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Effect of NLRP3 inhibition in allergic rhinitis mice model

알레르기비염 쥐모델에서 NLRP3 억제의 영향 분석

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Effect of NLRP3 inhibition in allergic rhinitis mice model

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Abstract

Objectives: This study aimed to identify the activation of NLRP3 inflammasome signaling pathway in OVA-induced allergic rhinitis (AR) mice model and to validate the effect of specific inhibitor of the NLRP3, MCC950.

Methods: Mice were divided into 3 groups and each group consisted of 10 mice (Saline group, which was negative control group; OVA group, which was OVA-induced allergic rhinitis model group; and OVA+MCC group, which was OVA group with 10mg/kg MCC950 treatment group). The OVA group was constructed as conventional OVA/Alum protocol. MCC950 was challenged intraperitoneally every second day. Multiple parameters of allergic rhinitis including NLRP3, caspase-1, IL-1β, and IL-18 were evaluated from serum and spleen supernatant ELISA, nasal tissue real-time PCR, nasal tissue histopathology, and immunohistochemistry.

Results: OVA-induced allergic rhinitis model was well-constructed in based on symptom score, serum immunoglobulin (Ig) levels, systemic cytokine levels, and mRNA levels of nasal tissue cytokines. In addition, the mRNA and protein levels of NLRP3, caspase-1, IL-1β and IL-18 were upregulated in the OVA group compared with the Saline group. MCC950 significantly inhibited the mRNA and protein levels of NLRP3, caspase-1, IL-1β and IL-18 in nasal tissue. Further, AR symptoms and eosinophil count were normalized after MCC950 treatment. However, OVA-specific IgE was not restored in the OVA+MCC group.

Conclusion: NLRP3 inflammasome signaling pathway may be an alternate pathway to induce AR symptoms in OVA-induced allergic rhinitis model. MCC950 is a specific inhibitor of NLRP3 cascade,

which attenuates AR symptoms regardless of IgE.

Keyword : NLRP3, Inflammasome, Allergic rhinits, MCC950

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Chapter 1. Introduction

Allergic diseases have become an important public health problem worldwide in recent decades. Allergic diseases affect millions. Despite many advances in our understanding of the immune system in the last century, the physiological basis for the presence of allergy still remains a mystery (1). Between 20% and 30% of residents in the developed countries suffer from at least one type of allergy-related disease (2). And allergic rhinitis (AR) was present in 15% to 18% of the western population (3).

Recently accumulated human and mouse experimental evidence has demonstrated that Nod-like receptor family pyrin domain-containing proteins (NLRP)3 inflammasome is closely involved in the development of allergic diseases. NLRP3 inflammasome is a multiprotein complex known to be associated with many inflammatory diseases (4). In response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), NLRP3 changes its conformation to enable the assembly of NLRP3 inflammasome complex, which activates caspase-1. Active caspase-1 cleaves the precursors of the inflammatory cytokines interleukin (IL)-1β and IL-18 (5). IL-1β is secreted mainly by macrophages. IL-1β can promote the differentiation of T helper (Th)17 cells, maintain the production of Th17-related cytokines, and accelerate allergic reactions (6). IL-18 can induce T cells, mast cells, and basophils to secrete Th2 cytokines like IL-4 and IL-13, and also enhance Th1 and Th2 cell-mediated immune reactions (7). Leaker et al. investigated the whole genome expression profile of AR patients and revealed upregulation of type 2 inflammation involving IL-1β (8). Verhaeghe et al. reported elevated levels of IL-18 in nasal secretions of seasonal and persistent AR patients, indicating the association of IL-18 with AR (9). Bogefors et al. found that the expression of NLRP3 in nasal mucosal epithelium increased significantly in AR patients, but decreased as symptoms were controlled (10). Oh et al. reported the increase of IL-1β and caspase-

1 in the serum or nasal mucosa in the OVA-induced AR mice compared to those in the control mice. IL-1β and caspase-1 expression were decreased after the administration of anti-allergic drugs, and the allergic symptoms were attenuated (11). Thus, NLRP3 is an important pathogen recognition receptor (PRR) within the cytoplasm and the application of specific NLRP3 inflammasome pathway inhibitors has been demonstrated in various animal studies (12).

IL-1β blockade by anti-IL-1β immunoglobulin (Ig) yolk could be a promising strategy for preventing and treating AR (13). The targeted inhibition of NLRP3 by microRNA-133b reduced the expression levels of caspase-1, apoptosis-associated speck-like protein containing a CARD (ASC), IL-1 and IL-18 and strongly attenuated the pathological alterations and eosinophil and mast cell infiltration in nasal mucosa from mice with AR (14). Specific caspase-1 inhibitor Belnacasan treatment ameliorated the development and progression of AR with favorable outcomes in OVA-induced AR mice model (15). Therefore, these inhibitors may represent potential therapeutic methods for the management of clinical allergic disorders (7).

