Hataedock Treatments for *Dermatophagoides Farinae*-induced Atopic Dermatitis in NC/Nga Mice Treated with High-fat Diet

Sang Hyun Ahn[1], Hee Yeon Kim[2,3], In Jun Yang[4], Han Sol Jeong[5], Kibong Kim[2,3]*

1 : Department of Anatomy, College of Korean Medicine, Semyung University,
2 : Department of Korean Pediatrics, School of Korean Medicine, Pusan National University,
3 : Department of Korean Pediatrics, Korean Medicine Hospital, Pusan National University,
4 : Department of Physiology, College of Korean Medicine, Dongguk University,
5 : Division of Applied Medicine, School of Korean Medicine, Pusan National University

Hataedock (HTD) treatment is a traditional preventive therapy for the fetal toxicosis- the acute allergic disease after childbirth, mainly manifested by a variety of skin allergies such as scab, phlegm. The aim of this study was to investigate the efficacy of HTD treatments for the alleviation of inflammation in *Dermatophagoides farinae*-induced obese NC/Nga mice. 20 mg/kg of Coptidis Rhizoma, Glycyrrhizae Radix (CRGR) extracts as a remedy of HTD treatments were orally administered to NC/Nga mice. We induced obesity in the mice by high-fat diet. To induce skin allergies, the extracts of *Dermatophagoides farinae* were topically applied on the NC/Nga mice at 4th-6th and 8th-10th weeks. Structural and molecular changes in the skin tissues were measured by immunohistochemical staining. HTD treatment decreased the atopic dermatitis (AD)-like symptoms including hemorrhage, erythema, erosion, edema, and dryness. HTD treatment suppressed the mast cell activation confirmed by reduction of FcεRI, substance P, and serotonin. The expression of several inflammatory mediators including nuclear factor-kappa B (NF-κB) p65, inducible nitric oxide synthase (iNOS), vascular endothelial growth factors (VEGFs) was also decreased by HTD treatment. HTD treatment suppressed the allergic, inflammatory responses in the skin tissues of the NC/Nga mice by reducing mast cells and down-regulating several inflammatory mediators.

keywords : Hataedock, Atopic dermatitis, NC/Nga obese mice, Inflammation, Mast cell

Introduction

Childhood obesity has increased remarkably worldwide. In South Korea, the prevalence of obesity in infants and preschool-aged children also increased from 1.4% in 2008 to 2.8% in 2015 and the prevalence of obesity in school-aged children (aged 7-18) increased from 8.36% in 2008 to 14.3% in 2016[1)]. Recently, many studies have shown that obesity increases the incidence of inflammatory diseases such as atopic dermatitis (AD) and asthma. Several studies have shown that obesity induces low-grade systemic inflammation by upregulation of several inflammatory mediators such as tumor necrosis factor-α (TNF-α)[2)], nuclear factor-kappa B (NF-κB)[3)], and Th2 cytokines[4)]. Furthermore, population studies suggested that increased incidence of pediatric obesity raises the risk of inflammatory diseases[5)]. Thus, it is important to control inflammatory responses especially in the case of obese children.

Symptoms of the AD in infants such as eczema and pruritus were considered to be related to unhealthy dietary habits during pregnancy, such as excessive fat intake[6)]. Hataedock (HTD) treatments is traditional treatment of Korean medicine that administers herbal extracts orally to neonates for the prevention of neonatal inflammatory diseases caused by mother's dietary habits during pregnancy. Among medications, a decoction made from herbal medicines such as Coptidis Rhizoma, Glycyrrhizae Radix (CRGR) is commonly used. These herbs have the potential to regulate Th2 differentiation. Prior studies have shown that CRGR down-regulate interleukin (IL)-4, IL-13, and signal transducer and activator of transcription 6 (STAT6), which play an important roles in Th2 differentiation[7,8)].

