

Suppressive Effect of Fluticasone Propionate on MMP Expression in the Nasal Mucosa of Allergic Rhinitis Patients *In Vivo*

YOSHIYUKI KYO¹, KEN-ICHI KANAI¹, KAZUHITO ASANO²,
TADASHI HISAMITSU² and HARUMI SUZAKI¹

*Departments of ¹Otorhinolaryngology and ²Physiology, School of Medicine,
Showa University, Shinagawa-ku, Tokyo 142-8666, Japan*

Abstract. The effects of intranasal corticosteroids on matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of metalloproteinases (TIMPs)) in the nasal mucosa of patients with allergic rhinitis (AR) are not known. Nasal mucosa biopsy specimens were obtained from AR patients, with or without the administration of fluticasone propionate (FP) nose drops, and from healthy volunteers as controls. The specimens were analyzed by immuno-histochemistry and ELISA for MMP-2, MMP-9, TIMP-1 and TIMP-2. The MMP-9 levels in nasal mucosa extracts in the AR patients were significantly higher than in the controls. A significant suppressive effect of FP on the MMP-9 levels was shown. The control subjects showed no MMP- or TIMP-positive cells, whereas such positive cells were clearly present in the AR patients. No MMP- or TIMP-positive cells were detected after topical application of FP. These results suggest that the suppressive effect of FP on MMP expression is, in part, responsible for its clinical efficacy in AR.

Allergic rhinitis (AR) is an inflammatory disorder of the nasal mucosa caused by an allergen-IgE interaction in sensitized individuals, which is characterized by nasal itching, sneezing, watery rhinorrhea and nasal obstruction (1, 2). These symptoms arise from the interaction between mediators secreted by effector cells and neural, vascular and glandular structures in the nasal mucosa (2). Histological examination of nasal mucosa biopsy specimens from patients with seasonal and perennial AR has revealed eosinophil accumulation within the lamina propria and

epithelium and an increase in tissue mast cells/basophils (3). Larger numbers of eosinophils and higher levels of mediators from inflammatory cells have been found in nasal lavage fluid of AR patients than of non-AR individuals (4).

The recruitment and migration of circulating inflammatory cells (e.g., eosinophils and basophils) to sites of inflammatory reactions involves traversing capillary walls and the interstitium (5). To traverse these barriers, it is necessary to degrade the extracellular matrix (ECM) proteins and the secretion of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which specifically degrade collagen, native Type IV and V collagen, and elastin (6). Thickening of the basement membrane of the nasal mucosa and sloughed-off epithelium with areas of epithelial metaplasia are also observed in AR (7) and these pathological changes, which are caused by MMPs secreted by epithelial cells, fibroblasts, and inflammatory cells, are referred to as tissue remodeling (8, 9). These MMPs are also responsible for increased microvascular permeability, which leads to edema and cell migration as well as ECM remodeling at the site of inflammation (8), suggesting that reducing MMP expression at sites of allergic inflammation may be an important strategy for treating symptoms of AR.

The management of AR includes avoidance of allergens (if possible), treatment with intranasal corticosteroids (CSs), short-term decongestants, oral H1 receptor antagonists and anticholinergic agents and allergen immunotherapy (10). Topical application of CSs was concluded to be more effective than other medical treatments for controlling nasal symptoms, especially nasal obstruction and rhinorrhea (10). Our previous study clearly showed that fluticasone propionate (FP) suppresses MMP production by nasal fibroblasts induced by inflammatory stimuli *in vitro* (11). However, there is no direct evidence that CSs suppress the expression of MMPs in nasal mucosa administered topically in patients with AR. The present study was undertaken to investigate the effect of CSs on MMP expression in the nasal mucosa obtained from AR patients.

