의학 석사 학위논문

알레르기비염 마우스 모델에서 응고 및 섬유소용해계 인자의 발현양상 분석

Analysis of the coagulation cascade and fibrinolysis system components in allergic rhinitis murine model

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홍 승 노

A thesis of the Master's degree

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Otorhinolaryngology Head and Neck Surgery,
Seoul National University
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이 논문을 **의학석사학위논문으로** 제출함

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이비인후과학 전공
홍 승 노

홍승노의 의학석사학위논문을 인준함

2015年 12月

위 원 장 김 현 직 (인)
부위원장 김 동 영 (인)
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Analysis of the coagulation cascade and fibrinolysis system components in allergic rhinitis murine model

by Seung-No Hong

A thesis submitted to the Department of Otorhinolaryngology Head and Neck Surgery in partial fulfillment of the requirement of the Degree of Master at Seoul National University College of Medicine

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Approved by Thesis Committee:

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ABSTRACT

Analysis of the coagulation cascade and fibrinolysis system components in allergic rhinitis murine model

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Background

Dysregulation of the coagulation cascade and fibrinolysis system may play an etiologic role in many diseases. Allergic diseases such as bronchial asthma, atopic dermatitis, and conjunctivitis are also associated with fibrin accumulation caused by the change in hemostasis. Nevertheless there has been only a few studies dealing with the relation between allergic rhinitis (AR) and the coagulation system.

Objective

This study was conduct to investigate the change of coagulation and fibrinolysis cascade components in allergic rhinitis murine model

Methods

BALB/c mice were sensitized and challenged with ovalbumin. Multiple parameters related to coagulation cascade and fibrinolysis system such as Factor II, V, VII, X, XIII, tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), plasminogen activator inhibitor-1 (PAI-1), and fibrin were compared between the allergic rhinitis model group and the control group.

Results

The symptom scores and eosinophil counts were higher in the allergic rhinitis model group than the control group ($p < .01$). The mRNA expression level of u-PA ($p = .040$), PAI-1 ($p = .054$), Factor II ($p = .038$), Factor X ($p = .036$) was significantly higher in the allergic rhinitis model group. Most of the fibrinolysis system and coagulation cascade components were localized at the epithelium, endothelium and submucosal glands of the nasal mucosa on immunohistochemical staining and there was a down regulation of u-PA in AR group while fibrin deposition was much prominent in the AR model group.

Conclusion

In allergic rhinitis condition, the coagulation cascade was up-regulated and the fibrinolysis system was down-regulated which seemed that the change of both components lead to fibrin deposition in allergic rhinitis mouse model.

Key words: Coagulation factor, Fibrinolysis, allergic rhinitis, Pathogenesis, mice

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LIST OF ABBREVIATIONS

AR: Allergic rhinitis

t-PA: Tissue-type plasminogen activator

u-PA: Urokinase-type plasminogen activator

PAI-1: Plasminogen activator inhibitor-1

CRS: Chronic rhinosinusitis

OVA: Ovalbumin

PBS: Phosphate-buffered saline

GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

IHC: Immunohistochemistry

INTRODUCTION

Allergic rhinitis (AR) which is one of the most common chronic allergic diseases represents a substantial socio-economic burden. The prevalence is still increasing as societies are becoming more industrialized. Moreover, in spite of the medical science advance to diagnose and control symptom, still the current treatment strategies for AR does not provide a complete cure. Therefore, efforts investigating for a novel efficient approach to manage the pathophysiology of AR are required.

Recently the hemostasis process which is composed of coagulation cascade and fibrinolysis system is known to be related with many inflammation diseases such as asthma, rheumatoid arthritis, delayed-type hypersensitivity and chronic rhinosinusitis.

For instance, deposition of fibrin, an end product of hemostasis, and abnormalities in the coagulation and fibrinolysis pathways in the distal airways of the lung were reported to contribute to airway hyperresponsiveness and airway closure in asthma.[1] The reduction of a fibrinolysis system component, tissue-type plasminogen activator (t-PA) was found in chronic rhinosinusitis (CRS) patients and fibrin depositions in the submucosa of nasal polyp as a consequence are also thought to play a critical role in the pathogenesis of CRS.[2]

AR shares many clinical characteristics with asthma in accordance with the unified airway concept. Moreover, AR has many common pathophysiologic similarities with CRS which is also an upper airway mucosal disease. However, there are only a few studies dealing the role of coagulation cascade and fibrinolysis system with allergic rhinitis[3,4] and the underlying pathogenic mechanism of the correlation between inflammation diseases and coagulation is still unclear.

Therefore, this study was conducted to investigate the expression change and the localization of coagulation cascade components and fibrinolysis cascade components in

allergic rhinitis murine model.

MATERIALS AND METHODS

Murine allergic rhinitis model

Four-week-old female BALB/c mice weighing 20 to 30 g were used in the experiments. This animal study was approved by the Institutional Animal Care Committee of the Clinical Research Institute of Seoul National University Hospital.

Mice were divided into two groups, an AR group and a control group, consisted of 20 mice each. The control group was sensitized and challenged with phosphate-buffered saline (PBS), and the AR group with ovalbumin (OVA). The procedures for allergen sensitization and treatment are summarized in **Figure 1**. Briefly, mice were sensitized with an intraperitoneal injection of 25 μg of ovalbumin (OVA; grade V; Sigma, St. Louis, MO, USA) and 1 mg of aluminum hydroxide gel on days 0, 7, and 14. From day 22, 2% OVA droplet was administered into the nasal cavity on 7 consecutive days.

Evaluation of allergic responses

Nasal symptom scores were evaluated at 15 min after the final OVA challenge with 200 μg of OVA on day 27. The frequencies of sneezing and rubbing were counted for 10 minutes by a blinded observer. Mice were then sacrificed 24 h after the last OVA challenge. After perfusion with 4 % paraformaldehyde, the heads of 10 mice from each group were removed en bloc and then fixed in 4 % paraformaldehyde. For evaluation of nasal histology, nasal tissues were decalcified, embedded in paraffin, and sectioned coronally (4 μm thick) approximately 5 mm from the nasal vestibule. Each section was stained with hematoxylin and eosin, and the number of eosinophils on both sides of the septal mucosa was counted. The

number of eosinophils in the submucosal area of the whole nasal septum was counted under a light microscope (400 X magnification).

Real-time reverse transcription-PCR

From the nasal mucosa homogenates of 5 mice from each group, real-time reverse transcription (RT)-PCR was done. Total RNA was prepared from the nasal mucosa using the TriZol reagent (Invitrogen, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized using superscript reverse transcriptase (Invitrogen) and oligo (dT) primers (Fermentas, Burlington, ON, Canada).

The sequences of the specific PCR primers used to amplify the fibrinolysis system component including urokinase-type plasminogen activator (u-PA), tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor-1 (PAI-1) and coagulation cascade components including factor II, V, VII, X, XIIa are given in Table 1. Then real-time quantitative PCRs were performed by using a SYBR® Green RT-PCR kit (Applied Biosystems, CA, USA) and a QuantStudio 3 system (Applied Biosystems) according to manufacturer's instructions. RNA integrity and the success of the RT reaction were monitored by PCR amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts. Amplification of each components and GAPDH cDNA was carried out in MicroAmp optical 96-well reaction plates (Applied Biosystems). The average transcript levels of genes were then normalized. Experiments were carried out with duplicates for each sample. Each PCR run included the five points of the standard curve, a no template control, and unknown patient cDNAs. The fluorescence intensity of each target gene was normalized with that of GAPDH. Additionally, amplified PCR products were resolved in 2% agarose gels and photographed under ultraviolet light.

