

Suppressive activity of fexofenadine hydrochloride on metalloproteinase production from nasal fibroblasts *in vitro*

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Summary

Background Allergic rhinitis (AR) is an inflammatory disease characterized by nasal wall remodelling with intense infiltration of eosinophils and mast cells/basophils. Matrix metalloproteinases (MMPs), MMP-2 and MMP-9, are the major proteolytic enzymes that induce airway remodelling. These enzymes are also important in the migration of inflammatory cells through basement membrane components.

Objective We evaluated whether fexofenadine hydrochloride (FEX), the carboxylic acid metabolite of terfenadine with selective H₁-receptor antagonist activity, could inhibit MMP production from nasal fibroblasts (NFs) in response to TNF- α stimulation *in vitro*.

Methods NFs were established from nasal polyp-derived fibroblasts (PFs) taken from patients with AR. Nasal mucosal fibroblasts (MFs) were also induced from nasal mucosal tissues from septal deformity patients without allergy. PF and MF (2×10^5 cells/mL, each) were stimulated with TNF- α in the presence of various concentrations of FEX. After 24 h, culture supernatants were obtained and assayed for MMP-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 levels by ELISA. The influence of FEX on mRNA expression of MMPs and TIMPs in 4 h-cultured cells was also evaluated by real-time RT-PCR. Furthermore, nuclear factor- κ B (NF- κ B) activation in fibroblasts treated with FEX for 4 h was examined by ELISA.

Results FEX at more than 350 ng/mL inhibited the production of MMP-2 and MMP-9 from both PF and MF in response to TNF- α stimulation, whereas TIMP-1 and TIMP-2 production was scarcely affected by FEX. FEX also inhibited MMP mRNA expression and NF- κ B activation in PF and MF after TNF- α stimulation.

Conclusion The present data suggest that the attenuating effect of FEX on MMP-2 and -9 production from NFs induced by inflammatory stimulation may underlie the therapeutic mode of action of the agent on allergic diseases, including AR.

Keywords fexofenadine, matrix metalloproteinases, mRNA, nasal fibroblasts, NF- κ B

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Introduction

Allergic rhinitis (AR) is an inflammatory condition of the nasal mucosa caused by an allergen–IgE interaction in sensitized individuals and is characterized by the clinical symptoms of sneezing, itching, congestion, rhinorrhoea, and nasal blockage, which are responsible for difficulties in breathing through the nose [1, 2]. Immunohistochemical studies of nasal biopsies show accumulation of eosinophils, basophils/mast cells within the lamina propria and epithelium in both seasonal and perennial AR [3, 4]. Furthermore, nasal lavage also reveals elevated levels of eosinophil cationic protein and tryptase in AR [5], indicating that the accumulated cells in the nasal mucosa are in an activated state.

The recruitment and migration of circulating inflammatory cells, such as eosinophils and basophils, to sites of inflam-

matory reactions involves traversing both the capillary walls and the interstitium [6]. To traverse these barriers, inflammatory cells adhere and degrade extracellular matrix (ECM) proteins [6]. The degradation of ECM proteins by inflammatory cells is accomplished, in part, by the secretion of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which specifically degrade denatured collagen, native type IV and V collagens, and elastin [7, 8]. Furthermore, structural abnormalities such as fibrosis, thickening of the basement membrane, and sloughed-off epithelium with areas of epithelial metaplasia have been well described in AR [9, 10]. These pathological changes are called tissue remodelling, which is caused by MMPs secreted from epithelial cells and fibroblasts in addition to infiltrating inflammatory cells [11–13]. These MMPs are also responsible for microvascular permeability leading to oedema and cell migration, and ECM remodelling at the site of inflammation [8, 11]. From these reports, it is reasonable to speculate that manipulation of MMP production from inflammatory cells, epithelial cells and fibroblasts by anti-allergic agents may

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be an important strategy for treating symptoms of allergic diseases including AR.

Several studies have demonstrated that corticosteroids, which are commonly used in the management of allergic airway diseases including AR, can attenuate the expression and release of MMPs from airway epithelial cells *in vivo* and *in vitro* [14–16]. However, despite their potent anti-inflammatory properties, the use of this class of drugs may be limited because of concerns over unwanted side-effects [17]. In contrast, H₁-antihistamines are commonly used as the first-line treatment for AR [17–19]. There is much evidence that certain H₁-antihistamines may possess anti-inflammatory activities in addition to blocking the action of histamine at the receptors, thus conferring a therapeutic advantage in the treatment and management of allergic diseases [20, 21]. However, to our knowledge, there is no evidence showing the influence of anti-allergic agents, especially H₁-antihistamines, on MMP production. In the present study, we have examined the effect of fexofenadine hydrochloride (FEX), the carboxylic acid metabolite of terfenadine with selective H₁-receptor antagonist activity [17], on MMP production from nasal fibroblasts (NFs) in response to inflammatory stimulation *in vitro*.

Materials and methods

Chemicals

FEX (Aventis Pharmaceutical Co., Ltd, Tokyo, Japan) as a preservative-free pure powder was dissolved in dimethyl sulfoxide (DMSO; Wako Pure Chemical Co., Ltd, Tokyo, Japan) at 2.0 mg/mL and diluted with antibiotics-free RPMI-1640 medium (Sigma Chemicals, Co., Ltd, St Louis, MO, USA) supplemented with 10% heat-inactivated foetal calf serum (RPMI-FCS; Flow Laboratories, North Ride, Australia) at 10.0 µg/mL. This solution was sterilized by passing through a 0.22 µm filter and stored at 4 °C as a stock solution. All dilutions used in this study were prepared from this stock solution. Our previous experiments revealed no harmful effects of 0.1% DMSO on fibroblast functions, such as MMP production after TNF-α stimulation [22]. Recombinant TNF-α (preservative-free) was purchased from Chemicon International, Inc. (Temecula, CA, USA) and diluted with RPMI-FCS to give a concentration of 50.0 ng/mL [22, 23].

