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Original Article

Ovalbumin으로 유도한 아토피성 피부염의 마우스 종별 차이에 관한 예비연구

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Ovalbumin Induces Atopic Dermatitis-like Skin Lesions in Different Species of mice: pilot study

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Objectives: Atopic dermatitis (AD) is an easily recurrent inflammatory skin disease. Since AD has complex pathology, people have been investigating it on different aspects with various experimental models. One of them is in vivo model which has spontaneously developed AD-like skin lesions by various inducers.

Methods: In this study, two kinds of mouse species were applied in the experiment; BALB/c and C57BL/6 mice. We compared features among the animal species making AD mouse model with protein allergen, ovalbumin. AD-like skin lesions were induced by ovalbumin on two kinds of scheme and were evaluated with the histological results and size of spleen which is a critical immunological organ. Also, we measured the level of immunoglobulin E in serum. In addition, we investigated the results of ovalbumin induced-AD-like skin lesions along with obesity.

Results and Conclusion: We evaluated weight of organs and thickness of epidermis. These results suggest the possibility of the appropriate in vivo experimental model for AD or the comorbidity with obesity.

Keywords : Moebius syndrome; facial palsy; eye abduction; MRI; treatment; acupuncture

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INTRODUCTION

Atopic dermatitis (AD) is a highly prevalent inflammatory skin disease accompanied by severe itch and relapsing features[1]. Though AD has symptoms which lower people's quality of life, there is no apparent therapeutic agent. It is caused by complicated pathology of AD. People have been investigating AD with a variety of experimental models[2]. One of them is in vivo study which is involved in many species of mice including NC/Nga, BALB/c, or C57BL/6. Also, there are several inducers to make AD-like skin lesions mimicking AD symptoms in human. Things to lead AD are such as house dust mite, 2,4-dinitrochlorobenzene (DNCB), or ovalbumin. In present study, we compared the results of ovalbumin-induced AD-like skin lesions in BABL/c and C57BL/6 mice[3]. Not only different species, but also two kinds of experimental protocol were performed.

As a protein allergen, ovalbumin is chicken egg albumin, which results in arise in total and ovalbumin-specific Immunoglobulin E (IgE) and causes the development of AD. It is characterized by infiltration of immune cells including CD3(+) T cells, eosinophils, or neutrophils[4] Also, a single exposure of ovalbumin on mice to aerosolize could induce eosinophilia in the bronchoalveolar lavage fluid in the development of allergic asthma[5] Ovalbumin acts as an allergen sensitizer to skin to airway which possibly leads atopic march. Therefore, ovalbumin itself could be an appropriate inducer of provoking disease in a view of comorbidity.

Comorbid condition is defined any distinct additional entity which has existed or various occur in the clinical course of a patient who has the index disease under study[6]. Since AD is one of chronic inflammatory diseases, it has potentials to cause another disease or worsen the inflammatory symptoms such as atopic march[7],[8]. That is the reason we focused the comorbidity with obesity which is a major disease involved in accompanied by various diseases.

MATERIALS AND METHODS

1. Reagents

Ovalbumin (OVA; grade V; Sigma Aldrich, MO, USA) and aluminum hydroxide adjuvant (Al(OH)3; ImjectTM Alum Adjuvant; Thermo ScientificTM, IL, USA) were purchased from Sigma Aldrich and Thermo Fisher Scientific, respectively.

2. Animals

Female mice (BALB/c) and male mice (C57BL/6) were used for OVA-induced AD model. They were 6 weeks old and obtained from Daehan Biolink Co. (Daejeon, Republic of Korea). Animals were kept under standard conditions according to the guidelines adopted and promulgated by Sangji University in accordance with the requirements of the National Institutes of Health. Ahead of the experiments, the Institutional Animal Care and Use Committee (IACUC) of Sangji University approved all the experimental protocols (IACUC animal approval protocol no. 2018-24). Mice were housed (four to five mice per a cage), acclimatized to the animal room, and fed standard laboratory chow. For ani

