol Chem 279: 15396-15401

36. Wei SH, Rosen H, Matheu MP, Sanna MG, Wang SK, Jo E, Wong CH, Parker I, Cahalan MD (2005) Sphingosine 1-phosphate type 1 receptor agonism inhibits transendothelial migration of medullary T cells to lymphatic sinuses. Nat Immunol 6: 1228-1235.

37. Olivera A, Rivera J (2005) Sphingolipids and the balancing of immune cell function: Lessons from the mast cell. J Immunol 174: 1153-1158

38. Sakai S, Sugawara T, Kishi T, Yanagimoto K, Hirata T (2010) Effect of glucosamine and related compounds on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. Life Sci 86: 337-343

39. Tomobe YI, Morizawa K, Tsuchida M, Hibino H, Nakano Y, Tanaka Y (2000) Dietary docosahexaenoic acid suppresses inflammation and immune responses in contact hypersensitivity reaction in mice. Lipids 35: 61-69

40. Nilsson Å (1968) Metabolism of sphingomyelin in the intestinal tract of the rat. Biochim Biophys Acta 164: 575–584

41. Sugawara T, Kinoshita M, Ohnishi M, Nagata J, Saito M (2003) Digestion of maize sphingolipids in rats and uptake of sphingadienine by Caco-2 cells. J Nutr 133: 2777–2782

42. Ono J, Kinoshita M, Aida K, Tamura M, Ohnishi M (2010) Effects of dietary glucosylceramide on dermatitis in atopic dermatitis model mice. Eur J Lipid Sci Tech 112: 708-711

43. Oskeritzian CA, Milstien S, Spiegel S (2007) Sphingosine-1-phosphate in allergic responses,

asthma and anaphylaxis. Pharmacol Ther 115: 390-399

44. Ma Y, Pitson S, Hercus T, Murphy J, Lopez A, Woodcock J (2005) Sphingosine activates protein kinase A type II by a novel cAMP-independent mechanism. J Biol Chem 280: 26011–26017

45. Spiegel S, Milstien S (2003) Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat Rev Mol Cell Biol 4: 397-407

46. Pettus BJ, Kitatani K, Chalfant CE, Taha TA, Kawamori T, Bielawski J, Obeid LM, Hannun YA (2005) The coordination of prostaglandin E2 production by sphingosine-1-phosphate and ceramide-1-phosphate. Mol Pharmacol 68: 330-335

47. Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 3: 745-756

48. Tartaglia LA, Goeddel DV (1992) Two TNF receptors. *Immunol Today* 13: 151-153

49. Oppenheim JJ (2001) Cytokines: past, present, and future. Int J Hematol 74: 3-8

50. Borish LC, Steinke JW (2003) Cytokines and chemokines. J Allergy Clin Immunol 111: 460-475

51. Snider AJ, Orr Gandy KA, Obeid LM (2010) Sphingosine kinase: Role in regulation of bioactive sphingolipid mediators in inflammation. Biochimie 92: 707-715

52. Cruz MT, Duarte CB, Gonçalo M, Figueiredo A, Carvalho AP, Lopes MC (2002) Differential activation of nuclear factor kappa B subunits in a skin dendritic cell line in response to the strong sensitizer 2,4-dinitrofluorobenzene. Arch Dermatol Res 294 : 419–425

53. Ryu KR, Lee B, Lee IA, Oh S, Kim DH (2010) Anti-scratching Behavioral Effects of *N*-Stearoyl-phytosphingosine and 4-Hydroxysphinganine in Mice. Lipids 45: 613-618

54. Inoue R, Otsuka M, Nishio A, Ushida K (2007) Primary administration of *Lactobacillus johnsonii* NCC533 in weaning period suppresses the elevation of proinflammatory cytokines and CD86 gene expressions in skin lesions in NC/Nga mice. FEMS Immunol Med Microbiol 50: 67–76