MCC950, a potent, selective, small-molecule inhibitor of NLRP3, blocks canonical and noncanonical NLRP3 activation (16). MCC950 directly interacts with the Walker B motif within the NLRP3 NACHT domain, blocking ATP hydrolysis and inhibiting NLRP3 activation and inflammasome formation (17). Kim et al. reported the inhibition of NLRP3 with MCC950 had more potent effects than inhibiting caspase-1 in severe, steroid-resistant asthma mice model. They concluded that there may be additional therapeutic benefits of inhibiting NLRP3 directly, instead of targeting inflammasome-mediated caspase-1 or IL-1β activity (18).

On the basis of the above results, I hypothesized that the inhibition of NLRP3 directly in AR might also present better therapeutic outcome compared to the inhibition of caspase-1, IL-1β or IL-18. I investigated the effects of MCC950 in OVA-induced AR mice model.

Chapter 2. Materials and Methods

2.1. Animal model

Thirty female BALB/c mice (4 weeks of age) were involved as experimental animals (Koatech, Pyeongtaek, Korea). Each mouse weighed 19–21 g and was maintained under specific pathogen–free conditions. All animal experiments in the present study followed the guidelines and ethics of the Institutional Animal Care and Use Committee of the Biomedical Research Institute of Seoul National University Hospital (IACUC No.: 18–0026–S1A0(3)) Mice were divided into 3 groups as follows: The saline group (n = 10) as a negative control group, the OVA group (n = 10) as a positive control group, and the OVA+MCC group (n = 10) as a MCC950 (AdipoGen, San Diego, CA, USA) treatment group. The mice were sensitized by an intraperitoneal injection of 25 μg of ovalbumin (OVA; Sigma–Aldrich, St. Louis, MO, USA) and 1 mg of aluminum hydroxide (Alum; ThermoFisher Scientific, Waltham, MA, USA) mixture on days 0, 7, and 14. The mice were then subjected to intranasal challenges with 100 μg of OVA on 7 consecutive days from days 21 to 27. The negative control mice were intraperitoneally injected and intranasally challenged with phosphate–buffered saline (PBS), instead of OVA following the same schedule until day 27 at which mice were intranasally challenged with OVA. The OVA+MCC group mice were intraperitoneally injected with 10mg/kg of MCC950 every second day from days 21 to 27 immediately after intranasal OVA challenge. The saline group and OVA group mice were intraperitoneally injected with PBS as the same manner (Fig. 1).

2.2. Symptom score

On day 27, after intranasal allergen provocation with 100 μg of OVA, the numbers of sneezing and nose rubbing bouts were counted for 15 minutes to evaluate early allergic responses by blinded observers.

2.3. Tissue preparation

The mice were euthanized 24 hours after the last OVA challenge. Serum and nasal lavage fluid samples from each mouse were obtained. Spleens were harvested for cell culture. The head of 5 mice from each group were removed en bloc and then fixed in formaldehyde solution. Nasal mucosa was obtained from the 5 other mice for a real time-polymerase chain reaction (RT-PCR) and then was immediately immersed in liquid nitrogen and stored at -70°C until use.

2.4. RT-PCR analysis of cytokines in the nasal mucosa

Total RNA was isolated from the nasal mucosa using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Gendepot, Katy, TX, USA). For the analysis of NLRP3 (Mm00840904_m1), caspase-1 (Mm00438023_m1), IL-1β (Mm00434228_m1), IL-18 (Mm00434226_m1), IL-4 (Mm00445259_m1), IL-5 (Mm00439646_m1), IL-10 (Mm00439616_m1), IL-13 (Mm00434204_m1), IFN-γ (Mm99999071_m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Mm 99999915_g1), probes were purchased from ThermoFisher Scientific. The amplification of NLRP3, caspase-1, IL-1β, IL-18, IL-4, IL-5, IL-10, IL-13, IFN-γ and GAPDH cDNA was performed in MicroAmp optical 96-well reaction plates (Applied Biosystems, Foster City, CA, USA). The reaction was performed using a QuantStudio™ 3 RT-PCR System (Applied Biosystems). The average transcript levels of genes are normalized to GAPDH expressed as ΔΔCT.

2.5. Nasal histology and immunohistochemistry (IHC)

For the evaluation of nasal histology, the heads of mice in each group were fixed with 10% formaldehyde solution. The nasal tissues were decalcified with ethylenediaminetetraacetic acid (EDTA) solution, embedded in paraffin, sectioned coronally into 4-μm slices and stained with hematoxylin and eosin (H&E). Under a light microscope (×400 magnification), eosinophils were counted in 5 fields of the nasal septal mucosa by a single-blind observer.

IHC was undertaken using anti-NLRP3 antibody, anti-caspase-1 antibody, anti-IL-1β antibody and anti-IL-18 antibody, which were purchased from Abcam (Cambridge, UK). The total area and stained area were measured in 4 fields of the nasal septal mucosa, and calculated the percentage of the stained area using ImageJ software.

