

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/340033367>

Effect of dexamethasone injection into Zusanli (ST 36) acupoint on ovalbumin-induced allergic rhinitis

Article · June 2019

CITATIONS

5

READS

43

8 authors, including:



Na-Ra Han

Kyung Hee University

82 PUBLICATIONS 1,092 CITATIONS

[SEE PROFILE](#)



Phil-Dong Moon

Kyung Hee University

115 PUBLICATIONS 2,040 CITATIONS

[SEE PROFILE](#)

RESEARCH ARTICLE

Effect of dexamethasone injection into Zusanli (ST 36) acupoint on ovalbumin-induced allergic rhinitis

Na-Ra Han, Seung-Jun Han, Phil-Dong Moon, Sungwei Hong, Hyeongjin Kim, Yanhua Li, Hyung-Min Kim, Hyun-Ja Jeong

Na-Ra Han, Phil-Dong Moon, Hyung-Min Kim, Department of Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul 02447, Republic of Korea

Seung-Jun Han, Department of Korean Medicine, Graduate School of Wonkwang University, Iksan 54538, Republic of Korea

Sungwei Hong, Hyeongjin Kim, Department of Pharmacology, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea

Yanhua Li, Department of Clinical Korean Medicine, Graduate School, Kyung Hee University, Seoul 02447, Republic of Korea

Hyun-Ja Jeong, Department of Food Science & Technology and Research Institute for Basic Science, Hoseo University, Chungnam 31499, Republic of Korea

Supported by Basic Science Research Program through the National Research Foundation of Korea Funded by the Ministry of Education (2016R1D1A1B03931625)

Correspondence to: Prof. Hyung-Min Kim, Department of Pharmacology, College of Korean Medicine, Kyung Hee University, Seoul 02447, Republic of Korea. hmkim@khu.ac.kr;

Prof. Hyun-Ja Jeong, Department of Food Science & Technology and Research Institute for Basic Science, Hoseo University, Chungnam 31499, Republic of Korea. hjj-eong@hoseo.edu

Telephone: +82-2-961-9448; +82-41-540-9681

Accepted: July 23, 2018

Abstract

OBJECTIVE: To investigate the effects of acupuncture with dexamethasone (A. Dex) on allergic rhinitis (AR) by injecting dexamethasone into the Zusanli (ST 36) acupoint.

METHODS: Thirty 6-week-old female BALB/c mice were sensitized on days 1, 5, and 14 by intraperitoneal

injection of 100 μ g of ovalbumin (OVA). The mice were then randomly divided into six groups ($n = 5$ in each group). Five groups were sensitized intranasally with 2 μ L of 1.5 mg of OVA for 10 consecutive days, while one group was sensitized intranasally with PBS in a similar manner as a negative control group. One hour before each administration of intranasal OVA, two groups were orally administered either a control vehicle (distilled water; AR control group) or 200 μ g/kg Dex (O. Dex group), while three groups received A. Dex at Zusanli (ST 36) with Dex concentrations of 2, 20, and 200 μ g/kg for each group, respectively. AR symptoms were evaluated by measuring the rubbing score, which comprised the number of nose, ear, and eye rubs that occurred in the initial 10 min after OVA intranasal provocation on the 10th day. We isolated spleen, serum, and nasal mucosal tissue after measuring the rubbing score. Spleen weight was measured using an electronic microbalance. The levels of IgE, thymic stromal lymphopoietin, tumor necrosis factor- α , intercellular adhesion molecule-1, and macrophage-inflammatory protein-2 in serum or nasal mucosal tissue were measured using enzyme-linked immunosorbent assays. The serum histamine levels of OVA-sensitized AR mice were measured using O-phthaldialdehyde spectrofluorometry. Western blot analysis was performed on nasal mucosal tissue extracts.

RESULTS: A. Dex significantly reduced the rubbing score, spleen weight, serum IgE, and serum histamine in OVA-sensitized mice. A. Dex significantly decreased the serum levels of inflammatory cytokines (thymic stromal lymphopoietin and tumor necrosis factor- α) in OVA-sensitized mice. A. Dex sig-

nificantly reduced the nasal mucosal levels of inflammatory markers (intercellular adhesion molecule-1 and macrophage-inflammatory protein-2) in AR mice. A. Dex effectively attenuated the expression of caspase-1 and receptor interacting protein-2 in nasal mucosal tissue.

CONCLUSION: A. Dex may be a new and useful therapy for AR.

© 2019 JTCM. All rights reserved.