Induction of fibroblasts

To induce fibroblasts from nasal polyps, five patients (three females and two males; 36.6 ± 9.4 years of age) with AR were recruited at Otolaryngology of Showa University Hospital. They were non-smokers and not treated with oral anti-allergic agents for at least 2 months. Nasal polyps were obtained during surgical operation from five patients under written informed consent, which was approved by the Ethics Committee of Showa University. The tissues were rinsed several times with RPMI-FCS that contained 500 U penicillin, 500 µg/mL streptomycin and 5.0 µg/mL amphotericin B and cut into small pieces (approximately 1 mm²). Diced specimens were plated at a density of 10 pieces in 100 mm tissue culture dishes and covered with a cover slip adhered to

the dish with sterile Vaseline. The dishes were then placed in a humidified atmosphere containing 5% CO₂ at 37 °C. When a monolayer of fibroblast-like cells was found to be confluent, the explanted tissues were removed. The cells were then trypsinized, and replated at a concentration of 5 × 10⁵ cells/mL into 100 mm tissue culture dishes with a final volume of 10.0 mL. The medium was changed every 3 days for 2–3 weeks until confluence was attained. Subsequently, the cells were split 1:2 at confluence and passaged [22, 23]. The cells were characterized with antibodies against vimentine, cytokeratin and fibronectin, using fluorescent microscopy (Model No. IX 70, OLYMPUS Co., Ltd, Tokyo, Japan) [23]. Fibroblast purity was more than 99% and used for polyp-derived fibroblasts (PFs). After characterization, aliquots of cells at each passage were frozen and stored in liquid nitrogen. To establish mucosal fibroblasts (MFs), five patients (two females and three males; 39.2 ± 8.4 years of age) with septal deformity were recruited at Otolaryngology of Showa University Hospital. They were non-smokers, non-allergic, and had not received any medications for at least 2 months. Nasal mucosa was obtained during plastic reconstruction of the septum under written informed consent, which was approved by the Ethics Committee of Showa University. Fibroblasts were induced in a similar manner, characterized and used as MF. Since our preliminary experiments revealed that the ability of cells to produce MMP-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 in response to TNF-α stimulation were not different between the third and eighth passages of cells (data not shown), PFs and MFs at seven to 10 passages were used for the following experiments.

Cell culture

Fibroblasts were washed several times with RPMI-FCS, introduced into each well of 24-well culture plates in triplicate at a concentration of 2 × 10⁵ cells/mL in a volume of 1.0 mL and allowed to adhere for 2 h. After removing unattached and dead cells by gentle washing of the wells with RPMI-FCS, the remaining cells were stimulated with 25.0 ng/mL TNF-α [22] in the absence or presence of various concentrations of FEX in a final volume of 2.0 mL. After 24 h, the culture medium was removed, and stored at –40 °C until use. In cases of examining transcription factor activation and mRNA expression, cells were cultured in a similar manner for 4 h and stored at –80 °C until use. In all cases, FEX was added to cell cultures at the start of TNF-α stimulation. The medium that contained TNF-α and 0.1% DMSO is used for solvent control.

Assay for MMP and TIMP

MMP-2, MMP-9, TIMP-1 and TIMP-2 levels in culture supernatants were assayed using commercially available human MMP and TIMP ELISA test kits (Amersham Biosciences, Bucks, UK) according to the manufacturer's recommendation. The activities of MMP-2 and MMP-9 in culture supernatants were also examined by ELISA test kits (Amersham Biosciences) according to the manufacturer's instructions. The results are expressed as the mean ng/mL ± standard error (SE) of duplicate assays for five subjects.

Real-time polymerase chain reaction

mRNA was extracted from fibroblasts using μ MACS mRNA isolation kits (Milteny Biotec GmbH, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The first-strand complementary DNA (cDNA) synthesis from 1.0 μ g mRNA was performed using the SuperScript Preamplification System for cDNA synthesis (GIBCO BRL, Gaithersburg, MD, USA). PCR was then carried out using a GeneAmp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). PCR mixture consisted of 2.0 μ L of sample cDNA solution (10.0 ng/ μ L), 25.0 μ L of SYBR-Green Mastermix (Applied Biosystems), 0.3 μ L of both sense and antisense primers, and distilled water to give a final volume of 50 μ L. The reaction was conducted as follows: 4 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. β -actin was amplified as an internal control. mRNA levels were calculated by using the comparative parameter threshold cycle (Ct) and normalized to β -actin. Oligonucleotide sequences of the primers used were shown in Table 1 [24–26].

Assay for NF- κ B activity

Nuclear factor- κ B (NF- κ B) activity was analysed with commercially available ELISA test kits (Active Motif, Carlsbad, CA, USA) that contained sufficient reagents and monoclonal antibodies against p50 and p65, according to the manufacturer's recommended procedure. In brief, nuclear extract (5.0 μ g protein) from NF was introduced into each well of 96-well microtitre plates pre-coated with oligonucleotide containing the NF- κ B consensus site (5'-GGGAC TTTCC-3') in a volume of 20.0 μ L, and incubated for 1 h at 25 °C. After washing three times, 100 μ L of monoclonal antibody against p50 or p65 was added to the appropriate wells, and incubated for a further 1 h at 25 °C. Anti-IgG horseradish peroxidase-conjugate in a volume of 100 μ L was then added and incubated 1 h at 25 °C. Absorbance at 450 nm was measured after the addition of tetramethylbenzidine solution.

Statistical analysis

The statistical significance of the difference between the control and experimental data was analysed using ANOVA followed by Fisher's PLSD test. A *P*-value <0.05 was accepted as statistically significant.

Results

Suppressive activity of FEX on the production of MMP-2, MMP-9, TIMP-1 and TIMP-2 from nasal fibroblasts

The first set of experiments was designed to examine the influence of FEX on MMPs production from PF in response to TNF- α stimulation *in vitro*. PF (2×10^5 cells/mL) was stimulated with 25.0 ng/mL TNF- α in the presence of either 0, 125, 250, 350, 500, or 750 ng/mL of FEX for 24 h. MMP levels in culture supernatants were assayed by ELISA. As shown in Fig. 1a, addition of FEX at quantities lower than 250 ng/mL did not cause the suppression of MMP-2 production from PF, which was increased by TNF- α stimulation. However, FEX significantly suppressed the ability of cells to produce MMP-2 after TNF- α stimulation when the agent was added into cell cultures at 350 ng/mL and higher (Fig. 1a). We next examined the influence of FEX on MMP-9 production from PF. As shown in Fig. 1b, low doses (125 and 250 ng/mL) of FEX did not affect MMP-9 production from PF in response to TNF- α stimulation: MMP-9 levels in experimental culture supernatants were nearly identical (not significant) to that in control supernatants (TNF alone). However, addition of FEX at concentrations of 350 ng/mL and higher significantly inhibited MMP-9 production from PF induced by TNF- α stimulation (Fig. 1b). The third experiments were carried out to examine the influence of FEX on the enzymatic activities of MMPs. The data in Figs 1c and d clearly showed that addition of FEX into cell cultures at more than 350 ng/mL could significantly suppress the activity of both MMP-2 and MMP-9 in culture supernatants. The fourth experiment was undertaken to examine the influence of FEX on the production of TIMP-1 and TIMP-2 from PF.