Measurement of fibrinolysis system component in nasal lavage fluids

On day 28, the nasal lavage fluids were collected 24 hours after the last intranasal challenge. After partial tracheal resection, a plastic catheter was inserted into the tracheal opening toward the upper airway, and saline wash was collected from the nostril. The nasal lavage fluids underwent centrifuge preparation, and the supernatant was assayed by enzyme-linked immunosorbent assay (ELISA). The concentration of t-PA, u-PA, PAI-1 was measured in the lavage fluid with an ELISA kit (Molecular Innovations Inc., Novi, MI, USA) according to the manufacturer's instructions. Optical density (OD) at 450 nm was measured using a microplate reader. The sensitivity of t-PA, u-PA and PAI-1 was 0.035, 0.00998 and 0.031 ng/mL, respectively.

Western blot analysis

Factor V, Factor VII, u-PA, PAI-1 and β-actin were immunoblotted with a primary mouse monoclonal anti–Factor V Ab (Santa, Santa Cruz, CA), anti–Factor VII Ab (Abcam, Cambridge, U.K.), anti–u-PA Ab (Abcam), anti–PAI1 Ab (Abcam) and anti- β-actin Ab (Cell Signaling Technology, Inc., Danvers, MA), respectively. Quantification of Western blots was performed using the image analysis software program (Amersham, Arlington Heights, IL).

Immunohistochemical staining

Specimens embedded in paraffin were cut into sections 5mm thick and floated onto aminoalkylsilane-coated slides (Polysciences, PA, USA). After deparaffinization in xylene, sections were rehydrated with ethanol. For antigen unmasking, sections were incubated at microwave for 15 minutes with antigen unmasking solution (Vector laboratories, CA, USA).

After microwave treatment, the sections were treated with 0.3% hydrogen peroxide in methanol for 15 min to inhibit endogenous peroxidase activity of blood cells, and with 1% bovine serum albumin (BSA) in 0.05 M phosphate, 0.1 M NaCl, pH 7.4 (PBS) containing blocking reagent for another 10 min at 25C. An appropriate non-immunized 2.5% normal horse serum was used for the blocking reagent. Appropriate primary and secondary antibodies against Factor V, Factor X were purchased from Santa (Santa Cruz, CA), Factor II, Factor VII, Factor VIIIa, t-PA, u-PA and PAI-1 (Abcam) and Fibrin (Sekisui, Stamford, CT, USA). The treated sections were incubated with the primary antibodies (same antibodies used in western blot analysis) for t-PA (1:500, ab157469), u-PA (1:500, ab133563), PAI-1 (1:1000, ab28207), Factor V (1: 500, sc292858), Factor VII (1: 500, ab97614), Factor X (1: 500, sc20673) and Fibrin (1:50, a350) at the appropriate concentrations in blocking reagent for 10 h at 4C. After extensively washing with PBS containing Tween 20, each sample was incubated with the secondary antibodies at the appropriate concentrations (Impress anti-rabbit IgG, vector) in blocking reagent for 1 h at 24°C. After washing again, immunoreactive sites were visualized with peroxide substrate kit 3, 3'-diaminobenzidine (DAB) (Vector laboratories, CA, USA), and then counterstained with hematoxylin. Control sections were incubated with 5mg/ml non-immune rabbit IgG (Impress) instead of the primary antibodies, respectively. Image J software (U.S. National Institutes of Health, Bethesda, MD) was used to quantify the strength of the immunohistochemical staining in each section.

Statistical analysis

Results are shown as the mean ± SD. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). A Mann–Whitney U test was used to compare results between negative and positive controls and between treatment group and positive control. A P

value less than 0.05 was deemed to indicate statistical significance.

RESULTS

Allergic rhinitis model preparation

The nasal symptom score showed a statistically significant difference between AR and control groups and the number of rubbing (p=.029) and sneezing (p=.001) was higher in the AR group. In H & E staining, a lot of eosinophil infiltration was recognized in the nasal mucosa of the AR group which was significantly different from that of the control group (p< .001) **(Fig. 2)**.

Measurement of the mRNA level in fibrinolysis system and coagulation cascade component in nasal mucosa

Quantitative real-time RT-PCRs were performed for fibrinolysis system and coagulation cascade component. The mRNA expression was increased in most of the coagulation cascade components. Factor II (p=0.038) and Factor X (p=0.036) showed a statistically significant increase in mRNA level in the AR group while Factor V (p=0.645), Factor VII (p=0.121), Factor XIII (p=0.878) did not show any difference **(Fig. 3)**. For fibrinolysis system components, the u-PA level was significantly decreased in AR group compared to control group (p=0.040) while PAI-1 level was significantly increased in AR group (p=0.054). The mRNA level of t-PA did not show any difference between both group (p=0.888) **(Fig. 4)**.

Fibrinolysis system components in nasal lavage fluid

The level of t-PA concentration in nasal lavage fluid did not show any statistical difference. However, there was a statistically significant difference in u-PA (p=0.029) and PAI-1 level

between the AR and control group. The average level of u-PA was higher in the control group (0.093 ± 0.029 ng/mL) than the AR group (0.064 ± 0.029 ng/mL). For PAI-1, ELISA failed to detect most of the control group lavage samples while more than half of the samples were detected in the AR group (0.084 ± 0.052 ng/mL) **(Fig. 5)**.

Protein expression in nasal mucosa of fibrinolysis system and coagulation cascade

Western blot analysis was performed on u-PA, PAI-1for fibrinolysis system component and Factor V, Factor VII for coagulation cascade component **(Fig. 6)**. Although there were no statistical difference, the average level of u-PA expression was lower in AR group (ratio against β-actin: 1.16, same as follows) compare to control group (1.55) and the level of Factor VII level expression was higher in AR group (1.38) compared to control group (1.13). The protein expression level of PAI-1 and Factor V did not show any trend **(Fig. 7)**.

Immunohistochemical localization in murine nasal mucosa

On immunohistochemical staining, the components of fibrinolysis system and coagulation cascade were mainly detected on the epithelium, submucosal glands and the endothelial lining of vessels in the nasal mucosa. Fibrin-immunoreactive material was localized predominantly in the interstitial area of submucosa and the epithelium of nasal mucosa **(Fig. 8A-I)**.

Among the components, quantification amount of u-PA staining was lower in the AR group than that of the control group ($p=0.041$). Other components did not show statistically significant difference. In Fibrin staining, the staining amount was higher in the AR group compare to control group ($p=0.026$) **(Fig. 8J)**.

DISCUSSION

The coagulation cascade and fibrinolysis system is well known for its contribution to inflammatory process. Though allergic rhinitis is one of the most common allergic inflammatory diseases, studies dealing with the relation with coagulation and fibrinolysis system are rather rare compare to the other inflammatory disease such as asthma, chronic rhinosinusitis. Moreover most of the studies only deal with, either the coagulation cascade components or the fibrinolysis components. In this study we report changes and location of expression in both coagulation cascade and fibrinolysis system, skewing toward fibrin accumulation in the condition of allergic rhinitis.

Plasmin which dissolves the fibrin clot are activated by plasminogen activators and between the two types of plasminogen activators, t-PA is believed to play the main role rather than u-PA.[5] The plasminogen activator inhibitors (PAIs) are members of the serine protease inhibitor superfamily and they inhibit plasminogen activators.[6] Previous studies have shown the close relation between fibrinolysis system components and inflammatory disorders. It was demonstrated that in chronic rhinosinusitis, the t-PA levels was down-regulated in the patient group, inhibiting the conversion of plasminogen to plasmin in the presence of Th2 cytokines.[2] Increased synthesis of PAI-1 in airways of asthma patients, which seemed to contribute to the development of airway hyperresponsiveness, was found in another study.[7] The other study reported that the level of t-PA was decreased in allergic rhinitis model which increased collagen deposition and gland hyperplasia leading to nasal mucosa matrix reconstruction.[3]

In this study, not only the expression of t-PA mRNA but also the immunohistochemical staining intensity difference was not prominent between AR and control group. Instead the mRNA level of u-PA and PAI-1 showed a significant difference between both groups. These results share similar aspects with other studies. Takayuki et al. demonstrated an increased

level of u-PA and PAI-1 mRNA in allergic nasal tissues while t-PA did not show a significant difference in human nasal mucosa. It was also reported that most of the plasminogen activators were localized at epithelium and submucosal glands which was consistent with our immunohistochemical findings.[8] However, the increased level of u-PA in allergic rhinitis differs with our study result. Larsen and his team also found a shift toward a higher u-PA/t-PA activity ratio in inflammatory process which is explained by the biological role of u-PA as tissue remodeling and inflammatory cell migration.[9] Alicja et al. could not found a clear difference in circulating levels of u-PA and its inhibitor between allergic rhinitis patients and healthy participants.[4] In contrast, it is known that plasminogen activator and its cellular receptor provide a functional unit that controls inflammation by regulating plasma contact system.[10] Therefore, down-regulation of u-PA in this study may suggest a dysregulation of inflammatory process which leads allergic condition.