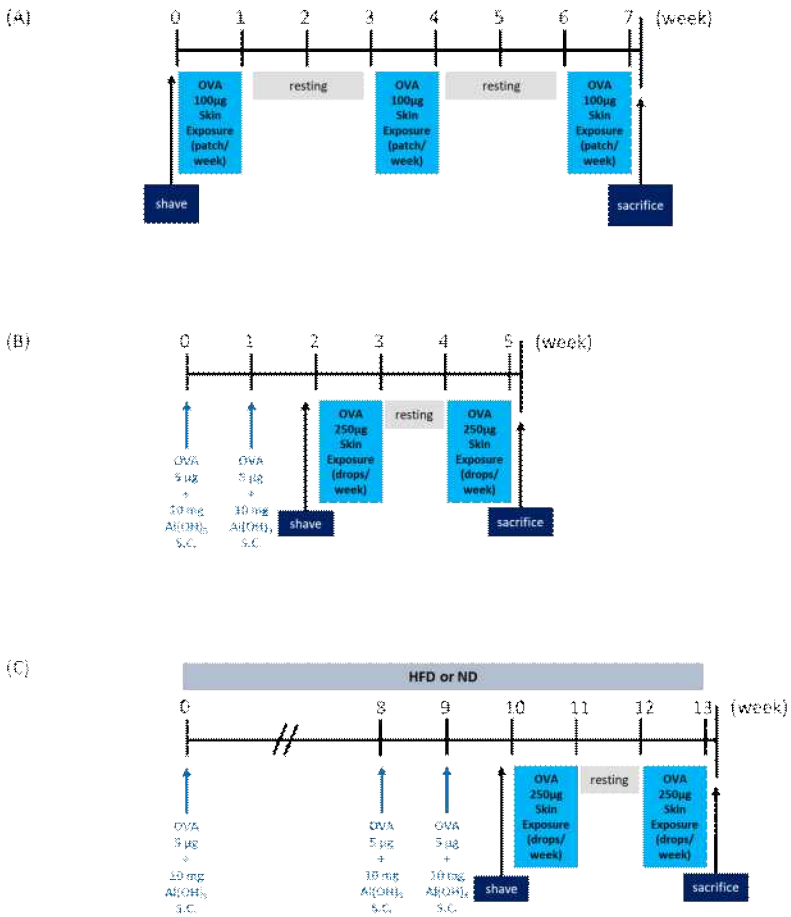

Figure 1. Experimental scheme for atopic dermatitis (AD) induced by ovalbumin (OVA) in mice. (A) Mice were exposed to OVA (100 μg) or saline applied in 100 μL to a sterile patch. The patch was applied for 1 week and then removed. It was repeated twice 2 weeks later to the same skin with identical patch. The sacrifice was performed after third sensitization. (B) Mice were immunized with a subcutaneous injection of OVA (5 μg) and the aluminum hydroxide adjuvant (Al(OH)3) (10 mg/mL). On day 14 to 18, and 28 to 32, shaved dorsal skin of animals were challenged with OVA (250 μg). Animals in control group received only sterile saline. (C) C57BL/6 mice were immunized with OVA (5 μg) and Al(OH)3 (10 mg/mL) after high-fat diet (45 kcal%)-induced obesity for 8 weeks. Mice were divided into four groups; (1) normal diet (ND) + PBS, (2) ND + OVA, (3) HFD + PBS, (4) HFD + OVA. After the 24 h of the last OVA challenge, mice were euthanized for further analysis.

mal involved in comorbidity of obesity, 45 kcal% high fat diet (HFD) was provided. The high fat diet was purchased from Research Diets, Inc. (D12451; NJ, USA). C57BL/6 mice were fed HFD for 8 weeks and then followed the protocol of Figure 1B to induce AD-like skin lesions.

3. Sensitization for AD

The schematic experimental procedures are described in Figure 1. Female BALB/c mice were

subjected to skin with ovalbumin-patch containing 100 μgovalbumin. This epicutaneous sensitization procedure was repeated thrice (Fig. 1A)[4].

