

The effects of Baekho-tang on 1-Fluoro-2, 4-dinitrofluorobenzene-induced allergic contact dermatitis in BALB/c mice

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Abstract Allergic contact dermatitis (ACD) is a cutaneous inflammatory response that is characterized skin lesion, increased cytokines secretion and splenomegaly. Baekho-tang (BHT) has been clinically used for the treatment of heat-related illnesses in Korea. Recently, it was reported that has an effect on epidemics such as hepatitis B, pneumonia, epidemic cerebrospinal meningitis and Japanese encephalitis. However, the effects of BHT on ACD is not yet fully studied. Therefore, the aims of this study was to determine the anti-allergic effects of BHT on 2, 4-dinitrofluorobenzene (DNFB)-induced ACD mice by investigating histopathologic examination of DNFB-induced inflamed tissues. Also, cytokines secretion in serum was determined using multiplex assay. The topical repeated application of DNFB on the dorsal skin of BALB/c mice were manifested the typical ACD. Treatment of different dose of BHT (20 or 80 mg/kg) attenuated DNFB-induced inflamed tissues such as erythema and crust. In addition, there is a trend for decreased serum cytokines levels in the treatment of high dose of BHT. Our results recommend that the high dose of BHT enhances the DNFB-induced ACD in mice.

Keywords Baekho-tang · Anti-allergic effect · Allergic contact dermatitis · 1-Fluoro-2, 4-dinitrobenzene · DNFB

Introduction

Contact dermatitis (CD), divided into irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD), is defined as an inflammatory process affecting the skin (Usatine and Riojas 2010). ACD is the symptom of an allergic response induced by contact with a substance in industrialized countries (Kimber et al. 2002). Although ICD is a common forms of CD, ACD can be considered the most prevalent symptom of immunotoxicity in humans. The presence of the rash or skin lesion at the skin where is exposure by a substance is the first sign of ACD. Other symptoms are itching, skin redness or inflammation (Choi et al. 2013; Honda et al. 2013). ACD may related with the other allergic disease such as allergic rhinitis and asthma (Mortz et al. 2001). Well-known causes of ACD are nickel allergy, Balsam of Peru and chromium (Hausen 2001; Kimber et al. 2002). Recently, didecylidimethylammonium chloride (DDAC), an antimicrobial agent used as a preservative in household products, has been reported that one of causes of CD (Kwon et al. 2015). The strong hapten 2, 4-dinitrofluorobenzene (DNFB) is mediated by CD8⁺ cytotoxic T cells and downregulated by CD4⁺ T cells (Bonneville et al. 2007). Many studies have provided that various cytokines and chemokines are required on the regulation of the process in ACD (Gober and Gaspari 2008; Kondo and Sauder 1995).

Nowadays, numerous researches in reducing the risk of ACD have been steadily studied (Atarashi et al. 2008; De Vry et al. 2005; Letko et al. 1999; Reitamo et al. 2002; Wolff and Stuetz 2004; Worm 2002). However, because of the adverse effects of ACD treatment agents (Fisher 1995;

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Ingber 2002), there is a great demand for the progress of effective therapies for ACD without side effects.

Baekho-tang (BHT), composed by *gypsum*, *Anemarrhena asphodeloides* Bunge, *Glycyrrhiza uralensis* Fisch, and *Oryza sativa* L, is prescribed to treat the heat-related illnesses in Korea. Also, BHT has been used for epidemics including hepatitis B, pneumonia, epidemic cerebrospinal meningitis and Japanese encephalitis (Kim et al. 2015a). Currently, BHT was determined to have an effect on alloxan-induced diabetes and rheumatoid arthritis (Hwang et al. 1990; Ryu and Shin 1995).

The aims of this study, therefore, are to assess the effect of BHT on ACD in BALB/c mice. We examined the effects of different dose of BHT on DNFB-induced dorsal skin swelling and erythema. Using multiplex assay, the cytokines secretion levels: interleukin (IL)-5, 9, 13 and tumor necrosis factor- α (TNF- α) and eotaxin, were determined. The effect of BHT on weights in spleen tissues were assessed.

