Original Article

Effect of a thromboxane A$_2$ receptor antagonist, ramatroban (BAY u 3405), on inflammatory cells, chemical mediators and non-specific nasal hyperreactivity after allergen challenge in patients with perennial allergic rhinitis

Nobuhisa Terada, Takayuki Yamakoshi, Masaya Hasegawa, Hirokazu Tanikawa, Hiroshi Nagata, Ken-ichi Maesako and Akiyoshi Konno

Department of Otorhinolaryngology, Chiba University School of Medicine, Chiba, Japan

ABSTRACT

In some clinical studies performed in patients with perennial allergic rhinitis, ramatroban, a new thromboxane A$_2$ receptor antagonist, significantly improved nasal symptoms. As yet the mechanism of action of this drug has not been fully elucidated. In the present study we investigated the effects of ramatroban on changes in nasal reactivity and levels of inflammatory cells and mediators in nasal lavage fluid after allergen challenge. Ramatroban was administered orally at a daily dose of 150 mg (b.i.d.) for 4 weeks to 11 patients with perennial allergic rhinitis exhibiting positive responses to nasal allergen challenge with house dust mite. Analysis of variance revealed that there was a significant decrease in eosinophil counts and eosinophil cationic protein levels in nasal lavage fluid when compared with values immediately before allergen challenge before and after ramatroban treatment. Histamine, tryptase and albumin levels were significantly decreased in analysis of variance before and after ramatroban treatment. The degree of nasal reactivity to histamine was also significantly decreased after the ramatroban treatment. These findings indicate that ramatroban decreases important pathogenic factors in allergic rhinitis, resulting in an improvement in nasal symptoms.

Key words: eosinophil, nasal allergen challenge, nasal mucosal reactivity, perennial allergic rhinitis, ramatroban, thromboxane A$_2$ receptor antagonist.

INTRODUCTION

Recent research has indicated that various mediators may participate in the pathogenesis of allergic rhinitis in addition to histamine, which is known to play an important role. These include leukotrienes (LT) and other inflammatory mediators, such as prostaglandins (PG), thromboxane A$_2$ (TXA$_2$) and platelet-activating factor (PAF). Prostaglandin D$_2$ (PGD$_2$) causes swelling of the nasal mucosa.[1] Thromboxane A$_2$ causes constriction of lower airway smooth muscle and increases vascular permeability and airway hyperreactivity.[2-4] In the nasal mucosa, where smooth muscle is localized in blood vessels, TXA$_2$ probably plays an important role in the aggravation of nasal allergic symptoms by increasing vascular permeability and airway hyperreactivity.

Ramatroban (BAY u 3405)[5] is a TXA$_2$ receptor antagonist synthesized by Bayer AG (Wuppertal, Germany). Ramatroban has been reported to inhibit allergen-induced increases in vascular permeability in the nasal mucosa, an elevation of nasal airway resistance and eosinophil infiltration in the nasal mucosa in the

experimental rhinitis model of sensitized guinea pigs.[6] In some clinical studies performed in patients with perennial allergic rhinitis, ramatroban significantly improved nasal symptoms, including nasal obstruction.[7] As yet the mechanism of action of this drug has not been fully elucidated.

In the present study we investigated the effects of ramatroban on changes in nasal reactivity and levels of inflammatory cells and mediators in nasal lavage fluid after allergen challenge in patients with perennial allergic rhinitis and discussed how TXA_2 is involved in the pathogenesis of allergic rhinitis.

METHODS

Subjects

The present study was performed in patients with perennial allergic rhinitis who exhibited positive responses to nasal allergen challenge with house dust mite and positive responses to an intracutaneous reactivity test (or RAST/MAST/CAP) or positive detection of eosinophils in nasal secretions. Eleven patients (10 male and one female), ranging in age from 18 to 36 years (mean ($\pm$ SD) 27.2 $\pm$ 6.4 years) participated in the study. During a 1 week run-in period, nasal symptoms were evaluated, using Okuda's criteria,[8] as severe for two patients, moderate for eight patients and mild for one patient. Two patients who skipped the scheduled visit for nasal allergen challenge testing 4 weeks after treatment were excluded from analysis for nasal allergen challenge. One patient whose nasal symptoms were evaluated as mild in the run-in period was excluded from analysis for clinical efficacy of nasal symptoms.

The present study was performed from October 1995 to December 1995 with the approval of the Institutional Review Board of Chiba University School of Medicine Affiliated Hospital and in compliance with Japan's Good Clinical Practice (GCP).