2.6. Serum levels of total and OVA-specific immunoglobulins

Serum samples from each mouse were obtained at the time of sacrifice. Serum levels of total IgE and OVA-specific IgE, IgG_1 and IgG_{2a} were measured by enzyme-linked immunosorbent assay (ELISA). For the analysis of total IgE, 96-well flat-bottom plates were coated with purified rat anti-mouse IgE (#553413; BD Bioscience, San Jose, CA, USA). A purified mouse IgE isotype (#557079; BD Bioscience) was used as a standard. Nonspecific antigen-antibody reactions were blocked with 3% bovine serum albumin (BSA). To detect total IgE, horseradish peroxidase (HRP)-conjugated anti-mouse Ig (#1130-05; SouthernBiotech, Birmingham, AL, USA) was added to the wells. For the analysis of OVA-specific IgE, serum samples were added to OVA-coated 96-well flat-bottom plates. After washing, 100 μL of biotin-conjugated rat anti-mouse IgE mAb (#553414; BD Bioscience) was added, followed by the addition of streptavidin-HRP (#554066; BD Bioscience). For the analysis of OVA-specific IgG_1 and IgG_{2a}, serum samples were added to OVA-coated 96-well flat-bottom plates. After washing, biotinylated rat anti-mouse IgG_1 and IgG_{2a} (#553341

and #553388, respectively; BD Bioscience) were added to each well and incubated. After washing, streptavidin-HRP was added to each well. The reactions were developed using 3,3′,5,5′-tetramethylbenzidine(TMB; SeraCare, Milford, MA, USA) and terminated by adding 1 MHCl. The absorbance was measured in a microplate reader at 450 nm.

2.7. Measurement of cytokines in the spleen cell culture

Spleen single-cell suspensions from each mouse were plated in 24-well cell culture plates at a final concentration of 5 × 106 cells/well using Roswell Park Memorial Institute 1,640 containing 10% fetal bovine serum (FBS) supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco, Grand Island, NY, USA). The cells were incubated in a CO2 incubator at 37° C for 72 hours and stimulated with OVA for 72 hours. The culture supernatant was collected and stored at -70° C until cytokines were measured. Cytokine levels in the culture supernatant were assayed using a DuoSet ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. After measuring the absorbance at 450 nm, the concentrations of IL-4, IL-5, IL-10 and IFN-γ were determined by interpolation from the respective standard curves; all data are expressed in pg/mL.

2.8. Statistics

The data are presented as mean ± standard error mean. Mann-Whitney U test was used to compare results between the negative and positive control groups and between the treatment and positive control groups. P values of <0.05 were considered statistically significant. Statistical analysis was performed using SPSS 22.0 software (IBM Co., Armonk, NY, USA) and GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA).

Chapter 3. Results

3.1. Symptom score

The mean numbers of nose rubbing, sneezing, and sum of symptoms in the OVA group (positive control) were significantly higher than those in the saline group (negative control) (p=0.0005, 0.0007, and 0.0007, respectively). The OVA+MCC group (MCC950 treatment group) showed downregulation of the numbers of nose rubbing, sneezing, and sum of symptoms than those in the OVA group (p=0.0021, 0.0352, and 0.0136, respectively) (Fig. 2).

3.2. Total and OVA-specific immunoglobulins in the serum and IL-4, 5, 10, and IFN-γ in the spleen supernatant

From serum ELISA, significant elevation of total IgE, OVA-specific IgE, IgG_{2a} and IgG_1 levels were identified in the OVA group, indicating well-constructed AR mice model (p= <0.0001, <0.0001, 0.0015, and 0.0011, respectively). But these effects were not attenuated by intraperitoneal MCC950 treatment (p=0.9766, 0.8534, 0.1333, and 0.7394, respectively) (Fig. 3). From ELISA of spleen supernatant, the increased levels of IL-4, 5, 10, and IFN-γ were found in the OVA group (p=0.0005, 0.0008, 0.0002, and 0.0006, respectively), which were not restored despite of intraperitoneal MCC950 treatment (p=0.8665, 0.0721, 0.2319, and 1.0000, respectively) (Fig. 4).

3.3. The mRNA levels of NLRP3, caspase-1, IL-1β, IL-18, IL-4, IL-5, IL-10, IL-13, and IFN-γ in the nasal tissues

NLRP3, caspase-1, IL-1β, and IL-18 mRNA levels were significantly elevated in nasal tissues from the OVA group compared

with the Saline group (p=0.0095, 0.0303, 0.0079, and 0.0079, respectively) and they were reduced in the OVA+MCC group (p=0.0286, 0.0357, 0.0079, and 0.0159, respectively) (Fig. 5A-D). The mRNA levels of IL-4 and IL-5 were upregulated in the OVA group compared with the Saline group (p=0.0025, and 0.0159, respectively), which were downregulated after MCC950 treatment without statistical significance (p=0.1508, and 0.4206, respectively) (Fig. 5E, F). This trend, increase in the OVA group and restoration in the OVA+MCC group also showed in IL-10, IL-13, and IFN-γ mRNA levels, but there was no statistical significance (Fig. 5G-I). I then calculate IL-4/IFN-γ and IL-5/IFN-γ ratio to validate Th2/Th1 balance. As indicated in Fig. 5J and K, the mRNA levels of IL-4/IFN-γ and IL-5/IFN-γ ratio were significantly increased in the OVA group (p=0.0159, and 0.0159, respectively), and they were decreased in the OVA+MCC group (p=0,0286, and 0.0286, respectively)

3.4. The H&E stain, and IHC stain of NLRP3, caspase-1, IL-1β, and IL-18 in the nasal tissues

From the histology, the increased eosinophils were identified in the OVA group compared with the Saline group, and eosinophil infiltration was suppressed in the OVA+MCC group with statistical significance (p=0.0049, and 0.0160, respectively) (Fig. 6).