Materials and methods

Preparation of aqueous extract of BHT

BHT was purchased from the Kyung Hee Oriental Herbal Medicine Research Center (Seoul, Korea). BHT was extracted with distilled water (DW) for 3 h at 100 °C as described in literatures (Choi et al. 2015; Kim et al. 2015b; Shin et al. 2014). Filtration and evaporation were then conducted with a rotary vacuum evaporator (N-N series, EYELA, Japan) at 60 °C. Freeze drying was performed in order to acquire the BHT extract. BHT was diluted with phosphate buffer saline (PBS, Corning Inc., NY, USA) to an appropriate concentration. The compositions and dosage of BHT was provided in Table 1.

Animal

Female BALB/c mice (seven-week-old) were obtained from the Daehan Bio Link (Daehan Bio Link Co., Ltd., Eumseong-gun Chungcheonbuk-do, Korea). All animal studies were performed according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Kyung Hee University (confirmation number: KHUASP(SE)-14-046). Mice were housed in the animal room where maintains the condition of a 12 h light-dark cycle at moderate temperature

(21–23 °C) and humidity levels (55–60%). All mice were acclimated for one week before the experiment. During the acclimation and experiment period, all mice were fed with the standard rodent chow [28.507% of protein, 13.496% of fat and 57.996% of carbohydrates (LabDiet, MO, USA)] and water ad libitum. After acclimation period, eight-week-old mice were divided into 4 groups: naïve, DNFB treatment as a control, DNFB + low dose of BHT treatment or DNFB + high dose of BHT treatment group.

DNFB-induced ACD in mice

After acclimation period, all groups of dorsal skin of the mice were shaved and treated with a depilatory prior for the experiment. At the first week, mice were sensitized with 150 μ l of DNFB (0.15% v/v) in acetone:olive oil (AOO, ratio of 4:1) applied to the dorsal skin twice per a week. After a week for sensitization, the percentage of DNFB was changed to 0.2% and mice were applied 150 μ l of DNFB every other day for 5 weeks. Naïve group (naïve) was painted vehicle (acetone:olive oil, AOO, 4:1). When the experiment was reached at 5 week, BHT (20 mg/kg or 80 mg/kg) was orally administrated every day for 2 weeks until the end of the experiment (Fig. 1).

Measurement the degree of the skin lesion and weighing the body and spleen

On the last day of the experiment, all mice were sacrificed and the dorsal skins of each mouse were observed using a digital camera (Canon, Japan) in order to observe the overall degree of ACD. The body weight of each mouse was measured right before mice were killed. The spleen were collected and weighed using Ohaus Adventurer™ (Ohaus Co., NJ, USA). The weight index refers to the weight of a spleen (mg) divided by the total body weight (g).

Histopathological examination

After mice were sacrificed, the dorsal skin of each mouse was resected and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed to the sectioned dorsal skin for investigating histopathological data. H&E stained samples were observed and images were taken using an Olympus BX51 microscope equipped with a digital camera (DP71; Olympus Co., Japan) (Yao et al. 2015) (Hwang et al. 2015).

Blood sampling

Blood samples were collected via the retro-orbital plexus before mice were sacrificed. The collected blood samples were allowed to clot at room temperature for 45 min. After which,

Table 1 The compositions and dosage of Baekho-tang

Herbal medicine	Dosage
<i>Gypsum</i>	20 g
<i>Anemarrhena asphodeloides</i> Bunge	8 g
<i>Glycyrrhiza uralensis</i> Fisch	2.8 g
<i>Oryza sativa</i> L	1.2 g

