Original Article

Efficacy of Hataedock Treatments for Maintenance and Formation of Lipid Barrier in Obese NC/Nga Mice with *Dermatophagoides Farinae*-Induced Atopic Dermatitis

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Objectives: HTD treatment is a traditional preventive therapy for neonatal inflammatory diseases such as AD. The aim of this study was to investigate the efficacy of HTD treatments for the maintenance and formation of lipid barrier in *Dermatophagoides farinae*-induced obese NC/Nga mice.

Methods: 20 mg/kg of CRGR extracts as HTD treatments were orally administered to NC/Nga mice. To induce obesity, high fat diet was served. *Dermatophagoides farinae* extracts was applied on the 4th-6th and 8th-10th weeks to induce AD-like skin lesions in NC/Nga mice. Changes of skin conditions in mice were observed by histochemistry and immunohistochemistry.

Results: The results showed that HTD treatments effectively maintained and formed the lipid barrier. In the experimental groups, restorations of LASS2 expression and distributions of filaggrin, involucrin, loricrin, ASM, and LXR means that HTD treatments maintained and generated the lipid barrier. In the dermal papillae, HTD treatments reduced PKC production accompanied by epidermis damage. Furthermore, levels of IL-4, and STAT6 was low. HTD treatment may be effective for preventing inflammation induced by Th2-skewed condition by suppressing the main pathway of Th2 differentiation.

Conclusions: HTD treatment alleviated the inflammatory damage in the skin tissues of the NC/Nga mice by maintaining the lipid barrier and suppressing Th2 differentiation.

Key Words : Hataedock, atopic dermatitis, NC/Nga obese mice, lipid barrier, Th2 differentiation

Introduction

The prevalence of obesity in children and adolescents continues to increase worldwide. The prevalence of

obesity in infants and preschool children in South Korea has increased about 2 times in 10 years¹⁾. Recently, several studies have reported that obesity affects the incidence of inflammatory diseases²⁾. Furthermore,

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studies have suggested that obesity induces reduction of the moisturizing and barrier functions of the stratum corneum (SC)³⁾.

Atopic dermatitis (AD) is the most common inflammatory disease of the skin based on the skin barrier dysfunction characterized by pruritus, dryness, and erythematous eczema⁴⁾. Recently the hypothesis that the pathogenesis of AD is caused by the destruction of the skin barrier system is emerging⁵⁾. The defects of skin barrier induced a various abnormalities in lipid composition and the extent of the permeability barrier abnormality in AD⁶⁾. Thus the abnormalities of the skin barrier are not simply epiphenomenon of AD, but in preference the driver for inflammation of AD⁷⁾. Damages to skin barrier function have highly interrelation with inflammatory skin disease induction. Recently, Interleukin (IL)-31 that is a cytokine associated with Th2 differentiation in AD, has been shown to reduce the expression of filaggrin, a major constituent of the skin barrier⁸⁾. In addition, reported that the mutated or reduced filaggrin can cause inflammation by damaging the skin barrier⁹⁾.

Within traditional Korean medicine, AD is regarded as related to a fetal heat or fetal poisoning, which are caused by unhealthy dietary habits such as excessive fat intake during pregnancy¹⁰⁾. Hataedock (HTD) is traditional treatments of Korean medicine in which herbal extracts are orally administered to neonates to prevent inflammatory diseases caused by mother's dietary habits during pregnancy. *Coptidis Rhizoma*, *Glycyrrhizae Radix* (CRGR) are a representative herbal medicine of HTD treatments. Our previous study suggested that HTD treatment maintains and fortifies the lipid barrier¹¹⁾. Based on that study, we sought to study the effect of HTD treatment on the maintenance of lipid barrier in obese conditions that could promote an inflammatory response.

In this study, CRGR was administered orally to

3-week-old NC/Nga mice as the HTD treatments and pediatric obese condition was induced by high fat diet. To investigate the lipid barrier maintenance effect of HTD treatment, *Dermatophagoides farinae* extract (DfE) was applied to NC/Nga mice to induce AD-like skin lesions. In a series of pathway related to provoking AD, HTD treatment is anticipated to assist in maintenance of the lipid barrier. Thus, we investigated the maintenance effects of HTD treatments on the lipid barrier disrupted by DfE in AD-induced obese NC/Nga mice.