Several studies demonstrated the role of coagulation cascade components in inflammatory disease. They suggest that activation of the coagulation system occurs during the allergic response and significant concentrations of thrombin were found in nasal secretion after allergic provocation in allergic patients.[11] They also reported that thrombin may play a role in nasal polyp formation by stimulating VEGF production from airway epithelial cells.[12] Tetsuji and his colleague demonstrated that the overproduction of FXIII-A by M2 macrophages might contribute to the excessive fibrin deposition in the submucosa of NP, which might contribute to the tissue remodeling and pathogenesis of chronic rhinosinusitis with nasal polyps.[13] Kazuhiko et al. found that Factor X transcript levels and factor X activity ~~were~~ increased in lungs of asthmatic mice challenged with OVA and suggested that factor X may function in airway remodeling.[14] In the present study there was a significant increase of thrombin (Factor II) and Factor X mRNA level which was consistent with the previous

reports and other factors also showed a tendency to be activated in allergic rhinitis mouse model.

Unlike the results of mRNA level, the protein expression showed some discrepancy between western blotting analysis and ELISA. Though all of the components fail to show any statistically significant difference between AR group and control group in on western blotting analysis, we could found a tendency of increased coagulation cascade component (factor VII) and decreased fibrinolysis system component (u-PA). In addition, the ELISA result, which was done with nasal lavage fluid, showed the same expression pattern with mRNA level analysis. The u-PA concentration was significantly lower in the AR group. But PAI-1 concentration did not show any statistical difference. While significant concentration of PAI-1 were not measured in most of the samples from control group, a certain portion of the AR group samples were detected on ELISA. We assumed that this finding reflects the difference in concentration of PAI-1 between two groups.

Both up-regulation of coagulation cascade components facilitating fibrin formation and down regulation of fibrinolysis components reducing the degradation of fibrin yields a common consequence of fibrin accumulation. Fibrin, the end product of coagulation cascade, is not only essential to clotting blood and repairing vessel injury but also is thought to play a critical role in host defense and modulate the inflammatory response by affecting leukocyte migration and cytokine production under inflammatory condition.[15] However, many previous studies found out that the accumulation of fibrin due to the dysregulation of the coagulation cascade or the fibrinolysis system could cause diverse chronic inflammatory diseases such as rheumatoid arthritis, severe asthma, glomerulonephritis, delayed-type hypersensitivity, and Crohn's diseases.[13] Accumulated fibrins are assumed to retain the plasma protein resulting in mucosal edema which is one of the most famous histopathological characteristics of allergic

rhinitis.[2,16] It is also demonstrated that fibrin has its own proinflammatory properties. Fibrin can directly induce production of the chemokines by endothelial cells and fibroblasts, promoting the migration of leukocytes and macrophages.[10,17] In this study, as previous studies described, the deposition of fibrin was observed at the epithelial and the submucosal area with a prominent staining in the allergic rhinitis group than the control group.

These findings of the change in coagulation cascade and fibrinolysis system components has clinical significance in terms of investigating a novel treatment target for allergic rhinitis. It was demonstrated that by applying an aerosolized fibrinolytic agent, t-PA, significantly diminished mucosal hyperresponsiveness in mice with allergic airway inflammation.[1] The attenuated effect of fondaparinux, a factor X inhibitor, was proved in airway remodeling.[14] Also, previous studies reported that removal of fibrin can diminish disease development and symptoms.[18,19]

This study has some limitations. First, there are limitations to studying exclusively rodents in animal models of human diseases. Moreover, though each part of the sinonasal mucosa may exhibit different immunological response, sampling of specific parts and compare to each other is technically difficult in mouse sinonasal mucosa unlike human study. Second, the number of samples was not large enough to draw a statistical significance in some experiments. Third, only the association of each components and allergic status was assessed which makes it difficult to figure out the causal relationship. In future study, to overcome these limitation, investigation of human sampling in large number is needed and analyzing the clinical effects of agents which reduce the formation of fibrin may provide a clue with the etiological relationship.

CONCLUSION

The present study provides the report on behavior of both coagulation cascade and fibrinolysis system components in nasal mucosa of allergic rhinitis murine model. Overall, the components of coagulation cascade was up-regulated and the fibrinolysis system was down-regulated, it seems that the change of the components both lead to fibrin deposition in allergic rhinitis condition. These findings suggest that dysregulation of hemostatic components in nasal mucosa may be related to the pathogenesis of allergic rhinitis.

Table 1 Reverse transcription polymerase chain reaction primer sequences for coagulation cascade, fibrinolysis system components and glyceraldehyde-3-phosphate dehydrogenase.

Primer	Forward	Reverse	size
GAPDH	TGTCCGTCGTGGATCTGAC	CCTGCTTCACCACCTTCTTG	74bp
F II	TGACCGGAAGGGGAAATACG	CCACCCACACACCTATCCAAA	97bp
F V	CCTGGTCAGCGCAACATCTA	GCCTGCATCCCAGCTTGATA	105bp
F VII	GACCATGTAGGGACCAAGCG	TTCTCCCACACGGGTACTCA	103bp
F X	CATCTGACCTGACACCCATGC	GCTACCCTTATCCAGAACTGCCA	129bp
F XIIIA	GAAGTACCCAGAGGCACACAG	TGGAGTTATTGGGCGGGAC	137bp
t-PA	AGAGATGAGCCAACGCAGAC	CAACTTCGGACAGGCACTGA	134bp
u-PA	AAATTCCAGGGGGAGCACTG	GTGTTGGCCTTTCCTCGGTA	86bp
PAI-1	CAAGGGGCAACGGATAGACA	AAGCAAGCTGTGTCAAGGGA	81bp

t-PA = Tissue-type plasminogen activator, u-PA = urokinase-type plasminogen activator, PAI-1 = plasminogen activator inhibitor-1, glyceraldehyde-3-phosphate dehydrogenase = GAPDH, F = Factor

Figure legends

Figure 1. Experimental protocol for allergen sensitization and challenge. BALB/c mice were sensitized on days 0, 7, and 14 by intraperitoneal injection of ovalbumin and 1 mg of aluminum hydroxide gel. On days 22 through 29 after the initial general sensitization, the mice were challenged with OVA intranasally. The control group were sensitized and challenged with phosphate-buffered saline. AR, Allergic rhinitis; PBS, phosphate-buffered saline; OVA, ovalbumin; alum, aluminum hydroxide gel.