Correspondence to: Harumi Suzuki, MD, Professor and Chairman, Department of Otorhinolaryngology, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan. Tel: +81 3 3784 8676, Fax: +81 3 3784 3757, e-mail: h-suzaki@med.showa-u.ac.jp

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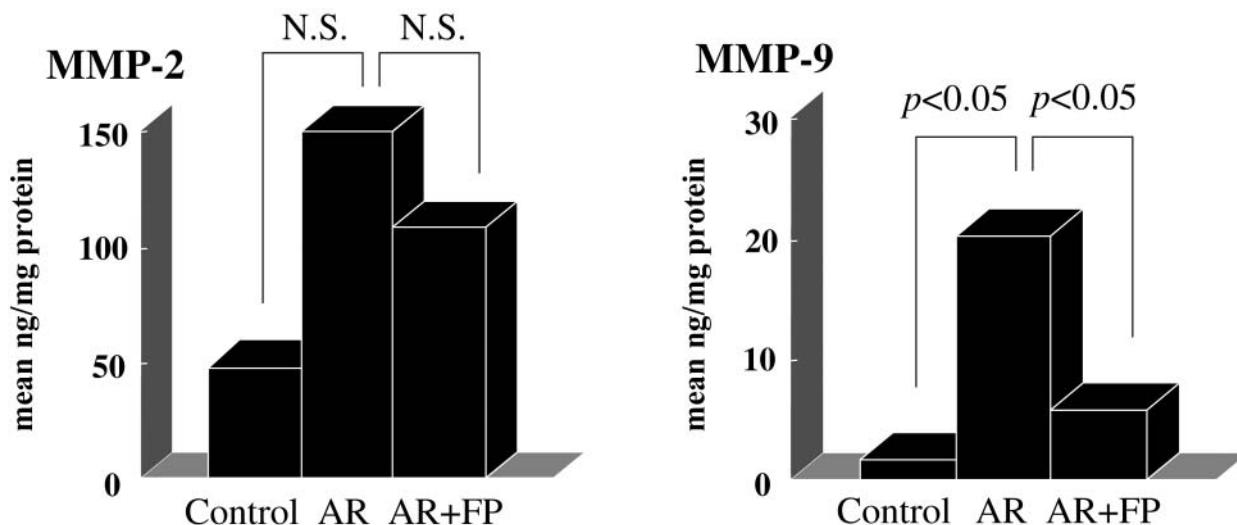


Figure 1. Concentrations of MMP-2 and MMP-9 (mean ng/mg protein) in the nasal mucosa extracts from patients with allergic rhinitis, treated (AR+FP) or untreated (AR) with FP and control: The difference between control and AR, or AR and AR+FP was not significant. The MMP-9 levels were much higher than in the control group ($p<0.05$). The MMP levels were significantly suppressed after intranasal application of fluticasone propionate (FP) ($p<0.05$).

Materials and Methods

Subjects. Nineteen patients with perennial mite-sensitive AR (10 females and 9 males, aged 18 – 58 years, mean 34.8 years), diagnosed according to the Japanese Association of Otorhinolaryngology criteria (1), were enrolled in this study. All patients were treated in the Department of Otorhinolaryngology, Showa University Hospital. Five non-allergic healthy volunteers (1 female and 4 males, 24 – 63 years, mean 43.4 years) were also selected and served as controls. The AR patients were divided into two groups: a group of nine patients not given any medication for two weeks and a group of 10 patients whose only treatment was the administration of fluticasone propionate nose drops (FP; Glaxo Smith Kline Co. Ltd., Tokyo, Japan) twice daily for two weeks. All nasal mucosa biopsy specimens were collected after obtaining signed informed consent. This study was approved by the Ethics Committee of Showa University.

Immunohistochemistry. Nasal mucosa biopsy specimens obtained from the inferior turbinate were fixed with 15% neutral formalin, embedded in paraffin and cut into 3-μm-thick sections. The sections were then deparaffinized and rehydrated with graded alcohol. After washing with phosphate-buffered saline (PBS) three times, the sections were exposed to the primary antibodies for 12 h at 4°C, washed with PBS three times and incubated with the secondary antibody for 2 h at room temperature (25°C). Then, the sections were treated with AEC (3-amino-9-ethyl carbazole) liquid substrate chromogen (DAKO Corporation, Carpinteria, CA, USA). The controls for the immunohistochemistry experiments were prepared by using human immunoglobulin G as the primary antibody and omitting the secondary antibody.