Materials and Methods

1. Preparation of extracts

In this study, extracts of *Coptidis Rhizoma* (*Coptis Japonica*) and *Glycyrrhizae Radix* (*Glycyrrhiza uralensis*) were used for HTD treatment. The CRGR extract was prepared as follows: 1) *Coptidis Rhizoma* (100 g) and *Glycyrrhizae Radix* (100 g) were decocted in 1 L of distilled water for 3 hours and then filtered; 2) The decoction was concentrated to 50 mL by a rotatory vacuum evaporator, and the filtrate was freeze-dried. As a result, we obtained 31 g of extract (yield: 15.5%).

2. Experimental animals and induction of AD

Male 3-week-old NC/Nga mice, which were 14.3 ± 0.3 g in weight, were obtained from Central Lab. Animal Inc. (Seoul, Republic of Korea). For diet-induced obesity, diet composed of 60% fat, 20% carbohydrates, and 20% protein was administered. All mice were allowed to eat freely throughout the experiment.

Each 10 mice were allocated to three groups (total 30 mice) as follows: the high fat diet group (Ctrl group), high fat diet and AD-induced group (AE group), and high fat diet and AD-induced group after HTD treatment with CRGR extract group (CGT group). In CGT group, with 3-week-old mice, HTD treatments

that CRGR extracts (20 mg/kg each) are orally administered to each group on the 1st, 2nd, and 3rd days was performed. For AD-like skin lesion, we skinned the backs of mice and swabbed the skinned areas 20 times with 1 mL of 5% sodium dodecyl sulfate (SDS; Sigma-Aldrich, St. Louis, MO, USA) using a cotton swab to remove the lipid lamella of the SC. Then, 100 mg of DfE (Biostir Inc., Kobe, Japan) was applied to the mice two times per week. The first exposure was conducted on the 4th, 5th, and 6th weeks. After 1 week, the second exposure was conducted on the 8th, 9th, and 10th weeks. On the 11th week, mice were sacrificed with sodium pentobarbital. The overall procedure is depicted in Fig 1.

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Pusan National University (IACUC number: PNU-2015-0924). We also followed the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals throughout this study.

3. Tissue sample preparation and histochemistry

After sacrificing the mice, we obtained dorsal skins and then fixed them in 10% neutral-buffered formalin

(NBF) at room temperature for 24 hours. The fixed tissues were embedded in paraffin for serial sectioning with 5- μ m thickness. We conducted H&E (Hematoxylin and Eosin) staining to investigate histological changes including epithelial hyperplasia, capillary distribution, and collagen fiber distribution of the tissue samples.

4. Immunohistochemistry

Skin tissue slices were soaked in 20 μ g/mL of proteinase K solution for 5 minutes for proteolysis. The proteolyzed slices were incubated in 10% normal goat serum for 4 hours, which was used as blocking serum. Next, the slices were treated with primary antibodies (All antibodies used in the experiment were purchased at Santa Cruz Biotechnology, Dallas, TX, USA), including goat anti-Lass2 (1:100), goat anti-filaggrin (1:100), goat anti-involucrin (1:50), goat anti-loricrin (1:50), goat anti-ASM (1:50), goat anti-liver X receptor (LXR; 1:200), goat anti-protein kinase C (1:100), goat anti-IL-4 (1:100), and goat anti-STAT6 (1:100) for 72 hours in a 4 °C humidified chamber.

Then, the slices reacted with the primary antibodies were treated with biotinylated rabbit anti-goat IgG (1:100) secondary antibody for 24 hours at room temperature. After reaction with the secondary antibody,

Fig. 1. Experiment design.

Prior to induce AD, the CRGR extracts for HTD treatment were orally administered to the CGT group on the 1st, 2nd, and 3rd days. Mice (per group n=10) were exposed to DfE on the 4th, 5th, and 6th weeks at the first time. On the 8th, 9th, and 10th weeks, the mice were exposed DfE again. Abbreviations: HFD: high fat diet, HTD: Hataedock, and DfE: Dermatophagoides farinae extract.

the slices were treated with an avidin-biotin complex kit (Vector Laboratories, Burlingame, CA, USA) for 1 hour at room temperature. As a final step, the slices were developed with 0.05M tris-HCl buffer solution (pH 7.4) consisting of 0.05% 3,3'-diaminobenzidine (DAB) and 0.01% HCl and then counterstained with hematoxylin.