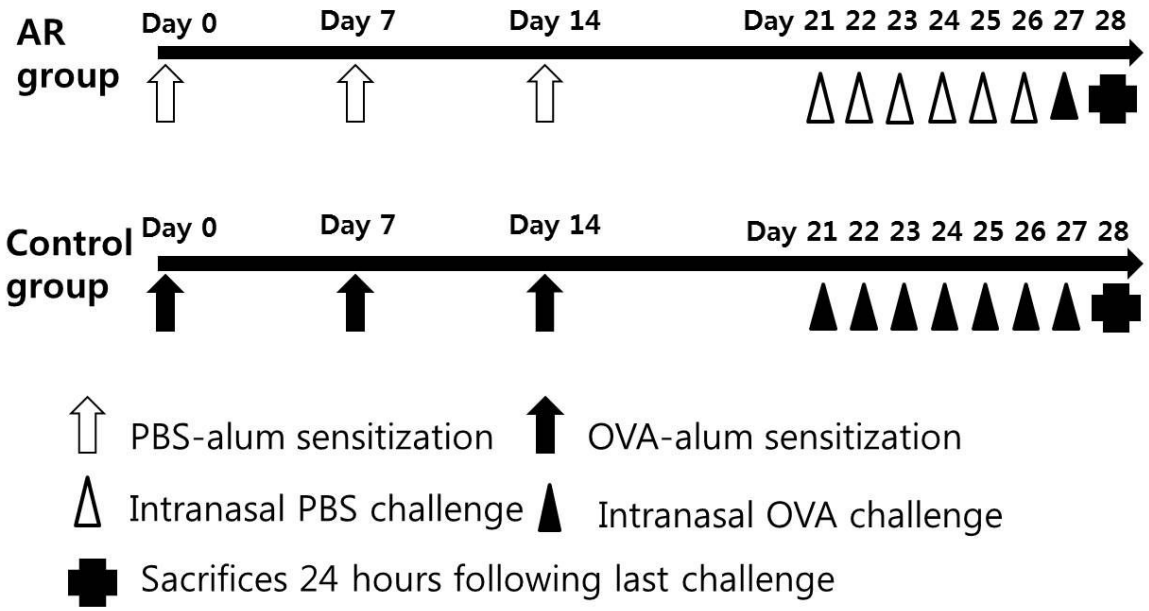


Figure 2. Symptom score and eosinophil infiltration. (a) Rubbing symptom score, (b) sneezing symptom score and (c) the number of eosinophils in the nasal mucosa of each group at a magnification of x400. Values are expressed as mean ± SEM. *P< 0.05.

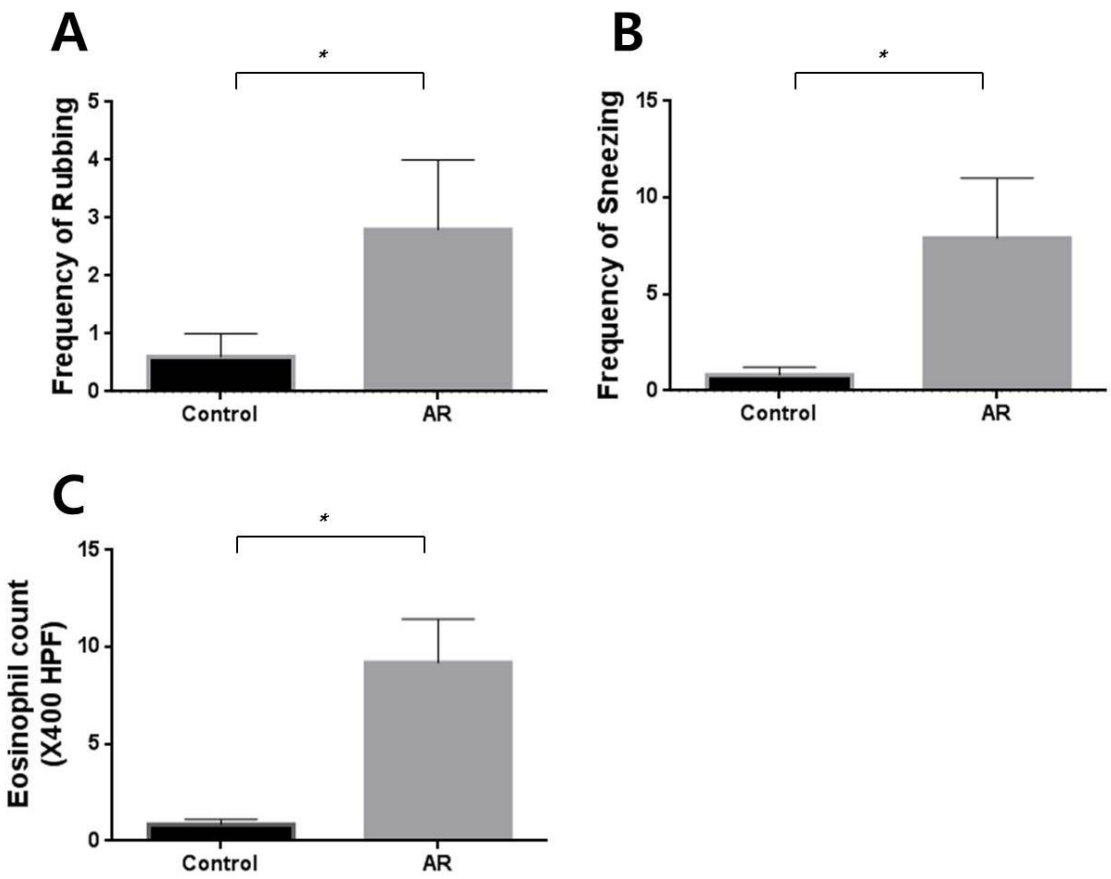


Figure 3. The mRNA expression of coagulation cascade components in mouse nasal tissues by quantitative real-time RT-PCR. (a) Factor II, (b) Factor V, (c) Factor VII, (d) Factor X and (e) Factor VIII. Values are expressed as mean ± SEM. *P< 0.05.