From the immunohistochemical staining of nasal tissues, significant upregulation of NLRP3, caspase-1, IL-1β and IL-18-stained areas was noted in the OVA group (p=0.0025, 0.0043, 0.0025, and 0.0022, respectively). They were significantly diminished after MCC950 treatment, indicating MCC950 efficiently inhibited the NLRP3 inflammasome pathway (p=0.0079, 0.0043, 0.0087, and 0.0022, respectively) (Fig. 7).

Chapter 4. Discussion

AR is characterized by nasal obstruction, nasal pruritus, rhinorrhea, and sneezing, and is caused by inhaled allergen which induces IgE-mediated inflammatory reaction. This allergic immune response is mediated by allergen-specific Th2 cells that induce the activation of B cell and IgE class switching, which is called as type I hypersensitivity. The activation and degranulation of mast cell and basophil lead to acute AR symptoms. Further, activated Th2 cells produce IL-4, IL-5, and IL-13 that induce eosinophil infiltration. Still, the mechanisms of AR are unclear and required further investigations (19, 20). NLRP3 inflammasome activation has been described in diverse infectious and nonmicrobial diseases of intestine and lung, gout, autoimmune disease, Alzheimer's disease, diabetes, and so on (21). The correlation with allergic diseases was also demonstrated, including asthma, dermatitis, rhinitis, and conjunctivitis (7). NLRP3 is recently considered the innate immune complex that activates caspase-1, which accelerates the production of IL-1β and IL-18 by various signals. Inflammatory stimuli such as PAMPs and DAMPs induces innate immune system, including PRRs in monocytes and macrophages, which produces pro- IL-1β and pro-IL-18. Monocytes and macrophages secret activated IL-1β and IL-18 by caspase-1 from NLRP3 inflammasome. The molecular mechanism of NLRP3 inflammasome assembly is still unveiled. To date, 3 classical mechanisms have been reported, including changes of K^+ influx/efflux, Ca^{2+} induced lysosomal damage, and production of reactive oxygen species (5).

Recently, one research was published, which was similar to this report. Zhang et al. reported MCC950 had ameliorative effect in an OVA-induced allergic rhinitis mouse model. MCC950 treatment showed the significant decrease in the allergic symptom, mRNA and protein levels of cytokines, which were close with the effects of dexamethasone (22). In this study, however, the upregulated total IgE, OVA-specific IgE, OVA-specific IgG_1, and OVA-specific IgG_{2a}

in serum, and systemic cytokines including IL-4, IL-5, IL-10, and IFN-γ in spleen supernatant were not decreased in the OVA+MCC group in comparison to the OVA group. On the contrary, the mRNA levels of IL-4, IL-5, IL-10, and IFN-γ in nasal tissue were attenuated by MCC950 treatment. Local, rather than systemic, downregulation of Th1, Th2, and regulatory T cell reactions was distinct after intraperitoneal administration of MCC950 in this study. Yang et al. also reported the activation of NLRP3 inflammasome signaling pathway promoted the development of allergic rhinitis (15). In their study, nasal lavage fluid and nasal mucosal tissue from AR patients were evaluated and identified the protein levels of caspase-1, IL-1β and IL-18 were upregulated. After then, OVA-induced allergic rhinitis mouse model was constructed by similar protocol of this study, which showed upregulated allergic symptoms. The protein level of IL-1β from nasal lavage fluid and nasal tissue was identified. Second, NLRP3 knockout mice were induced by OVA and exhibited decreased AR symptoms and the levels of caspase-1 and IL-1β compared with the control group. The mRNA levels of some proinflammatory cytokines and chemokines related with allergic rhinitis were decreased in NLRP3 knockout group. However, there was no difference in serum IgE level between control and NLRP3 knockout group. After treatment of Belnacasan, the specific inhibitor of caspase-1, the attenuation of AR symptoms, the protein levels of caspase-1 and IL-1β in nasal tissue, and the mRNA levels of some cytokine and chemokine in nasal tissue were identified. But still, serum IgE level was not restored after Belnacasan treatment. In other study, Xiao et al. presented the upregulation of microRNA-133b suppressed NLRP3 inflammasome activation. MicroRNA-133b directly targeted NLRP3, and reduced AR symptoms, serum OVA-specific IgE, the levels of cytokines such as IL-4, 5, and IFN-γ from cervical lymph nodes cell suspensions and serum, and the mRNA level of AR cytokines from nasal mucosa tissue. From immunohistochemistry, microRNA-133b overexpression alleviated NLRP3 inflammasome signaling pathway including NLRP3, caspase-1, ASC, IL-1β and IL-18 (14). In result, there is a debate that the