Our previous study suggested that HTD treatment alleviates AD-like skin lesions in NC/Nga mice by

* Corresponding author
Kibong Kim, Korean Medicine Hospital, Pusan National University, Geumo-ro 20, Mulgeum-eup, Yangsan-si, 50612, Korea
·E-mail : kkb@pusan.ac.kr ·Tel : +82-55-360-5952
·Received : 2018/11/19 ·Revised : 2018/12/13 ·Accepted : 2018/12/17

pISSN 1738-7698 eISSN 2288-2529 http://dx.doi.org/10.15188/kjopp.2018.12.32.6.396
Available online at https://kmpath.jams.or.kr

regulating Th2 differentiation[9,10]. In particular, maintenance of the stratum corneum (SC) and reduced protein kinase C (PKC) activation were notable results. Based on that study, we sought to study the effect of HTD treatment for alleviation of inflammation relieving in obese conditions that could promote an inflammatory response.

In this study, we applied CRGR extracts as the HTD treatments, which have shown potential for regulating inflammatory cytokines. High fat diet was administered to 3-week-old NC/Nga mice to induce pediatric obese condition and then used *Dermatophagoides farinae* extract (DfE) to induce the AD-like skin lesions. Allergens such as house dust mites are known to infiltrate the impaired epidermal lipid barrier and accelerate inflammatory pathways, resulting in AD[11]. Thus, we investigated the alleviation effects of HTD treatments on the epidermal lipid barrier disrupted by DfE in AD-induced obese NC/Nga mice.

Materials and Methods

1. Preparation of extracts

For HTD treatment, this study used extract of Coptidis Rhizoma (Coptis Japonica) and Glycyrrhizae Radix (Glycyrrhiza uralensis). 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 (14.3 ± 0.3 g in weight) were obtained from Central Lab. Animal Inc. (Seoul, Republic of Korea). To induce obesity, high-fat diet (composed of 60% fat, 20% carbohydrates, and 20% protein) was administered to the mice.

Total 30 mice were divided into three groups (10 mice per each group) as follows: the high-fat diet group (Ctrl group), high-fat diet and AD-induced group (AE group), and HTD treatment with CRGR extracts before the high-fat diet and AD-induction group (CGT group). In CGT group, HTD treatment that CRGR extracts (20 mg/kg each) are orally administered to each group on the 1st, 2nd, and 3rd days was performed. At the 4th week of the experiment, the body weight of Ctrl group and AE group was 32.3 ± 1.0 g and the CGT group was 25.9 ± 0.4 g.

For the induction of AD-like skin lesion, we removed the dorsal hairs the mice and swabbed the hair removed 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 topically applied to the dorsal skin of mice two times per week. The first exposure was conducted on the 4th, 5th, and 6th weeks. 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.

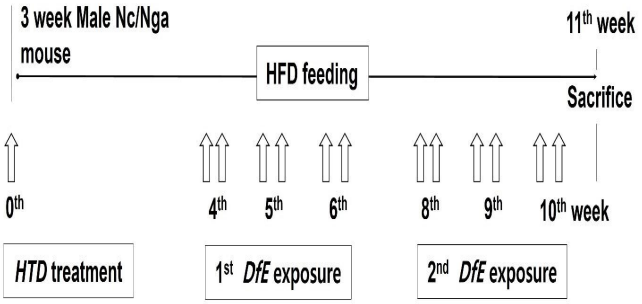

Fig. 1. Experiment design. Prior to induce AD, the herbal extracts for HTD treatment were orally administered to the CGT groups on the 1st, 2nd, and 3rd days. Mice (per group n=10) were exposed to DfE on the 4th, 5th, and 6th weeks at the first time. On the 8th, 9th, and 10th weeks, the mice were exposed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

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-μm thickness.

We conducted Masson's trichrome staining to investigate histological changes including epithelial hyperplasia, capillary distribution, and collagen fiber distribution of the tissue samples. Masson's trichrome staining was proceeded as follows: 1) The samples were fixed with Bouin's fluid at 50-60 °C for 1 hour; 2) Picric acid was removed by 70% ethanol; 3) The fixed samples were incubated in Weigert's iron hematoxylin solution for 10 minutes to stain the nuclei; 4) The collagen fiber was stained blue by Biebrich scarlet-acid fuchsin solution and phosphomolybdic-phosphotungstic acid for 15 minutes

each; 5) The samples were finally treated with aniline blue solution for 5 minutes.