Prior to the study, written informed consent to participate in the study was obtained individually from patients after provision of detailed information about the test drug, contents of the study and subjects' human rights.
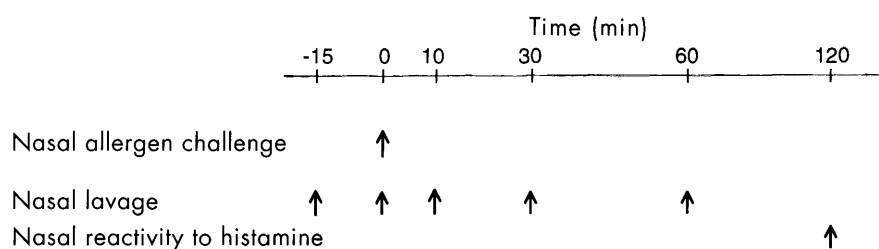
Study schedule

After a 1 week run-in period, a ramatroban 75 mg tablet was administered twice daily, after breakfast and before bedtime, for 4 weeks. Patients were subjected to nasal allergen challenge on the first day (before the first dose) and the last day (day 29; after the last dose) of treatment. Nasal lavage was performed 15 min and immediately before allergen challenge and then 10 and 30 min and 1 h after allergen challenge. In addition, nasal reactivity to histamine was examined 2 h after allergen challenge (Fig. 1).

Nasal allergen challenge

After a patient blew his/her nose at rest, two house-dust allergen discs (Allergen Disc®; Torii Pharmaceutical Co. Ltd, Tokyo, Japan) were placed on the anterior end of the right and left inferior conchal mucosa for 10 min to induce nasal mucosal reactivity.

Nasal lavage

With a patient bent forward at the head in a sitting position, a nozzle, connected to a syringe by a tube, was inserted into a nostril to close the opening tightly and the nasal cavity was washed with 20 mL physiological saline warmed at 37°C. The syringe was then inserted into the other nostril and the same process was repeated, alternating between the left and right nostrils, 10 times on

Fig. 1 The study schedule. Nasal allergen (house dust) challenge and nasal lavage were performed at 15 and 0 min before and then 10, 30 and 60 min after allergen challenge, and nasal reactivity to histamine was examined at 2 h after allergen challenge before and after 4 weeks treatment with ramatroban (150 mg/day) The last tablet of ramatroban was administered 2 h before nasal allergen challenge.

each side. Lavage fluid recovered was measured, mixed thoroughly, filtered through a nylon filter net (Nippon Rikagaku Kikai Co., Tokyo, Japan) to remove mucin and then centrifuged for 20 min at 4°C at 400 g. The supernatant was stored frozen at −80°C until assayed for chemical mediator levels. The sediment was subjected to inflammatory cell count measurement.

Levels of mediators in nasal lavage fluid were measured by a third party, blinded to the purpose and details of the study (Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, Japan). Eosinophil cationic protein (ECP), histamine, tryptase and albumin were assayed using a Pharmacia ECP radioimmunoassay (RIA) kit (Pharmacia, Uppsala, Sweden), a Histamine CT EIKEN kit (Eiken Chemical, Tokyo, Japan), a Pharmacia Tryptase RIA CT® (Pharmacia) and a Superior Microalbumin kit (Japan DPC, Tokyo, Japan), respectively.

Cell counts were performed separately by two scientists. Eosinophils were counted by a hemato-cytometer after staining cells with Hinkelman's stain. Mean values of the counts obtained by two scientists were used for data analysis.

Nasal reactivity to histamine

Physiological saline solution and 0.001, 0.01, 0.1, 0.25, 0.5 and 1% concentrations of histamine hydrochloride were prepared. Using a micropipette, physiological saline and the histamine solutions were infused, at a volume of 20 μL each, near the anterior end of the median plane of the right and left inferior conchae with increasing concentrations of histamine hydrochloride. Nasal provocation by histamine titration was continued for all patients up to 0.25% histamine. Furthermore, nasal provocation was continued in patients in whom sneezing had not been evoked once or more and/or in whom the amount of nasal secretion produced had not exceeded

0.5 g or more, until these symptoms were attained. The number of sneezes and the weight of nasal secretions were measured during 10 min following application of each dilution. Nasal secretion was collected as follows: the secretion was wiped off with tissue paper of a known weight during 10 min. Then, a patient was asked to blow his or her nose with the tissue paper after 10 min and the weight of the nasal secretion collected in the tissue was measured.