Another protocol for sensitization was with Al(OH)3 administration. Female BALB/c mice were immunized on day 0 and day 7 with a subcutaneous injection of ovalbumin (5 μg) and Al(OH)3 (10 mg/mL) mixture according to previous studies[11]. After the administration, the dorsal skin of mice was challenged every day with drops containing 250 μg ovalbumin. It was repeated one more time after 1 week of resting time (Fig. 1B).

4. Histological analysis of skin lesions

Skin samples from the dorsal area were isolated after euthanasia. The samples were fixed in 10 % buffered formalin and then embedded in paraffin. Next, they were sectioned into 8 μM slices and stained with hematoxylin and eosin (H&E). Pathological changes, such as hyperkeratosis, dermal edema, epidermal and dermal hyperplasia, vesicular formation, parakeratosis, and inflammation were found. Images were captured under an optical microscope (Leica DFC 295, Wetzlar, Germany) using Leica software.

5. Cytokine analysis

Blood was collected from the orbital sinus of each mouse at the end of the experiments. Total serum was obtained by centrifugation at 1700x g for 30 min and kept at -80 ℃ till analysis. The serum level of IgE was evaluated with mouse IgE ELISA kit following the instructions for use (BD OptEIATM, BD Science, CA, USA).

6. Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Comparisons among groups were carried out using one-way ANOVA followed by Dunnett's post-hoc test. P-values of less than 0.05 were considered statistically significant.

RESULTS

1. AD-like skin lesions induced by repeated application of ovalbumin with different applying ways

We tried to set the mouse model induced by ovalbumin following previous studies[12),3)]. For first time, we used patch with ovalbumin putting it on the dorsal skin of mice for a week with resting time of 2 weeks and it was repeated for three times[4),11),13)]. However, the skin lesions were not significantly induced with bare eyes. Therefore, we studied another experimental protocol changing the way of exposing ovalbumin patch to drops on the skin adding Al(OH) 3 administration. The topical application of 1 week was performed every day on BALB/c twice with 1 week of interval. We could compare the AD mouse model induced ovalbumin based on the previous studies[11),14)]

2. AD-like skin lesions and their histopathological changes in ovalbumin-induced AD in two species of mice

Hematoxylin and eosin (H&E) staining was performed to present the thickness of epidermis and dermis. Though the thickness of epidermis was slightly increased than ones in control group (Figure 2B and 2C), AD-like skin lesions were not significantly visible (Figure 2A). Therefore, we evaluated another way of ovalbumin exposure to