5. Image analysis and statistical analysis

To obtain numerical data from immunohistochemistry, image analysis was performed by Image Pro Plus (Media cybernetics, Rockville, MD, USA). In the image analysis of 400x-magnified exposure photography, positively reacted particle as pixel cells (intensity range: 80-100) were counted from 10 randomly selected fields from each group. Total pixel cells were 20,000,000 depending on the results of immunohistochemistry such as non-specific structure and artificiality. Data were presented as the mean $\pm$ standard error (mean $\pm$ SE). One-way ANOVA and Post-Hoc test were used to analyze statistical significance of the differences with a significance level of $P < 0.05$. SPSS 23 software (IBM Corp, Armonk, NY, USA) was used for statistical analysis.

Results

1. Alleviating effect on symptoms of AD

H&E staining was used to observe changes caused by edema including collagen fiber distribution and epithelial hyperplasia. The AE group exhibited pathological change such as reduction in collagen fiber and an increase in epithelial hyperplasia. These results represent the typical histological appearance of inflammatory skin damage induced through persistent application of DfE. In contrast, the CGT group showed less pathological changes than the AE group in most areas. The CGT group exhibited low epidermal hyperplasia and maintenance of collagen fiber density (Fig 2).

2. Maintenance of lipid barrier

To investigate the protective effect of HTD treatment on the epidermal barrier, Lass2-, filaggrin-, involucrin-, and, loricrin- positive reactions were measured.

Lass2-positive reactions were used to detect the ceramide synthesis of the epidermal lipid layer. The Lass2 level of the AE group was $8,317 \pm 221/20,000,000$ pixel which was decreased in the SC by 90% compared

Fig. 2. Alleviating effect on symptoms of AD.

HTD treatments treated to the CGT group alleviated the characteristic lesion of AD. In the results of H&E staining, hyperplasia of the epithelium increased in the AE group but decreased in the CGT group (bar size: 50 μ m). Abbreviations: Ctrl: high fat diet-fed mice, AE: high fat diet-fed and AD-induced mice but no treatment, CGT: high fat diet-fed and AD-induced mice after treated with CRGR extract, EP: epidermis, DE: dermis, and H&E: Hematoxylin and eosin stain.

with that of the Ctrl group. Compared with the AE group, Lass2 levels of the CGT group was increased significantly. The Lass2 level of the CGT group was $34,041 \pm 1320/20,000,000$ pixel which was increased by 309% ($P < 0.05$) compared with that of the AE group (Fig 3).

Fig. 3. Maintenance of lipid barrier.

In the results of each immunohistochemistry, both HTD treatments contributed to the maintenance of epidermal barrier. Image analysis data for Lass2-, filaggrin-, involucrin-, loricrin-, ASM-, and LXR-positive reactions (arrows indicating dark brown spot) decreased in the AE group compared with Ctrl group (per group $n=10$) (bar size: $50 \mu\text{m}$). In contrast, both HTD treatments showed that Lass2-, filaggrin-, involucrin-, loricrin-, ASM-, and LXR-positive reactions significantly increased in the CGT group (per group $n=10$) compared with that of AE group ($P < 0.05$). Abbreviations: Ctrl: high fat diet-fed mice, AE: high fat diet-fed and AD-induced mice but no treatment, CGT: high fat diet-fed and AD-induced mice after treated with CRGR extract, FLG: filaggrin, INV: involucrin, LOR: loricrin, SC: stratum corneum, EP: epidermis, DE: dermis, and #: $P < 0.05$ compared with the AE group.

Filaggrin-positive reactions were mainly found in keratohyalin granule of SC. The filaggrin level of the AE group was $5,511 \pm 390/20,000,000$ pixel which was decreased by 49% compared with that of the Ctrl group.

However, both HTD treatments recovered filaggrin levels in the CGT group. The levels of filaggrin in the CGT group was respectively $8,840 \pm 218/20,000,000$ pixel which were increased by 60% ($P < 0.05$) compared with that of the AE group (Fig 3).

Involucrin-positive reactions were observed in cornified layer of SC. The involucrin level of the AE group was $4,417 \pm 93/20,000,000$ pixel which was decreased by 87% compared with that of the Ctrl group. In contrast, HTD treatments improved involucrin levels in the CGT group. The levels of involucrin in the CGT group was $14,342 \pm 419/20,000,000$ pixel which were significantly increased by 225% ($P < 0.05$) compared with that of the AE group (Fig 3).

The results for loricrin-positive reaction were similar to the results for involucrin. Compared with the Ctrl group, the loricrin level of the AE group was $6,501 \pm 227/20,000,000$ pixel which was decreased by 93%. After both HTD treatments, the loricrin levels increased in the CGT group. The loricrin levels of the CGT group was $24,602 \pm 1090/20,000,000$ pixel which was increased by 283% ($P < 0.05$) compared with that of the AE group (Fig 3).