NLRP3 pathway in OVA-induced AR mice model is really associated with IgE-mediated reaction (14, 22) or not (15). Classical type I hypersensitivity in AR is induced by mast cells and basophils cross-linked with antigen-specific IgE. IgE-independent response in AR can be explained as the induction of mast cell/basophil-independent histamine release from endotoxin-containing allergen exposure (23). The application of endotoxin-containing OVA triggered a type 1 hypersensitivity-like symptoms in mice, which was dependent on CD4 T cell, histamine, and monocytes/macrophages. Like NLRP3 inflammasome signaling pathway, monocytes and macrophages are considered the major effector cells in IgE-, and mast cell/basophil-independent nasal hypersensitivity. In addition, severe, steroid-resistant asthma mice model which induced by Chlamydia and Haemophilus infection and showed the upregulation of NLRP3 inflammasome, caspase-1, and IL-1β, presented increased airway neutrophils and macrophages without eosinophilia (18). In this reason, the mechanism of NLRP3-related allergic reaction may be associated with IgE-independent monocyte/macrophage-dominant hypersensitivity rather than classical allergic reaction. In conclusion, NLRP3 inflammasome activation in nasal mucosa may contribute to the AR progression through the regulation of caspase-1, IL-1β, and IL-18 signals. In addition, MCC950 can be an effective treatment option to relieve AR symptoms. I suspect that the conventional OVA-induced AR mice model may not be a satisfactory animal model to validate the molecular mechanisms and therapeutic outcomes associated with NLRP3. The assembly of inflammasome complexes is induced by PAMPs, and lipopolysaccharide (LPS) and double-stranded DNA or RNA are typical PAMP molecules. Thus, LPS- or double stranded DNA or RNA, such as polyinosinic-polycytidyic acid, -induced rhinitis mice models may be better mice models which presented lower eosinophils and IgE, and higher neutrophils (24,25). To elucidate the action of NLRP3 inflammasome in the nasal microenvironment, a proper animal model should be constructed at first. In this study, local Th2 cells were suppressed after inhibition of NLRP3, but local Th1 cells were not. This result was unexpected

because I hypothesized NLRP3 might be linked closely with Th1-related inflammation. IgE-independent allergic rhinitis models showed IL-17 dominancy (24,25), which can explain the possible correlation between NLRP3 and Th17 rather than Th1. The limitation of this study was insufficiency of pharmacologic consideration of MCC950 instillation intraperitoneally. I did not optimize the proper dose of MCC950 and the route of instillation, and just followed the previous study (16,17). Intranasal administration of MCC950 may be more effective, because the activation of NLRP3 and inhibition by MCC950 were predominant at nasal mucosa. The small number of groups was also the significant factor to cause obscure results. Further study requires the evidence of correlation between NLRP3 and IgE-independent, Th17-related allergic reaction, with optimized methods.

Chapter 5. Conclusion

Well-constructed conventional OVA-induced AR mice model was associated with the activation of NLRP3 inflammasome signaling pathway. Specific inhibition of NLRP3 by MCC950 treatment attenuated NLRP3, caspase-1, IL-1β and IL-18, but not restored OVA-specific IgE elevation.

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Figure 1. Experimental protocol for the OVA-induced rhinitis model and MCC950 treatment

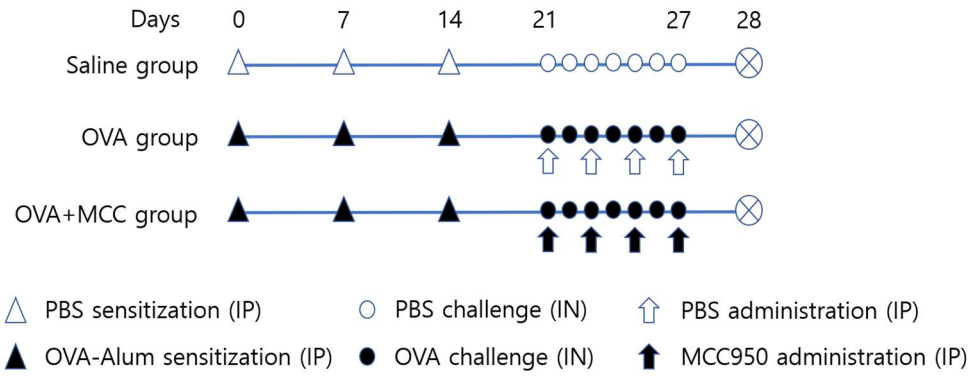


Figure 2. (A–C) Symptom score calculated from the frequencies of nasal rubbing and sneezing. *P<0.05, **P<0.01, ***P<0.001.