Table 1. Primer sequences used for real-time PCR

Primer sequences	Position	Product size (bp)	References
MMP-2			
Sense-5'-AGATCTTCTTCTTCAAGGACCGTT-3'	1740–1764	440	[24]
Antisense-5'-GGCTGGTCAGTGGCTTGGGGTA-3'	1964–1943		
MMP-9			
Sense-5'-TGGACGATGCCTGCAACGTG-3'	1554–4573	454	[25]
Antisense-5'-AGTGTAGGTCTTGGTGAAGCC-3'	2008–1986		
TIMP-1			
Sense-5'-CACCCACAGACGGCCTTCTGCAAT-3'	150–173	345	[25]
Antisense-5'-AGTGTAGGTCTTGGTGAAGCC-3	494–474		
TIMP-2			
Sense-5'-CTCGCTGGACGTTGGAGGAAAGAA-3'	602–625	433	[26]
Antisense-5'-AGCCCATCTGGTACCTGTGGTTCA-3'	756–733		
β-actin			
Sense-5'-ACCCACACTGTGCCCATCTA-3'	551–570	239	[24]
Antisense-5'-CGGAACCGCTCATTGCC-3'	733–717		

MMP, matrix metalloproteinases; TIMP, tissue inhibitor of metalloproteinase.

The data in Figs 2a and b clearly showed the negative suppressive effect of FEX on the production of both TIMP-1 and TIMP-2 from PF: culture supernatant obtained from cells stimulated with TNF- α in the presence of 750 ng/mL FEX contained similar levels (not significant) of TIMP-1 and TIMP-2 to those from control cell cultures (TNF alone). The second part of experiments was performed to examine whether FEX could also suppress the production of MMPs from MF after TNF- α stimulation as in the case of PF. The data in Figs 3a and b clearly showed the suppressive activity of FEX on the production of both MMP-2 and MMP-9 at more than 350 ng/mL. It is also showed that FEX at more than 350 ng/mL could significantly suppress the activity of both MMP-2 and MMP-9 in culture supernatants (Figs 3c

and d). We finally examined the influence of FEX on TIMP-1 and TIMP-2 production from MF induced by TNF- α stimulation. As shown in Fig. 4, addition of FEX into cell cultures scarcely affected the ability of MP to produce TIMP-1 and TIMP-2: culture supernatants from cells stimulated with TNF- α in the presence of 750 ng/mL FEX contained almost identical (not significant) levels of TIMP-1 and TIMP-2 to those in control culture supernatants (TNF alone).

Influence of FEX on MMP and TIMP mRNA expression in nasal fibroblasts

The third set of experiments was undertaken to examine the possible mechanisms by which 350 ng/mL FEX could

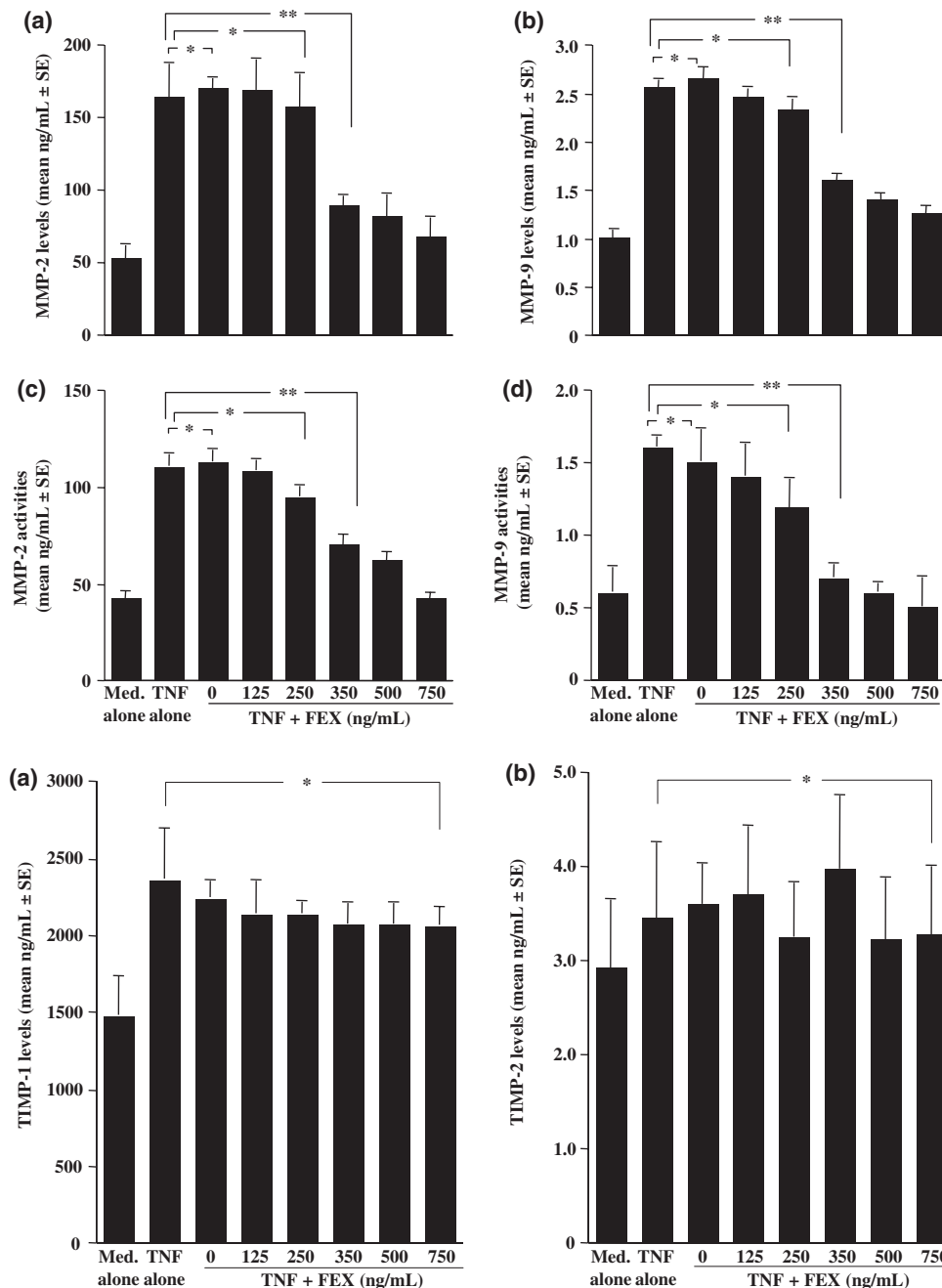


Fig. 1. Influence of fexofenadine hydrochloride (FEX) on the production of matrix metalloproteinases (MMPs) from nasal polyp fibroblasts in response to TNF- α stimulation and on their activities. Cells were stimulated with TNF- α (TNF) in the presence of various concentrations of FEX for 24 h. MMP levels in culture supernatants were examined by ELISA. Data are expressed as the mean ng/mL $\pm$ SE of five different subjects of one of two different experiments, which gave reproducible results. (a) MMP-2 levels; (b) MMP-9 levels; (c) MMP-2 activities; (d) MMP-9 activities; *not significant; **significant ($P < 0.05$).

