

Original Article

Effect of ramatroban, a thromboxane A₂ antagonist, in the treatment of perennial allergic rhinitis

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ABSTRACT

Background: It is known that the symptoms of allergic rhinitis can significantly reduce the quality of life of the patient. One of such typical symptoms of allergic rhinitis is nasal obstruction. Nasal obstruction is currently thought to be closely related to the presence and abundance of lipid mediators, such as leukotriene and thromboxane (TX) A₂. The novel drug ramatroban, a TXA₂ receptor antagonist, which has been developed by Bayer Yakuhin Ltd. (Osaka, Japan), has been demonstrated, in clinical trials, to improve nasal obstruction in the treatment of patients with allergic rhinitis and it has recently become commercially available.

Methods: In the present study, ramatroban was administered for 28 days to 10 patients who were diagnosed with perennial allergic rhinitis but were untreated. Changes in self-reported symptom scores and *in vivo* allergic reaction parameters were assessed during three observational periods.

Results: From baseline scores, all three symptom scores after 28 days treatment with ramatroban declined clearly in all patients, except for one patient who suffered a cold during the study period and had aggravated rhinorrhea and nasal obstruction as a result. The concentrations of histamine and TXB₂ (a metabolite of TXA₂) in the nasal fluid induced by antigen challenge after the 28 day treatment period also decreased in most subjects compared with concentrations during the pretreatment period. The symptom scores for nasal obstruction during the pretreatment

period were correlated with the concentration of TXB₂ in antigen-induced nasal fluid.

Conclusions: The present study reconfirmed the clinical efficacy of a post-marketed drug, namely ramatroban, in the treatment of allergic rhinitis. In addition, the results suggest that ramatroban suppressed the secretion of chemical mediators in nasal that are thought to be involved in the allergic reaction in patients with perennial allergic rhinitis.

Key words: allergic, histamine, nasal obstruction, perennial, ramatroban, rhinitis, thromboxane A₂ receptor.

INTRODUCTION

The first step in an allergic reaction is the release of histamine and various lipid mediators derived from arachidonic acid via antigen-stimulated mast cells in mucous membranes. Although histamine is predominantly responsible for sneezing and nasal discharge, various substances, such as lipid mediators, are involved in nasal obstruction in a complex manner.¹ The reaction site is considered to be blood vessels in the nasal mucosa.²

One study³ that investigated the mediators involved in the development of nasal obstruction reported that when various mediators were sprinkled on the nasal mucosa, the resistance of the nasal cavity started to increase. The major mediators that were identified in the study as contributors to nasal obstruction included histamine, leukotriene, platelet-activating factor and prostaglandin D₂. In other studies,^{4,5} it was reported that thromboxane (TX) A₂ also played an important role in the development process of nasal obstruction. It is also known that lipid mediators are released not only from mast cells, but also from eosinophils and that they are involved in the late-phase inflammatory reaction.¹

Ramatroban is a selective TXA₂ receptor antagonist that was developed by Bayer AG (Leverkusen, Germany)

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and is now licensed for use in the treatment of allergic rhinitis by Bayer Yakuhin Ltd (Osaka, Japan) in Japan. Pharmacologically, ramatroban suppresses the promotion of vascular permeability and infiltration of eosinophils and its efficacy has been proven clinically for treating patients with allergic rhinitis.⁶

METHODS

The clinical study was conducted at Sekino Clinical Pharmacology Institute (CPI). Prior to the study, the study protocol was reviewed and approved by the institutional review board at Sekino CPI.

Subjects

Ten patients were enrolled into the study. These patients were diagnosed with perennial allergic rhinitis but were

untreated at diagnosis. All patients gave written informed consent to participate in the study. The diagnosis of perennial allergic rhinitis was confirmed on the basis of a positive history of typical symptoms of allergic rhinitis, +2 RAST scores and a positive allergic response to house dust mite in any of an anterior rhinoscope examination, nasal eosinophilia test, skin test or nasal provocation test.