Luna's staining was performed to investigate the distribution and morphological changes of mast cells activated by neuropeptides. We stained the granules of mast cells with aldehyde fuchsin solution for 30 minutes, followed by treatment with Weigert's iron hematoxylin solution for 10 minutes. Methyl orange solution was used for counterstaining for 5 minutes.

4. Immunohistochemistry

Skin tissue slices were soaked in 20 μg/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-high-affinity IgE receptor (FcεRI; 1:100), goat anti-substance P (1:100), goat anti-serotonin (1:200), goat anti-NF-κB p65 (1:500), goat anti-inducible nitric oxide synthase (iNOS; 1:200), and goat anti-vascular endothelial growth factors (VEGFs; 1:200) 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, 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 ± standard error (mean ± 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

Sensitization with DfE induced AD-like skin lesions on the dorsal skin of NC/Nga obese mice. However, HTD treatments showed palliative effects on AD symptoms. In the AE group, lesions were the most severe among the groups and showed various pathological features such as hemorrhage, erythema, erosion, edema, and dryness. In contrast, the CGT groups showed alleviation of symptoms compared with the AE group(Fig. 2).

An angiogram was used to compare AD-related angiogenesis among the groups. As shown in Fig. 2, DfE increased the angiogenesis on the dorsal skin of the mice, while HTD treatment reduced the development of blood vessels on the dorsal skin.

Masson's trichrome 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).

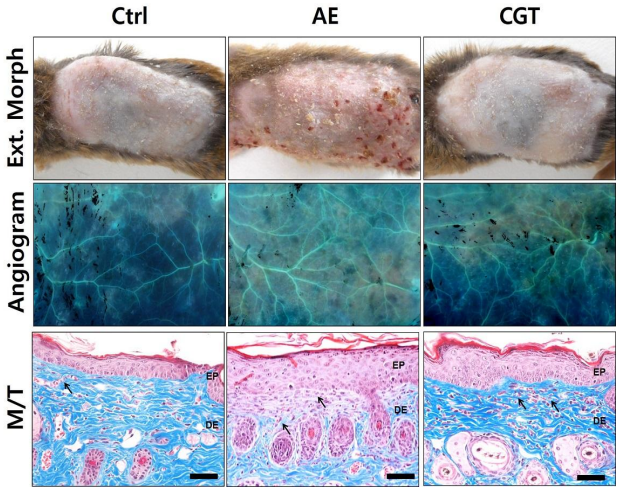

Fig. 2. Alleviating effect on symptoms of AD. HTD treatments treated to the CGT groups alleviated the characteristic lesion of AD. Although the distribution of capillary increased in the AE group, the CGT group (per group n=10) showed reduction of distribution (4x magnification). In the results of Masson's trichrome staining, spongiosis increased in the AE group but decreased in the CGT group (bar size: 50 μm). 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, Ext. Morph: external morphology, and M/T: Masson's trichrome staining.

2. Regulatory effect on mast cells

The Luna's staining results demonstrate that the HTD treatments affected the distribution of granulated mast cells. In the CGT group, there were many granulated mast cells from the dermal papilla to the subcutaneous layer. In contrast, the number of de-granulated mast cells in the AE group was relatively higher than that of the CGT group(Fig. 3).

The regulatory effect of HTD treatment on mast cell activation was observed by measuring positive reactions for FcεRI, substance P, and serotonin in the dermal papilla. HTD treatment remarkably reduced positive reactions for FcεRI, substance P, and serotonin compared with the AE group. In AE group, the level of FcεRI was 501,030 ± 4106 /20,000,000 pixel which was increased by 613% compared with that of the Ctrl group. Compared with the AE group, levels of FcεRI in the CGT group was 183,586 ± 2849 /20,000,000 pixel which was decreased by 63% ($p < 0.05$, Fig 3). For substance P, the level in the AE group was 225,430 ± 4790 /20,000,000 pixel which was increased by 957% compared with that of the Ctrl group. In contrast, levels of substance P in the CGT group was 64,263 ± 1121 /20,000,000 pixel which was decreased by 71% ($p < 0.05$), compared with that of the AE group (Fig. 3). For serotonin, the level in the AE group was 342,714 ± 6484 /20,000,000 pixel which was increased by 410% compared with that of the Ctrl group. In contrast, levels of serotonin in the CGT group was 97,626 ± 2656 /20,000,000 pixel which was decreased by 71% ($p < 0.05$), compared with that of the AE group(Fig. 3).