Fig. 2. Influence of fexofenadine hydrochloride (FEX) on the production of tissue inhibitor of metalloproteinase (TIMP)-1 (a) and TIMP-2 (b) from nasal polyp fibroblasts in response to TNF- α stimulation. Cells were stimulated with TNF- α (TNF) in the presence of various concentrations of FEX for 24 h. TIMP levels in culture supernatants were examined by ELISA. Data are expressed as the mean ng/mL $\pm$ SE of five different subjects of one of two different experiments, which gave reproducible results. *Not significant.

suppress MMP-2 and MMP-9, but not TIMP-1 and TIMP-2 production from NFs, PF and MF, in response to TNF- α stimulation. Cells were cultured with 25.0 ng/mL TNF- α in the presence of either 0, 250, 350, or 500 ng/mL FEX for 4 h. Levels of mRNA expression was evaluated by real-time PCR. Addition of FEX at more than 350 ng/mL significantly suppressed MMP mRNA expression in both PF (Figs 5a and b) and MF (Figs 6a and b), which were enhanced by TNF- α stimulation. However, FEX could not reduce levels of TIMP mRNA expression in PF (Figs 5c and d) and MF (Figs 6c and d).

Influence of FEX on NF- κ B activation by TNF- α stimulation in nasal fibroblasts

The final set of experiments was designed to examine the influence of FEX on NF- κ B activation in NFs, PF and MF, after TNF- α stimulation *in vitro*. Cells were stimulated with TNF- α in the presence of various doses of FEX for 4 h. The nuclear extracts were prepared, and NF- κ B activity was assessed by measuring both p50 and p65 activities with ELISA. Addition of FEX at 125 ng/mL into cell cultures did not affect NF- κ B activation in both fibroblasts: optical

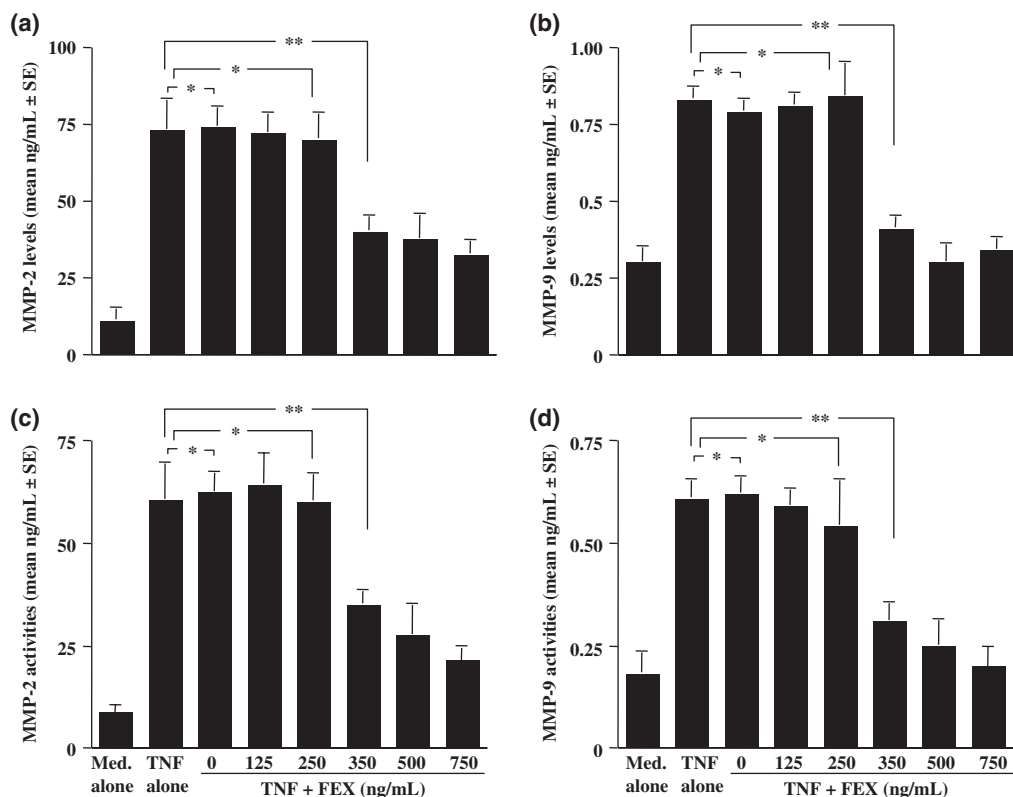


Fig. 3. Influence of fexofenadine hydrochloride (FEX) on the production of matrix metalloproteinases (MMPs) from nasal mucosal fibroblasts in response to TNF- α stimulation and on their activities. Cells were stimulated with TNF- α in the presence of various concentrations of FEX for 24 h. The activities of MMPs in culture supernatants were examined by ELISA. Data are expressed as the mean ng/mL $\pm$ SE of five different subjects of one of two different experiments, which gave reproducible results. (a) MMP-2 levels; (b) MMP-9 levels; (c) MMP-2 activities; (d) MMP-9 activities; *not significant; **significant ($P < 0.05$).