Keywords: Dexamethasone; Point ST36 (Zusanli); Pharmaceutical research; Acupuncture; Rhinitis, allergic; Ovalbumin; Thymic stromal lymphopoietin

INTRODUCTION

Allergic rhinitis (AR) is a common inflammatory disease that affects the nasal mucosa.¹ AR includes symptoms such as nasal congestion, nasal itching, sneezing, and rhinorrhea, which can reduce quality of life and involve considerable costs to society.² These clinical manifestations result from the release of histamine and other active substances from mast cells, which stimulate blood vessel dilation, irritation of nerve endings, and tear secretion.³ AR involves inflammatory IgE-mediated responses, activation of mast cells and eosinophils, and release of inflammatory mediators, such as thymic stromal lymphopoietin (TSLP) and tumor necrosis factor- α (TNF- α).⁴⁻⁶ Inflammatory cell infiltration is induced by the expression of intercellular adhesion molecule-1 (ICAM-1) in mucosa during persistent inflammation.⁷ Macrophage-inflammatory protein-2 (MIP-2) is a potent chemoattractant for immune cells.⁸ ICAM-1 and MIP-2 are considered to be critical markers of allergic inflammation.^{7,8}

Traditional Chinese Medicine (TCM) therapy is a popular treatment for patients with allergic diseases. Acupuncture is a therapeutic TCM modality in which fine needles are used to pierce specific acupoints, and this method has improved clinical results in patients with AR.⁹ Pharmaceutical acupuncture or herbal acupuncture (a combination of acupuncture and herbal therapies) is a new acupuncture therapy in TCM.¹⁰ Pharmaceutical acupuncture is reportedly much more effective than acupuncture alone, and its effect varies with the herb used.¹¹

One of the most commonly used acupoints is Zusanli (ST 36).¹² Zusanli (ST 36) is located on the stomach meridian, and is the main point for regulation of gastrointestinal function, promotion of detoxification, and protection of mucosal barriers.¹³ Moreover, the anti-inflammatory and anti-allergic properties of Zusanli (ST 36) intervention have been recently reported.^{14,15}

Various medical treatments are available for AR, including oral decongestants, anti-histamine drugs, mast cell stabilizers, and corticosteroids.¹⁶ Corticosteroids are the most effective AR treatment.¹⁷ Dexamethasone (Dex) is a synthetic glucocorticosteroid that has anti-inflammatory and immunosuppressant properties.¹⁸ As Dex has a glucocorticoid effect that is 25 times more potent than cortisol, Dex has been widely used in treating AR.¹⁹ We hypothesized that using a relatively novel acupuncture method of injecting small amounts of medicine into Zusanli (ST 36) could benefit patients with AR, especially those who experience side effects from oral Dex or cannot take oral Dex.

In the present study, Dex was injected into the Zusanli (ST 36) acupoint of mice with ovalbumin (OVA)-induced AR to investigate the effects of acupuncture with Dex (A. Dex) on AR.

MATERIALS AND METHODS

Materials

Dex, OVA, aluminum hydroxide, avidin peroxidase, bicinchoninic acid, and O-phthalaldehyde were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-mouse IgE/TNF- α antibodies were purchased from BD Biosciences Pharmingen (San Diego, CA, USA). Anti-mouse TSLP/ICAM-1/MIP-2 antibodies were purchased from R & D Systems (Minneapolis, MN, USA). Antibodies for caspase-1, receptor interacting protein 2 (RIP-2), and actin were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

The ovalbumin-induced allergic rhinitis animal model

All procedures involving experimental animals were approved by the animal care committee of Kyung Hee University [approval No. KHUASP (SE)-15-118]. We maintained 6-week-old female BALB/c mice (Charles River Laboratories, Inc., Wilmington, MA, USA) under pathogen-free conditions. After 1 week of acclimatization, the 30 mice were randomly assigned to six groups ($n = 5$ in each group). The mice were sensitized on days 1, 5, and 14 by intraperitoneal injection of 100 μ g of OVA emulsified with 20 mg of aluminum hydroxide in 100 μ L of phosphate-buffered saline (PBS). Five groups were then sensitized intranasally with 2 μ L of 1.5 mg of OVA once daily for 10 consecutive days,⁴ while one group was sensitized intranasally with PBS in a similar manner as a negative control group (normal mice). One hour before each administration of intranasal OVA, two groups were orally administered either a control vehicle (distilled water; AR control group) or 200 μ g/kg Dex (O. Dex group), while three groups received A. Dex (2, 20, and 200 μ g/kg) at Zusanli (ST 36). The location of Zusanli (ST 36) was defined as 2 mm lateral to the anterior tubercle of the tibia and 3 mm below the knee joint.²⁰ AR symptoms were evaluated by measuring the rubbing score, which

comprised the number of nose, ear, and eye rubs that occurred in the initial 10 min after intranasal provocation on the 10th day. The total numbers of rubs were assessed on video recordings.

Spleen weight

Spleen weight was analyzed using an electronic microbalance, as spleen enlargement usually indicates the presence of an immune response.²¹

Enzyme-linked immunosorbent assay

Pro-inflammatory cytokines are critical factors in the pathogenesis of AR.²² Thus, we investigated the regulatory effect of A. Dex on the serum inflammatory cytokines levels of AR mice. ICAM-1 and MIP-2 are chemotactic molecules for inflammatory cells.²³ Hence, we examined the regulatory effect of A. Dex on the levels of ICAM-1 and MIP-2 in the nasal mucosal tissues of AR mice. The IgE, TSLP, TNF- α , ICAM-1, and MIP-2 levels in serum or nasal mucosal tissue were measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BD Biosciences Pharmingen; R & D Systems). The absorbance was measured using an ELISA reader at 405 nm. The cytokine levels in nasal mucosa were presented as the value divided by the total protein concentration. The protein concentrations were estimated using a bicinchoninic acid protein assay.