Methodology

The study consisted of three observational periods: (i) a period of 7 days before initiating medication (pretreatment period: days 1–7); (ii) a period of 21 days with study medication (treatment period: days 8–28); and (iii) a period after completing medication (post-treatment period: days 29–32). During the treatment period, ramatroban

Table 1 Evaluation criteria for determination of the symptom score

	Score (points)				
	4	3	2	1	0
Sneezing attacks*	≥ 21	20–11	10–6	5–1	0
Nasal discharge†	≥ 21	20–11	10–6	5–1	0
Nasal obstruction	Complete nasal obstruction all day	Nasal obstruction severe and breathing through the mouth performed for a considerable time	Nasal obstruction marked and breathing thorough the mouth performed several times a day	No breathing through the mouth, but nasal obstruction present	No nasal obstruction

*Mean no. attacks per day.

†Mean no. nose blows per day.

Table 2 Symptom score

	Sneezing attack or secretion score				
	4	3	2	1	0
Nasal obstruction score					
4	4	4	4	4	4
3	4	3	3	3	3
2	4	3	2	2	2
1	4	3	2	1	1
0	4	3	2	1	0

Table 3 Evaluation criteria for the reactivity of nasal antigen challenge test

	Reactivity			
	+++	++	+	–
No. symptoms	3	3	2	0
No. sneezing episodes	≥ 6	≤ 5	Not counted	

Reactivity is evaluated based on the number of symptoms (sneezing, nasal discharge and nasal obstruction) and the number of sneezing episodes.

In determining the number of sneezing episodes, a single sneeze in 5 min was denoted as one episode in the challenge test.

(75 mg tablet, p.o.) was administered twice daily, once after breakfast and once in the evening. No concomitant drugs were allowed during the study period.

Evaluations

By using a patient diary, the severity of nasal symptoms, such as sneezing, rhinorrhea and nasal obstruction, was scored by patients each day during the study period in accordance with the Severity Classification of the Clinical Practice Guideline for Nasal Allergy and the Symptom Score (Table 1).⁷ The average scores for the three evaluation periods (days 1–7, 22–28 (the last 7 days of the treatment period) and 29–32) were used for evaluation analyses as representations of the symptom severity during the pretreatment, treatment and post-treatment periods, respectively. Changes in average scores for individual patients during the three evaluation periods were determined.

In addition to the patient diary for daily symptom scores, nasal antigen challenge tests were performed using house dust mite-extracted paper discs (dry weight 250 µg/mL). The tests were conducted with all subjects at the beginning of the pretreatment period (day 1) and at the end of the post-treatment period (day 32) to assess the effect of ramatroban on nasal reactions. Assessments were performed by comparing the two test results in terms of reactivity (Table 2), the volume of nasal fluid, nasal cavity resistance (Table 3) and the concentration levels of histamine and TXB₂ in the nasal fluid.

Five minutes after challenge, reactivity was assessed, nasal fluid was collected directly from both nostrils by suction and the resistance of the nasal cavity was measured with a rhinomanometer (Rhinorrheograph MPR-3100; Nihon Kohden, Tokyo, Japan).

The nasal fluid sampled was mixed with dithiothreitol (500 µL of 0.05% dithiothreitol in 0.01 mol/L phosphate-buffered saline) in order to resolve mucin and samples were centrifuged at 400 g for 10 min. Then, the cell-free supernatant was extracted and stored at –20°C for future analyses of histamine and TXB₂.

Histamine and TXB₂ assays^{8,9}

Histamine was quantified using radioimmunoassay by competitively binding acylated histamine and [¹²⁵I]-labeled acylated histamine to stabilized antihistamine mouse monoclonal antibody.