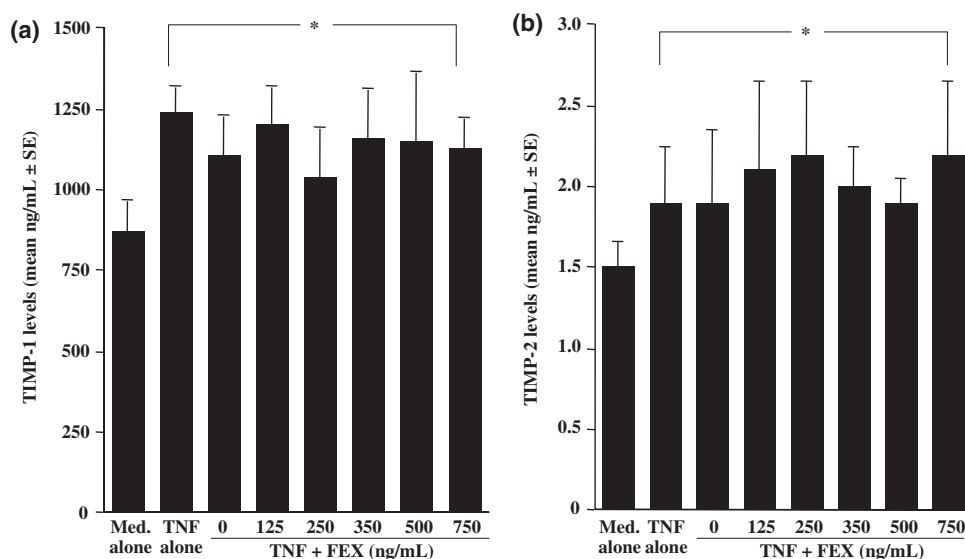


Fig. 4. Influence of fexofenadine hydrochloride (FEX) on the production of tissue inhibitor of metalloproteinase (TIMP)-1 (a) and TIMP-2 (b) from nasal mucosal fibroblasts in response to TNF- α stimulation. Cells were stimulated with TNF- α (TNF) in the presence of various concentrations of FEX for 24 h. TIMP levels in culture supernatants were examined by ELISA. Data are expressed as the mean ng/mL $\pm$ SE of five different subjects of one of two different experiments, which gave reproducible results. *Not significant.

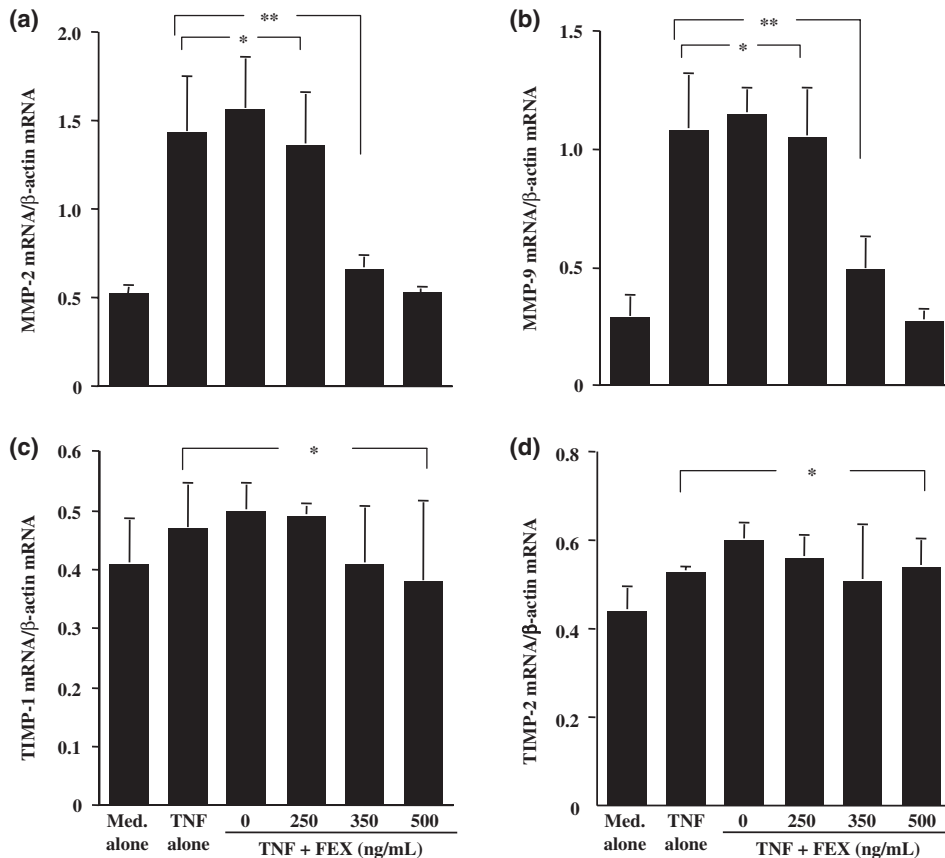


Fig. 5. Influence of fexofenadine hydrochloride (FEX) on mRNA expression of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMPs) in nasal polyp fibroblasts after $\text{TNF-}\alpha$ stimulation. Cells were stimulated with $\text{TNF-}\alpha$ (TNF) in the presence of various concentrations of FEX for 4 h. mRNA expression was examined by real-time RT-PCR. Data are expressed as the mean ratio $\pm$ SE of five different subjects of one of two different experiments, which gave reproducible results. (a) MMP-2; (b) MMP-9; (c) TIMP-1; (d) TIMP-2; *significant ($P < 0.05$); **not significant.

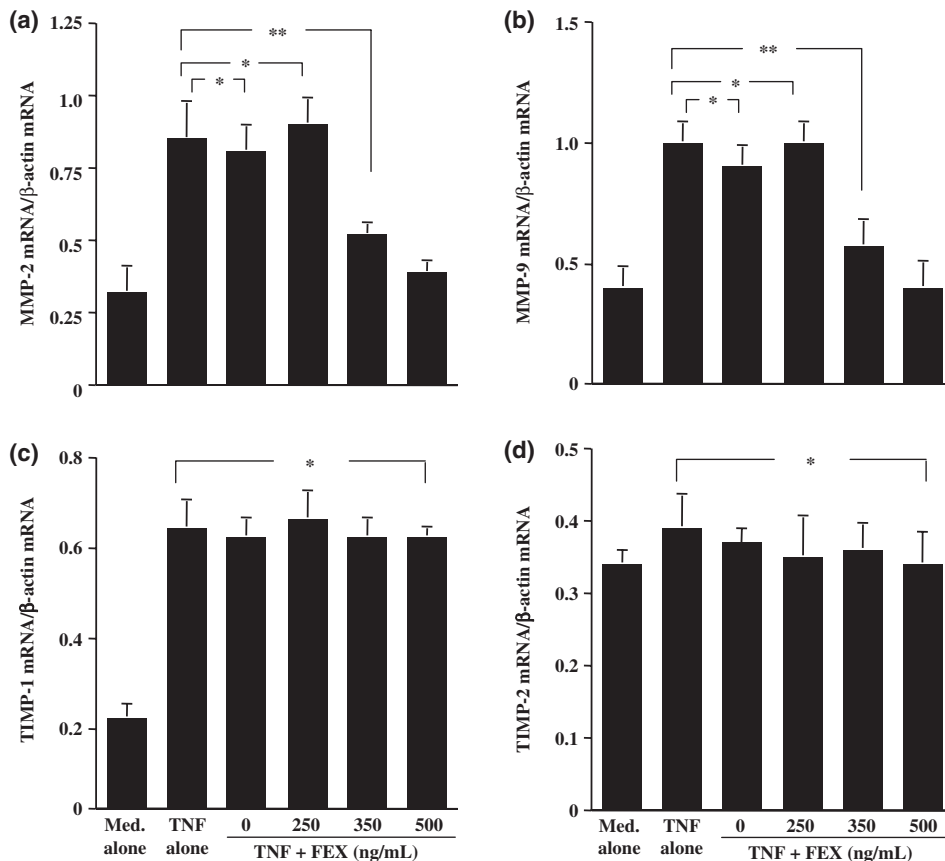


Fig. 6. Influence of fexofenadine hydrochloride (FEX) on mRNA expression of matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinase (TIMPs) in nasal mucosal fibroblasts after $\text{TNF-}\alpha$ stimulation. After the same stimulation of cells as described in Fig. 5, mRNA expression was examined by real-time RT-PCR. Each value is the ratio of MMPs and TIMPs to β-actin mRNA level. Results are presented as the mean of five different subjects $\pm$ SE. Experiments were performed twice with similar results. (a) MMP-2; (b) MMP-9; (c) TIMP-1; (d) TIMP-2; *significant ($P < 0.05$); **not significant.