Histamine assay

The serum histamine levels of OVA-sensitized AR mice were measured by the O-phthalaldehyde spectrofluorometric method. A spectrofluorometer was used to analyze the fluorescent intensities at 440 nm (excitation at 360 nm).

Western blot analysis

Caspase-1 plays a very important role in inflammatory responses,²⁴ while RIP-2 interacts with caspase-1 and is involved in immune response signaling.²⁵ Thus, the cas-

pase-1 and RIP-2 levels in the nasal mucosal tissue of AR mice were analyzed using Western blot analysis. The nasal mucosal tissue extracts were heated at 95 °C for 5 min, and then cooled on ice. After centrifugation, 50 μ g aliquots were resolved by 12% sodium dodecyl sulphate-polyacrylamide gelelectrophoresis. The resolved proteins were electrophoretically transferred to nitrocellulose membrane that had been presoaked with transfer buffer (25 Mm Tris, pH 8.5, 200 mM glycerol, and 20% methanol). The blots were blocked in blocking buffer (PBS containing 0.05% Tween 20 and 5% nonfat dry milk) for at least 2 h, and then incubated with primary caspase-1 and RIP-2 antibodies for 1 h at room temperature. Blots were developed by peroxidase-conjugated secondary antibodies, and detected by enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA).

Statistical analysis

Statistical analyses were performed by one-way analysis of variance *via* Tukey's multiple range tests using SPSS statistical software (SPSS 11.5; SPSS Inc., Chicago, IL, USA). Experimental values represent the mean $\pm$ standard error of mean (SEM). $P < 0.05$ was taken to indicate significance.

RESULTS

Rubbing score

Dex did not affect the rubbing score on the 3rd day after the OVA intranasal provocation (Figure 1A). However, the rubbing score in OVA-sensitized mice was significantly higher than that in PBS-sensitized mice on the 10th day of intranasal provocation (Figure 1A; $P = 0.000$). This elevation of the rubbing score was significantly inhibited by A. Dex ($P = 0.000$) or O. Dex ($P = 0.025$; Figure 1A). The mean rubbing score of A. Dex-treated AR mice was significantly lower than that of O. Dex-treated AR mice (Figure 1A; $P = 0.008$).

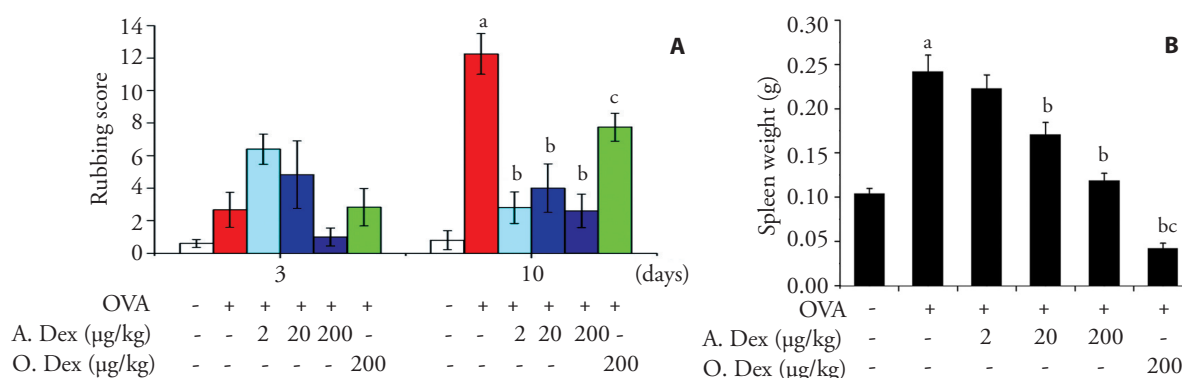


Figure 1 Effect of A. Dex on the rubbing score in AR mice

A: the rub number of nose, eyes, and ears was measured after intranasal provocation with OVA. White: OVA-unsensitized mice; Red: OVA-sensitized mice; Cyan: OVA-sensitized and A. Dex (2 μ g/kg)-administered mice; Blue: OVA-sensitized and A. Dex (20 μ g/kg)-administered mice; Yellow: OVA-sensitized and A. Dex (200 μ g/kg)-administered mice; Green: OVA-sensitized and O. Dex (200 μ g/kg)-administered mice. B: spleen weight was measured after the last OVA intranasal provocation. ^a $P < 0.05$ compared with mice not sensitized with OVA; ^b $P < 0.05$ compared with OVA-sensitized mice; ^c $P < 0.05$ compared with OVA-sensitized and A. Dex-administered mice. $n = 5$ mice in each group. OVA: ovalbumin; A. Dex: pharmaceutical acupuncture with dexamethasone; O. Dex: oral dexamethasone. Mice were administered A. Dex or O. Dex before intranasal OVA sensitization for 10 d.

Spleen weight

The mean spleen weights after OVA sensitization in the five OVA-sensitized groups were significantly higher than that in the PBS-sensitized mice (Figure 1B; $P = 0.000$). This increase in spleen weight was inhibited by treatment with A. Dex (20 $\mu\text{g/kg}$, $P = 0.003$; 200 $\mu\text{g/kg}$, $P = 0.000$; Figure 1B). The mean spleen weight of O. Dex-treated AR mice was significantly lower than that of A. Dex-treated AR mice (Figure 1B; $P = 0.000$).