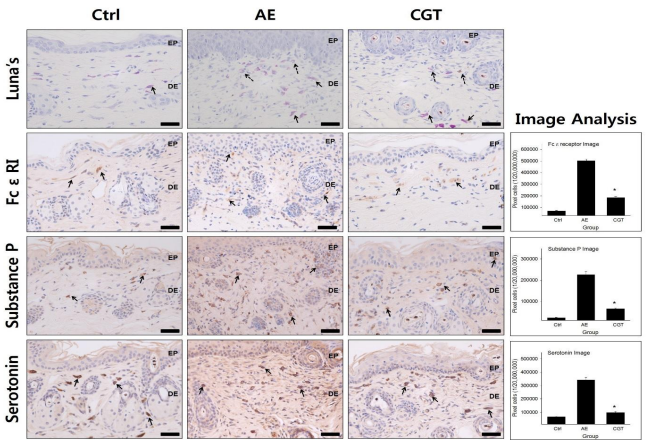

Fig. 3. Regulatory effect on mast cell. In the Luna's staining result, distribution of granulated mast cell (indicating arrows) increased in the AE group, but decreased in the CGT group (per group n=10) (bar size: 50 μm). Positive reactions for FcεRI, substance P, and serotonin in the CGT group compared with that of the AE group (bar size: 50 μm). Image analysis data for each neuropeptide 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, Luna's: Luna's staining, and #: $p < 0.05$ compared with the AE group.

3. Down-regulatory effect on inflammatory response

To investigate the anti-inflammatory effects of the HTD treatments, we measured the positive reactions for NF-κB p65, iNOS, and VEGFs in the cytoplasm of the stratum basale and dermal papilla by immunohistochemistry. In the results, after HTD treatments, inflammatory factors such as NF-κB p65, iNOS, and VEGFs were significantly decreased. The NF-κB p65 level in the AE group was 141,639 ± 1931 /20,000,000 pixel which was increased by 313% compared with that of the Ctrl group. In contrast, NF-κB levels in the CGT group was 88,480 ± 2151 /20,000,000 pixel which was significantly decreased by 38% ($p < 0.05$), compared with that of the AE group (Fig. 4). The iNOS level in the AE group was 124,163 ± 3684 /20,000,000 pixel which was increased by 1799% compared with that of the Ctrl group. However, HTD treatments also remarkably reduced iNOS levels in the CGT group by 49% ($p < 0.05$) compared with that of the AE group. The levels of iNOS was 63,721 ± 1912 /20,000,000 pixel in CGT group(Fig. 4).

The level of VEGFs in the AE group was 136,029 ± 3421 /20,000,000 pixel which was increased by 927%, compared with the Ctrl group. After both HTD treatments, the level of VEGFs in CGT group remarkably decreased. The VEGFs level of CGT group was 77,534 ± 1779 /20,000,000 pixel which was decreased by 43% ($p < 0.05$) compared with that of the AE group(Fig. 4).