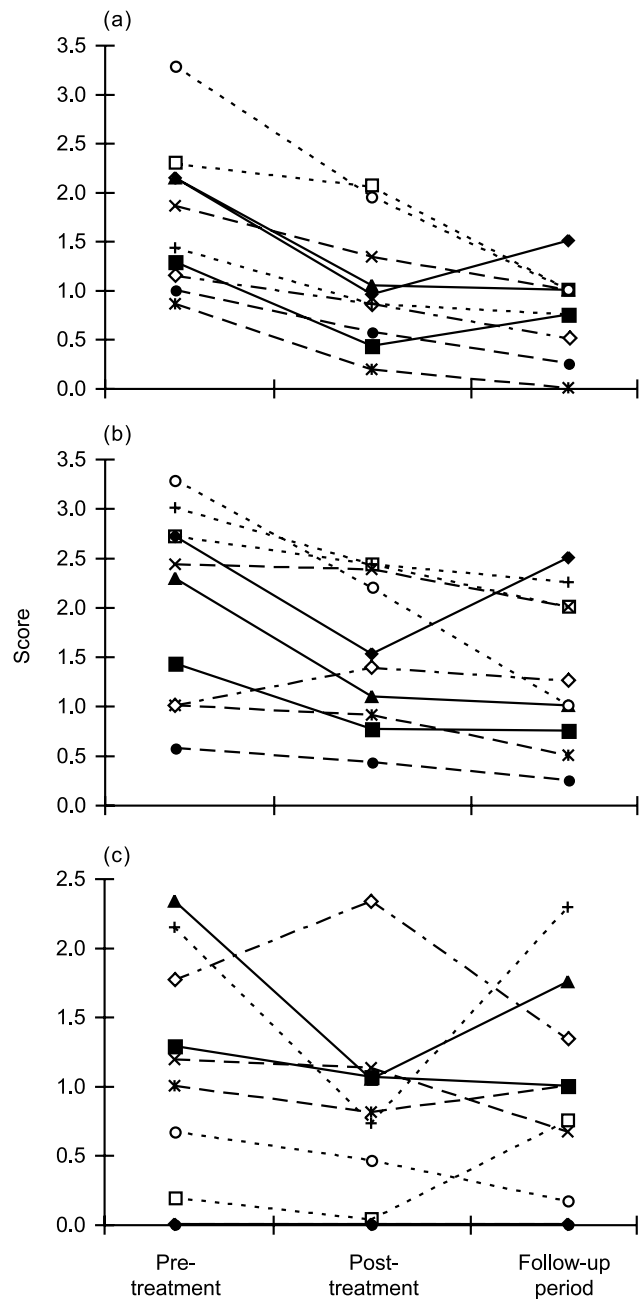


Fig. 1 Average symptom scores during the three observational periods for each individual patient for (a) sneezing, (b) rhinorrhea and (c) nasal obstruction. The three observational periods during the study consisted of: (i) a period of 7 days before initiating medication (pretreatment period: days 1–7); (ii) a period of 21 days with the study medication (treatment period: days 8–28); and (iii) a period after completing medication (post-treatment period: days 29–32).

The prostanoid in the samples was bound to an octadecylsilica silica carrier under acidic conditions and protein and lipids were removed.^{10,11} Prostanoid was

eluted from the carrier with ethyl acetate. Thromboxane B₂ antibody was added to the eluate to react with the antigen. The concentration of radioactivity in the antigen-antibody reaction product was used to determine concentration of TXB₂.

Statistical analysis

Statistical analysis was performed using the Wilcoxon signed-ranks test and all results are expressed as the mean $\pm$ SD.

RESULTS

Nasal symptoms

Changes in symptom scores during the three evaluation periods were assessed for each symptom in individual subjects (Fig. 1). Compared with the pretreatment period, the score for sneezing during the treatment period decreased in all subjects. In addition, except for one subject whose symptoms were aggravated due to a cold, in nine other subjects the same downward trend was observed for nasal obstruction and rhinorrhea scores during the treatment period.

After finishing treatment with ramatroban, in general, the symptoms that had been improved during the treatment period did not revert to levels seen during the pretreatment period, but remained as low as during the treatment period.