Assay for MMP and tissue inhibitors of metalloproteinases (TIMP). Nasal mucosa samples obtained from the inferior turbinate were homogenized in a teflon homogenizer for 5 min in an ice-cold water bath. After centrifugation at 13,000 rpm for 15 min, the

supernatants were collected and stored at -40°C until used. MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in the supernatants were measured with commercially available enzyme-linked immunosorbent assay (ELISA) test kits (Amersham Biosciences, Piscataway, NJ, USA). The sensitivity of the kits was 0.75 ng/ml for MMP-2, 0.6 ng/ml for MMP-9, 5.1 ng/ml for TIMP-1 and 3.0 ng/ml for TIMP-2. The protein concentration in the supernatants was measured with Bradford protein assay reagents (Bio-Rad Laboratories, Hercules, CA, USA) and the results were expressed as mean values in units of ng/mg protein.

Statistical analysis. The statistical significance of differences in data between the control and treated group was analyzed by ANOVA, followed by Fisher's PLSD test. A p value less than 0.05 was considered statistically significant.

Results

MMP and TIMP levels in nasal mucosa extracts from patients with AR. The concentration of MMP and TIMP in nasal mucosa extracts was measured by ELISA. As shown in Figure 1, the nasal mucosa extracts from the AR patients contained higher MMP2 levels than the extracts from the control group, but the difference was not significant. The MMP-9 levels, on the other hand, were much higher than in the control group ($p<0.05$). The data in Figure 1 show a significant suppressive effect of FP on MMP-9 levels in the tissue extracts ($p<0.05$). The TIMP-1 and TIMP-2 levels in nasal mucosa extracts were subsequently examined by ELISA. As shown in Figure 2, there were no significant differences between the AR patients and control group regarding the TIMP-1 or TIMP-2 levels in the nasal mucosa. A significant suppressive effect of FP on TIMP levels in nasal mucosa extracts was not observed.

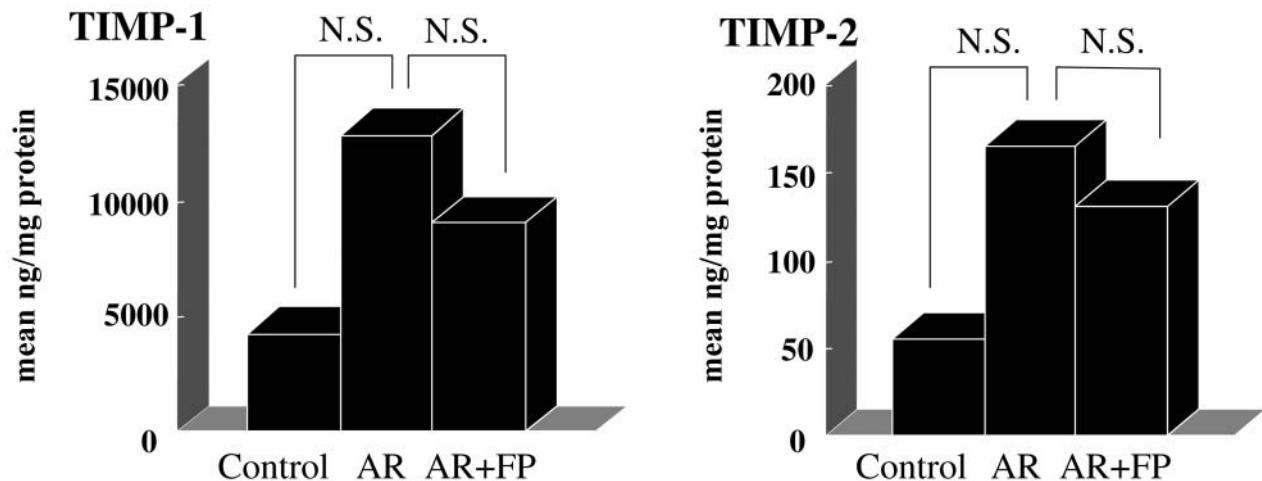


Figure 2. Concentration of TIMP-1 and TIMP-2 (mean ng/mg protein) in the nasal mucosa extracts from patients with allergic rhinitis treated (AR+FP) or untreated (AR) with FP and control. There were no significant differences between the patients with allergic rhinitis (AR) and control group. A significant suppressive effect of fluticasone propionate (FP) on the TIMP level in the nasal mucosa extracts was not observed.