3. Formation of lipid barrier

To investigate the generative effect of HTD treatment on the lipid barrier, ASM- and LXR-positive reactions were measured.

ASM-positive reaction in the stratum granulosum was $3,284 \pm 161/20,000,000$ pixel which decreased by 93% in AE group compared with that of the Ctrl group. However, HTD treatments remarkably increased ASM levels in CGT group. The ASM level of the CGT group was $11,198 \pm 345/20,000,000$ pixel which was increased by 240% ($P < 0.05$) compared with that of the AE group (Fig 4).

LXR-positive reactions were widely exhibited in the cytoplasm of cells in the SC and stratum granulosum.

Compared with the Ctrl group, the LXR level was $5,511 \pm 71/20,000,000$ pixel which was remarkably decreased by 49% in the AE group. In contrast, both HTD treatments significantly improved LXR levels in the CGT group similarly to that of the Ctrl group. The LXR level of the CGT group was $8,840 \pm 149/20,000,000$ pixel which was increased by 60% ($P < 0.05$) compared with that of the AE group (Fig 4).

Fig. 4. Formation of lipid barrier.

In the results of each immunohistochemistry, HTD treatments contributed to the formation of lipid barrier. Image analysis data for ASM- and LXR-positive reactions (arrows indicating dark brown spot) decreased in the AE group compared with Ctrl group (per group $n=10$) (bar size: $50 \mu\text{m}$). In contrast, both HTD treatments showed that ASM- and LXR-positive reactions significantly increased in the CGT group (per group $n=10$) compared with that of AE group ($P < 0.05$). Abbreviations: Ctrl: high fat diet-fed mice, AE: high fat diet-fed and AD-induced mice but no treatment, CGT: high fat diet-fed and AD-induced mice after treated with CRGR extract, SC: stratum corneum, EP: epidermis, DE: dermis, and #: $P < 0.05$ compared with the AE group.

4. Regulatory effect on Th2 differentiation

PKC-, IL-4-, and STAT6-positive reactions were measured to observe the regulatory effect of HTD treatment on Th2 differentiation in the dermal papilla.

PKC immunohistochemistry was used to detect PKC-positive reactions in damaged epidermis and basement of epidermis. In the AE group, the level of

PKC was $198,283 \pm 3590/20,000,000$ pixel which was markedly increased by 5385% compared with that of the Ctrl group. However, HTD treatments also reduced the levels of PKC in the CGT group by 90% ($P < 0.05$), compared with that of the AE group. The levels of PKC was $20,016 \pm 324/20,000,000$ pixel in the CGT group (Fig 5).

Fig. 5. Regulatory effect on Th2 differentiation.

In the immunohistochemistry for PKC-, IL-4-, and STAT6-positive reaction (arrows indicating dark brown spot) decreased both in the CGT group compared with those of the AE group (per group $n=10$) (bar size: $50 \mu\text{m}$). Image analysis data for PKC, IL-4, and STAT6 also showed significant decrease in the CGT group ($P < 0.05$). Abbreviations: Ctrl: high fat diet-fed mice, AE: high fat diet-fed and AD-induced mice but no treatment, CGT: high fat diet-fed and AD-induced mice after treated with CRGR extract, EP: epidermis, DE: dermis, and #: $P < 0.05$ compared with the AE group.

The HTD treatments also remarkably reduced the levels of IL-4 and STAT6 compared with the AE group. The IL-4-positive reaction was $556,945 \pm 5852/20,000,000$ pixel which was increased by 977% compared with that of the Ctrl group. In contrast, the levels of IL-4 in the CGT group was $114,731 \pm 4327/20,000,000$ pixel which was decreased by 79% ($P < 0.05$) compared with that of the AE group (Fig 5). Immunohistochemistry for STAT6 exhibited similar results as those for IL-4. In AE group, the STAT6-positive reaction was

117,672±3819/20,000,000 pixel which was increased by 210% compared with that of the Ctrl group. In contrast, levels of STAT6 in the CGT groups was 74,344±817/20,000,000 pixel which was decreased by 37% ($P<0.05$), compared with that of the AE group (Fig 5).