Serum biomarkers

As shown in Figure 2A, the serum IgE levels were significantly higher in AR mice than in PBS-sensitized mice ($P = 0.000$). The increased serum IgE levels in AR mice were significantly reduced by A. Dex ($P = 0.000$) or O. Dex ($P = 0.000$). The serum histamine levels were also significantly decreased by A. Dex (20 $\mu\text{g/kg}$, $P = 0.006$; 200 $\mu\text{g/kg}$, $P = 0.000$) and O. Dex (Figure 2B; $P = 0.000$). A. Dex ($P = 0.000$) and O. Dex ($P = 0.000$) significantly suppressed the serum levels of TSLP in AR mice (Figure 2C). A. Dex ($P = 0.000$) and O. Dex ($P = 0.000$) significantly suppressed the serum levels of TNF- α in AR mice (Figure 2D). The serum levels of TNF- α of O. Dex-treated AR mice was significantly lower than those of A. Dex-treated AR mice (Figure 2D; $P = 0.042$).

Inflammatory cytokines and chemokines levels in nasal mucosal tissue

The protein levels of TSLP ($P = 0.000$) and TNF- α

($P = 0.049$) in the nasal mucosal tissues of AR mice were significantly increased compared with those of the normal mice (Figure 3A, 3B). The levels of TSLP that were upregulated by OVA sensitization were significantly downregulated by A. Dex (20 $\mu\text{g/kg}$, $P = 0.000$; 200 $\mu\text{g/kg}$, $P = 0.000$; Figure 3A). The levels of TNF- α that were upregulated by OVA sensitization were significantly downregulated by A. Dex (200 $\mu\text{g/kg}$, $P = 0.010$; Figure 3B). The ICAM-1 levels that were increased by OVA sensitization were significantly downregulated by A. Dex ($P = 0.000$; Figure 3C). The MIP-2 levels that were increased by OVA sensitization were significantly downregulated by A. Dex ($P = 0.000$; Figure 3D). O. Dex also significantly decreased the levels of TSLP ($P = 0.000$), TNF- α ($P = 0.012$), ICAM-1 ($P = 0.000$), and MIP-2 ($P = 0.000$) in the nasal mucosal tissue (Figure 3). The serum levels of ICAM-1 of O. Dex-treated AR mice was significantly lower than those of A. Dex-treated AR mice ($P = 0.006$; Figure 3D).

Caspase-1/RIP-2 expression in nasal mucosal tissue

As shown in Figure 4, A. Dex inhibited the OVA-induced expression of caspase-1 and RIP-2 in the nasal mucosal tissue. O. Dex also suppressed the expression of caspase-1 and RIP-2 in the nasal mucosal tissue. O. Dex had a greater regulatory effect on caspase-1 expression than A. Dex (Figure 4).