55. Fujiwara R, Sasajima N, Takemura N, Ozawa K, Nagasaka Y, Okubo T, Sahasakul Y,

Watanabe J, Sonoyama K (2010) 2,4-Dinitrofluorobenzene-Induced Contact Hypersensitivity Response in NC/Nga Mice Fed Fructo-Oligosaccharide. J Nutr Sci Vitaminol 56:260-265

56. Wang M, Qin X, Mudgett JS, Ferguson TA, Senior RM, Welgus HG (1999) Matrix metalloproteinase deficiencies affect contact hypersensitivity: stromelysin-1 deficiency prevents the response and gelatinase B deficiency prolongs the response. PANS 96:6885-6889
57. Snider AJ, Orr Gandy KA, Obeid LM (2010) Sphingosine kinase: Role in regulation of bioactive sphingolipid mediators in inflammation. Biochimie 92: 707-715
58. Ishikawa J, Takada S, Hashizume K, Takagi Y, Hotta M, Masukawa Y, Kitahara T, Mizutani Y, Igarashi Y (2009) Dietary glucosylceramide is absorbed into the lymph and increases levels of epidermal sphingolipids. J Dermatol Sci 56: 220-222
59. Proksch E, Fölster-Holst R, Jensen JM (2006) Skin barrier function, epidermal proliferation and differentiation in eczema. J Dermatol Sci 43: 159-169
60. Piekutowska A, Pin D, Rème CA, Gatto H, Haftek M (2008) Effects of a topically applied preparation of epidermal lipids on the stratum corneum barrier of atopic dogs. J Comp Pathol 138: 197-203

Figure Legends

Fig. 1 Effect of orally administered GluCer on DNFB-induced ear swelling in BALB/c mice.

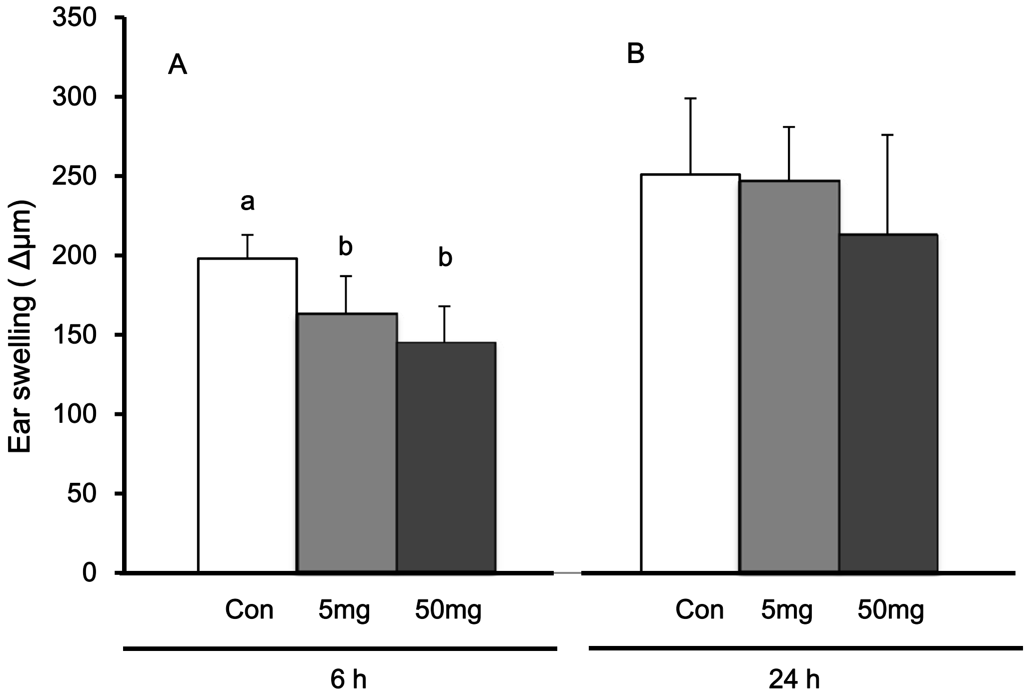
The thickness of the left ear of each mouse was measured both before and after the DNFB challenge. Ear swelling values are presented as the difference in thickness at 6 h (A) and at 24 h (B): Ear swelling = ear thickness after challenge (6/24 h) – ear thickness before challenge (0 h). Con, 6 days 0.5% CMC (vehicle) orally administered; Low, 6 days low dose (5 mg/day) maize GluCer orally administered; High, 6 days high dose (50 mg/day) maize GluCer orally administered. Values are means ± SD, n = 6. Values with different superscript letters are significantly different ($p<0.05$).