Discussion

In this study, we investigated the preventive effect of HTD treatment on AD-like skin lesions using DfE - induced obesity NC/Nga mice. H&E staining was observed to confirm the AD symptom relief effect of HTD treatments. HTD treatments was observed to alleviate capillary angiogenesis, edema and epithelial hyperplasia. We anticipated that relieved AD symptoms was due to well-maintained lipid barrier. To observe the effect of HTD treatments on maintaining the lipid barrier, changes of Lass2, filaggrin, involucrin, loricrin, ASM, and LXR levels were measured. HTD treatments helped to maintain the lipid barrier by increasing Lass2, filaggrin, involucrin, loricrin, ASM, and LXR levels. Moreover, we anticipated that HTD treatments relieved AD symptoms by contributing to the regulation of the Th2 predominant immune response, a major pathological mechanism of AD. To regulate Th2-skewed condition, HTD treatments affected Th2 differentiation by reducing PKC, IL-4, and STAT6 levels.

The hypothesis for etiopathogenesis of AD has shifted from “inside to outside” to “outside to inside”¹². The “inside to outside” hypothesis suggests that the epidermal barrier is secondarily damaged due to rising inflammation caused by the imbalance between Th1 and Th2 cytokines, which is known as “Th2-skewed condition”¹³. However, the recent “outside to inside” hypothesis focuses on filaggrin. Filaggrin plays an important role in maintaining the SC by modulating hydration and pH level¹⁴. The “outside to inside”

hypothesis proposes that the defective SC due to impaired filaggrin stimulates the Th2 response by promoting persistent secretion of pro-inflammatory cytokines^{13, 14}.

As mentioned earlier, obesity and inflammatory response are correlated. In particular, adipocytes produce and secrete cytokines such as TNF- α , IL-6, and monocyte chemoattractant activating factor (MCP)-1, which play a central role in the inflammatory response¹⁵. Actually in obese subjects, an increase in pro-inflammatory cytokines such as TNF- α and IL-6 in the blood and an increase in CRP (c-reactive protein) as a biomarker of inflammatory diseases are observed¹⁶. Furthermore, many macrophages accumulate in adipocytes, leading to inflammation of the obese tissue itself, leading to chronic inflammation¹⁷.

Thus, we tried to confirm the efficacy of treatment of HTD treatments on AD by observing the levels of protein constituting lipid barrier in the obese conditions promoting the inflammatory response.

1. Alleviating effect on symptoms of AD

AD is characterized by pruritic skin lesions, impaired epidermal lipid barrier function, imbalance of the immune system, and allergic reactions¹⁸. Macroscopically, the HTD treatments alleviated AD symptoms such as edema including collagen fiber distribution and epithelial hyperplasia in obese NC/Nga mice. Furthermore, HTD treatment reduced angiogenesis. Angiogenesis of capillaries promoted by VEGFs is a hallmark of chronic inflammatory diseases such as AD. Angiogenesis is closely related to inflammation response by molecular links that cells involved in the inflammatory process release factors acting on the vascular endothelial cells¹⁹. Moreover, angiogenesis maintains inflammation by supplying oxygen and nutrients to cells in the inflammatory area²⁰.

2. Maintenance of lipid barrier

Lass2, filaggrin, involucrin, and loricrin are closely related to maintenance of the epidermal lipid barrier. Ceramide is a typical substance to keep moisture and to form permeability barrier function in SC. Reduced ceramides in SC can cause skin abnormalities such as AD due to loss of protection against antigens, moisture loss from the epidermis, and dysfunction of barrier²¹. Among the Lass recently named ceramide synthase (CerS), Lass2 that catalyzes the synthesis of very long acyl chain ceramides is the most commonly expressed of all CerS and is most widely distributed in the human body²². Deficiency of ceramide synthesis containing heavy chain fatty acids by Lass2 causes severe skin diseases²³. A reduced level of ceramide and changes in ceramide subclass were observed in AD or psoriasis²⁴, and the replacement of ceramide to short chain lengths was reported in patients with AD²⁵.

Filaggrin mutations causes skin barrier damage in AD and promotes IgE sensitization due to damaged skin barrier²⁶. Although there are many causes for filaggrin reduction, particularly Th2-skewed condition contributes to reducing filaggrin levels in AD patient¹⁴. AD symptoms was relieved by improving the skin barrier due to increased expression of filaggrin²⁷. Involucrin and loricrin are down-regulated by IL-4 in keratinocytes that process plays an important role in AD pathogenesis²⁸. Particularly, decreased expression of involucrin and loricrin is inhibited in knock-out STAT6 mice, suggesting that down-regulation by IL-4 is mediated through a STAT6-dependent mechanism²⁹.