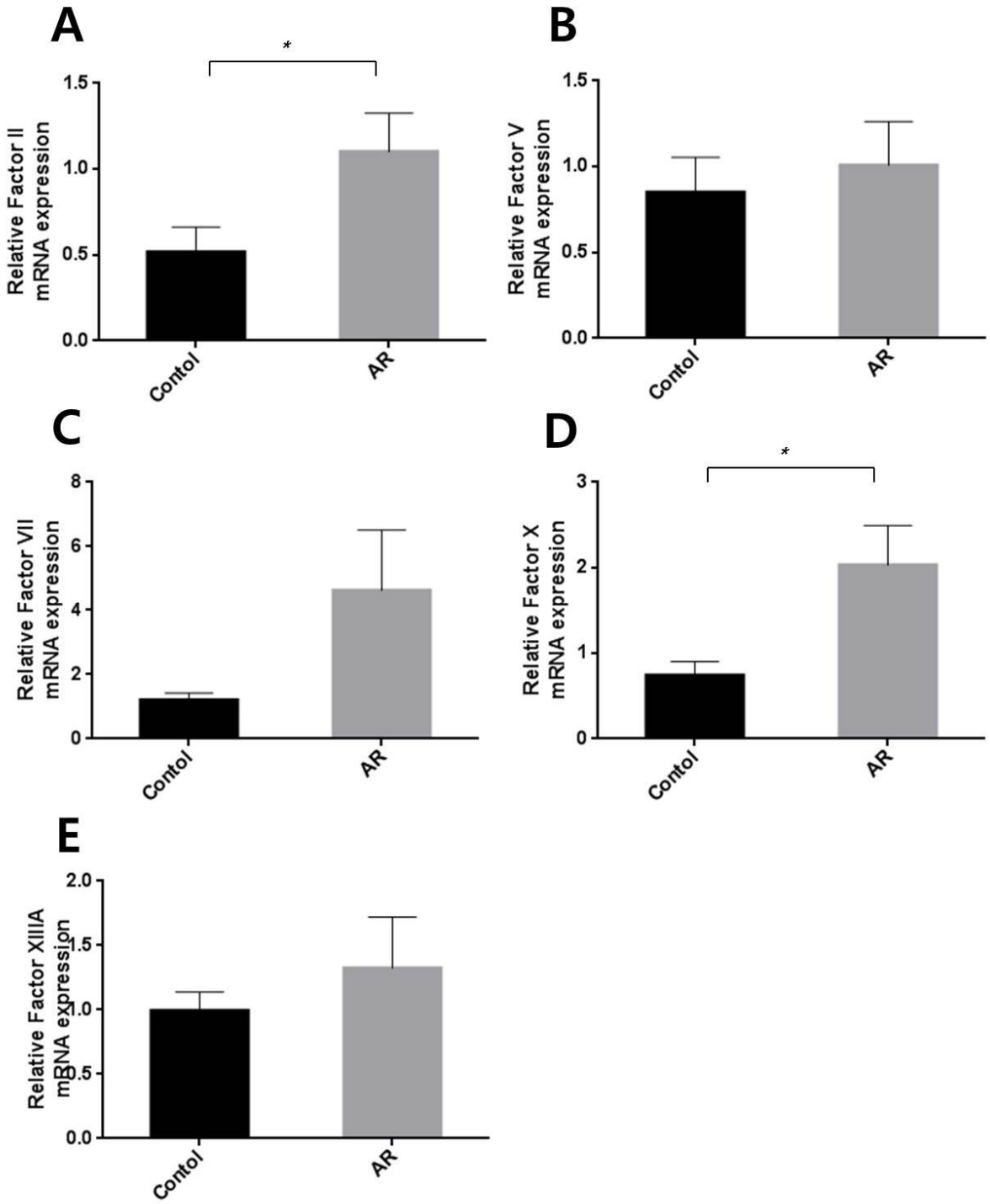


Figure 4. The mRNA expression of fibrinolysis system components in mouse nasal tissues by quantitative real-time RT-PCR. (a) The tissue-type plasminogen activator (t-PA) quantitative, (b) the urokinase-type plasminogen activator (u-PA) quantitative and (c) the plasminogen activator inhibitor-1 (PAI-1). Values are expressed as mean ± SEM. *P< 0.05.

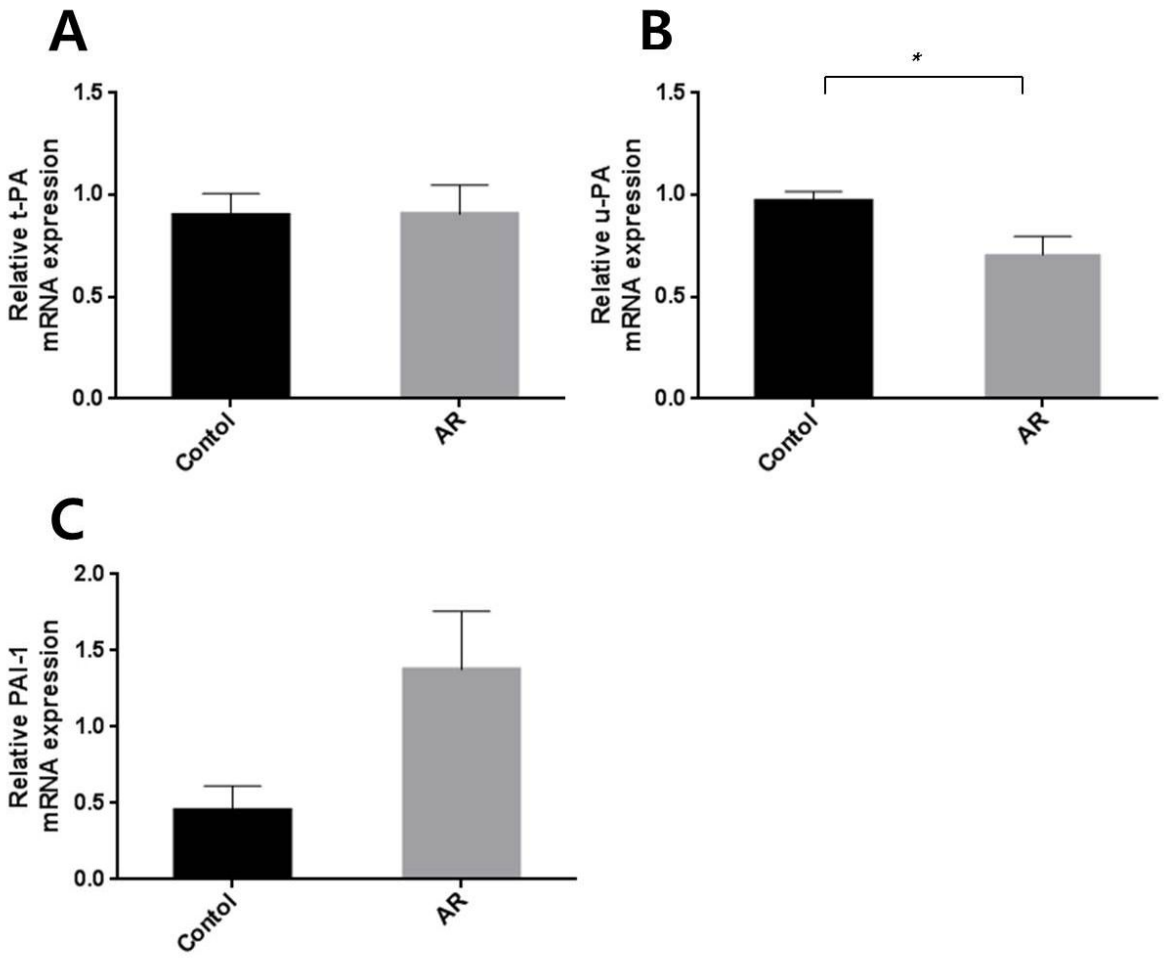


Figure 5. Enzyme-linked immunosorbent assay of fibrinolysis system components in nasal lavage fluid. Comparison of the concentration of (a) Tissue-type plasminogen activator (u-PA), (b) Urokinase-type plasminogen activator (u-PA), (c) plasminogen activator inhibitor-1 (PAI-1) between control and allergic rhinitis group. Values are expressed as mean ± SEM. *P< 0.05.