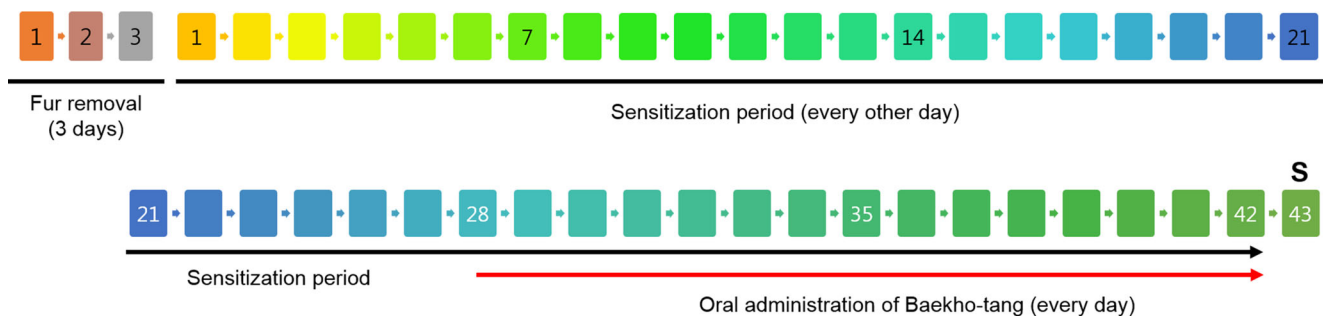


Fig. 1 Experimental design. The experimental groups were allowed to rest for three days just after the dorsal fur of each mouse was shaved. After three days, all groups, except the naïve group, were sensitized by painting with DNFB every other day for 6 weeks. The naïve group was

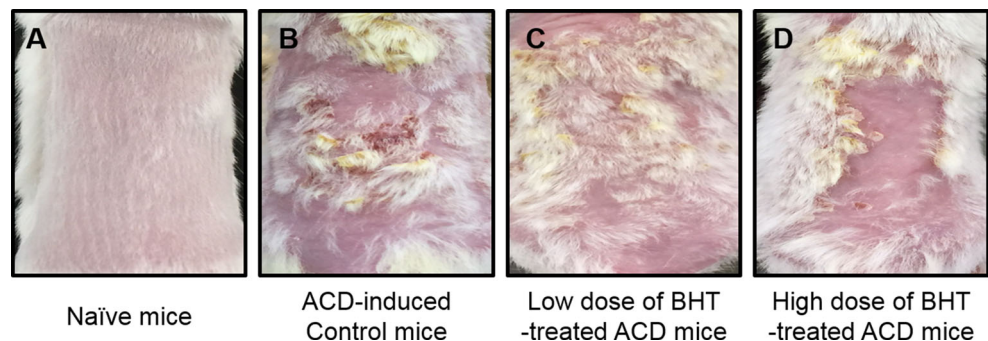
treated AOO in the same way. The BHT-treated group was orally administrated with BHT on the last 2 weeks. All mice were sacrificed on day 43

samples were centrifuged at $1000\times g$ for 15 min at 4 °C, the supernatant was transferred to a new tube. To completely remove platelets and precipitates, the transferred serum samples were centrifuged again at $10,000\times g$ for 10 min at 4 °C. Centrifuged samples were transferred to a new tube and stored at -80 °C before the experiment.

Measurement of cytokines concentration

Mouse Cytokine Group-1 23-plex panels involving IL-5, IL-9, IL-13, TNF- α , and Eotaxin multiplex assays (Bio-Rad, Hercules, CA, USA) were performed according to the manufacturer's guidelines. Briefly, an amounts of 10 μ l of each serum sample were diluted with 40 μ l of a sample dilution buffer (dilution factor 1:4). They were then transferred to separate wells which included mouse cytokine beads (IL-5, IL-9, IL-13, TNF- α , and Eotaxin). This was followed by shaking (850 ± 50) incubated for 30 min at RT. After 30 min, an amounts of 25 μ l of detection antibody mixed solution was added to each well and incubated for 30 min at room temperature. During the 30 min of incubation, streptavidin-Phycoerythrin (SA-PE) was diluted to an assay buffer (Bio-Rad, Hercules, CA, USA), after which an amount of 50 μ l of the SA-PE diluted solution was put into separate wells. This was followed by shaking incubation for 10 min at room temperature. Finally, 125 μ l of assay buffer was added to each well, with measurement by a Bio-Plex® MAGPIX™ Multiplex reader (Bio-Rad, Hercules, CA, USA). (Fan et al. 2016).

Fig. 2 Effects of BHT on skin lesions in ACD-induced mice. Skin conditions were observed using a digital camera: (a) naïve mice, (b) DNFB-treated ACD-induced mice, (c) low dose of BHT-treated ACD mice, and (d) high dose of BHT-treated ACD mice



Statistical analysis

Data were represented with the means $\pm$ SEM. The GraphPad Prism 5 software package (Graphpad Software, San Diego, CA, USA) was used to analyze the results statistically. The statistical significance of cytokines concentration and spleen weights graphs was measured by Mann-Whitney U test (one-tailed).