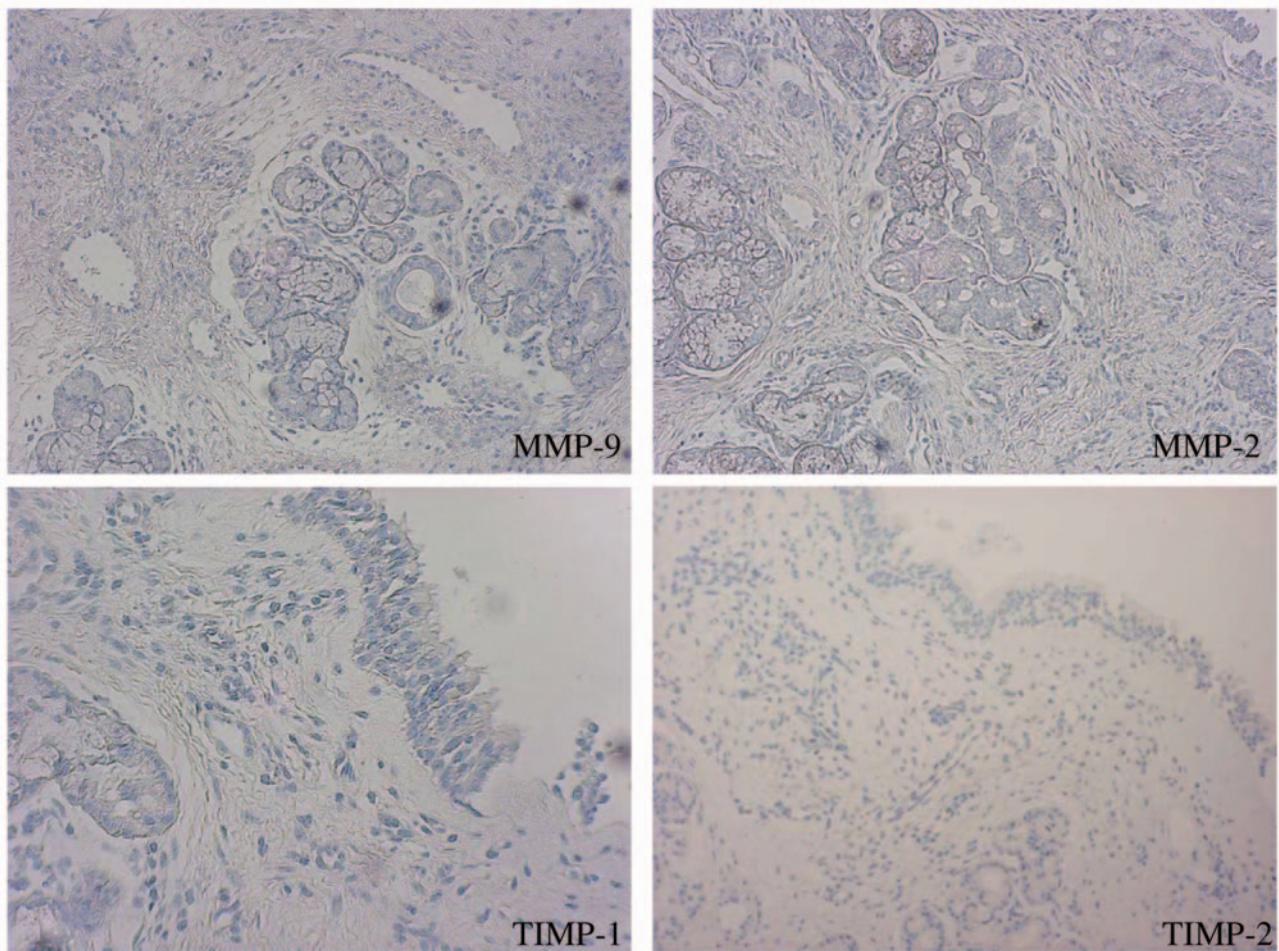


Figure 3. Expression of MMPs and TIMPs in the nasal mucosa of control subjects. Immunohistological staining of nasal mucosa from the control subjects showed no MMP- or TIMP-positive cells.