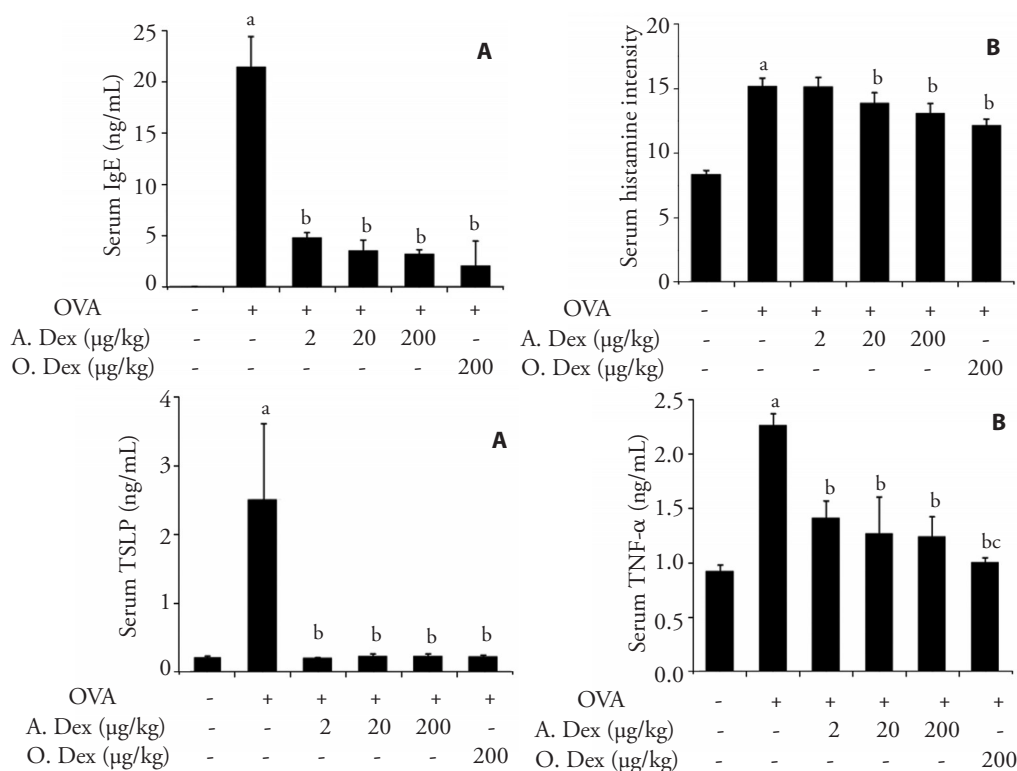


Figure 2 Effect of A. Dex on serum biomarkers in AR mice

A: serum IgE levels were measured by enzyme-linked immunosorbent assay. B: serum histamine levels were measured by histamine assay as described in the Methods section. C, D: serum TSLP and TNF- α levels were measured by ELISA. * $P < 0.05$, compared with mice not sensitized with OVA; ^b $P < 0.05$, compared with OVA-sensitized mice; ^c $P < 0.05$, compared with OVA-sensitized and A. Dex-administered mice. $n = 5$ mice in each group. OVA: ovalbumin; A. Dex: pharmaceutical acupuncture with dexamethasone; O. Dex: oral dexamethasone; TSLP: thymic stromal lymphopoietin; TNF- α : tumor necrosis factor- α ; ELISA: enzyme-linked immunosorbent assay. Mice were administered A. Dex or O. Dex before intranasal OVA sensitization for 10 d.

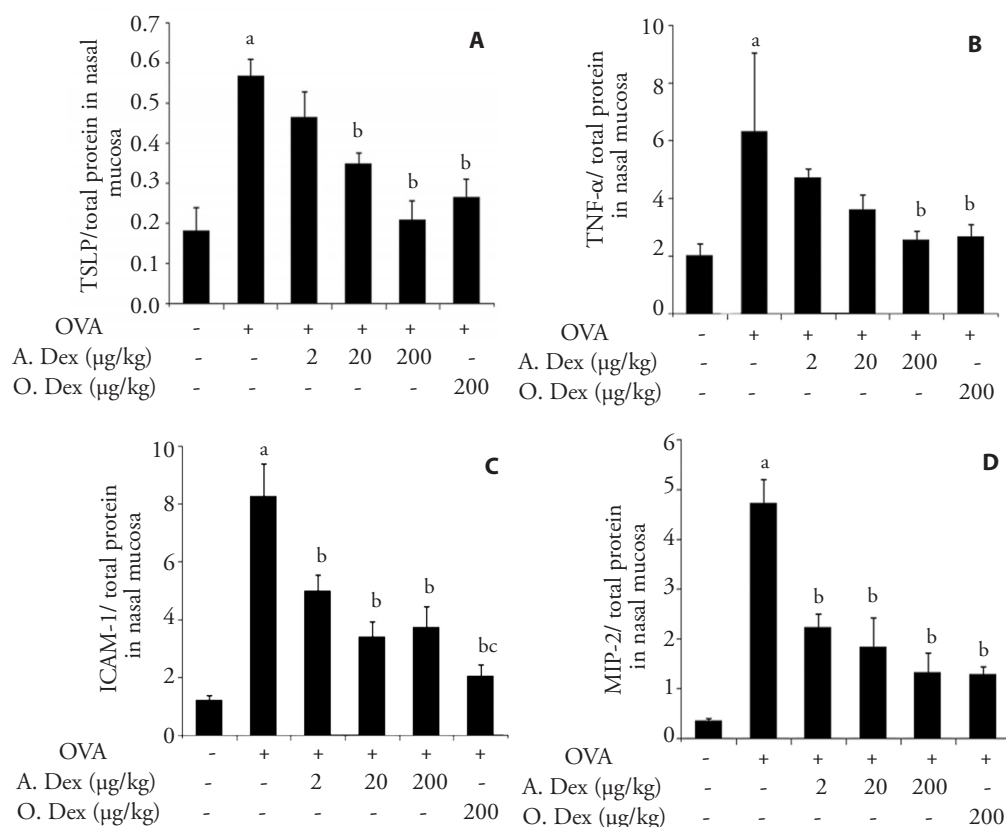


Figure 3 Effects of A. Dex on inflammatory cytokines and chemokines levels in the nasal mucosal tissues of AR mice

A: thymic stromal lymphopoietin (TSLP); B: tumor necrosis factor- α (TNF- α); C: intercellular adhesion molecule-1 (ICAM-1); D: macrophage-inflammatory protein-2 (MIP-2) levels in the nasal mucosal tissue homogenate were measured by ELISA. All parameters are shown as a ratio in accordance with total protein levels in the nasal mucosal tissue. ^a $P < 0.05$, compared with mice not sensitized with OVA; ^b $P < 0.05$, compared with OVA-sensitized mice; ^c $P < 0.05$, compared with OVA-sensitized and A. Dex-administered mice. $n = 5$ mice in each group. A. Dex: pharmaceutical acupuncture with dexamethasone; O. Dex: oral dexamethasone. Mice were administered A. Dex or O. Dex before intranasal OVA sensitization for 10 d.