Nasal reactivities to histamine before and after ramatroban treatment were evaluated using the following three parameters: (i) the threshold histamine concentration to induce one or more sneezes and to produce 0.5 g or more nasal secretion; (ii) the sum of the number of sneezes and the amount of nasal secretion induced by 0.001–0.25% histamine solution; and (iii) the degree of nasal reactivity, determined as a combination of the number of sneezes and the amount of nasal secretion induced by 0.001–0.25% histamine dilution, using the six grades shown in Table 1.

Clinical symptoms

Patients were given allergy diaries to record nasal symptoms during the study period. Scores for nasal symptoms were evaluated using Okuda's evaluation criteria,[8] with a four grade scale of 3+, 2+, + and −, at each scheduled visit. General clinical laboratory findings (hematology, biochemical profile and urinalysis) were examined before and after ramatroban treatment, together with results of investigation of subjective and objective adverse reactions.

Statistical analyses

The following analysis was made for cell counts and mediator levels by converting measured values to logarithms, as a study of the variance of measured values

Table 1. Criteria for the degree of nasal reactivity, obtained by combining the number of sneezes and the amount of nasal secretion induced by 0.001, 0.01, 0.1 and 0.25% histamine solution

| | Degree of nasal reactivity | | | | | |
	−	+	2+	3+	4+	5+
No. sneezes*	0	1–2	3–6	7–10	11–15	≤ 16
Amount of nasal secretion (g)†	≤ 0.7	0.8–1.0	1.1–3.0	3.1–6.0	6.1–10.0	≤ 10.1

When the degree determined for sneezing differed from that determined for nasal secretion, the higher degree was chosen and reduced by one grade.
*Sum of the number of sneezes induced by 0.001, 0.01, 0.1 and 0.25% histamine solution; †sum of the amount of nasal secretion induced by 0.001, 0.01, 0.1 and 0.25% histamine solution.

implied that any of them were close to log-normal distribution rather than normal distribution. Changes after allergen challenge against values immediately before allergen challenge were determined by the Wilcoxon signed rank test before ramatroban treatment and at 4 weeks after ramatroban treatment. Different logarithmic values and logarithmic change rates [ln (value after challenge/value immediately before challenge)] before and after ramatroban treatment were compared using an analysis of variance for repeated measures (ANOVA). When significant differences were noted, comparisons were made at every time point with the Wilcoxon signed rank test.

The Wilcoxon signed rank test was also used for comparison of nasal mucosal reactivity and nasal symptoms between before and after ramatroban treatment.

$P < 0.05$ was considered statistically significant. Measured values are expressed as the mean $\pm$ SEM and cell counts and mediator levels are expressed as the geometric mean ($\pm$ geometric standard error). Statistical analysis was made with the statistical software package SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

Changes in eosinophil counts in nasal lavage fluid

Before ramatroban treatment, eosinophil counts in nasal lavage fluid were 143 $\pm$ 1.55 and 116 $\pm$ 1.40 /mL 15 min before and immediately before nasal allergen challenge, respectively; the counts were significantly increased 30 and 60 min after challenge, being 189 $\pm$ 1.46, 536 $\pm$ 1.57 ($P = 0.0039$) and 803 $\pm$ 1.51 /mL ($P = 0.0039$) at 10, 30 and 60 min after challenge, respectively. After 4 weeks ramatroban treatment, the eosinophil counts in nasal lavage fluid were 236 $\pm$ 1.40 and 204 $\pm$ 1.29 /mL 15 min before and immediately before nasal allergen challenge, respectively. After challenge, a significant increase was noted only at 60 min after challenge, with counts of 323 $\pm$ 1.45, 234 $\pm$ 1.44 and 472 $\pm$ 1.24 /mL ($P = 0.0195$) at 10, 30 and 60 min after challenge, respectively.