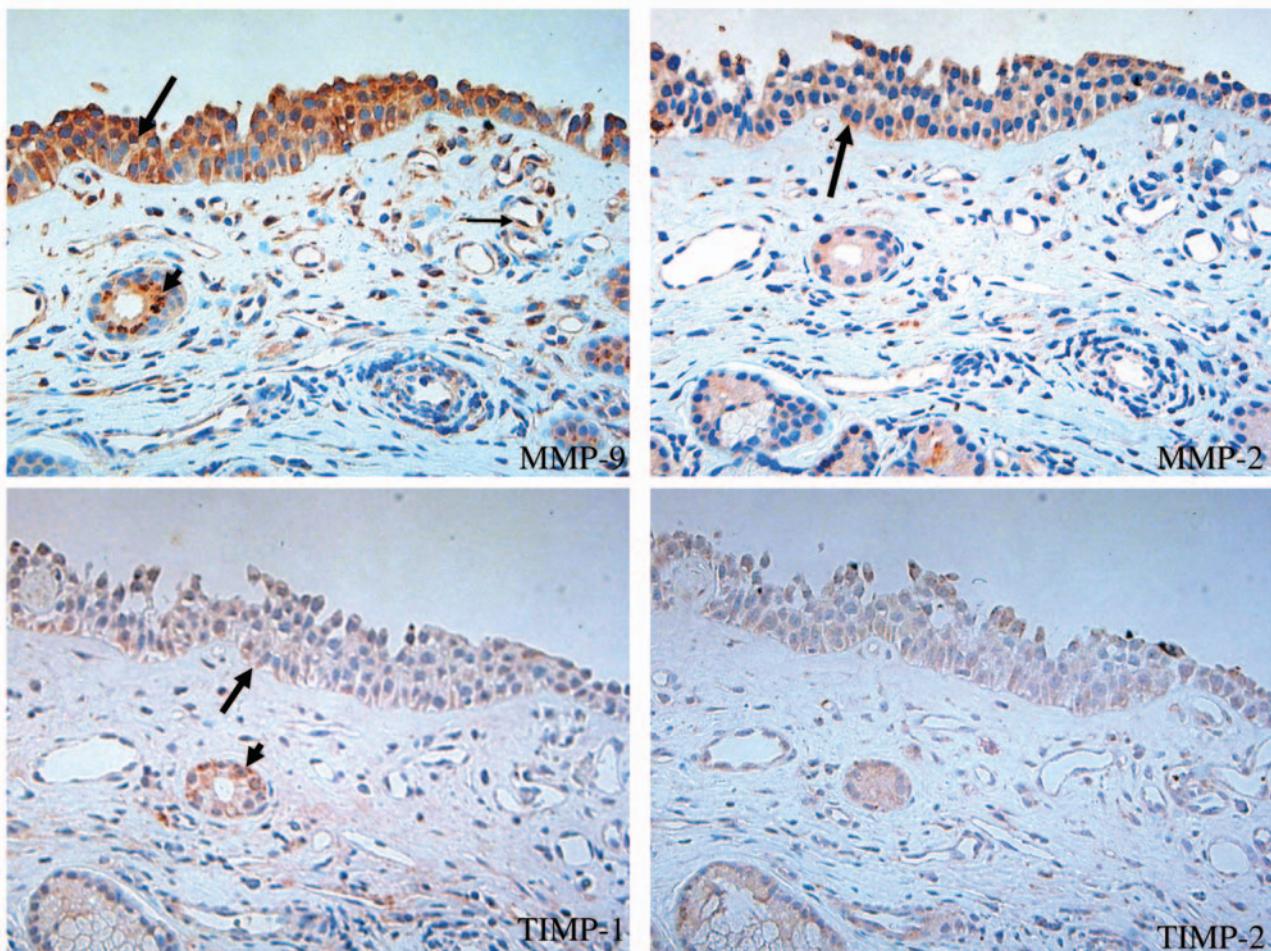


Figure 4. Expression of MMPs and TIMPs in the nasal mucosa of patients with allergic rhinitis (AR). MMP-9-positive cells were observed in the epithelial layer (arrow), blood vessels (thin arrow) and glands (short arrow), whereas MMP-2-positive cells were observed in the epithelial layer (arrow) alone. TIMP-1-positive cells but not TIMP-2-positive cells were observed in the basement of the epithelial layer (arrow) and glands (short arrow).