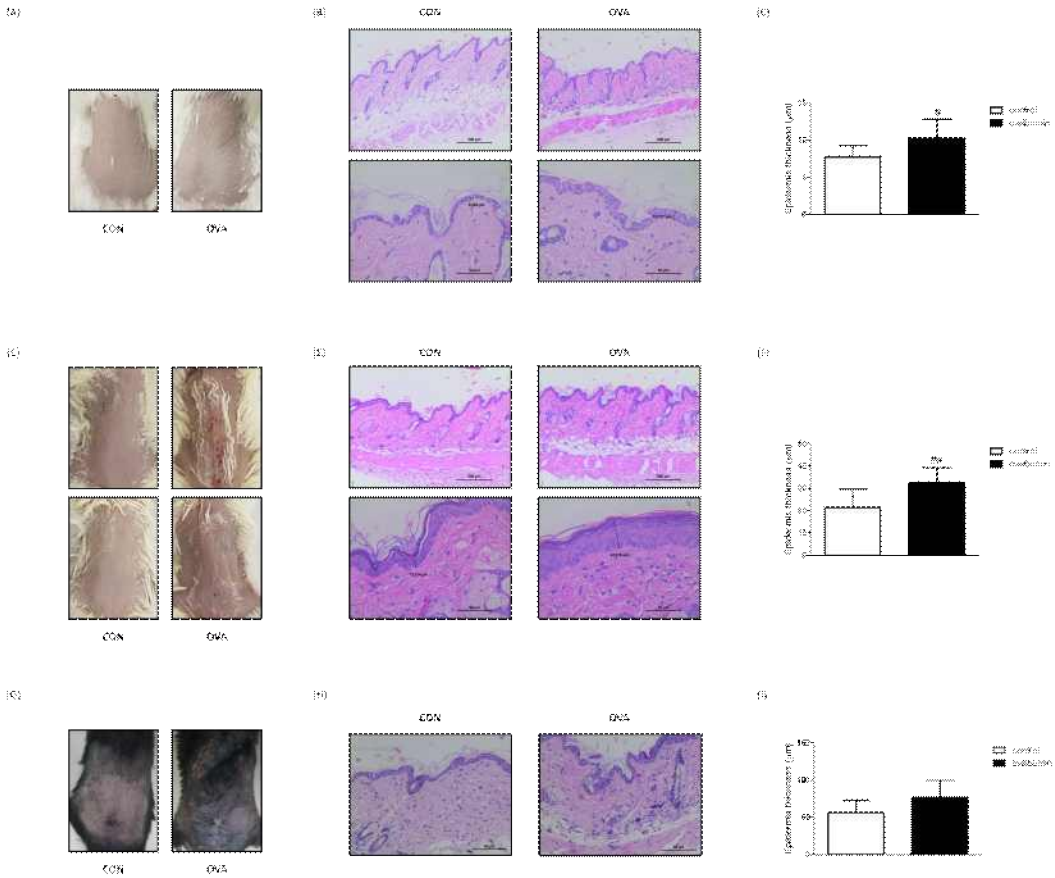

Figure 2. AD-like skin lesions induced by ovalbumin in BALB/c (A-F) and C57BL/6 mice (G-I). (A, D, G) Gross observation on dorsal skin in BALB/c (A, D) and C57BL/6 (G). (B, E, H) Histological examinations presented by hematoxylin and eosin (H&E) staining (100X, upper line; 400X, lower line and (H)) and thickness of epidermis in BALB/c (B, C, E, F) and C57BL/6 (H, I). Data are expressed as the mean ± standard deviation (SD). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ versus the only saline treated group by ANOVA and Dunnett's post-hoc test.

induce AD-like skin lesions following the protocol of figure 1B. After following the scheme in figure 1B, the mice skin condition seemed to have worsened with excoriation in the ovalbumin group (Figure 2D). Under the induction of AD-like skin lesions by ovalbumin and Al(OH)3, the dorsal skin and histopathological images were changed compare to the ones in control group (Figure 2E). The thickness of epidermis was apparently increased in ovalbumin-induced group compare to the control one (Figure 2F). In addition to the induction of AD in BABL/c, we applied the allergen, ovalbumin, in another mouse species, C57BL/6. The dorsal of mice seemed similar between control and ovalbumin treatment groups (Figure 2G). Also, it was seen in the histological analysis of epidermal thickness in the groups; control and ovalbumin-treated group (Figure 2H and 2I).

3. Immunological characteristics in ovalbumin-induced AD in two species of mice

Immunoglobulin E (IgE) is one of representative hallmarks in AD model[15] And spleen is secondary lymphoid organ[16]. We evaluated the serum IgE level and weight of spleen in two kinds of AD experiments induced by ovalbumin; exposure with patch in BALB/c (Fig. 3A and 3B), exposure with drops and Al(OH)3 immunization in BALB/c (Fig. 3C and 3D), and exposure with drops with Al(OH)3 immunization in C57BL/6 (Fig. 3E and 3F). In three kinds of models, levels of serum IgE were significantly increased in ovalbumin treated groups (Fig. 3A, 3C, and 3E). Especially the amount of IgE exposed in ovalbumin and Al(OH)3 was apparently increased. Also, the enlargement of spleens found in the ovalbumin treated groups. Mainly, the relative spleen weight to body weight was increased in the ovalbumin drops and