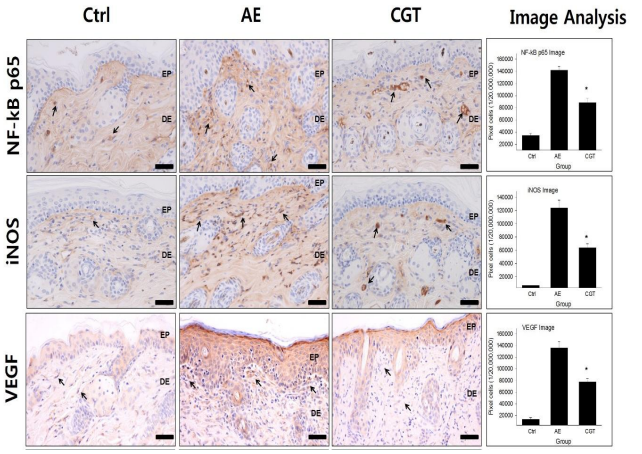

Fig. 4. Down-regulatory effect on inflammatory response. HTD treatments suppressed excessive inflammation by reducing NF-κB p65, iNOS, and VEGFs and promoting apoptosis. In the immunohistochemistry results, positive reactions for NF-κB p65, iNOS, and VEGFs (arrows indicating dark brown spot) remarkably decreased in the CGT group compared to those of the AE group (per group n=10) (bar size: 50 μm). Image analysis data for those results also exhibited 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.

Discussion

In this study, we confirmed the preventive treatment effect of both HTD treatments for AD using a DfE-induced obese NC/Nga mice. To confirm the AD symptoms relieving effect of HTD treatments, external morphology, angiogram, and Masson's trichrome staining. HTD treatments alleviated eczematous lesions, capillary angiogenesis, edema, and epithelial hyperplasia. Moreover, observation of mast cells activity, which plays an important role in induction of inflammation and in regulation of immune, using Luna staining confirmed that HTD treatment affects mast cell activity by reducing FcεRI, substance P, and serotonin levels. To investigate the effects of HTD treatment on anti-inflammatory responses, we examined levels of apoptosis and inflammatory cytokines. After HTD treatment, reduction of inflammatory cytokines including NF-κB p65 and iNOS and promotion of apoptosis were confirmed.

As mentioned earlier, obesity has a synergetic relationship with AD likely because excessive fat accumulation accelerates the inflammatory response. Excessive fat accumulation increases the overall burden of mast cells because the increase in adipose tissue is the source of mast cells[12,13]. Consequently, increased level of mast cell may lead to onset of inflammatory diseases such as AD[14]. In additions, hypertrophic adipocytes are characterized by permeation of macrophages and produce proinflammatory factors such as TNF-α, IL-6, monocyte chemotactic activating factor (MCP) -1, and plasminogen activator inhibitor (PAI) -1. These proinflammatory cytokines cause inflammation locally, and macrophages in adipocytes aggravate inflammations[15].

Thus, we tried to confirm the efficacy of treatment of HTD treatments on AD by observing the activities of mast cell and inflammatory cytokines 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 reactions16). Macroscopically, the both HTD treatments alleviated AD symptoms such as hemorrhage, erythema, erosion, edema, and dryness in obese NC/Nga mice. Furthermore, angiogram results showed that 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 cells[17]. Moreover, angiogenesis maintains inflammation by supplying oxygen and nutrients to cells in the inflammatory area[18]. Thus, this result suggests that the alleviation of AD-like symptoms may due to the anti-inflammatory activity of the HTD treatments.

2. Regulatory effect on mast cells

Mast cell is a major immune cell type in allergic diseases. Under allergic condition, antigen-specific IgE binds to FcεRI expressed on mast cells, and FcεRI activates mast cells by amplifying IgE signal. When mast cells are activated, various downstream pathways such as phosphoinositide 3-kinase (PI3K), extracellular signal-regulated kinase (ERK), Jun N-terminal kinases (JNK), NF-κB, and PKC are also activated and granules containing many proinflammatory mediators such as histamine, serotonin, TNFs, kinins (for example, substance P), and proteases are released[19,20]. If mast cells contact with activated T cells, MMP-9 and IL-6 are released from mast cells[21]. In our results, after HTD treatments, reductions of FcεRI, substance P, and serotonin levels are observed. Down-regulation of downstream mediators, including substance P, and serotonin suggest the inactivation of mast cells. Considering the importance of FcεRI in activating mast cells, the results suggest that HTD treatment may suppress FcεRI expression on the surfaces of mast cells. On the other hand, HTD treatments reduced skin edema, increased collagen fiber density, and suppressed epithelial hyperplasia according to the results of Masson's trichrome staining. Thus, reduction of edema is also the result of mast cell inactivation.