Nasal antigen challenge test

In eight of 10 patients, a lesser degree of reactivity to antigen challenge was recorded on day 32 compared with day 1. The volume of nasal fluid collected was also decreased or unchanged in the majority patients when results from day 32 and day 1 were compared. However, the resistance of the nasal cavity decreased in fewer patients than did reactivity and volume of nasal fluid (Table 4).

Concentration of mediators in nasal fluid

The concentrations of histamine and TXB₂ detected in the nasal fluid collected following antigen challenge tests in the post-treatment period were lower than concentrations in the nasal fluid during the pretreatment period in seven and eight patients, respectively (Fig. 2).

Table 4 Summary data for clinical efficacy and pre- and post-treatment nasal antigen challenge test

Patient no.	Age (years)	Sex	Clinical efficacy		Evaluation	Pre-treatment nasal antigen challenge test			Post-treatment nasal antigen challenge test		
			Pre-treatment symptom score	Post-treatment symptom score		Reactivity	Volume nasal fluid (mL)	Resistance of nasal cavity (p : 50)	Reactivity	Volume nasal fluid (mL)	Resistance of nasal cavity (p : 50)
1	24	Male	2.0	1.9	-	++	0.4	Off scale	+	0.3	Off scale
2	29	Male	1.4	1.0	+	++	1.3	Off scale	+	0.3	0.11
3	22	Male	3.0	1.0	++	++	0.6	6.74	-	0.1	Off scale
4	23	Male	1.9	1.3	+	+	0.4	1.10	+	0.4	0.32
5	24	Male	1.0	1.0	-	++	0.3	Off scale	+	0.9	Off scale
6	32	Male	1.0	0.1	+	++	0.3	0.19	++	0.6	0.28
7	25	Male	2.4	1.7	+	++	1.0	0.64	+	1.0	1.09
8	21	Male	2.1	1.9	-	+	0.2	0.26	-	0.1	Off scale
9	21	Male	2.9	1.6	++	++	1.3	Off scale	+	1.0	0.17
10	26	Male	1.9	3.0	-	++	0.4	0.12	+	0.2	0.12

Off scale indicates cases where no measurements were available due to excessive nasal obstruction with the resistance values $p > 100$ where p is Pascal.

The efficacy notations -, + and ++ corresponded to cases where the difference in symptom scores was <0.4 , <1.0 and >1.0 , respectively. For reactivity: +, ++, three symptoms (sneezing, nasal discharge and nasal obstruction) and six or more sneezing episodes; ++, three symptoms and five or fewer sneezing episodes; +, two symptoms and no counted sneezing episodes; -, no symptoms.