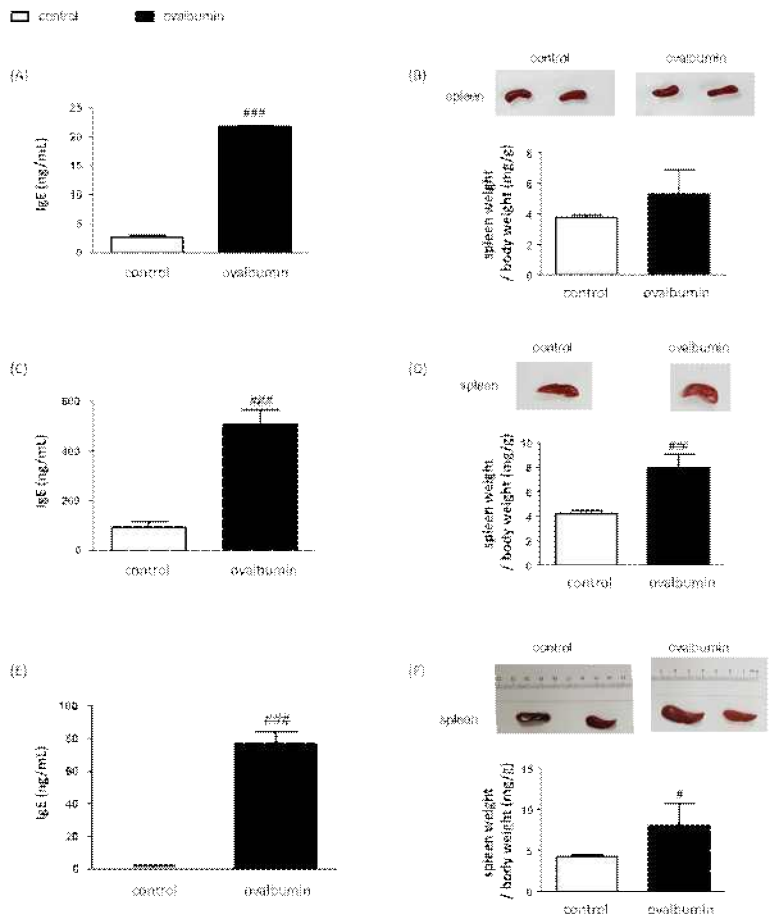

Figure 3. Evaluation of total serum immunoglobulin E (IgE) and spleen weight in ovalbumin-induced AD mice; BABL/c (A-D) and C57BL/6 (E, F). Data are expressed as the mean ± standard deviation (SD). # $p < 0.05$, and ### $p < 0.001$ versus the only saline treated group by ANOVA and Dunnett's post-hoc test.

Al(OH)3 induced AD model (Fig. 3D).

4. Study on the possibility of comorbidity between AD and obesity

In the aspect of comorbidity which is worsen the diseases, obesity is one of major risk factors for many medical problems[17]. We evaluated whether ovalbumin induced AD-like skin lesions and inflammatory changes. C57BL/6 mice were fed high fat diet (HFD) for 8 weeks and then sensitized with ovalbumin following the protocol in Figure 1C. After the experiment, the mice treated with ovalbumin had mild excoriation in dorsal skin. And the obesity was apparently induced which was shown in the abdominal fat content and liver (Fig. 4A). However, the weight

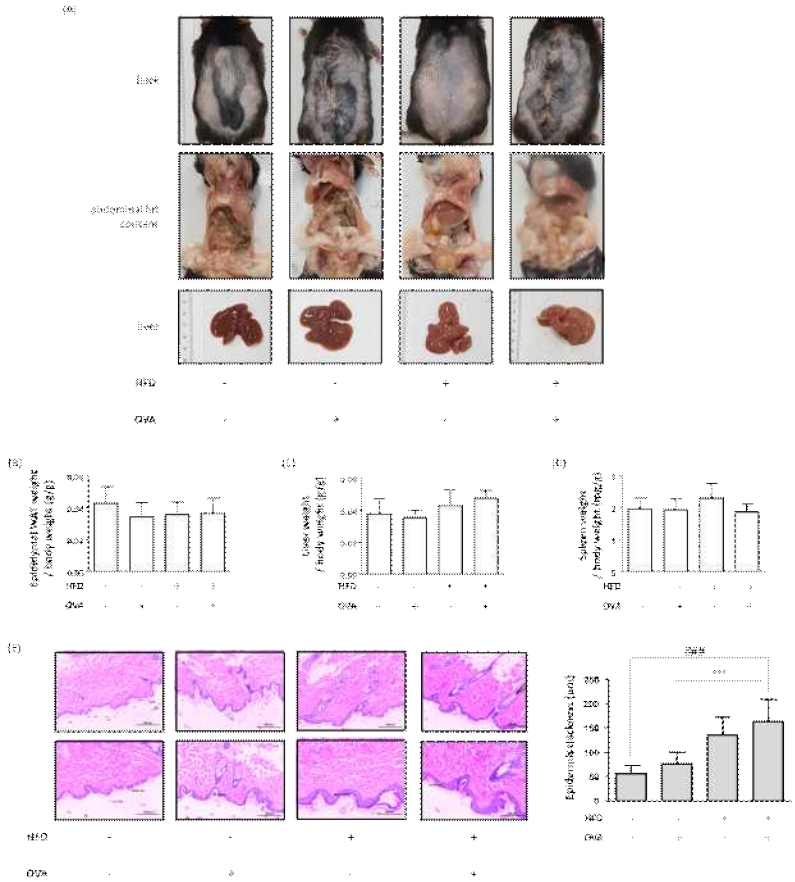

Figure 4. Results of comorbidity of HFD-induced obesity and OVA-induced AD in C57BL/6 mice. (A) Dorsal skin, abdominal fat content, and liver images are presented at the end of the experiment. Relative levels of epididymal WAT (B), liver (C), or spleen (D) weight compare to body weight. (E) Histological examinations presented by H&E staining (100X, upper line; 400X, lower line) and thickness of epidermis in C57BL/6 mice. Data are expressed as the mean ± standard deviation (SD). ### $p < 0.001$ versus the only saline and normal diet treated group and *** $p < 0.001$ versus the only OVA-induced AD and normal diet treated group by ANOVA and Dunnett's post-hoc test.

of epididymal white adipose tissue (WAT) and liver were not increased in HFD-treated group compare to the body weight (Fig. 4B and 4C). Spleen which is an immunological organ did not show the difference between the groups (Fig. 4D). But, there was a significant increase in the epidermal thickness in both HFD and ovalbumin treated group compare to the only ovalbumin or vehicle treated groups (Fig. 4E). It could suggest the possibility of vice cycle in the obesity and AD, one of major inflammatory diseases. Following the previous studies on AD mouse model[3], we compared the results of AD-like skin lesions between the mouse species. When we performed as described previously[18], AD-like skin lesions in histopathological aspect were shown thickening the epidermis in BABL/c (Fig. 2A and Fig. 3A). Though there was not evident AD-like skin lesions, the inflammatory markers such as serum IgE or spleen mass were increased dependent on the ovalbumin.

DISCUSSION

However, the way of sensitization with patch was not trouble-free. Patch was attached to mice with flexible bandage alleviating the difficulties from binding. However, mice put them off and it was not easy to apply the stable induction. To lead high reproducibility, we adjusted mediator into oil having higher concentration and the frequency (Fig. 1B). We could get the result of ovalbumin-induced AD-like skin lesions and the features of AD in two species of mice (Fig. 2B, 2C, 3B, and 3C).

AD-like skin lesions were apparently shown in C57BL/6 with repeated ovalbumin treatment in oil. It was accompanied by the histological and immunological changes. IgE is one of specifically increased mediators in ovalbumin induced inflammatory environment[19]. It was increased in two species with two protocols in this study. Since spleen is secondary lymphoid organ, it acts as one of biomarkers in immune responses[20][21]. We could have the swollen spleen with increased mass. Also, thickness of epidermis and the inflammatory cells including eosinophils and mononuclear cells are infiltrated in the ovalbumin groups compare to the controls. Number of inflammatory cells were increased epidermis and dermis of AD-like skin lesions[22]. It was presented in the histopathological images (Fig. 2B, 2H, 2E, and 4E).

CONCLUSION

Obviously, we need to study another experiment to investigate with reproducibility on same sex in the mouse species. Though the limitation, we could get the protocol of another AD-mouse model and possibility of study on the comorbidity with obesity. With these results, we would be able to evaluate potentials of other inducers in AD animal models.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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