3. Down-regulatory effect on inflammatory response

NF-κB p65, iNOS, and VEGFs are closely associated with the inflammatory response and apoptosis. NF-κB p65, which is a transcription factor, regulates inflammation by controlling pro-inflammatory cytokines induced by TNF-α [22]. iNOS, which acutely synthesizes nitric oxide (NO), inhibits TNF-α-induced apoptosis by inhibiting caspase activation and mitochondrial dysfunction[23]. As mentioned earlier, VEGFs is considered to be a potent stimulator of angiogenesis and epidermal permeability in the skin[24]. Excess-production of VEGFs has been proven in AD lesional keratinocytes and the production of VEGFs in AD lesions was nearly 25 times greater than in normal SC[25]. High levels of VEGF-A is measured in skin of AD patients

and related to the disease activity of AD[26]. Because there is a correlation between VEGFs levels and the severity of AD, down-regulation of VEGFs production may be targeted as a new therapeutic strategy for AD[27].

Our previous study demonstrated that HTD treatment reduces inflammation by regulating Th2 differentiation[9]. In addition, we suggested that HTD treatment helps to maintain the epidermal lipid barrier[28]. Based on these considerations, we hypothesize that HTD treatment may attenuate AD symptoms by down-regulating the mast cell and inflammatory cytokines in the context of obesity, which is an aggravating factor in inflammation. In conclusion, the HTD treatments used in this study contributed to down-regulation of mast cell and inflammatory cytokines in AD-induced obese NC/Nga mice, leading to the alleviation of AD symptoms. Considering that the prevalence of obesity is increasing, our results may contribute to the identification of an alternative treatment for preventing inflammatory diseases.

Conclusions

Obesity can exacerbate inflammation in the context of inflammatory diseases. However, in this study, HTD treatments with CRGR were down-regulated mast cell activation and inflammatory cytokines, resulting in alleviation of tissue damage caused by excessive inflammation in AD-induced obese NC/Nga mice.

Acknowledgements

This study was supported by the Basic Science Research program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No. NRF-2016R1D1A1B03930474).

References

1. Kang KS. Nutritional Counseling for Obese Children with Obesity-Related Metabolic Abnormalities in Korea. Pediatr Gastroenterol Hepatol Nutr. 2017;20(2):71-8.
2. Kelishadi R, Roufarshbaf M, Soheili S, Payghambarzadeh F, Masjedi M. Association of Childhood Obesity and the Immune System: A Systematic Review of Reviews. Child Obes. 2017;13(4):332-46.
3. Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. J Endocrinol. 2014;222(3):R113-27.
4. Guttman-Yassky E, Krueger JG, Lebwohl MG. Systemic immune mechanisms in atopic dermatitis and psoriasis with implications for treatment. Exp Dermatol. 2018;27(4):409-17.
5. Silverberg JI, Becker L, Kwasny M, Menter A, Cordoro KM, Paller AS. Central obesity and high blood pressure in pediatric patients with atopic dermatitis. JAMA Dermatol. 2015;151(2):144-52.
6. Romieu I, Torrent M, Garcia-Esteban R, Ferrer C, Ribas-Fito N, Anto JM, et al. Maternal fish intake during pregnancy and atopy and asthma in infancy. Clin Exp Allergy. 2007;37(4):518-25.
7. Moon M, Huh E, Lee W, Song EJ, Hwang DS, Lee TH, et al. Coptidis Rhizoma Prevents Heat Stress-Induced Brain Damage and Cognitive Impairment in Mice. Nutrients. 2017;9(10):1057.
8. Kim BB, Kim JR, Kim JH, Kim YA, Park JS, Yeom MH, et al. 7,3',4'-Trihydroxyisoflavone Ameliorates the Development of Dermatophagoides farinae-Induced Atopic Dermatitis in NC/Nga Mice. Evid Based Complement Alternat Med. 2013;2013:636597.
9. Jung AR, Ahn SH, Park IS, Park SY, Jeong SI, Cheon JH, et al. Douchi (fermented Glycine max Merr.) alleviates atopic dermatitis-like skin lesions in NC/Nga mice by regulation of PKC and IL-4. BMC Complement Altern Med. 2016;16(1):416.
10. Cha HY, Ahn SH, Cheon JH, Park IS, Kim JT, Kim KB. Hataedock Treatment Has Preventive Therapeutic Effects in Atopic Dermatitis-Induced NC/Nga Mice under High-Fat Diet Conditions. Evid Based Complement Alternat Med. 2016;2016:1739760.
11. Proksch E, Folster-Holst R, Brautigam M, Sepehrmanesh M, Pfeiffer S, Jensen JM. Role of the epidermal barrier in atopic dermatitis. J Dtsch Dermatol Ges. 2009;7(10):899-910.
12. Liu J, Divoux A, Sun J, Zhang J, Clement K, Glickman JN, et al. Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat Med. 2009;15(8):940-5.
13. Poglio S, De Toni-Costes F, Arnaud E, Laharrague P, Espinosa E, Casteilla L, et al. Adipose tissue as a dedicated reservoir of functional mast cell progenitors. Stem Cells. 2010;28(11):2065-72.
14. Zhang J, Shi GP. Mast cells and metabolic syndrome. Biochim Biophys Acta. 2012;1822(1):14-20.
15. Shoelson SE, Herrero L, Naaz A. Obesity, Inflammation, and Insulin Resistance. Gastroenterology. 2007;132(6):2169-80.
16. Kanchongkittiphon W, Gaffin JM, Phipatanakul W. Child