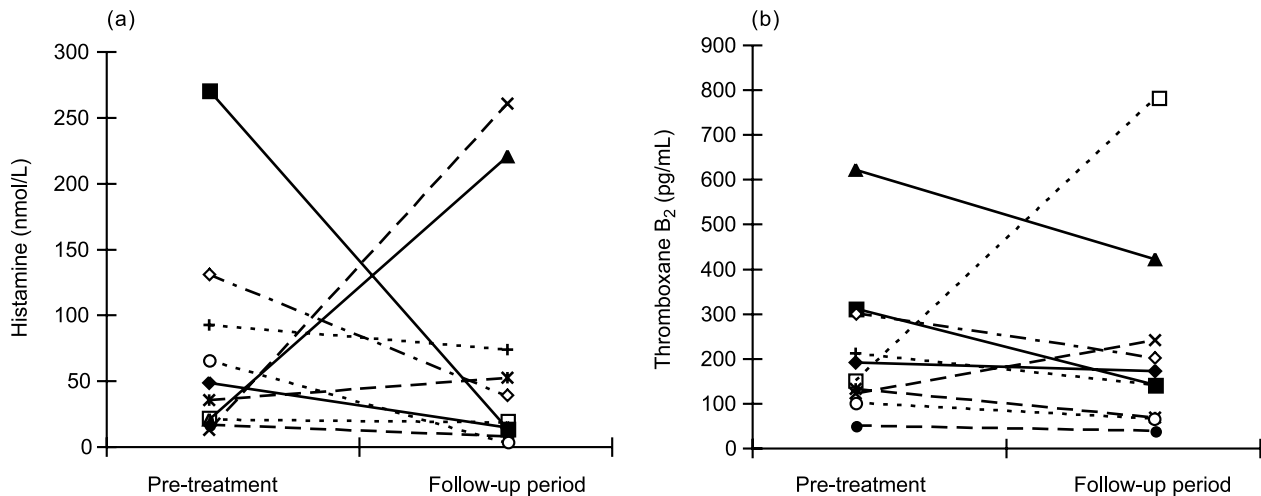


Fig. 2 (a) Histamine and (b) thromboxane B₂ concentrations in nasal discharge induced by nasal antigen challenge during the pretreatment and follow-up periods after ramatroban treatment.

The concentrations of histamine and TXB₂ in the nasal fluid sampled at two points are shown in Fig. 2. The concentrations of histamine and TXB₂ after treatment with ramatroban decreased from levels seen during the pre-treatment period in eight and seven subjects, respectively. Median values of the concentration for both histamine and TXB₂ show a downward trend, but no significant difference was detected ($P = 0.492$ and $P = 0.332$, respectively).

Correlation of three individual nasal symptoms with the concentration of histamine and TXB₂ was also investigated during the pre- and post-treatment periods, independently. Among six analyses, as shown in Fig. 3, a positive correlation was shown only for nasal obstruction and TXB₂ concentration during the pretreatment period. Other analyses did not reach statistical significance.

DISCUSSION

Ramatroban, a novel TXA₂ receptor antagonist, has been investigated in a series of clinical trials to confirm its efficacy in the treatment of patients with allergic rhinitis¹² and it is now available for daily use in Japan. The efficacy of ramatroban in improving nasal obstruction was of particular attraction to physicians, who often face difficulties in relieving patients from nasal obstruction.

Terada *et al.*,⁶ who conducted antigen challenge tests with allergic rhinitis patients treated with ramatroban and

measured changes in nasal cavity volume, reported that ramatroban significantly suppressed nasal obstruction within 2 h after administration. They also reported that the albumin concentration in the nasal fluid and a vascular permeability index were decreased following the administration of ramatroban. In another study,¹³ which examined the migration of cells involved in nasal obstruction, it was confirmed that ramatroban lessened both the eosinophil count and the concentration of eosinophil cationic protein in nasal wash water. These results strongly support the clinical efficacy of ramatroban in improving nasal obstruction.

To further investigate the effects of ramatroban, we performed a clinical investigation with two major objectives. One of the objectives was to reconfirm the efficacy of ramatroban by comparing symptom levels during the pretreatment period with those after 4 weeks treatment with ramatroban. The other objective of the study was to determine the relationships between symptoms and the secretion of chemical mediators in the nasal fluid, namely histamine and TX, which are known to provoke allergic reactions in patients with allergic rhinitis.

Of 10 untreated male patients with moderate to severe perennial allergic rhinitis who were administered ramatroban for 28 days, nine experienced an improvement in nasal obstruction. An interesting observation was that the scores for nasal obstruction came were reduced at an earlier stage of the treatment period than expected (data not shown). In parallel with the improvement in nasal obstruction, scores for sneezing and a runny nose