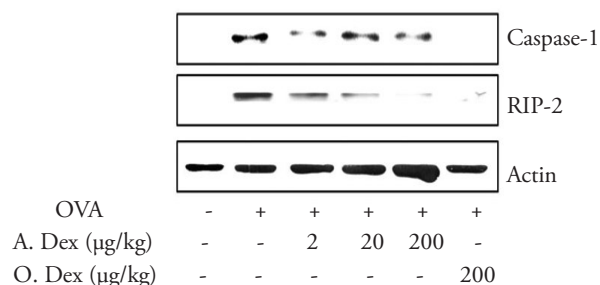


Figure 4 Effect of A. Dex on caspase-1/RIP-2 expression in the nasal mucosal tissues of AR mice

Mice were administered A. Dex or O. Dex before intranasal OVA sensitization for 10 d. Caspase-1 and RIP-2 expression in the nasal mucosal tissues were evaluated by Western blotting. The images are examples from representative tissue samples. OVA: ovalbumin; A. Dex: pharmaceutical acupuncture with dexamethasone; O. Dex: oral dexamethasone; RIP-2: receptor interacting protein 2.

DISCUSSION

The present study showed that A. Dex suppressed the levels of IgE, histamine, inflammatory cytokines, caspase-1, and RIP-2 in the serum or nasal mucosal tissue of mice with OVA-induced AR.

AR is an upper airway disease resulting from IgE-mediated inflammatory reactions to allergen exposure.¹ Al-

lergen-IgE-dependent activation of mast cells causes the production of active mediators, such as histamine and cytokines, which induce rhinorrhea, sneezing, and itching in the early phase of the inflammatory response.²⁶ Epithelial accumulations of mast cells, eosinophils, and basophils occur in the late phase of AR.²⁷ The recruitment of inflammatory cells results in the further release of histamine, as well as releases of pro-inflammatory cytokines and chemokines, which sustain the allergic response and promote the late phase response.^{4, 27} Pro-inflammatory cytokines released from mast cells, such as TNF- α , participate in allergic inflammation;²⁸ TSLP and TNF- α induce inflammatory reactions in the nasal mucosal tissues in AR.^{6, 29} ICAM-1 and MIP-2 have been proposed as chemotactic factors and markers of allergic inflammation, as the expressions of ICAM-1 and MIP-2 are increased in the nasal mucosal epithelium of patients with AR.²³

The symptoms of AR can be relieved using medications. Oh *et al.*³⁰ reported that rosmarinic acid has a beneficial effect on AR, as it inhibits the levels of IgE, histamine, and inflammatory cytokines, and reduces the number of nasal, ear, and eye rubs in OVA-sensitized mice. The anti-inflammatory effects of glucocorticosteroids in AR are reportedly mediated by inflammatory cells, such as mast cells.³¹ Smith *et al.*³² reported

that glucocorticosteroids down regulate a function of mast cells and reduce the production of inflammatory cytokines and recruitment of inflammatory cells. Corticosteroids also directly affect the epithelial synthesis of ICAM-1,³³ resulting in a reduced number of eosinophils or neutrophils in the nasal mucosal tissue in AR.³⁴ MIP-2 levels in tissue injury of AR were suppressed by glucocorticosteroid administration.³⁵ In the present study, A. Dex inhibited the levels of IgE, histamine, TSLP, TNF- α , ICAM-1, and MIP-2 in AR mice. Therefore, we suggest that A. Dex has an anti-allergic inflammatory effect, and we speculate that these effects of A. Dex in AR may be induced through a mechanism similar to that of glucocorticosteroid administration. Dex has direct inhibitory effects on human mast cell maturation and on mature mast cell function.³² Consequently, we can speculate that the inhibition of mast cell development by Dex would lead to fewer mast cells in tissues and this could lead to a reduced allergic inflammatory reaction. In addition, the capacity of glucocorticosteroids to inhibit cytokine production in mature mast cells suggests that they might reduce the recruitment, activation and survival of cells involved in the late phase of allergic reactions or in airway remodelling, such as eosinophils. Caspase-1 mediates inflammatory responses.²⁴ Caspase-1 levels are significantly increased in patients with allergic asthma compared with normal individuals,³⁶ and caspase-1-deficient mice show decreased TNF- α levels during inflammatory reactions.³⁷ TSLP is expressed and produced through caspase-1 activation in mast cells,³⁸ while RIP-2 promotes caspase-1 activation and induces cytokine stimulation.²⁵ Furthermore, RIP-2 is reportedly associated with the development and severity of allergic airway inflammation in a mouse model.³⁹ In the present study, A. Dex inhibited caspase-1/RIP-2 expression in AR mice. Thus, we suggest that the inhibitory effect of A. Dex on inflammatory cytokine production might be derived from the blockade of the caspase-1/RIP-2 pathway. Acupoint treatment reportedly ameliorates the allergic reaction in mice.⁴⁰ Furthermore, acupuncture modulates immune responses in the upper airways to improve clinical results in patients with AR,⁹ and significantly reduces AR symptoms in patients with AR.⁴¹ In particular, stimulation of Zusanli (ST 36) decreases inflammation and edema in an inflammation-induced rat model,¹⁴ and acupuncture at Zusanli (ST 36) suppresses inflammation in OVA-induced allergic reaction.¹⁵ In the present study, stimulation of Zusanli (ST 36) with Dex reduced the allergic inflammatory reaction in AR mice. A. Dex had an inhibitory effect similar to that of O. Dex on AR. Therefore, we suggest that A. Dex can be used as a new drug delivery system. However, further study is needed to compare the effect

of stimulation at Zusanli (ST 36) with the effect of pharmaceutical acupuncture with Dex in AR.