with atopic dermatitis. Ann Allergy Asthma Immunol. 2015;114(1):6-11.
17. Benelli R, Lorusso G, Albini A, Noonan DM. Cytokines and chemokines as regulators of angiogenesis in health and disease. Curr Pharm Des. 2006;12(24):3101-15.
18. Charo IF, Taubman MB. Chemokines in the pathogenesis of vascular disease. Circ Res. 2004;95(9):858-66.
19. Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B, et al. Mast cells and inflammation. Biochim Biophys Acta. 2012;1822(1):21-33.
20. Suzuki H, Miura S, Liu YY, Tsuchiya M, Ishii H. Substance P induces degranulation of mast cells and leukocyte adhesion to venular endothelium. Peptides. 1995;16(8):1447-52.
21. Baram D, Vaday GG, Salamon P, Drucker I, Hershkoviz R, Mekori YA. Human Mast Cells Release Metalloproteinase-9 on Contact with Activated T Cells: Juxtacrine Regulation by TNF-alpha. J Immunol. 2001;167(7):4008-16.
22. Chen TL, Zhu GL, Wang JA, Zhang GD, Liu HF, Chen JR, et al. Protective effects of isorhamnetin on apoptosis and inflammation in TNF-alpha-induced HUVECs injury. Int J Clin Exp Pathol. 2015;8(3):2311-20.
23. Li J, Bombeck CA, Yang S, Kim YM, Billiar TR. Nitric oxide suppresses apoptosis via interrupting caspase activation and mitochondrial dysfunction in cultured hepatocytes. J Biol Chem. 1999;274(24):17325-33.
24. Chen L, Marble DJ, Agha R, Peterson JD, Becker RP, Jin T, et al. The progression of inflammation parallels the dermal angiogenesis in a keratin 14 IL-4-transgenic model of atopic dermatitis. Microcirculation. 2008;15(1):49-64.
25. Zhang Y, Matsuo H, Morita E. Increased production of vascular endothelial growth factor in the lesions of atopic dermatitis. Arch Dermatol Res. 2006;297(9):425-9.
26. Genovese A, Detoraki A, Granata F, Galdiero MR, Spadaro G, Marone G. Angiogenesis, lymphangiogenesis and atopic dermatitis. Chem Immunol Allergy. 2012;96:50-60.
27. Samochocki Z, Bogaczewicz J, Sysa-Jedrzejowska A, McCauliffe DP, Kontny E, Wozniacka A. Expression of vascular endothelial growth factor and other cytokines in atopic dermatitis, and correlation with clinical features. Int J Dermatol. 2016;55(3):e141-6.
28. Kim HY, Ahn SH, Yang IJ, Kim KB. Effect of Skin Lipid Barrier Formation on Hataedock Treatment with Douchi. J Korean Med. 2017;38(2):41-52.