In our previous study, we suggested that HTD treatment significantly increased filaggrin, involucrin, loricrin, and ASM levels, which are critical to the differentiation of epithelial cells and epidermal barrier function³⁰. Consistent with previous findings, HTD treatments used in this study increased the level of Lass2, filaggrin, involucrin, and loricrin. Based on this

result, HTD treatments may help to fortify the epidermal lipid barrier.

3. Formation of lipid barrier

ASM and LXR are closely related to formation of the epidermal lipid barrier. ASM, an enzyme that converts phospholipids into ceramides, also plays an important role in the formation of ceramides for epidermal barrier function³¹. ASM activity has been reported to be significantly reduced in AD lesions³², and reduced ASM activity is thought to be responsible for the reduced ceramide level, which is specific found in AD³³. In addition, deficiency of ASM in AD inhibits recovery after damage to the skin barrier³² and the reduction of ASM activity in AD leads to skin barrier disorders by decreasing ceramide, involucrin, loricrin, and filaggrin³⁴. In skin, activation of LXR improves permeability barrier homeostasis by stimulating epidermal lipid synthesis³⁵, and promotes recovery of permeability barrier damage³⁶. In addition, LXR controls cutaneous inflammation by inhibiting secretion of cytokine such as TNF- α and IL-1 α that mediate the cutaneous inflammatory response³⁷.

In this study, we compared the levels of ASM and LXR in order to examine the generative effect of HTD treatment on epidermal lipid barrier. The result showed that HTD treatment affected production of the levels of ASM and LXR. Based on this result, HTD treatments may help to generate the epidermal lipid barrier.

4. Regulatory effect on Th2 differentiation

Infiltrated external allergens can exacerbate Th2-skewed condition by activating inflammatory cells, including Th2 cells. For Th2 differentiation during inflammation, STAT6 signaling is an important pathway, and IL-4 is upstream of STAT6³⁸. STAT6 is closely related to IL-4 and IL-13 signaling, regulates important gene expression to IL-4 function and Th2

differentiation^{39), 40)}. In a knock-out STAT6 mice model, Th2 response by IL-4 does not occur⁴¹⁾, and showed a loss of Th2 cell accumulation, which is one of the main features of asthma⁴²⁾. IL-4 is one of the key cytokines for Th2 differentiation and subsequent inflammation since it affects immune cells such as T cell, B cell, and macrophage through the α subunit of IL-4 receptor (IL-4R α)^{38, 43)}. CD4 cells of knock-out IL-4 mice were unable to produce Th2 cytokines⁴⁴⁾. In additions, PKC is absolutely essential to differentiation of naive T cells into Th2 cells⁴⁵⁾ and PKC regulates the IL-4/STAT6 signaling pathway in T cells by phosphorylating Janus kinase (Jak) 1⁴⁶⁾. PKC plays an important role in the Jak1/STAT6 signaling cascade associated with activation through IL-4 and Th2 differentiation. Moreover, PKC activation is important for epidermal inflammation since keratinocytes secreting inflammatory mediators such as TNF- α and cyclooxygenase-2 (COX-2) require PKC activation for appropriate differentiation⁴⁷⁾.

Based on these results, we compared the levels of IL-4, STAT-6 and PKC in order to examine the regulatory effect of HTD treatment on Th2 differentiation. In this study, HTD treatment affected reduction of the levels of IL-4, STAT6, and PKC. Down-regulation of IL-4 and PKC were also observed in our previous study⁴⁸⁾, but STAT6-inhibitory activity was reported here for the first time. Considering our results, HTD treatment may be effective for preventing inflammation induced by Th2-skewed condition by suppressing the main pathway of Th2 differentiation.

In previous studies, we demonstrated that HTD treatments reduce the inflammatory response by down-regulating Th2 differentiation⁴⁸⁾. In addition, we demonstrated that HTD treatments maintains and fortifies the lipid barrier¹¹⁾. Based on these results, we hypothesize that HTD could attenuate AD symptoms

by maintaining lipid barrier in obese conditions, an aggravating factor of inflammation. In conclusion, the HTD treatments contributed to down-regulation of Th2 differentiation in AD-induced obese NC/Nga mice, leading to the alleviation of AD symptoms by maintaining the lipid barrier. Considering that the prevalence of childhood obesity is increasing, these results suggest that HTD treatments may contribute as an alternative treatment for the prevention of inflammatory diseases.

Conclusions

Obesity can aggravate inflammatory diseases. However, in this study, CRGR HTD treatment alleviated the inflammatory damage in the skin tissues of the NC/Nga mice by maintaining the lipid barrier and suppressing Th2 differentiation.

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