MMP- and TIMP-positive cells in nasal mucosa. The presence of MMP- and TIMP-positive cells was examined by immunohistological techniques in nasal mucosa obtained from the inferior turbinates in the AR patients and the control subjects. As shown in Figure 3, immunohistological staining of nasal mucosa from the control subjects showed no MMP- or TIMP-positive cells, whereas MMP- and TIMP-positive cells were clearly present in the mucosa obtained from the AR patients (Figure 4). MMP-9-positive cells were observed in the epithelial layer, blood vessels and glands, whereas MMP-2-positive cells were observed in the epithelial layer alone (Figure 4). The data in Figure 4 also show a much higher number of MMP-9-positive cells than MMP-2-positive cells. In the case of TIMP-positive cells, a similar pattern to that of MMP-positive cells was observed: TIMP-1-positive cells, but not TIMP-2-positive cells, were observed in the basement of the epithelial layer and glands

(Figure 4). Finally, the presence of MMP- and TIMP-positive cells was examined in the nasal mucosa obtained from AR patients after FP treatment. As shown in Figure 5, no MMP- or TIMP-positive cells were detected after topical application of FP for two weeks.

Discussion

The results of this study clearly demonstrated that a large amount of MMP-9, but not MMP-2, was contained in the nasal mucosa obtained from AR patients as compared with that from non-allergic healthy subjects and that the topical application of FP twice daily for two weeks caused the disappearance of MMP-9 from the nasal mucosa.

AR is an inflammatory disorder of the nasal mucosa (1-3). Exposure of AR patients to allergens causes immune cell activation within the nasal mucosa as well as activation of the