Analysis of variance showed significant differences in logarithmic change rates of eosinophil cell counts between before and after ramatroban treatment ($P = 0.0382$; ANOVA). Logarithmic change rates against values immediately before allergen challenge exhibited a significant decrease ($P = 0.0391$) at 30 min after aller-

gen challenge after ramatroban administration in comparison with before administration (Fig. 2).
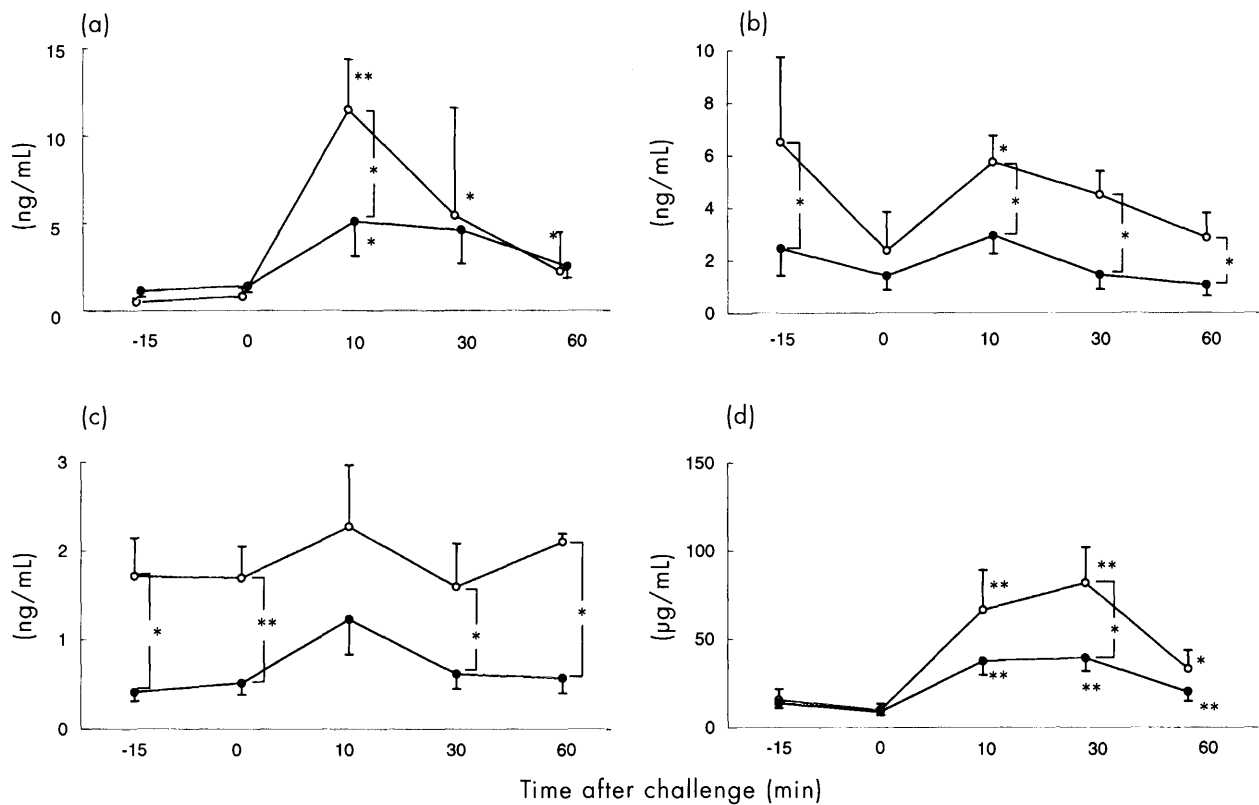
Mediator levels in nasal lavage fluid

Eosinophil cationic protein

Before treatment, ECP levels in nasal lavage fluid were significantly increased at 10 ($P = 0.0039$), 30 ($P = 0.0313$) and 60 min ($P = 0.0313$) after challenge. However, after ramatroban treatment, a significant increase in ECP levels was noted only 10 min after challenge ($P = 0.0391$).