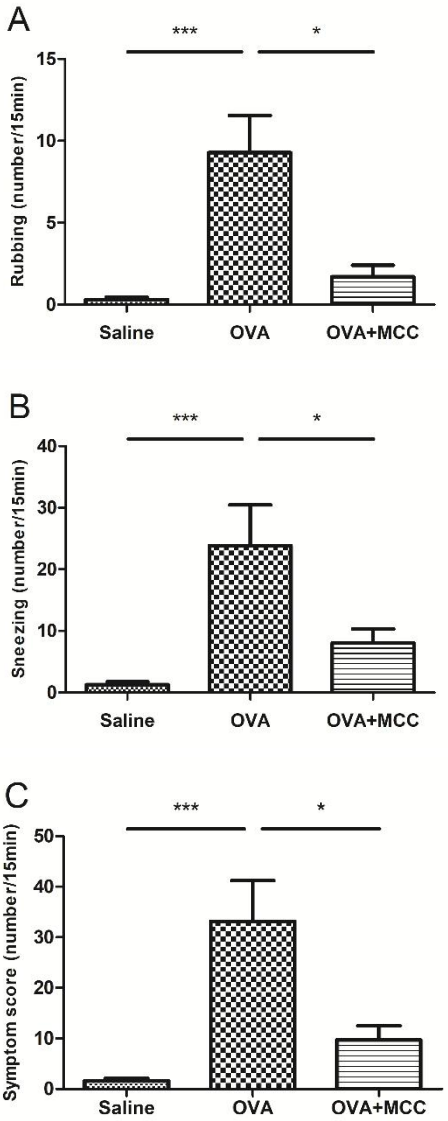


Figure 3. (A–D) Serum levels of total IgE, OVA–specific IgE, OVA–specific IgG_{2a}, and OVA–specific IgG_1 measured by ELISA. (E) OVA–specific IgG_1/IgG_{2a} ratio was calculated to identify the balance between T helper 1 and 2 cells. *P<0.05, **P<0.01, ***P<0.001.

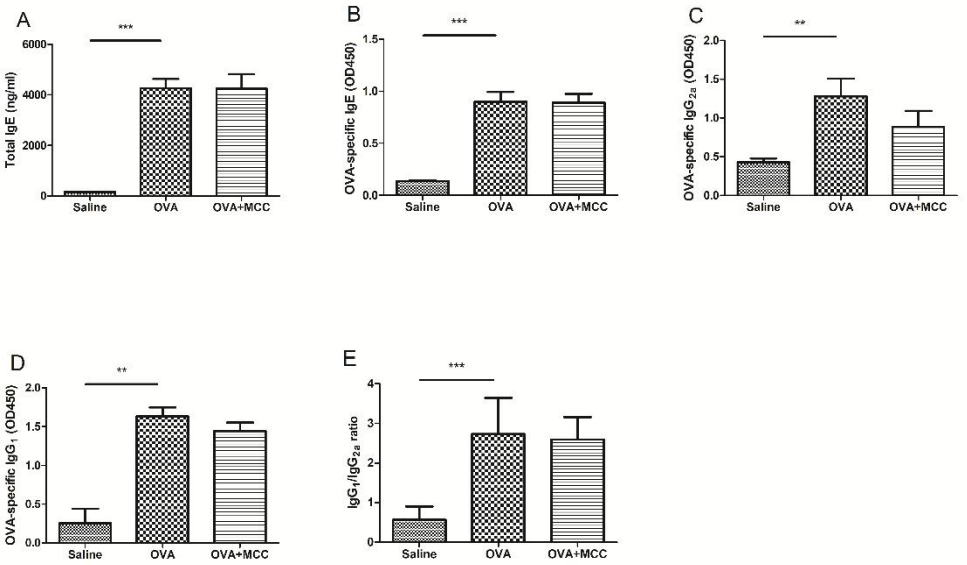


Figure 4. Systemic cytokine levels of IL-4, IL-5, IL-10, and IFN-γ in the spleen cell culture measured by ELISA. *P<0.05, **P<0.01, ***P<0.001.