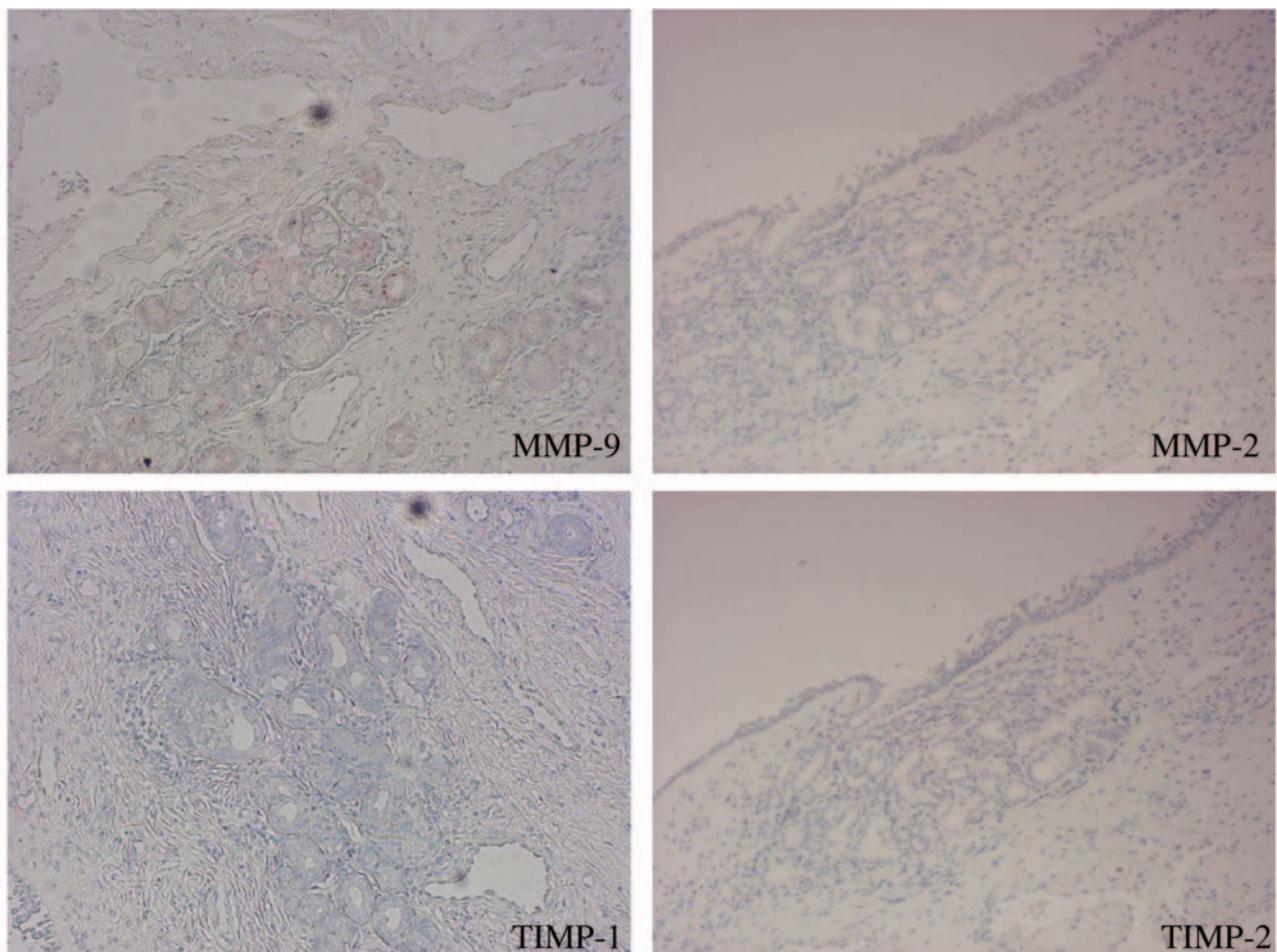


Figure 5. Expression of MMPs and TIMPs in the nasal mucosa of patients with allergic rhinitis (AR) treated with fluticasone propionate (FP). No MMP- or TIMP-positive cells were detected after topical application of FP for two weeks.

resident epithelial and endothelial cells (12). Histological observation revealed structural changes, including thickening of the basement membrane and fibrosis, associated with intense infiltration by inflammatory cells, including eosinophils and mast cells (7, 13). These changes are referred to as tissue remodeling and involve alteration of the ECM (8). Two groups of proteins, MMPs and their inhibitors, TIMPs, have been found to be factors important to the maintenance of ECM homeostasis. MMPs are a large family of zinc-containing enzymes with well conserved common structural domains that degrade a range of ECM proteins (6, 14). One of the MMPs, MMP-9, is capable of degrading native type IV collagen and denaturing collagen and elastin, which are the most important components of the ECM and basement membrane in nasal mucosa (6). MMP-9 is produced by numerous cell types, including inflammatory cells (e.g.; eosinophils and macrophages) and fibroblasts, in response to inflammatory stimuli and enhances inflammatory cell transmigration activity through the basement membrane

to propagate inflammation (6, 15, 16). MMPs, including MMP-9, are also responsible for increasing microvascular permeability, which leads to edema and increased cell migration (8). Taken together, the results of this study showing a suppressive effect of FP on expression of MMP-9 in the nasal mucosa of AR patients may be interpreted as meaning that some of the therapeutic effects of FP in AR depend on its suppression of MMP-9 expression in the nasal mucosa. The extracellular activity of MMP-9 is regulated by TIMP-1, which forms a 1:1 complex with MMP-9 (17). Our results clearly indicate that topical administration of FP to AR patients did not suppress TIMP-1 expression in the nasal mucosa, suggesting that MMP-9 secreted in small amounts during treatment is inactivated by TIMP-1, resulting in reduction of the symptoms caused by ECM remodeling in AR. In addition to its inhibitory effect on MMP-9 activation, the MMP inhibitor reduces the migration of inflammatory cells through the endothelial and epithelial basement membrane by suppressing the expression of intracellular

adhesion molecule-1 and vascular adhesion molecule-1 (5), which is over-expressed in AR (18). Based on these reports, the negative inhibitory effects of FP on TIMP-1 expression in the nasal mucosa may contribute to the relief of symptoms when administered topically to AR patients.

Our previous studies clearly showed that when nasal fibroblasts were exposed to FP, the drug suppressed the production of inflammatory mediators, including MMPs, induced by inflammatory stimuli through suppression of specific mRNA expression (11, 19). Although these reports suggest that topical application of FP to AR patients suppresses MMP-9 mRNA expression by nasal mucosal cells and results in the disappearance of MMP-9 in the nasal mucosa, further experiments are required to clarify the suppressive mechanism(s) of FP *in vivo*.