There were significant differences noted in logarithmic change rates between before and after ramatroban treatment by ANOVA ($P = 0.0118$). The logarithmic change rates tended to decrease before and after ramatroban treatment at 10 min after allergen challenge ($P = 0.0742$). Comparison by logarithm before and after administration exhibited significant differences at 10 min after allergen challenge ($P = 0.0195$; Fig. 3a).
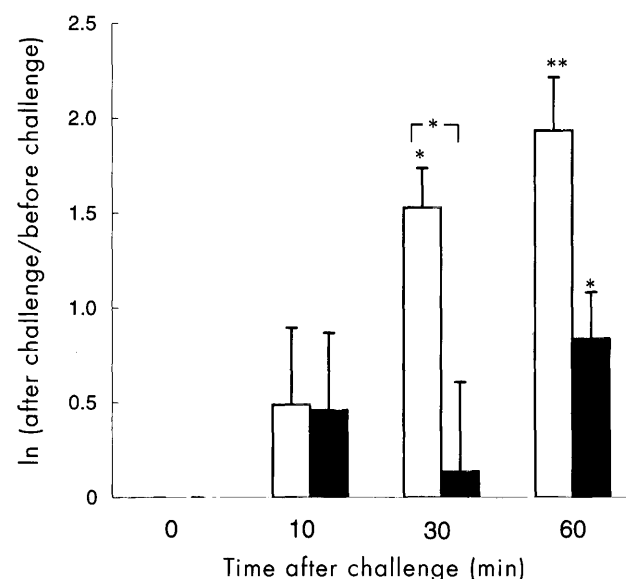
Fig. 2 Effect of ramatroban on the infiltration of eosinophils in nasal lavage fluid after nasal allergen challenge. Eosinophil counts were measured before (□) and after (■) 4 weeks ramatroban treatment. Values are the mean $\pm$ SEM of logarithmic change rate from values before challenge [ln (after challenge/before challenge)] in nine patients. *$P < 0.05$, **$P < 0.01$ compared with before challenge (0 min). ┌*┐ $P < 0.05$ compared with corresponding values before and after ramatroban treatment.

Fig. 3 Effect of ramatroban on chemical mediators in nasal lavage fluid after nasal allergen challenge. (a) Eosinophil cationic protein (b) histamine, (c) tryptase and (d) albumin were measured before (—O—) and after (—●—) 4 weeks ramatroban treatment. Values are the geometric mean ± geometric SEM in nine patients. *$P < 0.05$, **$P < 0.01$ compared with values for 0 min before challenge. ⫞ $P < 0.05$, ⫞* $P < 0.01$ compared with corresponding values before and after ramatroban treatment.

Histamine

Before ramatroban treatment, histamine levels in nasal lavage fluid were quite high, even 15 min before nasal allergen challenge. Although they decreased immediately before challenge due to the first nasal washing performed 15 min previously, they were significantly increased 10 min after challenge ($P = 0.0391$; Fig. 3b). Four weeks after ramatroban treatment, no significant increases were noted in histamine levels at any time point after allergen challenge.

Comparison of logarithms before and after ramatroban treatment exhibited significant differences by ANOVA ($P = 0.0026$). Histamine levels were significantly lower after ramatroban treatment at 15 min before challenge ($P = 0.0273$) and 10 ($P = 0.0117$), 30 ($P = 0.0117$) and 60 min ($P = 0.0273$) after challenge.

Tryptase

After allergen challenge, tryptase levels in nasal lavage fluid were somewhat, but not significantly, increased at all monitoring points both before and after ramatroban treatment.

Comparison of logarithms before and after ramatroban treatment exhibited significant differences by ANOVA ($P = 0.0003$). Tryptase levels were significantly lower after ramatroban treatment at 15 min before challenge, immediately before challenge and 30 and 60 min after challenge ($P = 0.0195, 0.0039, 0.0391$ and 0.0234, respectively; Fig. 3c).

Albumin

After allergen challenge, albumin levels in nasal lavage fluid were significantly increased ($P < 0.05$) at

(a)

(b)