Fig. 2 Histopathological analysis of orally administered maize GluCer on DNFB-induced ear swelling in BALB/c mice.

Morphological changes in the left ear of 6 h and 24 h after DNFB-challenged BALB/c mice were observed. Ear sections from control mice (A), low dose (5 mg/day) maize GluCer administered mice (B) and high dose (50 mg/day) maize GluCer administered mice (C) were stained with hematoxylin and eosin (H&E). Microvasular (asterisk marks) and leukocyte (arrowheads) were pointed out in the histological sections. Sections are representatives of more than five observations.

Fig. 3 TNF-α levels in the ears and IgE levels in the serum of DNFB- challenged BALB/c mice.

TNF-α (A,B) levels in the right ear homogenates and IgE levels in the serum (C,D) of control, low dose (5 mg/day) and high dose (50 mg/day) maize GluCer administered mice were measured both 6 (A,C) and 24 h (B,D) after the DNFB challenge. These data represent the means ± SD for groups of six mice. Values with different letters differ significantly ($p<0.05$).



350
300
250
200
150
100
50
0
Ear swelling (Δμm)
A
B
a
b
b
Con
5mg
50mg
Con
5mg
50mg
6 h
24 h

Fig. 1

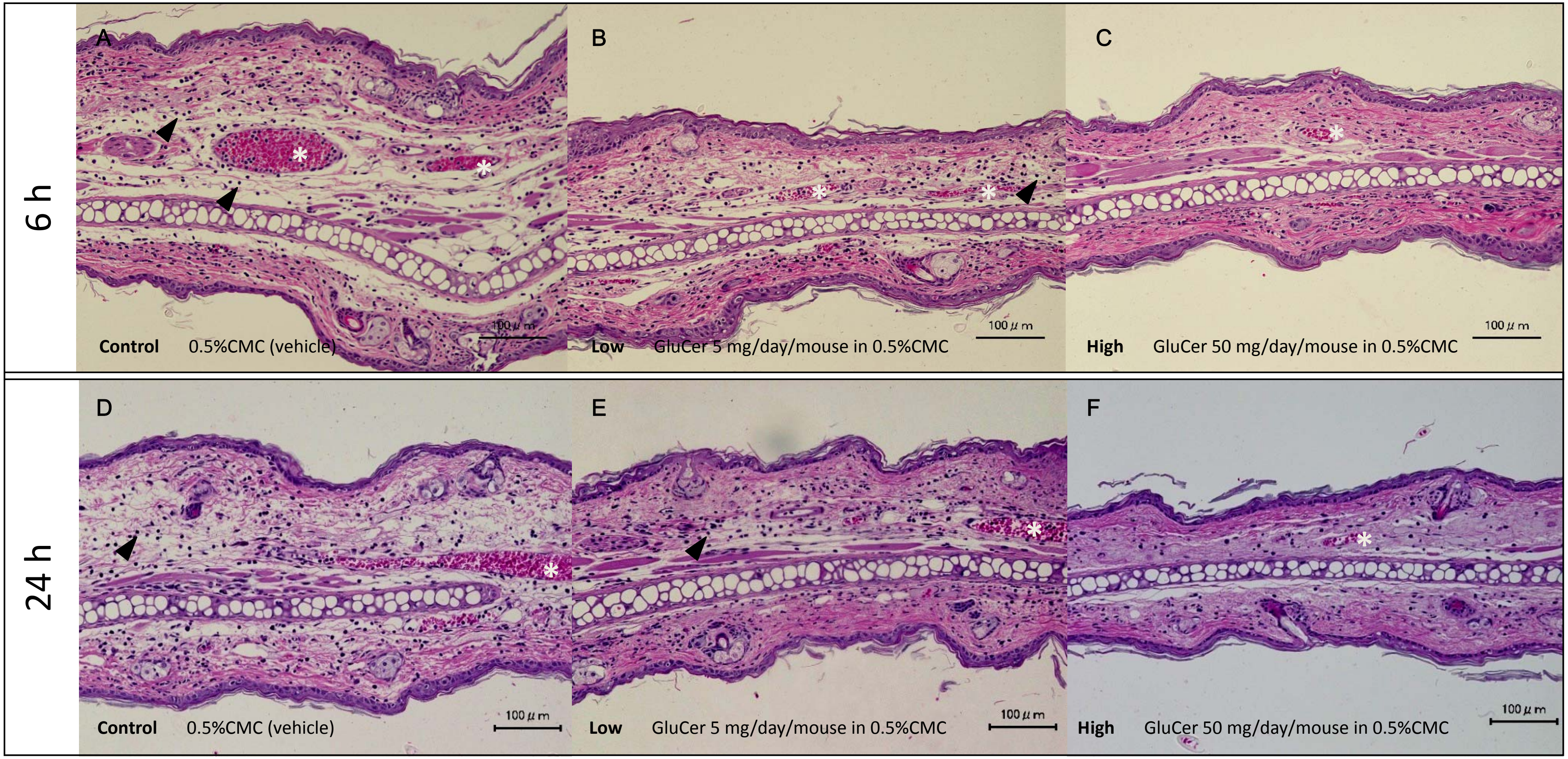

6 h
A
B
C
Control 0.5%CMC (vehicle)
Low GluCer 5 mg/day/mouse in 0.5%CMC
High GluCer 50 mg/day/mouse in 0.5%CMC
24 h
D
E
F
Control 0.5%CMC (vehicle)
Low GluCer 5 mg/day/mouse in 0.5%CMC
High GluCer 50 mg/day/mouse in 0.5%CMC