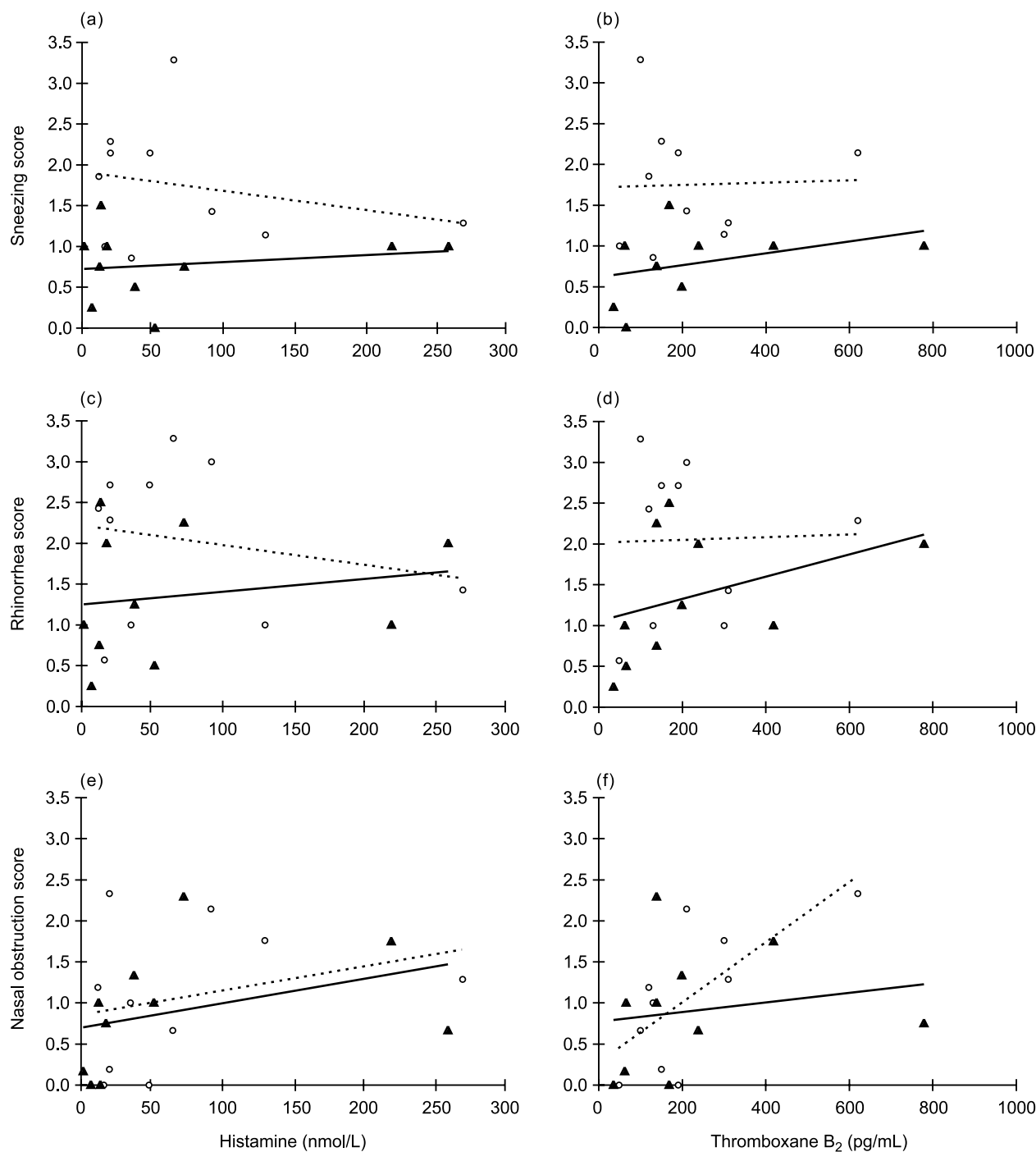


Fig. 3 Correlation of nasal symptoms, namely sneezing (a,b), rhinorrhea (c,d) and nasal obstruction (e,f), and concentrations of histamine (a,c,e) and thromboxane B₂ (b,d,f) in nasal discharge induced by nasal antigen challenge during the pretreatment period (○; ----) and during follow up (▲; —). The regression equations for the pretreatment and follow-up periods are as follows: (a) $y = -0.0023x + 1.9084$ ($R^2 = 0.0623$) and $y = 0.0008x + 0.7158$ ($R^2 = 0.0332$), respectively; (b) $y = 0.0001x + 1.7121$ ($R^2 = 0.001$) and $y = 0.0007x + 0.6105$ ($R^2 = 0.1423$), respectively; (c) $y = -0.0024x + 2.2134$ ($R^2 = 0.0399$) and $y = 0.0016x + 1.2414$ ($R^2 = 0.034$), respectively; (d) $y = 0.0002x + 2.0073$ ($R^2 = 0.0008$) and $y = 0.0014x + 1.0426$ ($R^2 = 0.1512$); (e) $y = 0.0029x + 0.8488$ ($R^2 = 0.0758$) and $y = 0.003x + 0.6888$ ($R^2 = 0.1339$), respectively; and (f) $y = 0.0037x + 0.2575$ ($R^2 = 0.5003$) and $y = 0.0006x + 0.76448$ ($R^2 = 0.0299$), respectively.

also started decreasing at around day 3 and became stable around day 10. As a result, similar score changes in terms of time to onset and improvement tendency were seen across the three symptoms. Another finding was that ramatroban was effective not only in improving nasal obstruction, which was more or less expected from its mode of action and the results of clinical trials already reported,¹² but also in relieving sneezing and rhinorrhea and that the effect of ramatroban on these two symptoms was maintained even during the post-treatment period in the majority of patients.

To investigate the relationships between symptoms and the secretion of chemical mediators in the nasal fluid, an antigen challenge test was conducted before and after the treatment period. Thromboxane is thought to be involved in the process of developing nasal obstruction in patients with perennial allergic rhinitis and, because ramatroban is a TXA₂ receptor antagonist, it was predicted that TXB₂ would have been more sensitively influenced than histamine by treatment with ramatroban. However, the concentration of histamine in antigen-induced nasal discharge decreased to the same extent as did the concentration of TXB₂, which was in contrast with the prediction prior to the study.⁵ With regard to the relationships of the concentrations of the mediators and clinical symptoms recorded in the patient diary, a statistical correlation was found only for nasal obstruction scores and TXB₂ concentration during the pretreatment period; no other combinations between any of the symptom scores and the concentration of histamine or TXB₂ during either the pre- or post-treatment period were correlated.