Time after treatment (weeks)
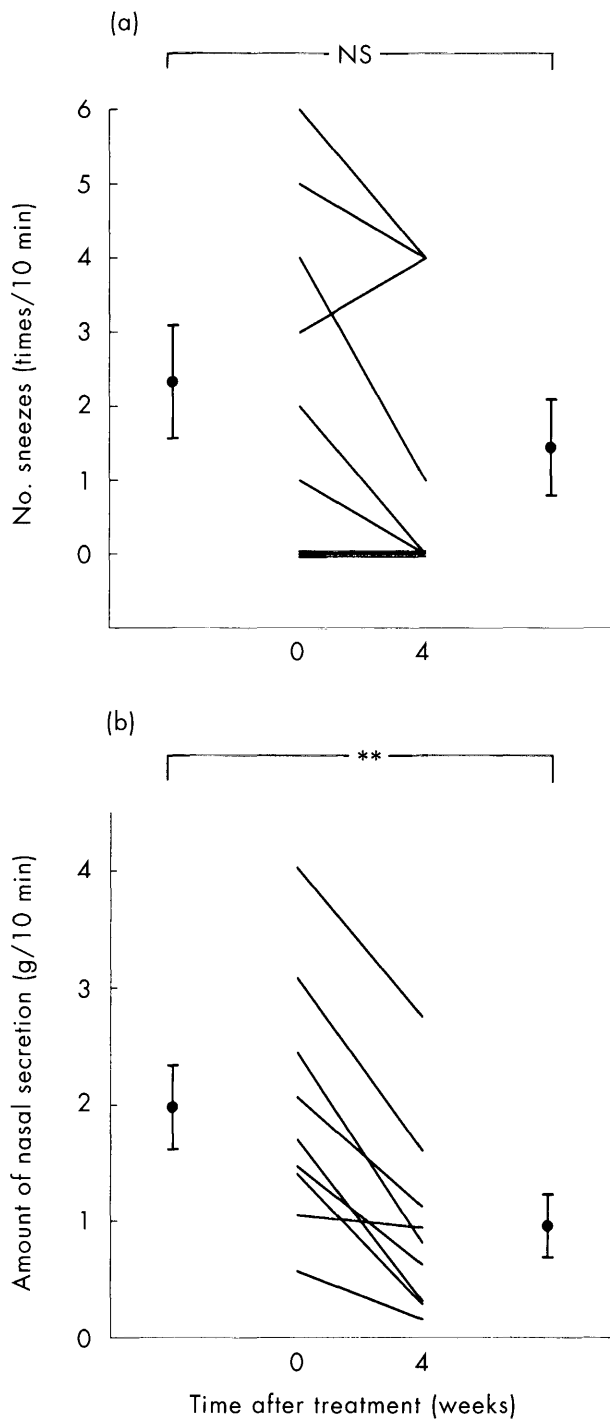
Fig. 4 Effect of ramatroban on nasal hyperractivity to histamine. The number of sneezes (a) and the amount of nasal secretions (b) were measured for 10 min after 0.25% histamine solution challenge before and after 4 weeks ramatroban treatment in nine patients. Values are individual values and are also given as the mean ± SE (●). **$P < 0.01$ between before and after ramatroban treatment. NS, not significant ($P \geq 0.1$).

all time points tested, both before and after ramatroban treatment.

There was a tendency towards a decrease in logarithmic change rates before and after ramatroban treatment by ANOVA ($P = 0.0688$). The logarithmic change rates were significantly lower after ramatroban treatment at 30 min ($P = 0.0391$) after allergen challenge. Significant differences were noted in logarithms before and after ramatroban treatment by ANOVA ($P = 0.0333$), particularaly at 30 min after allergen challenge ($P = 0.0273$; Fig. 3d).
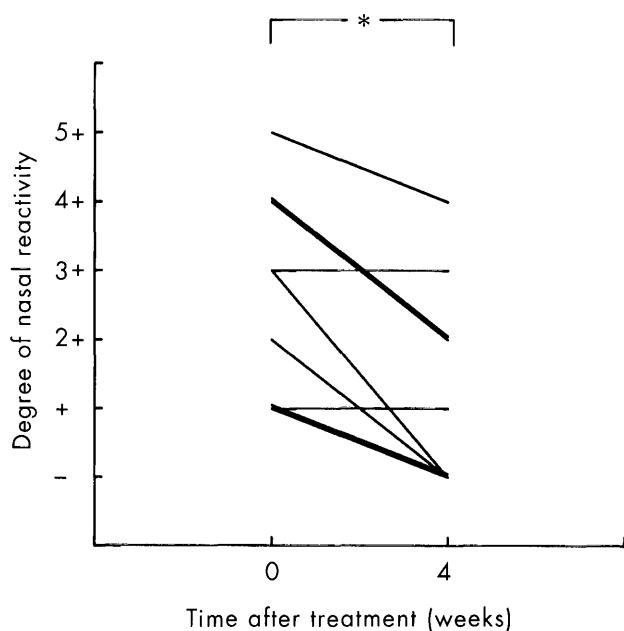
Nasal reactivity to histamine

Threshold concentrations of histamine [median (25–75% intervals)] were shifted from 0.01% (0.01–0.25%) before treatment to 0.1% (0.01–1%) after treatment in terms of sneezing, and from 0.1% (0.01–0.1%) to 0.1% (0.1–0.5%) in terms of nasal secretion, which tended to increase, although not significantly, after ramatroban treatment ($P = 0.0625$ for sneezing; $P = 0.0938$ for nasal secretion). The mean number of sneezes induced by local application of 0.25% histamine was decreased from 2.33 ± 0.76 times before treatment to 1.44 ± 0.65 times after ramatroban treatment; however, this decrease was not significant. In contrast, the mean amount of nasal secretion was significantly decreased ($P = 0.0039$) after ramatroban treatment, with values of 1.98 ± 0.36 and 0.96 ± 0.27 g before and after ramatroban treatment, respectively (Fig. 4). The degree of nasal mucosal reactivity to histamine was significantly decreased ($P = 0.0156$) after ramatroban treatment (Fig. 5).