Results

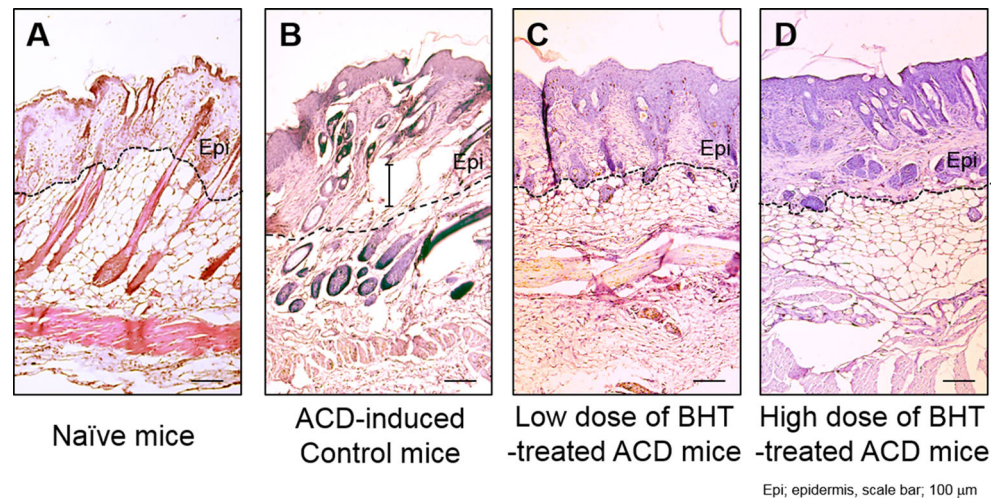
Effects of the BHT administration on the degree of skin lesions in ACD mice

The mice repeated painting with DNFB demonstrated skin lesions in AD such as erythema and crust (Fig. 2b). Twenty or eighty mg/kg of BHT-treated AD-induced mice demonstrated improved skin lesions compared to the ACD-induced control group (Fig. 2c, d).

Effects of the BHT administration on histopathological changes in ACD mice

No abnormal changes in skin tissue were observed in the native group (Fig. 3a). The epidermis in the control group represented the epithelial hyperplasia, and epidermis trauma DNFB-mediated inflammation (Fig. 3b). The BHT-treated

Fig. 3 Effects of BHT on histopathological changes in ACD mice. The dorsal tissues were stained with hematoxylin and eosin (H&E) after which tissues were observed using a light microscope: (a) naïve mice, (b) DNFB-treated ACD-induced mice, (c) low dose of BHT-treated ACD mice, and (d) high dose of BHT-treated ACD mice



group observed the decreased epithelial hyperplasia and moderated trauma in epidermis (Fig. 3c, d).

Effects of the BHT on concentration of inflammation-related cytokines in ACD-induced mice

To study the inflammation-related cytokines regulation effects of BHT, serum was isolated from the collected blood samples in each group. Serum interleukin (IL)-9 and -13 concentration levels were significantly reduced in high-dose BHT-treated mice compared to the ACD-induced control group (Fig. 4b,

c). There was no statistical difference between the groups treated with BHT and the ACD-induced control group, but there was a trend toward decreased serum IL-5 and tumor necrosis factor (TNF)- α concentration by low- and high-dose of BHT treatment (Fig. 4a, d).

Effects of BHT on the weights of the spleen / body weight ratio in ACD mice

In Fig. 4, the weights of the spleen / body weight ratio of each group are shown. The mice, repeated painting with DNFB