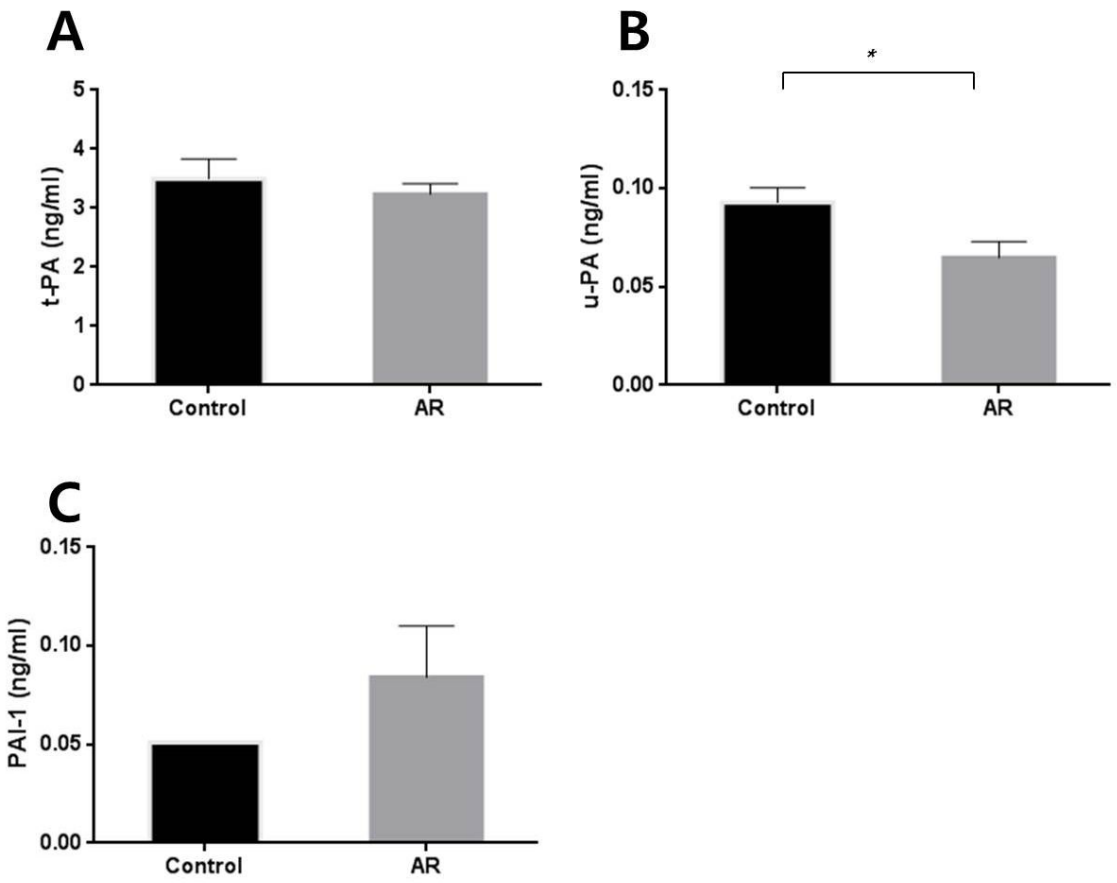


Figure 6. Western blotting analysis. (a) Urokinase-type plasminogen activator (u-PA), (b) plasminogen activator inhibitor-1 (PAI-1), (c) Factor V, (d) Factor XIII.

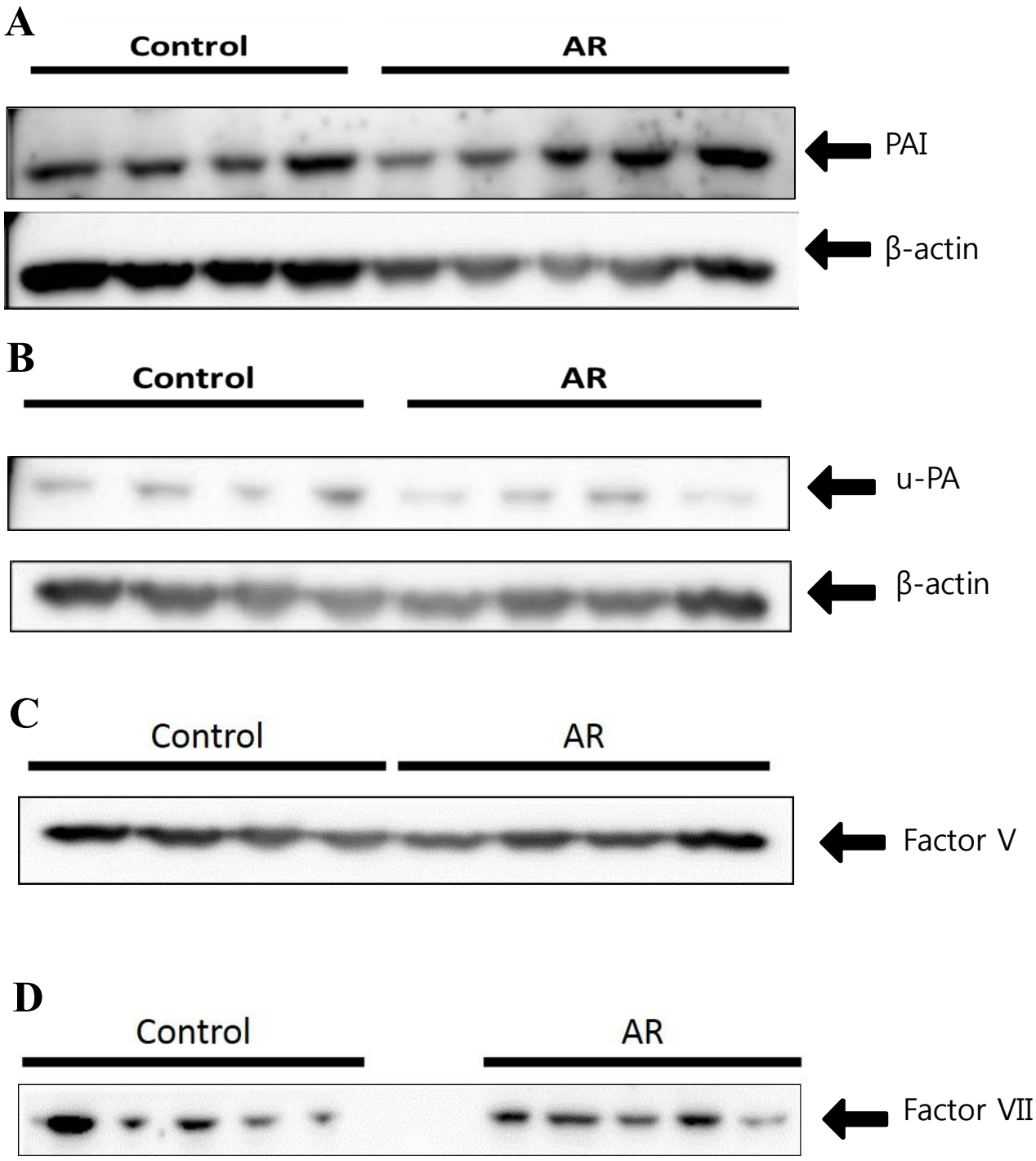


Figure 7. Relative density of protein expression level which were normalized to β-actin. (a) Urokinase-type plasminogen activator (u-PA), (b) plasminogen activator inhibitor-1 (PAI-1), (c) Factor V, (d) Factor XIII. Values are expressed as mean ± SEM. *P< 0.05.