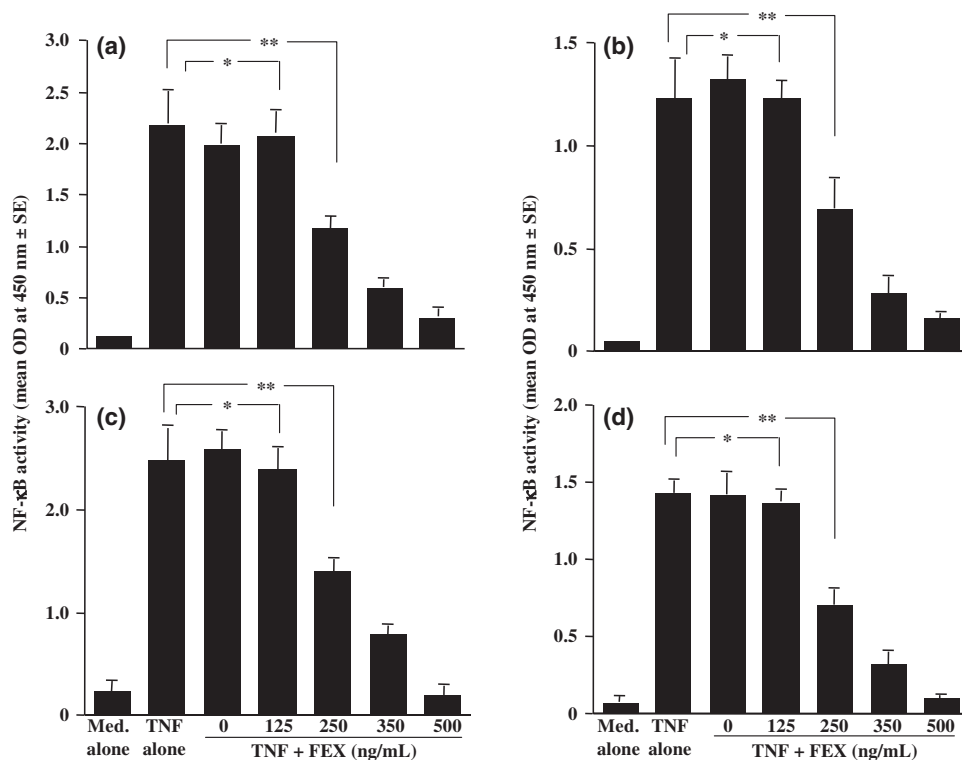


Fig. 7. Influence of fexofenadine hydrochloride (FEX) on nuclear factor (NF- κ B) activation in nasal fibroblasts after TNF- α stimulation. Cells were stimulated with TNF- α in the presence of various concentrations of FEX for 4 h. NF- κ B, p50 (a and c) and p65 (b and d), activities were assayed by ELISA. Upper panels (a and b) show the activities in polyp-derived fibroblasts, and lower panels in mucosal fibroblasts. Data are expressed as the mean optical density (OD) at 450 nm $\pm$ SE of five different subjects. *Significant ($P < 0.05$).

density at 450 nm in experimental groups was similar (not significant) to those in appropriate controls (Fig. 7). However, a higher dose (250 ng/mL) of FEX caused significant suppression of p50 and p65 activation in PF and MF (Fig. 7). This suppressive activity was further strengthened when FEX was added to cell cultures at 350 ng/mL and higher (Fig. 7).

Discussion

In the present study, we clearly demonstrated that FEX at 350 ng/mL, which is lower than therapeutic tissue levels [27], could inhibit the production of both MMP-2 and MMP-9 from NF with virtually no effect on the production of TIMP-1 and TIMP-2. In addition, this inhibitory action of FEX on MMP production is due, in part, to its suppressive activity on MMP mRNA expression through the inhibition of transcription factor-NF- κ B activation by TNF- α stimulation.

AR is an inflammatory disorder of the nasal mucosa and epithelium [1–3, 18, 22]. Aeroallergen exposure in sensitized individuals with AR results in immune cell activation within the nasal mucosa along with activation of the resident epithelial and endothelial cells [28, 29]. Histological observation also revealed structural abnormalities, including thickening of the basement membrane and structural fibrosis, which are associated with intense infiltration of several types of inflammatory cells such as eosinophils and mast cells/basophils [9, 10]. These cellular events are now called tissue remodelling, and involve extensive alteration of tissue ECM [14]. ECM is involved in tissue homeostasis and in pathologic conditions such as tumour invasion and inflammation. Two groups of proteins, MMPs and their counter-regulatory

inhibitors, TIMPs, are recognized to be important factors for maintenance of ECM homeostasis. The MMPs are a large family of Ca^{2+} -activated, Zn^{2+} -dependent endopeptidases that can degrade various components of the ECM and basement membrane [13, 30]. Among MMPs, MMP-2 and MMP-9 degrade native type IV collagen and denatured collagens, as well as elastin, which are the most important components of ECM and basement membrane in nasal walls [12]. These MMPs are produced by numerous cell types, including fibroblasts and macrophages in response to inflammatory stimulation, and mediate transmigration of the inflammatory cells (eosinophils and macrophages) through the basement membrane to propagate inflammation [11, 12, 30]. MMPs are also responsible for microvascular permeability leading to oedema and enhanced cell migration [11, 31]. From these reports, it is possible that the attenuating effect of FEX on MMP-2 and MMP-9 production from NF induced by inflammatory stimulation may underlie the therapeutic mode of action of the agent on allergic diseases, including AR. The extracellular activity of MMPs is regulated by TIMPs that form a 1:1 complex with MMPs [32]. The present results clearly showed that FEX could not inhibit the TIMP-1 and TIMP-2 production from NF in response to TNF- α stimulation, suggesting that MMPs secreted in small amounts during FEX treatment are inactivated by TIMPs, and resulting in modification of clinical symptoms derived from ECM remodelling and accumulation of inflammatory cells in AR. Experimental studies clearly showed that administration of MMP inhibitor into mice reduces the migration of inflammatory cells through the endothelial and epithelial basement membrane [33]. This inhibitory action of MMP inhibitor on cell migration is also reported to be because of its suppressive effect on the expression of intercellular adhesion molecule 1 and vascular adhesion

molecule-1 [6], which are essential molecules for cell migration into inflammatory tissues and are reported to be over-expressed in AR [21]. From these reports, the negative suppressive effect of FEX on TIMP-1 and TIMP-2 production may contribute, in part, to favourable modification of clinical symptoms, when FEX is administered into patients with AR.