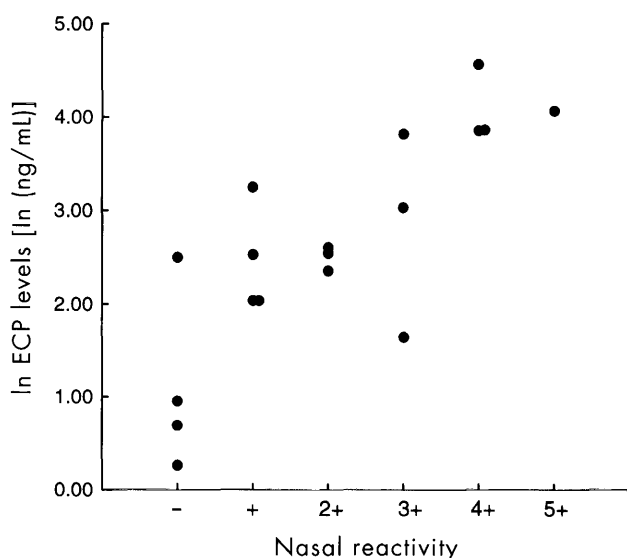## Correlation between ECP levels and nasal reactivity to histamine

Peak logarithmic ECP levels and degrees of nasal reactivity to histamine determined before and after ramatroban treatment were plotted to investigate their correlation. As shown in Fig. 6, a high correlation ($r = 0.8085$; $P = 0.0001$; Spearman's rank order correlation) was observed between ECP levels and the degree of nasal reactivity.

Clinical symptoms

Clinical efficacy was determined for 10 of 11 patients participating in the study, excluding one patient whose nasal symptoms were evaluated as mild in the run-in period. Scores for nasal obstruction were significantly

Fig. 5 Changes in the degree of nasal reactivity to histamine before and after 4 week ramatroban treatment in nine patients. The degree of nasal reactivity was judged on the basis of both the sum of the numbers of sneezes and the sum of the amount of nasal secretions induced by 0.001, 0.01, 0.1 and 0.25% histamine solution using the criteria shown in Table 1. *$P < 0.05$ between before and after ramatroban treatment.

Fig. 6 Correlation between peak eosinophil cationic protein (ECP) levels and the degree of nasal reactivity to histamine. $r = 0.8085$; $P = 0.0001$.

decreased from (mean ± SD) 1.8 ± 0.4 points before treatment to 0.9 ± 0.6 points after the 4 week ramatroban treatment ($P = 0.0078$).

Adverse reactions

No subjective/objective adverse reactions or abnormal changes in laboratory test results were observed.

DISCUSSION

Thromboxane A_2 and its receptors take part in the pathogenesis of allergic disorders via complex mechanisms involving other inflammatory mediators. For example, leukotriene (LT)C_4 and LTD_4 induce bronchoconstriction via cyclo-oxgenase products, including TXA_2[9,11] and platelet-activating factor (PAF)-induced airway reactivity is inhibited by OKY 046, a TXA_2 synthetase inhibitor.[12] Prostaglandin (PG)D_2 and $PGF_{2\alpha}$ induce airway constriction by binding to TXA_2 receptors.[13-16] In addition, ramatroban, a TXA_2 receptor antagonist, has been shown to inhibit bronchoconstriction induced by the TXA_2 analog U46619, PGD_2 and $PGF_{2\alpha}$ and even that by the allergens LTC_4 and LTD_4.[17] These findings indicate that blockade of TXA_2 receptor function by ramatroban can reduce levels of various mediators, such as LTC_4, LTD_4, PAF and PGD_2, which cause swelling of the nasal mucosa. In fact, ramatroban has been shown to improve various pathophysiological conditions in an experimental model of allergic rhinitis[6] and clinical findings in patients with perennial allergic rhinitis have confirmed the efficacy of ramatroban.[7]

In the present study, inflammatory mediator levels and eosinophil counts in nasal lavage fluid after allergen challenge were compared before and after treatment with ramatroban in patients with perennial allergic rhinitis. The effect of a TXA_2 receptor antagonist on nasal reactivity to histamine was also examined.