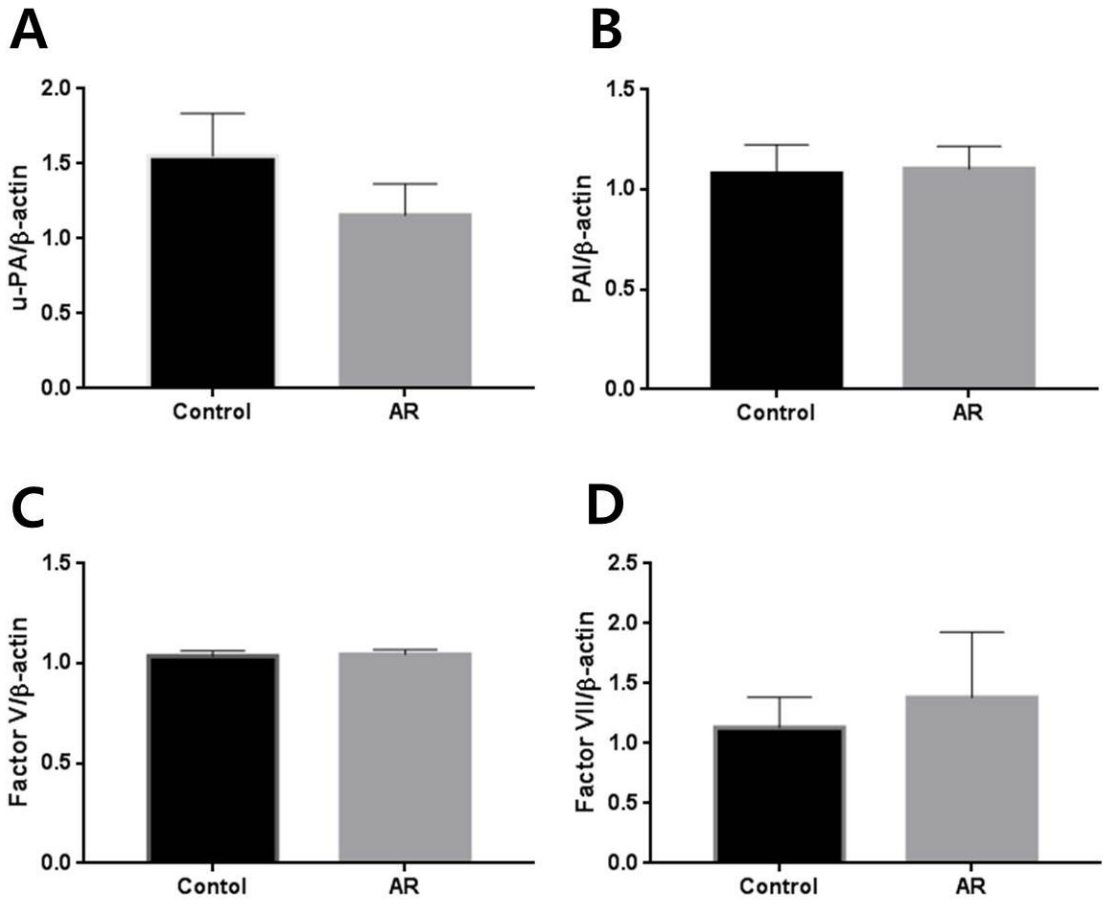
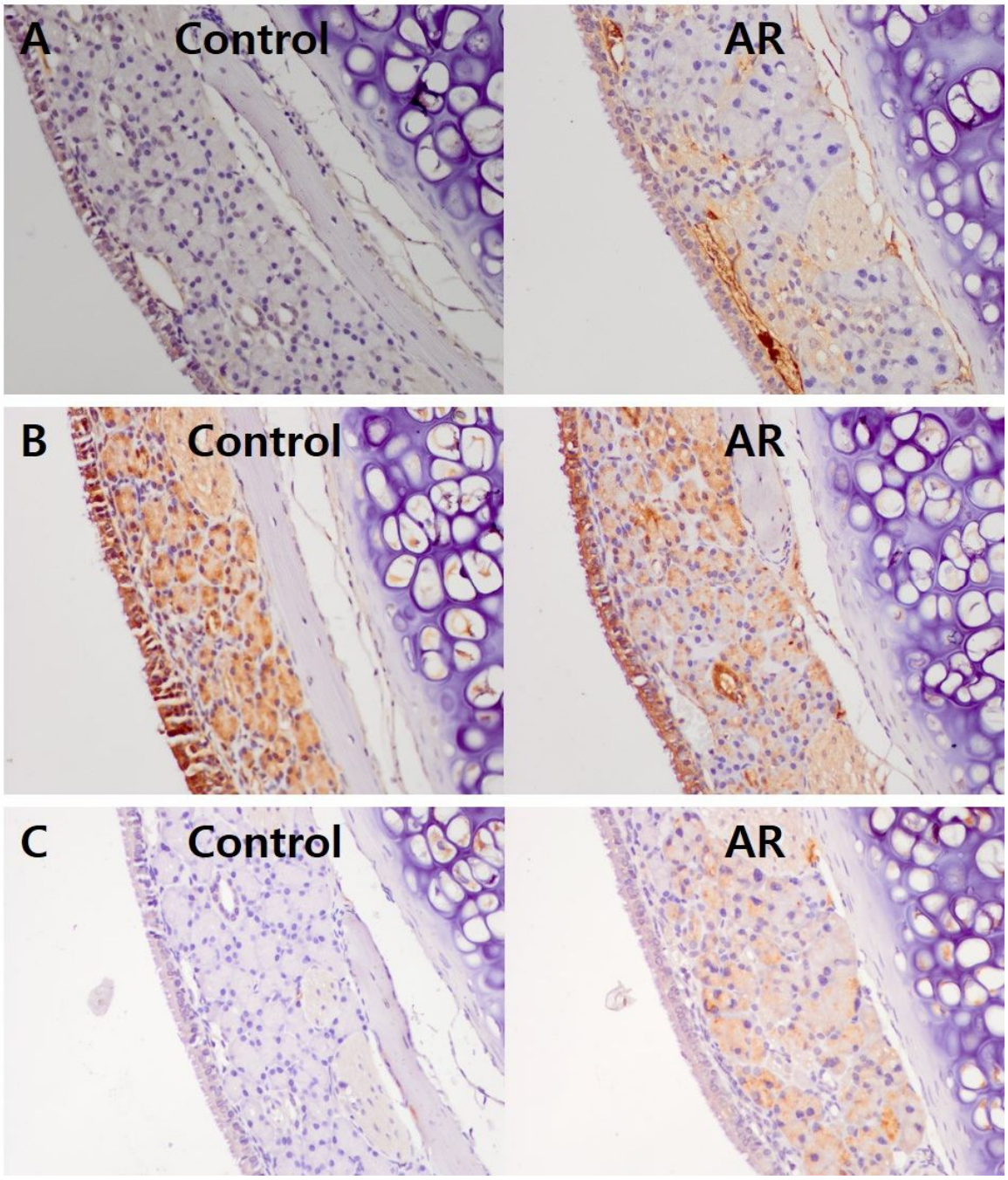
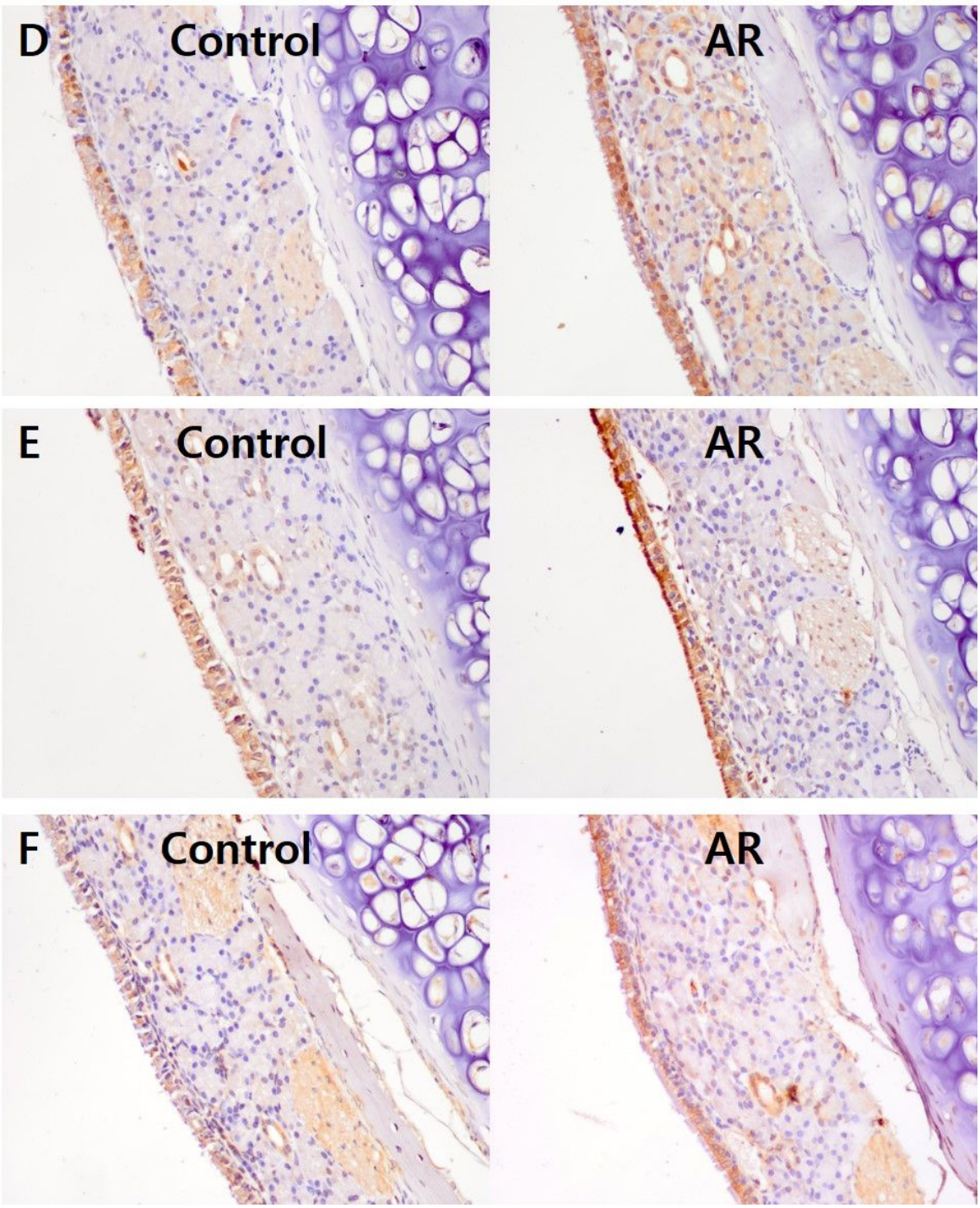


Figure 8. The immunohistochemical staining for fibrinolysis system components. Sections were immunostained with (A) anti-Fibrin, (B) anti-urokinase-type plasminogen activator, (C) anti-Factor V, (D) anti-Factor VII, (E) anti-Factor X, (F) anti-tissue-type plasminogen activator, and (G) anti-plasminogen activator inhibitor-1. (H) Quantification by means of image analysis of urokinase-type plasminogen activator and Fibrin. Values are expressed as mean ± SEM. *P< 0.05.