References

- 1 Howarth PH: Disease pathogenic mechanisms in allergy: a rational basis for treatment. *BMJ* 316: 757-761, 1998.
- 2 Howarth PH, Salagean M and Dokic D: Allergic rhinitis: not purely a histamine-related disease. *Allergy* 55: 7-16, 2000.
- 3 Bentley AM, Jacobson MR, Cumberworth V, Warkans JR, Maqbel R, Schwartz LB, Irani AM, Kay AB and Durham SR: Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increase in activated eosinophils and epithelial cells. *J Allergy Clin Immunol* 89: 877-883, 1992.
- 4 Wilson S, Lau L and Howarth PH: Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 28: 220-227, 1998.
- 5 Lee KS, Jin SM, Kim HJ and Lee YC: Matrix metalloproteinase inhibitor regulates inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression in murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 111: 1278-1284, 2003.
- 6 Dahlen B, Shute J and Howarth PH: Immunohistochemical localisation of the matrix metalloproteinases MMP-3 and MMP-9 within the airways in asthma. *Thorax* 54: 590-596, 1999.
- 7 Calderon MA, Lozewicz S, Prior A, Jordan S, Trigg CJ and Davies RJ: Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. *J Allergy Clin Immunol* 93: 635-643, 1994.
- 8 Hoshino M, Nakamura Y, Sim JJ, Shimoji J and Isogai S: Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthma airway inflammation. *J Allergy Clin Immunol* 102: 783-788, 1998.
- 9 Shaida A, Kenyon G and Devalia J: Matrix metalloproteinases and their inhibitors in the nasal mucosa of patients with perennial allergic rhinitis. *J Allergy Clin Immunol* 108: 791-796, 2001.
- 10 Weiner JM, Abramson MJ and Puy RM: Intranasal corticosteroids versus oral H1 receptor antagonists in allergic rhinitis: systemic review of randomized controlled trials. *BMJ* 317: 1624-1629, 1998.
- 11 Namba M, Asano K, Kanai K, Kyo Y, Watanabe S, Hisamitsu T and Suzuki H: Suppression of matrix metalloproteinase production from nasal fibroblasts by fluticasone propionate *in vitro*. *Acta Otolaryngol* 124: 964-969, 2004.
- 12 Baroody FM, Lim MC, Proud D, Kagey-Sobotka A, Lichtenstein LM and Naclerio RM: Effects of loratadine and terfenadine on the induced nasal allergic reaction. *Arch Otolaryngol Head Neck Surg* 122: 309-316, 1996.
- 13 Howarth PH: The cellular basis of allergic rhinitis. *Allergy* 50(Suppl. 23): 6-10, 1995.
- 14 Birkedal-Hansen H, Moore WGI, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A and Engler JA: Matrix metalloproteinase: a review. *Crit Rev. Oral Biol Med* 4: 197-250, 1993.
- 15 Shute JK, Parmar J, Holgate ST and Howarth PH: Urinary Glycosaminoglycan levels are increased in acute severe asthma – a role for eosinophil-derived gelatinase B? *Int Arch Allergy Immunol* 113: 366-367, 1997.
- 16 Ohno I, Ohtani H, Nitta Y, Suzuki J, Hoshi H, Honma M, Isoyama S, Tanno Y, Tamura G, Yamauchi K, Nagura H and Shiota K: Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 16: 212-219, 1997.
- 17 Nagase H: Activation mechanisms of matrix metalloproteinase. *Biol Chem* 378: 151-160, 1997.
- 18 Howarth PH: A comparison of anti-inflammatory properties of intranasal corticosteroids and antihistamines in allergic rhinitis. *Allergy* 55: 425-434, 2000.
- 19 Uchida J, Kanai K, Asano K, Watanabe S, Hisamitsu T and Suzuki H: Influence of fluticasone propionate on the production of vascular endothelial growth factor and basic fibroblast growth factor from nasal fibroblasts *in vitro*. *In Vivo* 18: 767-770, 2004.

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