The mechanisms and signal transduction pathways involved in the production of MMPs in fibroblasts are not well understood. Prostaglandins (PGs), especially PGE1 and PGE2, however, have been reported to up-regulate the production of MMPs in synoviocytes [34, 35] and gingival fibroblasts [36] after inflammatory stimulation *in vitro*. It is also observed the involvement of PGs in the production of MMPs from human pulp cells [37] and prostate epithelial tumour cells [38]. The finding that FEX, a potent H₁-receptor antagonist, inhibited MMP-2 and MMP-9 production suggests that PG-independent mechanism(s) may be implicated in MMP production in NF induced by TNF- α stimulation, since H₁-receptor antagonists are reported to increase the production and secretion of PGE2 in fibroblasts [39]. The signal transduction pathways involved in the production of MMP-2 and MMP-9 in fibroblasts stimulated with IL-1 β or TNF- α was studied by using tyrosine kinase and p38 MAP-kinase inhibitors. The tyrosine kinase inhibitor, herbimycin A, down-regulated MMP-2 and MMP-9 production, stimulated by IL-1 β and TNF- α [36, 37], indicating that tyrosine kinases are involved in the signal pathways of MMP-2 and MMP-9 production in fibroblasts. In addition, SB 203580, a potent inhibitor of p38 MAP-kinase, is also reported to down-regulated TNF- α -induced MMP gene expression and protein production in fibroblasts [40]. Extracellular signal-regulated kinases and c-Jun N-terminal kinases, which constitute together with p38 kinases the family of mitogen-activated protein kinases (MAPKs), have been implicated in the regulation of collagenase gene expression in cultured fibroblasts induced by IL-1 β and TNF- α [35]. Activation of tyrosine kinases and MAPKs after stimulation with TNF- α has been reported to regulate by transcriptional factor NF- κ B [41]. These reports strongly suggest that FEX may suppress NF- κ B activation, which is responsible for MMP mRNA expression and results in inhibition of MMP production. This speculation may be supported by the present observations showing that FEX could suppress MMP mRNA expression through the inhibition of NF- κ B activation induced by TNF- α stimulation. Different from MMPs, which contained binding sites for NF- κ B, TIMPs have activator protein-1 (AP-1) binding sites in their promoters and the transcription of TIMPs is AP-1 dependent [42, 43]. It is also reported that PEA3, a protooncogene-related transcriptional factor, is involved in the regulation of TIMP transcription [42, 44, 45]. From these reports, the present data may be interpreted that FEX could not inhibit AP-1 and PEA3 activation induced by TNF- α stimulation and results in negative suppressive effects on TIMP production from NF. Further experiments are required to clarify this point.

Since the concentration of FEX showing the suppressive effect on MMP-2 and MMP-9 production *in vitro* (350 ng/mL) is far below that in tissue levels (more than 500 ng/mL) [27], the efficacy of FEX in allergic inflammatory diseases

may be explained by these *in vitro* data suggesting down-regulating activity of the drugs on fibroblast functions related to inflammation and tissue remodelling.

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References

- 1 Howarth PH. Disease pathogenic mechanisms in allergy: a rational basis for treatment. *BMJ* 1998; 316:757–61.
- 2 Howarth PH, Salagean M, Dokic D. Allergic rhinitis: not purely a histamine-related disease. *Allergy* 2000; 55:7–16.
- 3 Bentley AM, Jacobson MR, Cumberworth V et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increase in activated eosinophils and epithelial mast cells. *J Allergy Clin Immunol* 1992; 89:877–83.
- 4 Okuda M, Otsuka H, Kawabori S. Studies on nasal surface basophilic cells. *Ann Allergy* 1995; 55:69.
- 5 Wilson S, Lau L, Howarth PH. Inflammatory mediators in naturally occurring rhinitis. *Clin Exp Allergy* 1998; 28:220–7.
- 6 Lee KS, Jin SM, Kim HJ, Lee YC. Matrix metalloproteinase inhibitor regulates inflammatory cell migration by reducing ICAM-1 and VCAM-1 expression in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 2003; 111:1278–84.
- 7 Okada Y, Gonoji Y, Naka K et al. Matrix metalloproteinase 9 (92-kDa gelatinase/type IV gelatinase) from HT 1080 human fibrosarcoma cells. *J Biol Chem* 1992; 267:21712–9.
- 8 Lechapt-Zalcman E, Coste A, d'Ortho MP et al. Increased expression of matrix metalloproteinase-9 in nasal polyps. *J Pathol* 2001; 193:233–41.
- 9 Calderon MA, Lozewicz S, Prior A et al. Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. *J. Allergy Clin Immunol* 1994; 93:635.
- 10 Howarth PH. The cellular basis of allergic rhinitis. *Allergy* 1995; 50 (Suppl. 23):6.
- 11 Hoshino M, Nakamura Y, Sim JJ, Shimoji J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998; 102:783–8.
- 12 Herouy Y, Mellos P, Bandemir E et al. Inflammation in stasis dermatitis upregulates MMP-1, MMP-2 and MMP-13 expression. *J Dermatol Sci* 2001; 25:198–205.
- 13 Shaida A, Kenyon G, Devalia J et al. Matrix metalloproteinases and their inhibitors in the nasal mucosa of patients with perennial allergic rhinitis. *J Allergy Clin Immunol* 2001; 108:791–6.
- 14 Hoshino M, Takahashi M, Takai Y et al. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *J Allergy Clin Immunol* 1999; 104:356–63.
- 15 Liu E, Thant AA, Kikkawa F et al. The ras-mitogen-activated protein kinase pathways is critical for the activation of matrix metalloproteinase secretion and the invasiveness in v-*crk*-transformed 3Y1. *Cancer Res* 2000; 60:2361–4.
- 16 Valencia AM, Beharry KD, Ang JG et al. Early postnatal dexamethasone influences matrix metalloproteinase-2 and -9, and their tissue inhibitors in the developing rat lung. *Pediatr Pulmonol* 2003; 35:456–62.