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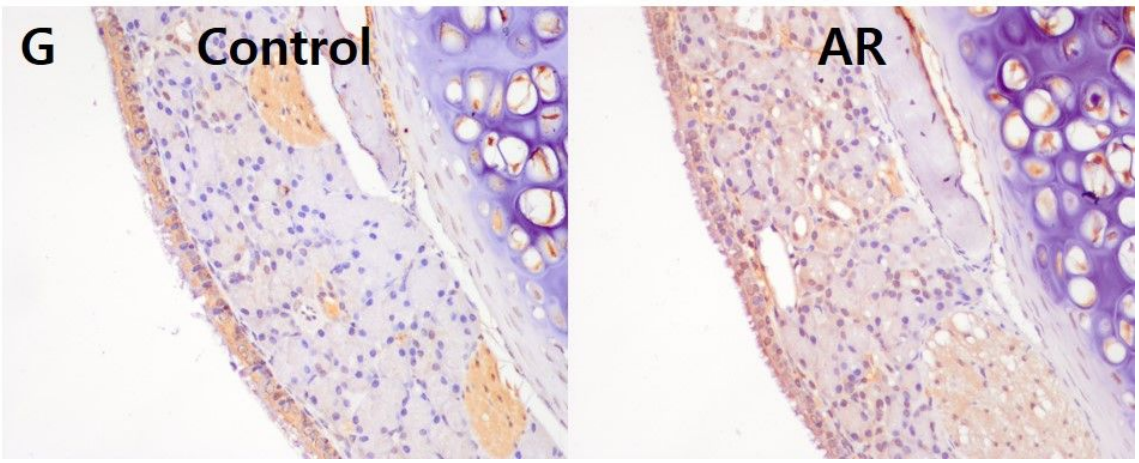
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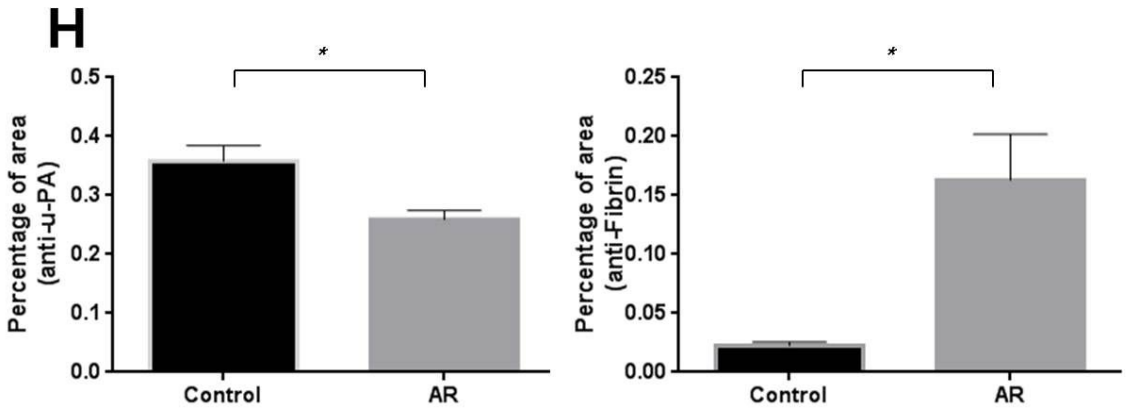
Control
AR


H
*
Percentage of area (anti-u-PA)
0.5
0.4
0.3
0.2
0.1
0.0
Control
AR
*
Percentage of area (anti-Fibrin)
0.25
0.20
0.15
0.10
0.05
0.00
Control
AR

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국문초록

서론: 응고 및 섬유소용해계의 조절장애(dysregulation)는 천식이나 아토피 피부염과 같은 다양한 염증성 질환과 연관이 높다고 알려져 있다. 이는 응고 및 섬유소용해계에 관여하는 인자들의 발현 양상 변화와 최종 산물인 피브린의 축적이 염증성 질환의 병태생리와 관련되어 있을 것으로 추측되지만 아직 뚜렷한 기전은 밝혀져 있지 않다. 알레르기비염은 대표적인 만성 염증성 질환임에도 불구하고 아직까지 응고 및 섬유소용해계와의 관련성에 대한 보고는 많지 않다. 본 연구는 알레르기 비염 마우스 모델에서 응고 및 섬유소용해계 인자들의 발현양상을 알아보고자 하였다.

방법: BALB/c 마우스를 대상으로 알레르기비염(AR)군과 대조군으로 나누어 비점막 조직 및 비세척액을 수집한 후 각 군간에 응고 및 섬유소용해계 인자들의 차이를 실시간역전사중합효소연쇄반응, 효소결합면역흡착측정법, 웨스턴블롯분석, 면역조직화학염색 등을 이용하여 비교 분석 하였다. AR 모델은 마우스의 복강에 난알부민을 주사하여 전신감작 시킨 후 경비강으로 난알부민 챌린지를 시행하여 제작하였다. 응고 인자로 Factor II, V, VII, X, XIII 를 비교하였고, 섬유소용해계 인자로는 tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), plasminogen activator inhibitor-1 (PAI-1)를 비교하였였으며 각 군에 최종 산물인 피브린의 축적 양상을 관찰하였다.

결과: 코비빔 동작과 재채기 횟수와 같은 알레르기비염 증상은 AR군에서 높았으며 비점막내 호산구의 침윤 정도도 AR군이 대조군에 비해 통계적으로 유의하게 높았다. 섬유소용해계 인자들 중 u-PA의 경우 AR군에서 낮았고, 반면

inhibitor인 PAI-1은 통계적으로 의미 있게 증가하였다. 응고 인자의 경우 대부분 AR군에서 높은 mRNA level이 보였으나 이중 Factor II와 X만 통계적으로 유의미한 차이를 보였다. 비세척액으로 시행한 ELISA 역시 t-PA는 차이를 보이지 않은 반면 AR군에서 낮은 u-PA와 높은 PAI-1 수치가 관찰되었다. 단백질 분석 결과 AR군에서 u-PA가 발현이 낮고 factor VII가 높은 경향을 보였으나 통계적으로 유의미하지는 않았다. 면역조직화학 염색 결과, 피브린은 AR군에서 더 높게 염색이 되었으며 고배율에서 피브린은 상피층과 점막하샘에서 주로 염색되었다.

결론: 본 연구는 알레르기비염 동물 모델을 통해 응고 및 섬유소용해계 인자들의 발현양상을 평가하였다. 알레르기비염 환경 하에서 응고 인자는 대체적으로 상향조절(up regulation)되고 섬유소용해계는 하향조절 되어 결국 피브린이 축척되는 방향으로 변화됨을 알 수 있었다.

주요어: 응고인자, 섬유소용해, 알레르기비염, 병태생리, 마우스

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