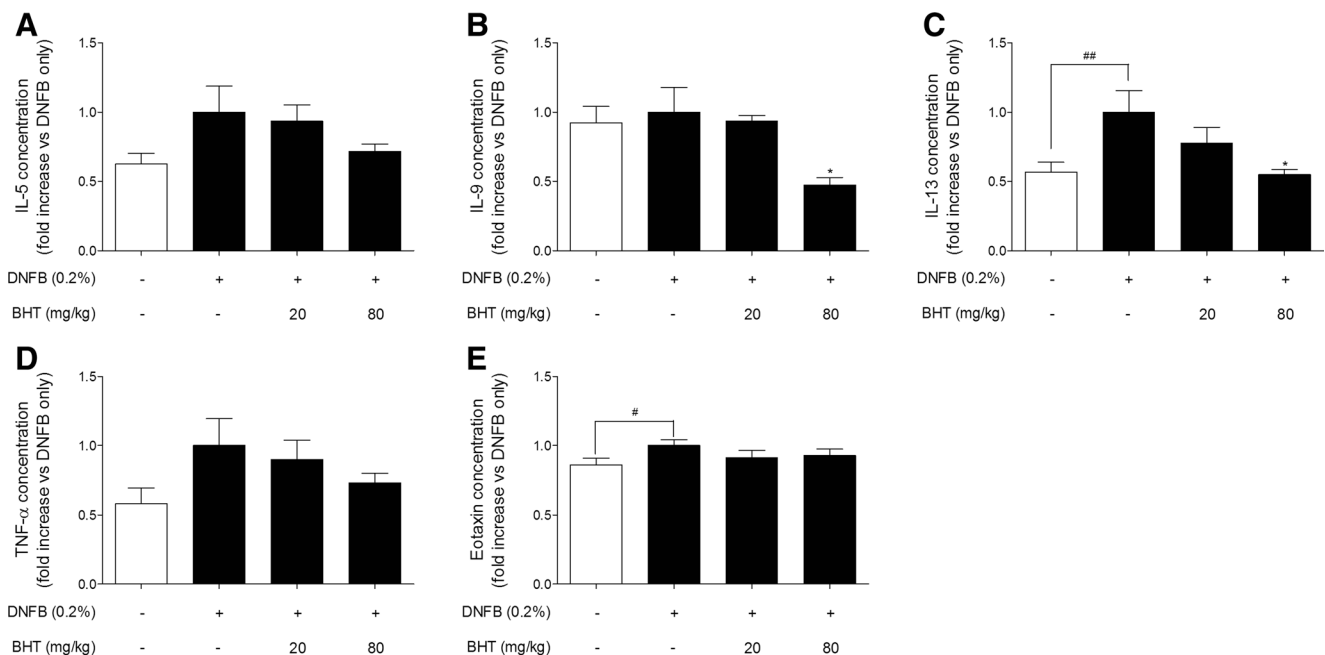


Fig. 4 Effects of BHT on inflammation-related cytokines regulation levels. Inflammation-related cytokines regulation levels by BHT were measured using a multiplex assays. (a) IL-5, (b) IL-9, (c) IL-13, (d) TNF- α and (e) eotaxin. IL-4 regulation levels were not detected. #,

$P < 0.05$; ##, $P < 0.01$; compared to the naïve group, *, $P < 0.05$; compared to the DNFB-treated group by Mann-Whitney U test (one tailed). The results are represented as means $\pm$ SEM. $n = 7$ –10 for each group

demonstrated an enlarged spleen compared to the naïve group (non-treated control group). The enlarged spleen was improved in low- and high-dose BHT-treated mice.

Discussion

ACD is the occupational skin diseases caused by an allergic reaction to material in contact with the skin (Kimber et al. 2002). Avoidance of the offending agent is the most effective etiologic treatment, however, the sensitizing allergens are common and difficult to avoid. The current drugs for ACD treatment such as corticosteroids usually tend to using repeatedly (Cohen and Heidary 2004). Despite the fact that their remedial efficacy, their clinical usage in cases of continuous application is limited at frequent intervals because of their serious side effects (Fisher 1995; Ingber 2002). Accordingly, it is necessary to develop the new drugs that minimize or alter usage of the corticosteroid. Recently, the herbal medicines has attracted the attention of its lower costs and more efficacy than corticosteroid. For examples, *Scrophularia buergeriana* (Kim et al. 2012b) and *Scrophora flavesces* Ation (Kim et al. 2012a) were determined to have anti-inflammatory effects in DNFB-induced ACD in mice.

In our animal model, the mice repeated application of DNFB were induced the epithelial hyperplasia and epidermis trauma in the dorsal skin of ACD mice (Fig. 3). Moreover, inflammation-related cytokines / chemokines including IL-5, 9, 13, TNF- α and eotaxin concentration levels tended to increased, IL-13 and eotaxin were significantly increased compared to the naïve group (repeated-AOO only treated mice, Fig. 4). The weight of spleen of ACD mice was also significantly increased (Fig. 5). These results imply that inflammatory reactions by repeated application of DNFB are believed to mimic the human ACD condition as well (Rose et al. 2012).

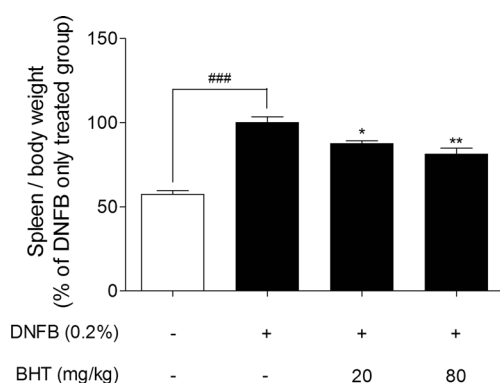


Fig. 5 Effects of BHT on spleen / body weight ratio in ACD mice. Spleen weights were measured on day 43. ###, $P < 0.001$; compared to the naïve group, *, $P < 0.05$; **, $P < 0.01$; compared to the DNFB-treated group by Mann-Whitney U test (one tailed). The results are represented as means $\pm$ SEM. $n = 7$ –10 for each group

In the present study, we investigated the anti-allergic effects of BHT on DNFB-induced ACD in mice. Daily oral administration of BHT extract effectively suppressed allergic reactions in the inflamed skin of ACD mice. Skin lesions, such as erythema and crust, were decreased by oral administration of BHT (Fig. 2). In the histopathological observation, the epidermal hyperplasia, also known as acanthosis that was related to inflammatory reactions with resident cells such as fibroblasts and keratinocytes (Kumar et al. 2005), was decreased by oral administration of BHT compared to the dorsal skin of DNFB-induced ACD in mice (Fig. 3). Moreover, epidermis trauma was diminished in the DNFB-induced ACD in mice, administrated BHT (Fig. 3). The skin lesions and histopathological data imply the use of BHT effectively prevented the inflammatory reactions in DNFB-induced ACD.

ACD mice induced by DNFB were reported that the secretion of cytokines / chemokines related to Th1 and Th2 were increased (Kitagaki et al. 1999; Kitagaki et al. 1997; Rose et al. 2012). Previous studies reported that the inhibition of pro-inflammatory cytokines such as TNF- α and IL-1 β might be useful for the treatment of ACD (Griffiths et al. 2005; Groves et al. 1995; Olmos et al. 2007; Watanabe et al. 2007). Cytokine and chemokine secreted by Th2 cells, IL-13 and eotaxin, were significantly stimulated in DNFB-treated group. Although there is no significant difference between naïve group and only DNFB-treated group, but there is a trend for increased serum IL-5, 9, and TNF- α . However, most of cytokines and chemokine in the high dose of BHT-administrated ACD mice were secreted similar to naïve group (Fig. 4). These results imply that the high dose of BHT plays as an anti-inflammatory agent, and may result in an improvement of skin lesions. The results of spleen / body weight ratio demonstrated that BHT effectively suppressed the immune response like cytokines data in ACD mice (Fig. 5).

In conclusion, BHT has significant improvement effects on immune response in DNFB-induced ACD in mice. The results of this study suggest that BHT may be a therapeutic agent for ACD patients through reducing skin lesions in inflamed tissues and suppressing cytokines regulation when more studies are demanded such as the immune response mechanism of BHT in vivo and in vitro.

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Compliance with ethical standards

Conflict of Interest The authors declare that there are no conflicts of interests.

Ethical Statement The research was carried out according to the rules governing the use of laboratory animals as acceptable internationally and the experimental protocol was approved by the Animal Ethics Committee, Kyung Hee University, Seoul, Korea (Registration number: KHUASP(SE)-14-046).

References

- Atarashi K, Kabashima K, Akiyama K, Tokura Y (2008) Skin application of the nonsteroidal anti-inflammatory drug ketoprofen downmodulates the antigen-presenting ability of Langerhans cells in mice. *Br J Dermatol* 159:306–313
- Bonneville M, Chavagnac C, Vocanson M, Rozières A, Benetiere J, Pernet I, Denis A, Nicolas JF, Hennino A (2007) Skin contact irritation conditions the development and severity of allergic contact dermatitis. *J Invest Dermatol* 127:1430–1435
- Choi EK, Cho YJ, Yang HJ, Kim KS, Lee IS, Jang JC, Kim KH, Bang JH, Kim Y, Kim SH, Cho YH, Yoon NY, Jang YP, Song MY, Jang HJ (2015) Coix seed extract attenuates the high-fat induced mouse obesity via PPAR gamma and C/EBP alpha a downregulation. *Mol Cell Toxicol* 11:213–221
- Choi JK, Oh HM, Lee S, Park JW, Khang D, Lee SW, Lee WS, Rho MC, Kim SH (2013) Oleanolic acid acetate inhibits atopic dermatitis and allergic contact dermatitis in a murine model. *Toxicol Appl Pharmacol* 269:72–80
- Cohen DE, Heidary N (2004) Treatment of irritant and allergic contact dermatitis. *Dermatol Ther* 17:334–340
- De Vry CG, Valdez M, Lazarov M, Muhr E, Buelow R, Fong T, Iyer S (2005) Topical application of a novel immunomodulatory peptide, RDP58, reduces skin inflammation in the phorbol ester-induced dermatitis model. *J Invest Dermatol* 125:473–481
- Fan W, Wang W, Zhang L, Qi L, Liu A (2016) Study on changes and mechanisms of cytokines for alloxan-induced hepatic injury by Cr3 + -treatment in mice. *Mol Cell Toxicol* 12:209–216
- Fisher DA (1995) Adverse effects of topical corticosteroid use. *West J Med* 162:123–126
- Gober MD, Gaspari AA (2008) Allergic contact dermatitis. *Curr Dir Autoimmun* 10:1–26
- Griffiths CE, Dearman RJ, Cumberbatch M, Kimber I (2005) Cytokines and Langerhans cell mobilisation in mouse and man. *Cytokine* 32:67–70
- Groves RW, Allen MH, Ross EL, Barker JN, MacDonald DM (1995) Tumour necrosis factor alpha is pro-inflammatory in normal human skin and modulates cutaneous adhesion molecule expression. *Br J Dermatol* 132:345–352
- Hausen BM (2001) Contact allergy to balsam of Peru. II. Patch test results in 102 patients with selected balsam of Peru constituents. *Am J Contact Dermat* 12:93–102
- Honda T, Egawa G, Grabbe S, Kabashima K (2013) Update of immune events in the murine contact hypersensitivity model: toward the understanding of allergic contact dermatitis. *J Invest Dermatol* 133:303–315
- Hwang S, Son L, Kim W (1990) A study for the effect of Bekhotang and Insambekhotang on the alloxan-diabetic rats. *J Eastern Physiology* 5: 31–48
- Hwang SM, Park S-M, Jeong J-S, Moon K-S, Kim Y-B, Yoon S, Oh J-H (2015) Comparative gene expression analysis in the liver, kidney and blood vessels during renal injury after repeated exposure to tacrolimus in Sprague-Dawley rats. *BioChip J* 9:202–214
- Ingber A (2002) What's new in contact dermatitis: allergy to topical steroids in Israeli patients with contact dermatitis. *Isr Med Assoc J* 4:867
- Kim H, Lee MR, Lee GS, An WG, Cho SI (2012a) Effect of *Sophora flavescens* Aiton extract on degranulation of mast cells and contact dermatitis induced by dinitrofluorobenzene in mice. *J Ethnopharmacol* 142:253–258
- Kim J, Yoo WJ, Kim NI (2015b) Medical historical Observations about Baekhotang. *Kyung Hee Univ*
- Kim JK, Kim YH, Lee HH, Lim SS, Park KW (2012b) Effect of *Scrophularia buergeriana* extract on the degranulation of mast cells and ear swelling induced by dinitrofluorobenzene in mice. *Inflammation* 35:183–191
- Kim KW, Lee IS, Lee WJ, Park J, Chung WS, Cho JH, Lee SL, Jang HJ, Chung SH (2015a) Osteogenic differentiation of human mesenchymal stem cells promoted by the crude extracts of the mixture of cortex mori radices, *Patrinia saniculaefolia*. *Mol Cell Toxicol* 11:475–482
- Kimber I, Basketter DA, Gerberick GF, Dearman RJ (2002) Allergic contact dermatitis. *Int Immunopharmacol* 2:201–211
- Kitagaki H, Kimishima M, Teraki Y, Hayakawa J, Hayakawa K, Fujisawa S, Shiohara T (1999) Distinct in vivo and in vitro cytokine profiles of draining lymph node cells in acute and chronic phases of contact hypersensitivity: importance of a type 2 cytokine-rich cutaneous milieu for the development of an early-type response in the chronic phase. *J Immunol* 163:1265–1273
- Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T (1997) Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. *J Immunol* 159:2484–2491
- Kondo S, Sauder DN (1995) Epidermal cytokines in allergic contact dermatitis. *J Am Acad Dermatol* 33:786–800
- Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS (2005) Robbins and Cotran pathologic basis of disease, 7th edn. Elsevier Saunders, Philadelphia
- Kwon DY, Kim E, Kwon JT, Lee DH, Park SY, Kim HM, Kim P, Choi K (2015) Ethylene glycol potentiated didecyltrimethylammonium chloride toxicity in human bronchial epithelial cells. *Mol Cell Toxicol* 11:161–166
- Letko E, Bhol K, Pinar V, Foster CS, Ahmed AR (1999) Tacrolimus (FK 506). *Ann Allergy Asthma Immunol* 83:179–189 quiz 189–190
- Mortz CG, Lauritsen JM, Bindsvlev-Jensen C, Andersen KE (2001) Prevalence of atopic dermatitis, asthma, allergic rhinitis, and hand and contact dermatitis in adolescents. The Odense adolescence cohort study on atopic diseases and dermatitis. *Br J Dermatol* 144:523–532
- Olmos A, Giner RM, Recio MC, Rios JL, Cerdá-Nicolas JM, Manez S (2007) Effects of plant alkylphenols on cytokine production, tyrosine nitration and inflammatory damage in the efferent phase of contact hypersensitivity. *Br J Pharmacol* 152:366–373
- Reitamo S, Remitz A, Kyllonen H, Saarikko J (2002) Topical noncorticosteroid immunomodulation in the treatment of atopic dermatitis. *Am J Clin Dermatol* 3:381–388
- Rose L, Schneider C, Stock C, Zollner TM, Docke WD (2012) Extended DNFB-induced contact hypersensitivity models display characteristics of chronic inflammatory dermatoses. *Exp Dermatol* 21:25–31
- Ryu KJ, Shin HD (1995) Effects of Baekhotang and Baekbokkakyujitang on Rheumatoid Arthritis and IL-8 of Human Monocyte Kyung Hee Univ
- Shin MH, Park YJ, Kim KS, Cho DH, Uh IJ, Kim KH, Ha IJ, Chung WS, Jung HJ, Jung SK, Jang HJ (2014) The anti-inflammatory effects of *Alisma herb* extract on allergic asthma mouse model. *Mol Cell Toxicol* 10:197–206
- Usatine RP, Riojas M (2010) Diagnosis and management of contact dermatitis. *Am Fam Physician* 82:249–255
- Watanabe H, Gaide O, Petrilli V, Martinon F, Contassot E, Roques S, Kummer JA, Tschopp J, French LE (2007) Activation of the IL-1beta-processing inflammasome is involved in contact hypersensitivity. *J Invest Dermatol* 127:1956–1963
- Wolff K, Stuetz A (2004) Pimecrolimus for the treatment of inflammatory skin disease. *Expert Opin Pharmacother* 5:643–655
- Worm M (2002) Novel therapies for atopic eczema. *Curr Opin Investig Drugs* 3:1596–1603
- Yao R, He Y, Zeng Z, Liang Z, Cao Y (2015) Protective effect of adiponectin on paraquat-induced pulmonary fibrosis in mice. *Mol Cell Toxicol* 11:247–255