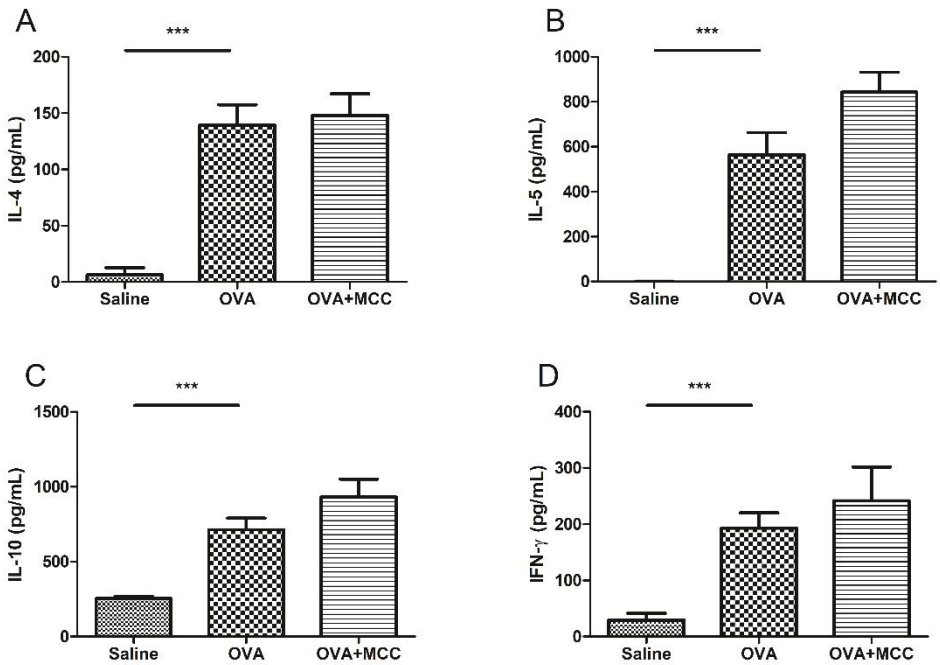


Figure 5. (A-I) The mRNA level of nasal cytokines measured by real-time PCR. (J-K) IL-4 or 5/IFN-γ ratio were calculated to identify the balance between T helper 1 and 2 cells. *P<0.05, **P<0.01, ***P<0.001.

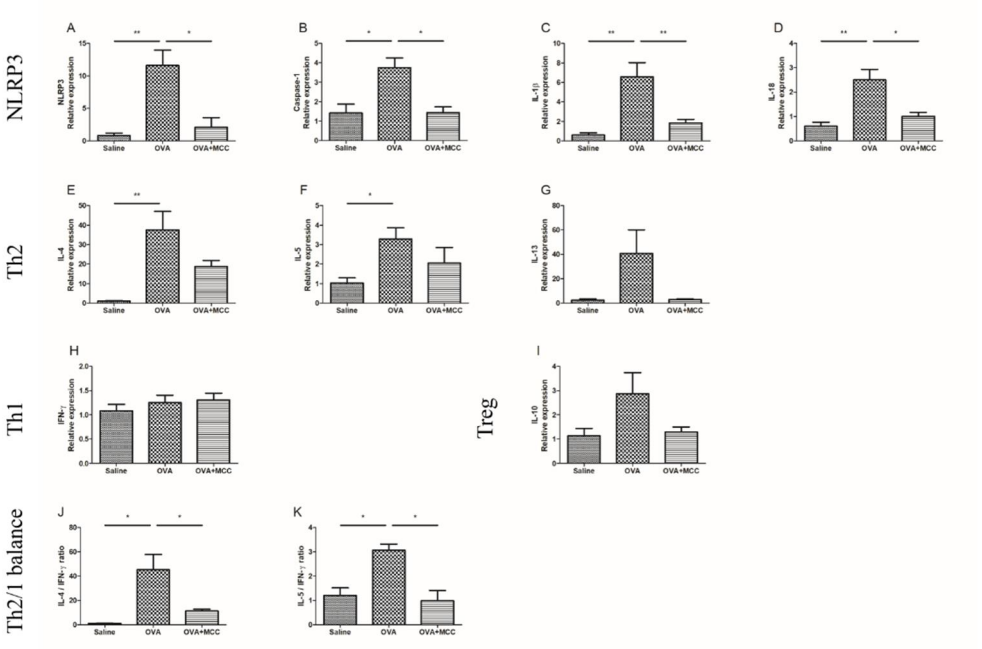


Figure 6. Nasal histology stained with H&E (x200 magnification). Eosinophil infiltration in the nasal septal mucosa was identified in the OVA group and the OVA+MCC group compared with the saline group. Eosinophil count was reduced after treatment of MCC950. *P<0.05, **P<0.01, ***P<0.001.