In conclusion, our findings indicate that A. Dex has an anti-allergic inflammatory effect in OVA-induced AR mice by ameliorating the number of nasal itching motions and inhibiting the release of allergic mediators. Furthermore, A. Dex suppressed the expression of caspase-1 and RIP-2. The present results suggest that A. Dex might be useful as a new drug administration method for the treatment of allergic inflammatory diseases, such as AR. However, further studies are needed to identify the distinct mechanisms of A. Dex prior to clinical use in humans.

ACKNOWLEDGEMENT

We thank Hyun-A Oh (Department of Pharmacology, College of Korean Medicine, Kyung Hee University) and Kyu-Yeop Kim (Department of Pharmacology, College of Korean Medicine, Kyung Hee University) for providing advice regarding this study.

REFERENCES

1. **Howarth PH**, Salagean M, Dokic D. Allergic rhinitis: not purely a histamine-related disease. *Allergy* 2000; 55(Suppl 64): 7-16.
2. **Jung JH**, Kang IG, Kim DY, Hwang YJ, Kim ST. The effect of Korean red ginseng on allergic inflammation in a murine model of allergic rhinitis. *J Ginseng Res* 2013; 37(2): 167-175.
3. **Whitcup SM**. Recent advances in ocular therapeutics. *Int Ophthalmol Clin* 2006; 46(4): 1-6.
4. **Jeong HJ**, Shin SY, Oh HA, Kim MH, Cho JS, Kim HM. IL-32 up-regulation is associated with inflammatory cytokine production in allergic rhinitis. *J Pathol* 2011; 224(4): 553-563.
5. **Johansson SG**, Hourihane JO, Bousquet J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56(9): 813-824.
6. **Miyata M**, Hatsushika K, Ando T, et al. Mast cell regulation of epithelial TSLP expression plays an important role in the development of allergic rhinitis. *Eur J Immunol* 2008; 38(6): 1487-1492.
7. **Montoro J**, Sastre J, Ja'uregui I, et al. Allergic rhinitis: continuous or on demand antihistamine therapy? *J Investig Allergol Clin Immunol* 2007; 17(Suppl 2): 21-27.
8. **Gupta S**, Feng L, Yoshimura T, Redick J, Fu SM, Rose CE Jr. Intraalveolar macrophage-inflammation peptide 2 induces rapid neutrophil localization in the lung. *Am J Respir Cell Mol Biol* 1996; 15(5): 656-663.
9. **McDonald JL**, Cripps AW, Smith PK, et al. The anti-inflammatory effects of acupuncture and their relevance to allergic rhinitis: a narrative review and proposed model. *Evid Based Complement Alternat Med* 2013; 2013: 591796.
10. **Shen FY**, Lee MS, Jung SK. Effectiveness of