The results of the present study indicate that the TXA₂ receptor antagonist ramatroban can be used not only for allergic rhinitis patients with nasal obstruction, but also for those patients with sneezing and a runny nose in addition to nasal obstruction. These findings in perennial allergic rhinitis are in line with a report by Suzaki *et al.* that ramatroban effectively improved severe sneezing and rhinorrhea in patients with seasonal allergic rhinitis in a randomized study.¹⁴ Based on the antigen challenge test results, it can be considered that the decrease in the concentration of TXB₂ produced by ramatroban may cause alterations in the nasal mucosa that resulted in a decrease in the total level of mast cell-related mediators and, consequently, an improvement in sneezing and rhinorrhea as well as nasal obstruction. It could be also suggested that ramatroban stabilizes mast cells or

decreases the metabolism of arachidonic acid so that the amount of mediators released decreases. In contrast, there is a report that TXA₂ receptors may be present only in blood vessels.² If this is the case, another possible explanation for the results of the present study is that the broad improvement in symptoms following ramatroban could be a result of the inhibitory effects on vascular permeability and eosinophil infiltration, which is consistent with the overall results of the antigen challenge test. However, the decrease in histamine concentration in the same antigen challenge test cannot be fully explained by this speculation. Therefore, for the time being, no evident explanation can be given for the broad beneficial effects of ramatroban on the clinical symptoms of allergic rhinitis, as well as hypersensitivity of the nasal mucosa. The detailed pharmacological mechanism and clinical outcomes of ramatroban should be investigated further.

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REFERENCES

- 1 Howarth PH, Salagean M, Dokic D. Allergic rhinitis: Not purely a histamine-related disease. *Allergy* 2000; **55**: 7–16.
- 2 Arimura A, