이학 석사 학위 논문

Effects of Chloromethylisothiazolinone/ Methylisothiazolinone (CMIT/MIT) in atopic dermatitis mice model

아토피피부염 마우스 모델에서
Chloromethylisothiazolinone/Methylisothiazolinone
(CMIT/MIT)의 영향

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의 학 과
고 한 나

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이 논문을 이학석사 학위 논문으로 제출함

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English Abstract

Background: Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by immune mediated inflammation and epidermal barrier dysfunction. Recently, exposure to many environmental factors such as air pollution, construction materials and harmful chemical substances has been found to cause AD. Mixture of chloromethylisothiazolinone (CMIT) and methylisothiazolinone (MIT) is widely used as a biocide, liquid detergent, and cosmetics, and used for humidifier disinfectants because of its strong bactericidal activity and low toxicity. CMIT/MIT exposure has been associated with allergic contact dermatitis, occupational asthma and lung injury. Despite of the association with allergic response, there was no study to investigate the effects of exposure to CMIT/MIT on the development of AD.

Objective: We aimed to investigate the influence of skin exposure to CMIT/MIT on major symptoms of AD and a biological mechanism through an assessment of immune responses in AD mice model.

Methods: BALB/C mice were exposed to skin with CMIT/MIT of 0.1875 mg/kg/day, 5 days/week for 3 weeks and then AD was developed by ovalbumin (OVA) epidermal sensitization. To assess AD symptoms, we measured transepidermal water loss (TEWL) and scored erythema, scaling, and excoriation in dorsum lesions. Total immunoglobulin (Ig) E and OVA specific IgE in serum and histopathological changes in skin were analyzed. To evaluate the immune response, the levels of T helper (Th) 2/Th17-related cytokines such as thymic

stromal lymphopoietin (TSLP), interleukin (IL) -4, IL-13 and IL-17A were detected using real-time PCR. The frequency of $CD4^{+}IL\text{-}4^{+}$ and IL-17A producing cells in skin draining lymph nodes was assessed using flow cytometry.

Results: CMIT/MIT skin exposure in mice significantly increased AD-like phenotypes (e.g., TEWL, clinical score, total serum IgE level and inflammatory cells) and skin mRNA expression level of Th2-related cytokines (TSLP and IL-13) significantly increased. In addition, CMIT/MIT skin exposure in mice significantly enhanced $CD4^{+}IL\text{-}4^{+}$ cells in skin draining lymph nodes. Mice exposed to CMIT/MIT in OVA challenge model had higher TEWL, clinical score, and skin inflammation than mice exposed to that. In addition, mice exposed to CMIT/MIT had higher total serum IgE, TSLP, IL-4 and IL-17A in skin mRNA expression and higher population of $CD4^{+}IL\text{-}4^{+}$ and IL-17A producing cells in skin draining lymph nodes than mice unexposed to that.

Conclusion: These findings demonstrated that CMIT/MIT acts as environmental antigen and enhances allergic inflammation by modulation of Th2/Th17 response in AD mice, suggesting that CMIT/MIT skin exposure might aggravate AD symptoms through dysregulation of immune response.

Key words: Atopic dermatitis, Chloromethylisothiazolinone, Methylisothiazolinone, Skin exposure, T helper 2, T helper 17

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Abbreviations

AD: Atopic dermatitis

TEWL: Transepidermal water loss

Ig: Immunoglobulin

Th: T helper

CMIT: Chloromethylisothiazolinone

MIT: Methylisothiazolinone

HD: Humidifier disinfectant

OVA: Ovalbumin

H&E: Hematoxylin and eosin

ELISA: Enzyme-linked immunosorbent assay

IL: Interleukin

TSLP: Thymic stromal lymphopoietin

DBP: Dibutyl phthalate

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease which manifests as eczematous skin include epidermal hyperplasia, spongiosis and infiltration of immune cells in the dermis [1, 2]. Epidermal barrier dysfunction is also an important feature of AD that cause transepidermal water loss (TEWL) and is associated with increased serum immunoglobulin (Ig) E levels and maintenance of AD pathogenesis [3-5]. In addition, most patients with AD exhibit systemic T helper (Th) 2-dominant immune response and upregulation of inflammatory mediators to cutaneous inflammation [6].

In recent years, the development of AD is known to be strong links to a number of environmental factors including exposure to allergens, air pollution and harmful chemical substances due to its high susceptibility to environmental exposure [7, 8]. Epidemiological studies have provided evidence for a possible relationship between environmental pollution exposure and the risk of AD [9-12]. Especially, the increased prevalence of morbidity of AD corresponds with the increase in chemical substances [8]. Therefore, the cause of the rapid increase in AD is generally thought to be changes in environmental factors rather than genetic factors.

Chloromethylisothiazolinone (CMIT) and Methylisothiazolinone (MIT) has been widely used in a biocide, paints, and cosmetics products such as shampoo, body lotion and skin care products [13, 14]. With increasing uses of CMIT/MIT, recent reports have revealed that exposure of CMIT/MIT has been associated with allergic contact dermatitis, which act as a

sensitizer [15-17]. In addition, CMIT/MIT exposure induces a systemic allergic reaction and decrease of lung function, resulting in development of occupational asthma [18-20]. Recently, experimental study has provided evidence of a biological basis for MIT as risk factor for allergic sensitization as indicated by enhanced skin inflammation, IgE production and immune response in mice [21]. Exposure of CMIT/MIT has the potential to may increase the potency of sensitization to allergens, and therefore may play a crucial part in the enhancement of allergic diseases. Despite the several evidence regarding the association between CMIT/MIT exposure and allergic immune response, no previous studies have investigated association with the development of AD.

Recently, in South Korea, Several chemical disinfectants including CMIT/MIT, polyhexamethylene guanidine phosphate (PHMG) and oligo (2-(2-ethoxy) ethoxyethyl guanidinium (PGH) were used for humidifier disinfectant (HD) because of its strong bactericidal activity and low toxicity [22]. However, in previous epidemiologic studies, HDs were later clinically confirmed to cause HD-associated lung injury (HDLI) [23-25]. In addition, people who exposed to HDs claims to develop allergic diseases after using HDs. Practically, recent evidence has shown that HDs exposure increase the risk for asthma in children [26]. However, until now, the development of AD on exposure of HDs remains unknown. Therefore, we aimed to investigate whether exposure to CMIT/MIT in normal mice has the ability to induce major symptoms of AD. We also investigated whether CMIT/MIT exposure affect AD development and immune responses in AD mice model.

Materials and Methods

1. Animal experiment

Five-weeks-old female normal mice (n=5 per group) that weighing 16-20g were purchased from Orient Bio (Seongnam, Korea) and housed under controlled humidity (40%), temperature (22 ± 2°C) and 12h light and dark cycle. All mice were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at Asan Medical Center and Ulsan University College of Medicine.

2. Skin exposure of CMIT/MIT and development of AD in mice.

Mice were lightly anesthetized with and their backs were shaved and then treated in the skin with CMIT/MIT at 0.1875 mg/kg/day, 5 days/week for 3 weeks. In the AD model, ovalbumin (OVA) sensitization was performed as previously described with a few modifications [27, 28]. Briefly, mice were intraperitoneally injected with 10 ㎍ of chicken OVA (grade V; Sigma, St Louis MO) mixed with 4 mg of aluminum hydroxide (Imject Alum; Pierce, Rockford, IL, USA) in a volume of 200 ㎕ 3 times at 1-week intervals (i.e., days 0, 7, and 14). The mice were epicutaneously sensitized with OVA patches on day 21. OVA (10㎍) repaired in 100 ㎕ of PBS was contained in a 1 X 1-cm sterile patch and attached to the tape stripped area with an adhesive tape. The OVA patch was changed once a week, and the skin was kept in contact with the OVA for 7 days. Epicutaneous sensitization was repeated once during the 1-week sensitization period, after which a 1-week resting period was allowed, and then a similar

sensitization treatment was again performed. All mice were sacrificed 24h following final OVA sensitization.

3. Estimation of clinical signs and skin barrier function in the AD mice model

The dorsum lesions were scored for erythema, scaling, and excoriation after each sensitization using a 0–3 scoring system, where 0 = no lesion, 1 = mild lesion, 2 = moderate lesion, and 3 = severe lesion. The same researcher performed all scoring evaluations throughout the study. To determine whether epidermal permeability barrier function was altered due to OVA-induced AD, we measured TEWL using a vapometer (SWL-3; Delfin Technologies Ltd., Kuopio, Finland). We assessed the baseline TEWL at the beginning of the experiment, and then performed one TEWL assessment after each sensitization. The dorsal skins of experimental mice were removed on the final day of the schedule and fixed in 10 % phosphate-buffered formalin before being embedded in paraffin for histopathological evaluation. Serial paraffin sections (5 μm thick) were stained with hematoxylin and eosin (H&E) for evaluation of skin inflammatory cell infiltration.

4. Quantitation of serum levels of immunoglobulin

Serum samples were obtained from blood taken during exsanguinations of the mice after sensitization treatments and stored – 80℃ until analyzed. Total serum IgE levels were detected using the Mouse IgE enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San

Diego, CA, USA). For the detection of OVA-IgE, the plates were coated overnight with OVA (100 μg in 1 ml carbonate-bicarbonate buffer; Sigma Chemical Co). Remaining binding sites were blocked, and plates were incubated with 100 μℓ of diluted serum (1/50 dilution in carbonate-bicarbonate buffer) per well. After washing, following substances were sequentially added, incubated and washed: peroxidase-labelled rat anti-mouse OVA-IgE (20 ng/100 μℓ, Acris Antibodies, Herford, Germany), and 100 μℓ of TMB solution (Sigma Chemical Co.) were added to per well. Then, determinations were performed in duplicate. The optical density was measured at 450 nm.

5. Real-time PCR

To measure the expression of interleukin (IL)-4, IL-13 and IL-17A and thymic stromal lymphopoietin (TSLP) in mice skin, RNA was extracted from the dorsal skin of experimental mice using the RNeasy kit (Qiagen, Valencia, CA, USA). Real-time PCR was performed using the TaqMan method on an ABI 7900 system (Applied Biosystems, Piscataway, NJ, USA). Each signal was normalized against the levels of GAPDH mRNA in the same sample.

6. Flow cytometry

Mouse IL-4 and IL-17A were collected from the skin draining lymph nodes (axillary and brachial lymph nodes) [29]. Skin draining lymph nodes cells were stained with using FITC-labeled anti-CD4, PerCP-eFluor 710-labeled anti-IL-4, PE-labeled anti-IL-17A and respective

isotype controls (eBioscience, San Diego, CA) were used to analyze $CD4^+IL\text{-}4^+$ and IL-17A expression in accordance with the manufacturer's directions. Lymphocytes were gated based upon expression of $CD4^+$ to identify IL-4 and IL-17A expression in the total $CD4^+$ fractions. Staining was analyzed by flow cytometry using FACS Calibur with CellQuest software (BD Biosciences, Mountain View, CA).

7. Statistical analysis

The ANOVA test and Tukey's multiple comparison test were used to assess difference in the measurements between multiple groups. Statistical analyses were performed using Graph Pad Prism 4.0 (San Diego, CA). Also, The independent samples t test was using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA).

Results

Skin exposure of CMIT/MIT induced AD-like skin inflammation and systemic immune response in normal mice

We first investigated whether CMIT/MIT exposure induce AD-like responses in normal mice. Mice were exposed to skin CMIT/MIT during 3 weeks sequentially (Fig. 1a). In histopathological evaluation, mice exposed to CMIT/MIT had higher inflammatory cell infiltrations and epidermis thickness in skin than mice exposed to PBS (Fig. 1b). In addition, mice exposed to CMIT/MIT had higher TEWL (Fig. 1c) as well as total serum IgE level (Fig. 2a) than mice exposed to PBS. In the assessment of immune response, mice exposed to CMIT/MIT had higher expression of TSLP and IL-13 in skin (Fig. 2b, c) and more populations of $CD4^{+}IL\text{-}4^{+}$ cells in skin draining lymph nodes (Fig. 3a, b) than mice exposed to PBS. However, mRNA expression of IL-4 and IL-17A in skin was non-detection (data not shown). These findings demonstrate that skin exposure to CMIT/MIT in normal mice induces a phenotype similar to AD and dysfunction of Th2.

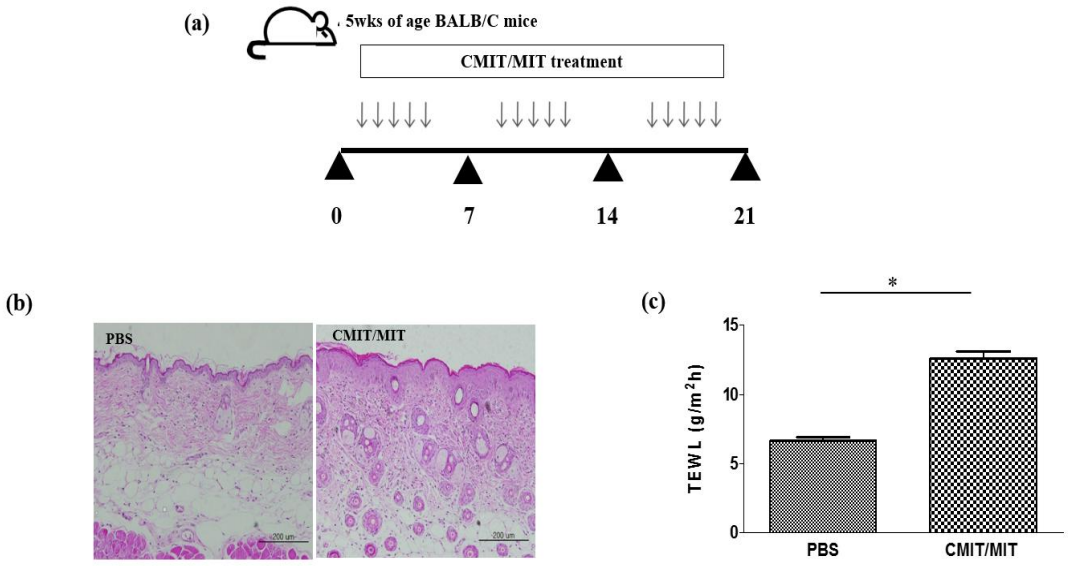

Fig.1 Effects of CMIT/MIT on skin histology and the epidermal permeability barrier in normal mice. (a) An experimental protocol for effects of CMIT/MIT exposure in normal mice, (b) H&E and (c) TEWL (* P < 0.01).

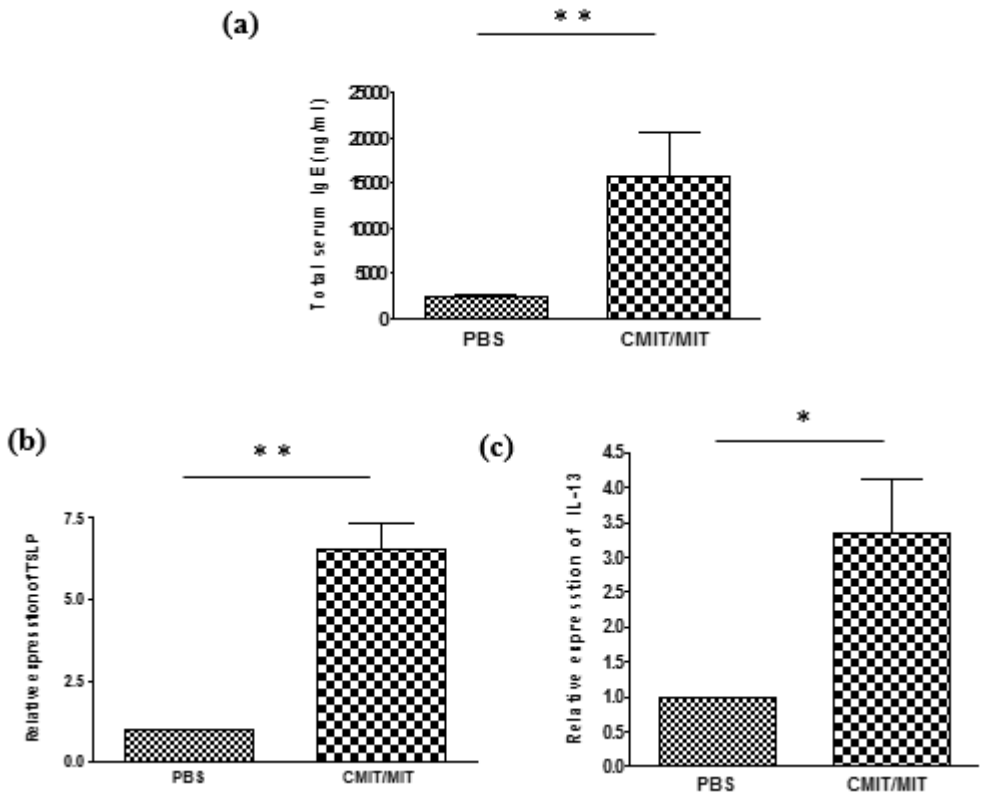

Fig. 2. Effects of CMIT/MIT on total IgE serum levels and Th2-relacted cytokines in normal mice. (a) Serum levels of total IgE were ELISA. Skin mRNA expression of (b) TSLP and (c) IL-13 was assessed by real-time PCR. Statistical significance was determined with t test. (* P < 0.05, * *P < 0.01).

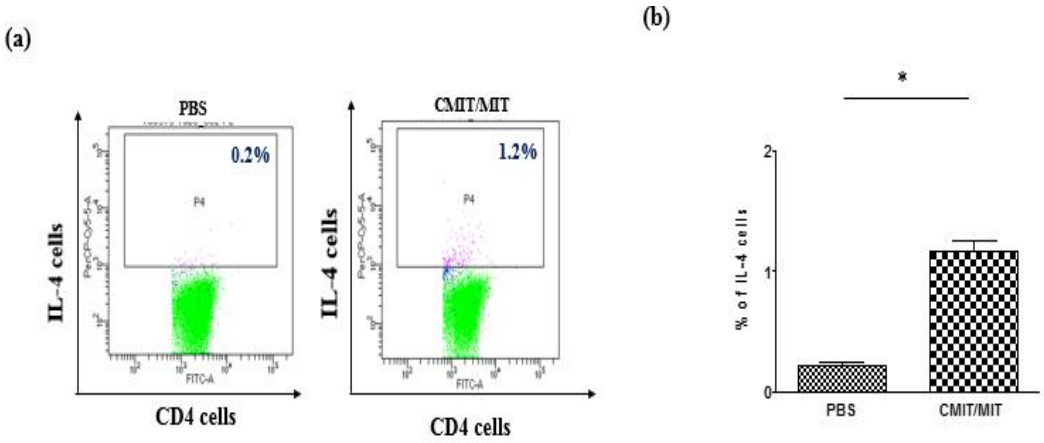

Fig. 3. Effects of CMIT/MIT on populations of $CD4^+IL\text{-}4^+$ cells in normal mice. (a) The frequency of IL-4 with $CD4^+$ cells was assessed by flow cytometry in skin draining lymph nodes (*p < 0.01).

Skin exposure of CMIT/MIT with OVA aggravated skin inflammation in AD mice model

We investigated whether skin exposure to CMIT/MIT during allergen sensitization enhances allergic AD-related consequences in AD mice model. Mice were exposed to CMIT/MIT during OVA intraperitoneal sensitization phase (Fig. 4). Regardless of OVA sensitization, CMIT/MIT exposure enhanced skin inflammation, TEWL and clinical score at 3 week after the last CMIT/MIT treatment than mice exposed to PBS alone. Furthermore, mice exposed to CMIT/MIT during OVA sensitization significantly increased inflammatory infiltrations, TEWL and clinical scores in skin compared to CMIT/MIT with PBS (Fig 5a-c). In addition, mice exposed to CMIT/MIT with OVA had higher inflammatory infiltration in skin, TEWL and clinical scores than mice exposed to OVA alone (Fig. 5a-c). However, there was no significant increase between them. This finding suggests that CMIT/MIT skin exposure during allergen sensitization enhances AD-like responses.

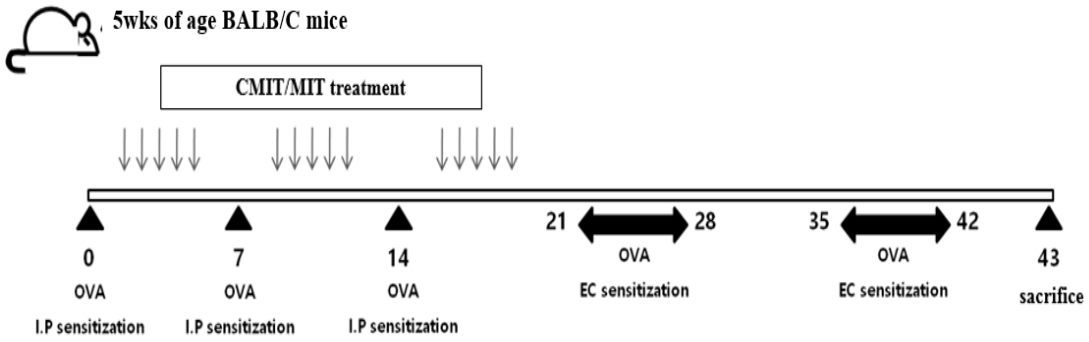

Fig.4. An experimental protocol for effects of CMIT/MIT exposure in AD mice model. Treatment was applied to skin for 3 weeks for 5 days a week. An AD mice model developed by OVA epidermal sensitization using normal mice. (I.P: Intraperitoneal, EC: Epicutaneous)

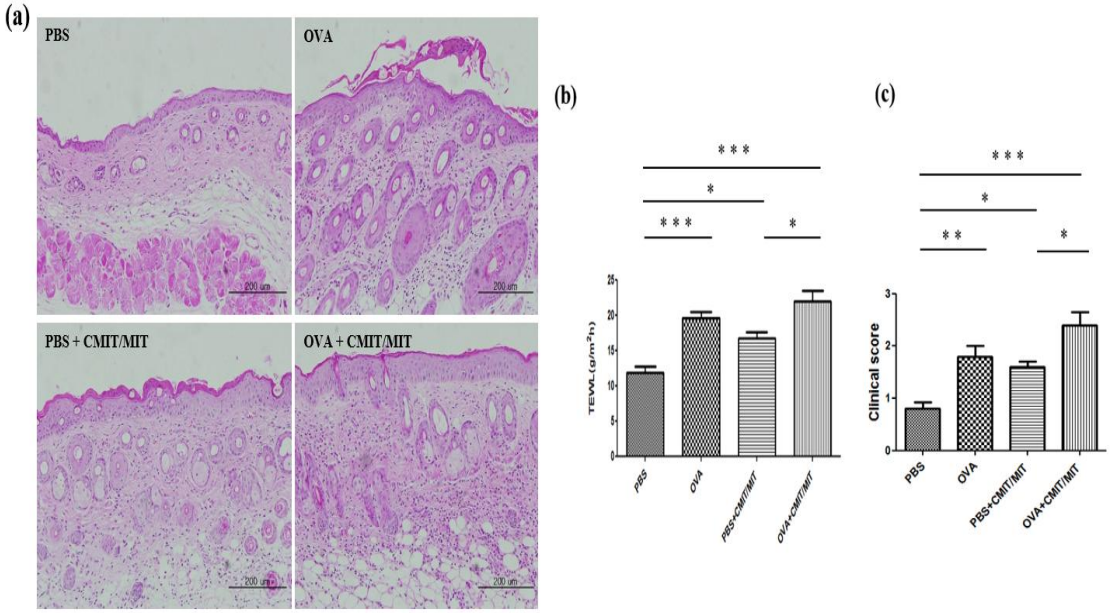

Fig. 5. Effects of CMIT/MIT exposure in AD mice model. (a) Histological from H&E-stained skin tissue, (b) TEWL and (c) Clinical score (*P < 0.05, **P < 0.01, ***P < 0.001).

Skin exposure of CMIT/MIT with OVA increased systemic immune response and Th2-Th17 response in the AD mice model

We investigated whether skin exposure to CMIT/MIT during allergen sensitization enhances systemic immune response in AD mice model. We observed that exposure of CMIT/MIT during OVA sensitization significantly increased serum total IgE levels than mice exposed to OVA alone (Fig. 6a). In addition, mice exposed to CMIT/MIT with OVA showed increased tendency of OVA-specific IgE compared to OVA alone which is not statistically significant (Fig. 6b).

Next, we measured the levels of the AD-like immune response in the skin and skin draining lymph node. TSLP expression in the skin was higher by exposure to CMIT/MIT with/without OVA than mice exposed to PBS alone (Fig. 6c). Furthermore, mice exposed to CMIT/MIT with OVA showed increased IL-4 and IL-17A expression in skin, not IL-13 than mice exposed to OVA alone (Fig. 6d-f). In addition, mice exposed to CMIT/MIT with OVA had more $CD4^{+}IL\text{-}4^{+}$ and $CD4^{+}$IL-17A producing cells in skin draining lymph nodes than mice exposed to OVA alone (Fig. 7). Interestingly, mice exposed to CMIT/MIT with PBS tended to have more there cells than mice exposed to OVA (Fig. 7). These findings indicate that CMIT/MIT exposure enhances allergic systemic response and dysregulation of Th2/Th17 in AD mice model.

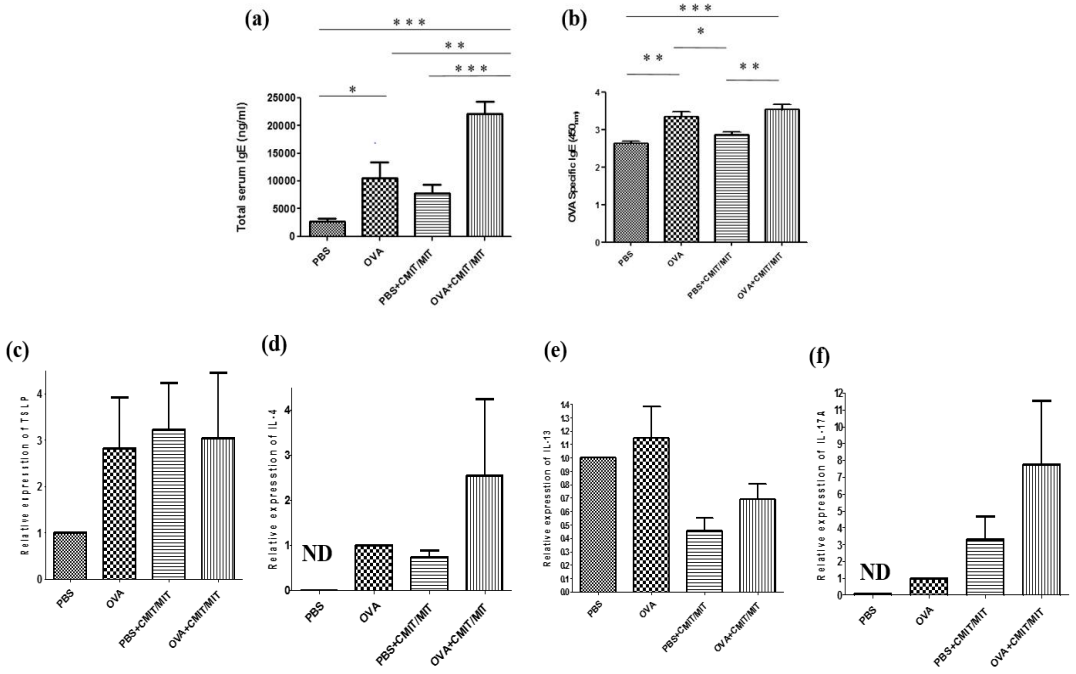

Fig. 6. Effects of CMIT/MIT exposure in AD mice model. (a) Concentration of total IgE in the serum and (b) Serum levels of OVA specific IgE were ELISA. Skin mRNA expression of (c) TSLP, Th2-related cytokines (d. IL-4; e. IL-13) and Th17-related cytokine (f. IL-17A) was assessed by real-time PCR, Statistical significance was determined with one-way analysis of variance (ANOVA) (*P < 0.05, **P < 0.01, ***P < 0.001). ND: non-detection.

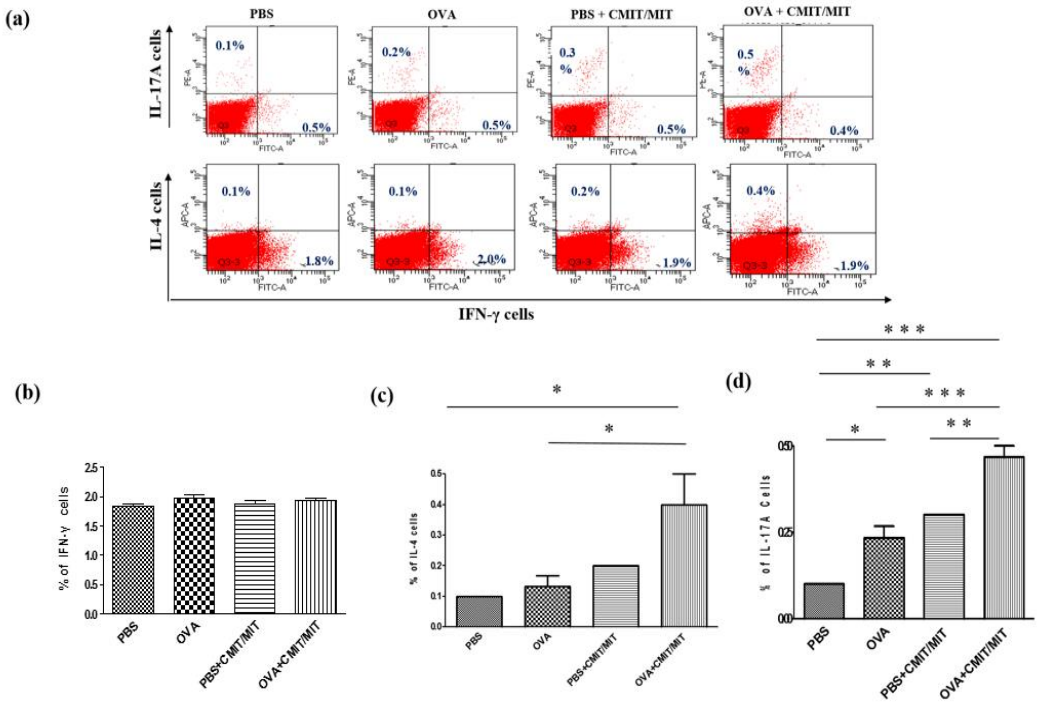

Fig. 7. Flow cytometric analysis of mice Th1/Th2/T17 phenotyping kit and effects of CMIT/MIT exposure in AD mice model. Statistical significance was determined with one-way analysis of variance (ANOVA). (*P < 0.05, **P < 0.01, ***P < 0.001).

Discussion

In the present study, we first demonstrated that repeated skin exposure to CMIT/MIT in normal mice significantly increased TEWL, serum IgE levels, infiltration of inflammatory cells and expression of TSLP and IL-13 into the skin and Th2 cell population in the skin draining lymph node. These findings suggest that skin exposure of CMIT/MIT in normal mice may induce AD-like skin inflammation and systemic immune responses. Moreover, CMIT/MIT skin exposure worsened the severity of subsequent OVA-induced AD mice, including TEWL, skin inflammation, serum total IgE levels, expression of Th2/Th17-related cytokines (IL-4 and IL-17A) in skin and Th2/Th17 cell population in the skin draining lymph node. These findings suggest that skin exposure of CMIT/MIT in AD mice may aggravate skin inflammation and systemic immune responses of AD. Therefore, the present study provides strong evidence that repeated skin exposure to CMIT/MIT may affect the development and aggravation of AD via the modulation of immune response in mice.

With the widespread use of chemical substances, the potential toxicity is getting increasing attention. Several studies have demonstrated that exposure to chemical substances such as dibutyl phthalate (DBP), di-(2-ethylhexyl)phthalate (DEHP) and CMIT/MIT can affect allergic inflammation in conditions with/without exposure to typical allergens [30, 31]. Exposure of DBP induced allergic skin inflammation and dermal eosinophil infiltration in mice [32]. Especially in the presence of antigen, DBP exposure rapidly aggravated allergic skin inflammation and AD-like symptoms [30, 33]. Similarly, our results showed that repeated skin

CMIT/MIT exposure induces allergic skin inflammation and enhances allergic skin inflammation during allergen sensitization in mice. In clinical studies, on physical examination, erythema and eczema was observed by skin exposure to CMIT/MIT through airborne [13, 15, 34, 35]. Also, CMIT categorized as an extreme skin sensitizer [36] and MIT proposed as a major contributor to systemic allergic reactions [37, 38]. These findings support that CMIT/MIT emerged as a potential systemic allergic inflammatory agent for the development of AD.

Our results also showed that expression of Th2 cytokine (IL-13) and Th2 cell population significantly increased immediately after exposure to CMIT/MIT even in normal mice. In addition, CMIT/MIT skin exposure during OVA sensitization increased expression of Th2/Th17-relatied cytokines (IL-4 and IL-17A) and Th2/Th17 cell population in AD mice. Interestingly, expression of IL-4 and IL-17A in normal skin mice was not detected immediately after CMIT/MIT exposure but was increased at 3 week after the last CMIT/MIT treatment without OVA sensitization. Our findings indicate that CMIT/MIT could cause allergic response via regulating Th2/Th17 immune function over time in chronic process.

Th2 cytokines (e.g., IL-4, IL-5 and IL-13) are known to play an important role in orchestrating the inflammation of AD through various routes [39]. Generally, allergen induces Th2 response and Th2 cell migration through stimulating the expression of Th2 cytokines, which in turn orchestrates the allergic-related responses [33]. Moreover, Th2 cytokines are an important biomarker in prolonged eosinophilia and play a key role in orchestrating the chronic

inflammation of AD by recruiting, activation, and promoting the eosinophils in the dermis [6]. The present study demonstrated that CMIT/MIT skin exposure increase Th2-related responses. Likewise, in experimental study, skin exposure of MIT induced increased T cell proliferation in mice [21]. Thus, it could be possible that exposure of CMIT/MIT induced and enhanced allergic AD-like consequences mediated via Th2-related responses in mice.

In addition to Th2 cells, recent studies have shown that Th17 immune response are also critical in mediating the initiation and progression of AD and differentiation of Th2 cells [39-41]. Also, TSLP might take part in activation of the Th17 responses. Several studies have shown that Th17 initiate signaling pathways to induce pro-inflammatory molecules which play a major role in recruiting neutrophils and tissue inflammation in patients with AD [42, 43]. Moreover, the activity of Th17 promote tissue fibrosis, chronicity of the inflammatory process, and the AD severity [44]. Our study showed that expression of IL-17A and Th17 cell population in the skin increased in CMIT/MIT exposure with/without OVA. Similarly, high expression levels of Th2/Th17-related cytokines (IL-4, IL-5, IL-13 and IL-17A) enhance skin inflammation and infiltration of inflammatory cells in DBP-induced mice [32]. These findings are suggestive that CMIT/MIT exposure could induces and enhances skin inflammation and allergic responses via Th2/Th17-mediated pathways in mice.

Our study has some limitations. First, we cannot discriminate the point of dermatitis whether it induces allergic contact dermatitis or AD by skin exposure of CMIT/MIT. Actually, allergic contact dermatitis and AD potentially share common cellular mechanisms and there is difficult

to distinguish between allergic contact dermatitis and AD in clinical diagnosis [45-46]. However, the increase in total IgE levels and TEWL is usually a feature of AD, not contact dermatitis [45]. Our results showed an increased serum IgE levels and TEWL after exposure to CMIT/MIT. Thus, it can be considered that the possibility of CMIT/MIT skin exposure may affect the development of AD. Second, although we show Th2/Th17 cytokines and cell population in mice, the detail functional effect of CMIT/MIT on Th2/Th17 cells will be needed. Thus, the ability of the CMIT/MIT to stimulate Th2/Th17 should also be investigated to identify the further mechanism. In addition, because little is known about the relationship between CMIT/MIT exposure and the development of AD in epidemiologic studies, further investigation is warranted.

The present study shows the AD-like effects of CMIT/MIT skin exposure in the normal and AD mice model and suggests the possible mechanism with Th2/Th17 immune response. More studies are need to confirm this interaction between immune responses and biological mechanism.

Conclusion

In conclusion, our findings suggest that skin exposure to CMIT/MIT could affect the development of AD via Th2/Th17-related activity in mice.

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Korean Abstracts

배경 및 목적: 아토피피부염은 만성 염증성 피부질환으로 유전적 요인과 함께 다양한 환경적인 요인의 상호작용이 면역체계에 영향을 주어 발생하는 것으로 알려져 있다. 최근에는 환경적인 요인, 특히 화장품 및 세정제 등에 이용되는 새로운 화학물질의 노출이 아토피피부염 증가의 주된 요인 임이 밝혀지고 있다. Chloromethylisothiazolinone/Methylisothiazolinone(CMIT/MIT)은 방부제와 살균제 용도로 이용되고 있는 화학물질 중 하나로 위생용품뿐 아니라 각종 화장품 및 세탁 용품에 이르기까지 다양한 제품에 광범위하게 이용되고 있다. 최근에는 CMIT/MIT가 경구, 피부 등의 경로로 쉽게 인체로 노출되며 피부 증감제와 자극물로 분류되어 알레르기 접촉성 피부염과의 관련성과 직업성 천식 증례도 보고되었다. 이는 CMIT/MIT가 알레르기 반응을 일으킬 가능성을 시사하여 주고 있다. 그러나 아직까지 CMIT/MIT가 아토피피부염 발생 및 악화에 영향을 미치는지에 대한 연구는 아직 부족하다. 따라서, 본 연구는 아토피피부염 동물 모델을 이용하여 지속적인 CMIT/MIT의 노출을 통해 아토피피부염 발생 및 악화에 대한 영향과 그에 따른 기전을 규명하고자 한다.

연구재료 및 연구방법: 5주령의 암컷 BALB/c 마우스 등에 털을 제거한 후 CMIT/MIT(0.1875mg/kg)를 하루에 1번씩 총 3주 동안 피부 도포를 통해 노출시켰다. 8주령이 되었을 때 난 알부민 (ovalbumin, OVA) 항원을 피부에 적용시켜 항원 감작을 통한 아토피피부염 유발을 시행하였다. 아토피피부염의 증상을 확인하기 위하여 피부의 수분 손실량 (TEWL)과 피부 발적 및 홍반 등

임상증상 정도를 점수화 (Clinical score) 하여 평가하였으며, 마우스의 피부 조직에서 H&E 염색을 통해 염증세포 침윤 정도를 확인하였다. 또한 전신 면역 유발 정도를 확인하기 위하여 혈청 내 총 immunoglobulin(Ig)E와 항원 특이 IgE를 Enzyme-linked immunosorbent assay (ELISA) 기법을 통해 측정하였고, 피부 내 염증 발생과 관련한 사이토카인인 Thymic stromal lymphopoietin (TSLP)와 T helper (Th)2 사이토카인인 interleukin(IL)-4, IL-13, Th17세포에서 분비되는 염증 사이토카인인 IL-17A를 qPCR을 이용하여 측정하였다.

연구결과: CMIT/MIT의 단독 노출의 영향을 정상 마우스에서 확인한 결과, PBS만 도포한 군에 비하여 CMIT/MIT 노출군에서 TEWL, 혈청 내 IgE 농도가 유의하게 증가함을 확인하였고 피부 조직 내 염증의 침윤 정도도 심해짐을 관찰하였다. 또한, CMIT/MIT 노출 군에서 피부 내 TSLP 및 IL-13의 발현량이 유의하게 증가하였으며, 피부 림프절에서 $CD4^{+}IL-4^{+}$의 유의한 증가를 보였다. 다음으로 아토피피부염 마우스 모델을 통해 CMIT/MIT의 영향을 확인하였다. OVA 단독으로 아토피피부염을 유발한 군에 비하여 CMIT/MIT 노출 후 아토피피부염을 유발한 군에서 TEWL, clinical score가 증가하였고, 피부 조직에서 표피층의 두께 증가 및 진피층까지 염증세포의 침윤 정도가 심해짐을 확인하였다. 또한, OVA 단독 아토피피부염 유발군에 비하여 CMIT/MIT가 노출된 아토피피부염 유발군에서 혈청 내 총 IgE의 농도가 유의하게 증가함을 확인하였으나 항원 특이 IgE 농도에서는 차이를 보이지

않았다. 다음으로 피부 내 염증과 관련된 사이토카인의 발현량을 확인한 결과, 아토피피부염 비유발군에 비하여 CMIT/MIT 노출군, 아토피피부염 유발군 및 CMIT/MIT 노출 후 아토피피부염 유발군 모두에서 TSLP, IL-4 및 IL-17A의 발현량이 증가함을 확인하였다, 특히, 단독 아토피피부염 유발군과 비교하여 CMIT/MIT 노출 후 아토피피부염 유발군에서 IL-4와 IL-17A의 발현량이 증가함을 확인하였으나 통계적으로 유의하지는 않았다. 또한 피부 림프절에서는 아토피피부염 유발군에 비하여 CMIT/MIT 노출 후 아토피피부염 유발군에서 $CD4^{+}IL-4^{+}$와 IL-17A 세포의 유의한 증가를 보였다.

결론: 본 연구결과, CMIT/MIT 노출은 환경항원으로 작동하여 면역 시스템에 영향을 끼쳐 Th2/Th17 반응을 유도한다. 아토피피부염 마우스 모델에서 CMIT/MIT의 지속적인 노출은 Th2/Th17 면역 반응과 관련하여 아토피피부염 악화에 영향을 줄 가능성이 있다.

중심단어: 아토피피부염, Chloromethylisothiazolinone, Methylisothiazolinone, Skin exposure, T helper 2, T helper 17