- 17 Abdelaziz MM, Devalia JL, Khair OA et al. Effect of fexofenadine on eosinophil-induced changes in epithelial permeability and cytokine release from nasal epithelial cells of patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 1998; 101:410–20.
- 18 Pipkorn U. Mediators and nasal allergy. *Clin Exp Allergy* 1989; 26:585–9.
- 19 Trigg CJ, Davies R. Local antihistamine. *Clin Exp Allergy* 1996; 26:1108–11.
- 20 Abdelaziz MM, Khair OA, Devalia JL. The potential of active metabolites of antihistamines in the management of allergic disease. *Allergy* 2000; 55:425–34.
- 21 Howarth PH. A comparison of anti-inflammatory properties of intranasal corticosteroids and antihistamines in allergic rhinitis. *Allergy* 2000; 62:6–11.
- 22 Kanai K, Asano K, Hisamitsu T, Suzaki H. Suppression of matrix metalloproteinase production from nasal fibroblasts by macrolide antibiotics *in vitro*. *Eur Respir J* 2004; 23:671–8.
- 23 Suzaki H, Asano K, Yu M, Hisamitsu T. Influence of roxithromycin on inflammatory cytokine production from nasal polyp fibroblasts *in vitro*. *Acta Otolaryngol* 2003; 123:637–42.
- 24 O-Charoenrat P, Phys-Evans P, Modjtahedi H et al. Overexpression of epidermal growth factor receptor in human head and neck squamous carcinoma cell lines correlates with matrix metalloproteinase-9 expression and *in vitro* invasion. *Int J Cancer* 2000; 86:307–17.
- 25 Yuan GH, Hongo K, Sakata M et al. The role of C–C chemokines and their receptors in osteoarthritis. *Arthritis Rheum* 2001; 44:1056–70.
- 26 Moore BA, Aznavoorian S, Engler JA, Windsor LJ. Induction of collagenase-3 (MMP-13) in rheumatoid arthritis synovial fibroblasts. *Biochim Biophys Acta* 2000; 1502:307–18.
- 27 Simons FER. H₁-antihistamines: more relevant than ever in the treatment of allergic disorders. *J Allergy Clin Immunol* 2003; 112 (Suppl. 4):S43–52.
- 28 Baroody FM, Lim MC, Proud D et al. Effects of loratadine and terfenadine on the induced nasal allergic reaction. *Arch Otolaryngol Head Neck Surg* 1996; 122:309–16.
- 29 Fasce L, Ciprandi G, Pronzato C et al. Cetirizine reduces ICAM-1 on epithelial cells during nasal minimal persistent inflammation in asymptomatic children with mite-allergic asthma. *Int Arch Allergy Immunol* 1996; 109:272–6.
- 30 Murphy G, Docherty AJ. The matrix metalloproteinases and their inhibitors. *Am J Respir Cell Mol Biol* 1992; 7:120–5.
- 31 Ohno I, Ohtani H, Nitta Y et al. Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1997; 16:212–9.
- 32 Nagase H. Activation mechanisms of matrix metalloproteinase. *Biol Chem* 1997; 378:151–60.
- 33 Lee YC, Song CH, Lee BH et al. A murine model of toluene diisocyanate-induced asthma can be treated with matrix metalloproteinase inhibitor. *J Allergy Clin Immunol* 2001; 108:1021–6.
- 34 Dibattista JA, Martel-Pelletier J, Fujimoto N et al. Prostaglandin E2 and E1 inhibit cytokine-induced metalloprotease expression in human synovial fibroblasts. Mediation by cyclic-AMP signaling pathway. *Lab Invest* 1994; 71:270–8.
- 35 Pillinger MH, Rosenthal PB, Tolani SN et al. Cyclooxygenase-2-derived E prostaglandins down-regulate matrix metalloproteinase-1 expression in fibroblast-like synoviocytes via inhibition of extracellular signal-regulated kinase activation. *J Immunol* 2003; 171:6080–9.
- 36 Domeij H, Yucel-Lindberg T, Modeer T. Signal pathways involved in the production of MMP-1 and MMP-3 in human gingival fibroblasts. *Eur J Oral Sci* 2002; 110:302–6.
- 37 Huang FM, Yang SF, Hsieh YS et al. Examination of the signal transduction pathways involved in matrix metalloproteinases-2 in human pulp cells. *Oral Surg Oral Med Oral Pathol* 2004; 97:398–403.
- 38 Attiga FA, Fernandez PM, Weeraratna AT et al. Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases. *Cancer Res* 2000; 60:4629–37.
- 39 Niisato N, Ogata Y, Furuyama S, Sugiyama H. Histamine H1 receptor-induced Ca²⁺ mobilization and prostaglandin E2 release in human gingival fibroblasts – Possible role of receptor-operated Ca²⁺ influx. *Biochem Pharmacol* 1996; 52:1015–23.
- 40 Barchowsky A, Frleta D, Vincenti MP. Integration of the NF-kappaB and mitogen-activated protein kinase/AP-1 pathway at the collagenase-1 promoter: divergence of IL-1 and TNF-dependent signal transduction in rabbit primary synovial fibroblasts. *Cytokine* 2000; 12:1469–79.
- 41 Antony P, Petro JP, Carlesso G et al. B-cell antigen receptor activates transcription factors NFAT (nuclear factor of activated T-cells) and NF-kappaB (nuclear factor kappaB) via a mechanism that involves diacylglycerol. *Biochem Soc Trans* 2004; 32:113–5.
- 42 Edward DR, Rocheleau H, Sharma RR et al. Involvement of AP1 and PEA3 binding sites in the regulation of murine tissue inhibitor of metalloproteinase-1 (TIMP-1) transcription. *Biochim Biophys Acta* 1992; 1171:41–55.
- 43 Hammani K, Blakis A, Morsette D et al. Structure and characterization of the human tissue inhibitor of metalloproteinases-2 gene. *J Biol Chem* 1996; 271:25498–505.
- 44 De Clerck YA, Darville MI, Eeckhout Y, Rousseau GG. Characterization of the promoter of the gene encoding human tissue inhibitor of metalloproteinases-2 (TIMP-2). *Gene* 1994; 139:185–91.
- 45 Sato T, Koike L, Miyata Y et al. Inhibition of activator protein-1 binding activity and phosphatidylinositol 3-kinase pathway by nobiletin, a polymethoxy flavonoid, results in augmentation of tissue inhibitor of metalloproteinases-2 production and suppression of production of matrix metalloproteinases-2 and -9 in human fibrosarcoma HT-1080 cells. *Cancer Res* 2002; 62:1025–9.