- pharmacopuncture for asthma: a systematic review and Meta-analysis. *Evid Based Complement Alternat Med* 2011; 2011.
- 11 **Strudwick MW**, Hinks RC, Choy ST. Point injection as an alternative acupuncture technique-an exploratory study of responses in healthy subjects. *Acupunct Med* 2007; 25 (4): 166-174.
 - 12 **Yang Q**, Xie YD, Zhang MX, et al. Effect of electroacupuncture stimulation at Zusanliacupoint (ST 36) on gastric motility: possible through PKC and MAPK signal transduction pathways. *BMC Complement Altern Med* 2014; 14: 137.
 - 13 **Wang X**, Shi H, Shang H, et al. Effect of electroacupuncture at Zusanli (ST 36) on gastric-related neurons in spinal dorsal horn and nucleus tractus solitarius. *Evid Based Complement Alternat Med* 2013; 2013: 912898.
 - 14 **Chae Y**, Hong MS, Kim GH, et al. Protein array analysis of cytokine levels on the action of acupuncture in carrageenan-induced inflammation. *Neurol Res* 2011; 29 (Suppl 1): s55-s58.
 - 15 **Wang Z**, Chen T, Long M, et al. Electro-acupuncture at acupoint Zusanli (ST 36) ameliorates inflammation and regulates Th1/Th2 balance in delayed-type hypersensitivity. *Inflammation* 2017; 40(2): 422-434.
 - 16 **Carson S**, Lee N, Thakurta S. Drug class review: newer antihistamines: final report update 2. Portland: OregonHealth & ScienceUniversity, 2010. May. Available from URL: <https://www.ncbi.nlm.nih.gov/books/NBK50554/> (accessed 2017. March. 01).
 - 17 **Baroody FM**, Naclerio RM. Nasal-ocular reflexes and their role in the management of allergic rhinoconjunctivitis with intranasal steroids. *World Allergy Organ J* 2011; 4(1 Suppl): S1-S5.
 - 18 **Vivian AE**, Ramez K, Syed AH. Neurosurgery: The Essential Guide to the Oral and Clinical Neurosurgical Exam. Kindle Edition. Boca Raton: CRC Press, 2014: 115.
 - 19 **Provan D**, Stasi R, Newland AC, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. *Blood* 2010; 115(2): 168-186.
 - 20 **Shi GX**, Wang XR, Yan CQ, et al. Acupuncture elicits neuroprotective effect by inhibiting NADPH oxidase-mediated reactive oxygen species production in cerebral ischaemia. *Sci Rep* 2015; 5: 17981.
 - 21 **Aswar U**, Shintre S, Chepurwar S, Aswar M. Antiallergic effect of piperine on ovalbumin-induced allergic rhinitis in mice. *Pharm Biol* 2015; 53(9): 1358-1366.
 - 22 **Grzelewska-Rzymowska I**, Pietrkowicz M. Role of tumor necrosis factor alpha in allergic inflammation and airway hyperresponsiveness. *Pol Merkur Lekarski* 2004; 16 (92): 173-178.
 - 23 **Spencer LA**, Szela CT, Perez SA, et al. Human eosinophils constitutively express multiple Th1, Th2, and immunoregulatory cytokines that are secreted rapidly and differentially. *J Leukoc Biol* 2009; 85(1): 117-123.
 - 24 **Martinon F**, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004; 117(5): 561-574.
 - 25 **Kobayashi K**, Inohara N, Hernandez LD, et al. RICK/Rip2/CARDIAK mediates signaling for receptors of the innate and adaptive immune systems. *Nature* 2002; 416 (6877): 194-199.
 - 26 **Jeong HJ**, Moon PD, Kim SJ, et al. Activation of hypoxia-inducible factor-1 regulates human histidine decarboxylase expression. *Cell Mol Life Sci* 2009; 66(7): 1309-1319.
 - 27 **Fuentes-Beltrán A**, Montes-Vizuet R, Valencia-Maqueda E, et al. Chemokine CC-ligand 5 production and eosinophil activation into the upper airways of aspirin-sensitive patients. *Clin Exp Allergy* 2009; 39(4): 491-499.
 - 28 **Iwasaki M**, Saito K, Takemura M, et al. TNF-alpha contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol* 2003; 112(1): 134-140.
 - 29 **Artis D**, Humphreys NE, Bancroft AJ, Rothwell NJ, Potten CS, Grecis RK. Tumor necrosis factor alpha is a critical component of interleukin 13-mediated protective T helper cell type 2 responses during helminth infection. *J Exp Med* 1999; 190(7): 953-962.
 - 30 **Oh HA**, Park CS, Ahn HJ, Park YS, Kim HM. Effect of *Perilla frutescens* var. *acuta* Kudo and rosmarinic acid on allergic inflammatory reactions. *Exp Biol Med* (Maywood) 2011; 236(1): 99-106.
 - 31 **Okano M**. Mechanisms and clinical implications of glucocorticosteroids in the treatment of allergic rhinitis. *Clin Exp Immunol* 2009; 158(2): 164-173.
 - 32 **Smith SJ**, Piliponsky AM, Rosenhead F. Dexamethasone inhibits maturation, cytokine production and Fc epsilon RI expression of human cord blood-derived mast cells. *Clin Exp Allergy* 2002; 32(6): 906-913.
 - 33 **Ciprandi G**, Buscaglia S, Iudice A, Pesce GP, Bagnasco M, Canonica GW. Protective effects of deflazacort on allergen-specific conjunctival challenge. *Eur J Clin Pharmacol* 1993; 45(Suppl 1): S35-S41.
 - 34 **Ciprandi G**, Tosca MA, Passalacqua G, Canonica GW. Intranasal mometasone furoate reduces late-phase inflammation after allergen challenge. *Ann Allergy Asthma Immunol* 2001; 86(4): 433-438.
 - 35 **Kimura M**, Sugaya N, Kimata K, et al. Effects of dexamethasone on tissue injury and reconstruction in ethanol/steroid injection therapy for allergic rhinitis. *J Allerg Ther* 2012; S5: 005.
 - 36 **Grzegorzczak J**, Kowalski ML, Pilat A, Iwaszkiewicz J. Increased apoptosis of peripheral blood mononuclear cells in patients with perennial allergic asthma/rhinitis: relation to serum markers of apoptosis. *Mediators Inflamm* 2002; 11(4): 225-233.
 - 37 **Furlan R**, Martino G, Galbiati F, et al. Caspase-1 regulates the inflammatory process leading to auto-immune demyelination. *J Immunol* 1999; 163(5): 2403-2409.
 - 38 **Moon PD**, Kim HM. Thymic stromal lymphopoietin is expressed and produced by caspase-1/NF-kB pathway in mast cells. *Cytokine* 2011; 54(3): 239-243.
 - 39 **Kim TH**, Park YM, Ryu SW, Kim DJ, Park JH, Park JH. Receptor interacting protein 2 (RIP2) is dispensable for OVA-induced airway inflammation in mice. *Allergy Asthma Immunol Res* 2014; 6(2): 163-168.
 - 40 **Jeong HJ**, Nam SY, Lee BJ, Kim MG, Kim JH, Kim

- HM. An alternative method to reduce anaphylaxis by
moxibustion. TANG 2014; 4(2): e12.
- 41 **van Oene CM**, van Reij EJ, Sprangers MA, Fokkens WJ.
Quality assessment of disease-specific quality of life
questionnaires for rhinitis and rhinosinusitis: a systematic
review. Allergy 2007; 62(12): 1359-1371.