Fig. 2

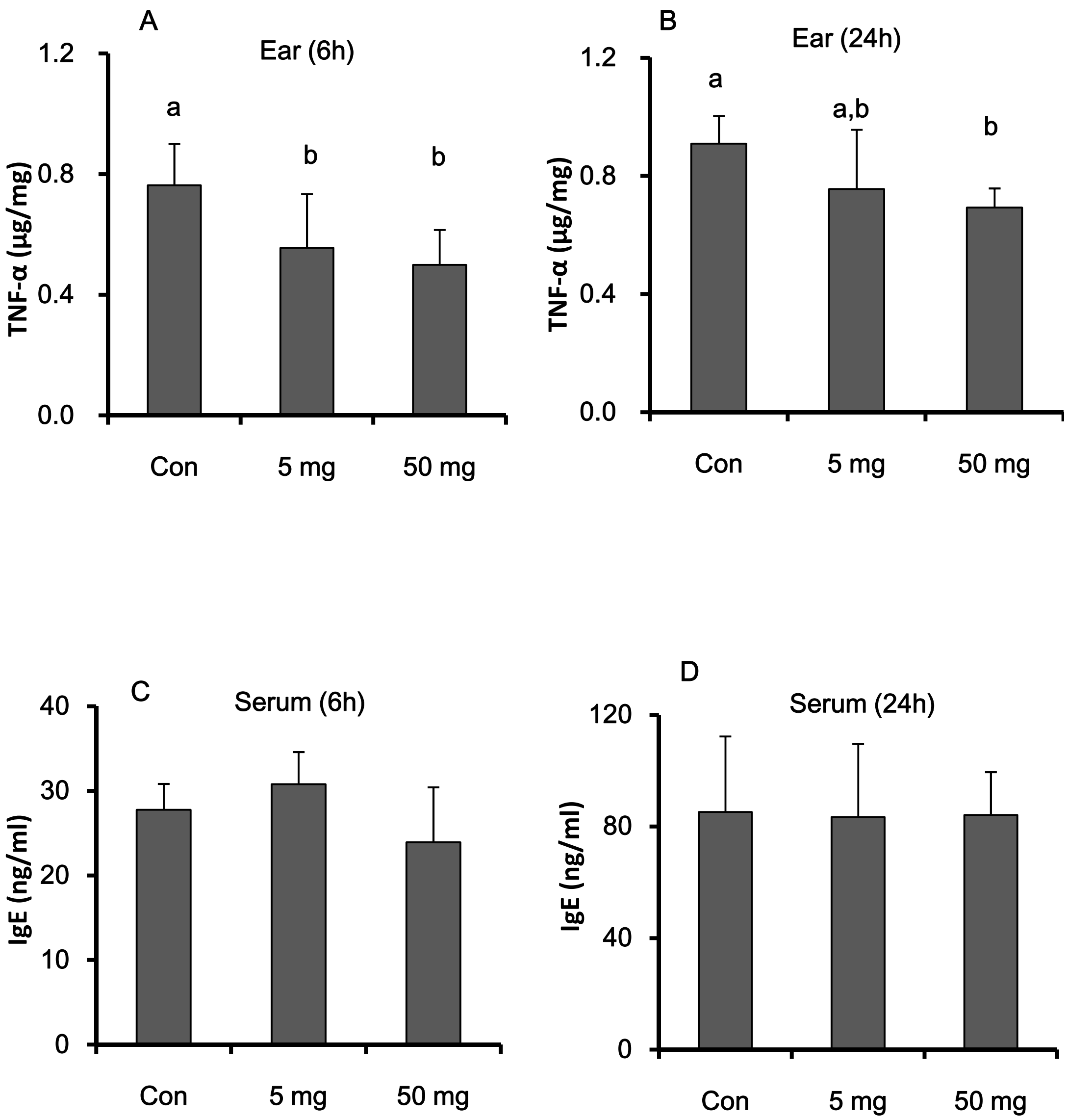

A
Ear (6h)
TNF-α (μg/mg)
1.2
0.8
0.4
0.0
a
b
b
Con
5 mg
50 mg
B
Ear (24h)
TNF-α (μg/mg)
1.2
0.8
0.4
0.0
a
a,b
b
Con
5 mg
50 mg
C
Serum (6h)
IgE (ng/ml)
40
30
20
10
0
Con
5 mg
50 mg
D
Serum (24h)
IgE (ng/ml)
120
80
40
0
Con
5 mg
50 mg

Fig. 3

Table 1. IFN-γ, IL-4 and IL-10 levels in ears and spleens of DNFB-challenged BALB/c mice.

Cytokines		**Ear**		**Spleen**	
		6 h	**24 h**	**6 h**	**24 h**
	mg/day	pg/mg protein			
IFN-γ	0	482.4±106.3	85.9±16.7	7.6±4.9	6.8±1.7
	5	362.0± 89.4	89.1±18.3	7.5±4.1	8.5±2.7
	50	373.2±107.1	92.1±13.1	5.9±2.2	6.0±2.9
IL-4	0	25.3±4.4	10.3±2.5	2.8±0.9	1.9±0.4
	5	30.6±6.5	10.7±2.3	4.0±1.0	1.8±0.2
	50	29.8±8.4	9.0±2.1	3.2±0.7	1.5±0.2
IL-10	0	3137.7±881.3 a	698.7±212.5	105.5±23.5	56.3± 7.8 a
	5	1326.7±297.7 a,b	543.0±158.9	131.0±37.9	72.0±33.1 a,b
	50	1578.5±352.3 b	618.0±264.9	170.2±47.6	110.4±48.7 b

These data represent the means ± SD for groups of six mice. Values with different superscript letters in the same series differ significantly ($p<0.05$).