As shown in the present study, 4 week ramatroban treatment significantly inhibited the increase in eosinophil counts in nasal lavage fluid 30 min after allergen challenge. It also reduced ECP levels after challenge. However, it has not been reported whether or not TXA_2 itself possesses an effect on eosinophil chemotaxis. It is unclear how ramatroban reduces allergen-induced eosinophil infiltration and ECP levels in the nasal mucosa. Its possible mechanisms of action include the following:

1. It has been observed that ramatroban inhibits the release of RANTES (regulated on activation normal T

expressed and secreted chemokine) induced by U46619 from human blood (S Sawada, pers. comm., 1996). It is known that RANTES promotes adhesion of eosinophils to vascular endothelial cells, increases the passage of eosinophils through vascular endothelial intercellular spaces and also has an eosinophil chemotactic effect.[18] Inhibition of eosinophil chemotaxis to nasal mucosa observed in the present study was possibly a result of inhibition of RANTES by ramatroban.

2. Eosinophils are considered to undergo chemotaxis from microvascular vessels to the nasal mucous membrane proper, while increased vascular permeability appears to promote eosinophil chemotaxis in the presence of eosinophil chemotactic factors. Ramatroban inhibits increases in vascular permeability, as confirmed in the present study by the finding that it significantly inhibited allergen-induced increases in albumin levels in nasal lavage fluid, resulting in inhibition of eosinophil infiltration.

The degree of nasal reactivity to histamine was significantly decreased after 4 weeks ramatroban treatment, with a tendency towards a decrease in the number of histamine-induced sneezes and a significant decrease in the amount of histamine-induced nasal secretion. These findings confirmed that ramatroban attenuates non-specific nasal hyperreactivity. The mechanism responsible for the reduction in nasal hyperreactivity includes the following possibilities:

1. We have previously reported a good correlation between ECP levels and nasal reactivity.[19] Inhibition of nasal reactivity by ramatroban may result from decreased ECP levels, because a high correlation was found between the degree of nasal reactivity and ECP levels.

2. It has been suggested that TXA_2 may act directly on histamine receptors in mucosal epithelial cells and vascular endothelial cells in the nasal cavity. Takehana *et al.* reported that inhalation of a TXA_2 analog (STA_2) resulted in increased reactivity to histamine in guinea pigs, with increases in the number of histamine receptors and muscarinic receptors in the lung.[12] A previous study showed that the expression of mRNA of the histamine H_1 receptor was up-regulated in epithelial cells isolated from human nasal mucosa following pretreatment with the TXA_2 analog U46619 for 6 h.[20] These reports suggest that the inhibition of nasal reactivity to histamine may result from suppression of up-regulation of histamine receptors by ramatroban.

3. The following findings suggest that ramatroban may inhibit acetylcholine release from Vidian nerves and suppress nasal reactivity. Thromboxane A_2 is known to

promote airway hyperreactivity.[3,4] Thromboxane A_2 synthetase inhibitors and TXA_2 receptor antagonists, including ramatroban, have been reported to suppress bronchial hyperreactivity in asthmatics.[21-23] Chung *et al.* have attributed airway hyperreactivity induced by TXA_2 to its promotion of acetylcholine release from nerve terminals.[24]

In the present study, ramatroban treatment decreased levels of the basophil cell-derived mediators histamine and tryptase. Levels of histamine and tryptase in nasal lavage fluid were significantly lower after treatment than before treatment, even before allergen challenge. Our findings indicate that not only allergen-induced but also spontaneous secretion of these mediators was inhibited by ramatroban treatment. Proliferation of mast cells is known to be enhanced by T cell-derived cytokines, such as interleukin-3, -4, -9 and -10.[25,26] It has also been reported that ramatroban inhibits lymphocyte infiltration.[27] The present study suggests that decreases in tryptase levels in nasal lavage fluid resulted from a decrease in the numbers of activated mast cells due to a decrease in lymphocyte infiltration into inflammatory tissue. It has been recently reported that RANTES also causes chemotaxis of mast cells.[28] Decreases in histamine and tryptase levels may result from decreases in numbers of mast cells due to the inhibition of production and release of RANTES, as they also result from decreases in eosinophil counts.

In conclusion, ramatroban, a TXA_2 receptor antagonist, attenuated non-specific nasal hyperreactivity and decreased eosinophil counts, ECP, histamine, tryptase and albumin levels in nasal lavage fluid. Our findings suggest that TXA_2 plays an important role in the pathogenesis of allergic rhinitis.

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