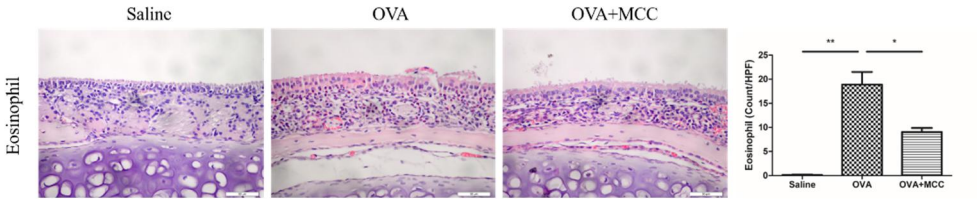
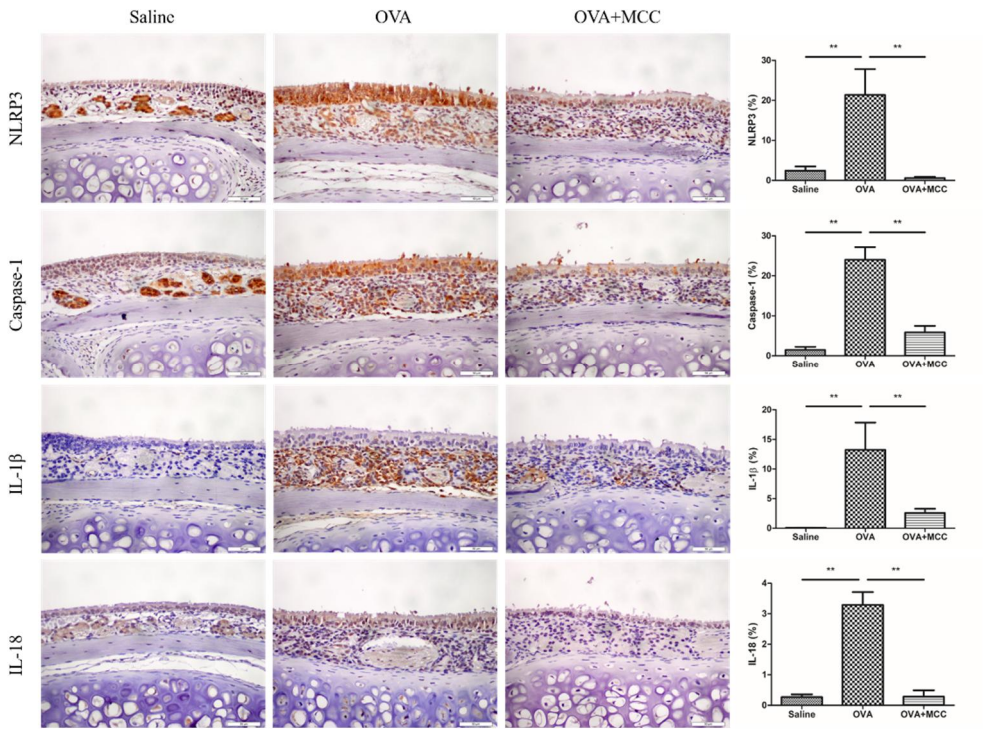


Figure 7. Immunohistochemical staining of nasal septal tissues for anti-NLRP3 antibody, anti-caspase-1 antibody, anti-IL-1β antibody and anti-IL-18 antibody (x200 magnification). The proportion of the stained area was calculated through ImageJ software. The stained areas of NLRP3, caspase-1, IL-1β and IL-18 increased in the OVA group. They were significantly diminished after MCC950 treatment. *P<0.05, **P<0.01, ***P<0.001.



국 문 초 록

알레르기비염 쥐모델에서 NLRP3 억제의 영향 분석

이민형

의학과 중개의학전공

목적: 본 연구는 OVA 유도 알레르기비염 쥐모델에서 NLRP3 인플라마좀 신호 전달 체계의 활성화 여부를 확인하고, NLRP3 를 특이적으로 억제하는 MCC950 의 역할을 평가하고자 한다.

방법: 전체 쥐를 3군으로 나누어 각 군별로 10마리씩 연구를 계획하였다. 음성대조군 (Saline 군), OVA 유도 알레르기비염 양성대조군 (OVA 군), OVA 유도 알레르기비염 모델에 10mg/kg 의 MCC950 을 처리한 치료군 (OVA+MCC 군) 으로 구성하였다. OVA 유도 알레르기비염 쥐모델 제작은 전통적인 OVA/Alum 프로토콜에 따랐다. MCC950 은 OVA 의 비강 주입 시기에 이틀에 한 번씩 복강내로 주입하였다. 알레르기비염 관련 싸이토카인 등과 함께 NLRP3, caspase-1, IL-1β, IL-18 를 평가하였다. 해당 검사를 위해 혈청 및 비장 상청액에서 효소결합면역흡착검사를, 비강 점막으로부터 실시간 연쇄중합반응검사와 병리조직학적 검사, 면역조직화학 검사를

시행하였다.

결과: 알레르기비염 관련 증상, 혈청 내 면역글로불린 수치, 전신 싸이토카인 수치, 비강 점막 내 전령RNA 수치가 증가하는 것에서 OVA 유도 알레르기비염 쥐모델이 잘 제작되었음을 확인하였다. 그리고 NLRP3, caspase-1, IL-1β, IL-18 의 비강 점막 내 전령RNA 와 단백질 수치가 Saline 군에 비해 OVA 군에서 증가됨을 확인하였다. 또한 MCC950 의 처리가 NLRP3, caspase-1, IL-1β, IL-18 의 비강 점막 내 전령RNA 와 단백질 수치를 감소시킴을 확인하였다. 그리고 MCC950 의 처리 후 알레르기비염 관련 증상과 호산구 개수가 회복됨을 확인하였다. 그러나 OVA 특이 면역글로불린E 는 MCC950 의 처리 이후에도 증가된 상태로 유지되었다.

결론: NLRP3 인플라마좀 신호 전달 체계는 OVA 유도 알레르기비염 쥐모델에서 알레르기비염 관련 증상을 유발하는 또다른 경로로 추정된다. MCC950 은 NLRP3 인플라마좀 신호 전달 체계의 시작점인 NLRP3 의 특이 억제제로 알레르기비염 관련 증상을 호전시킨다. 하지만 이는 전통적인 알레르기비염의 중요한 요소인 면역글로불린E 와 관련이 없는 경로로 추정된다.

주요어 : NLRP3, 인플라마좀, 알레르기비염, MCC950

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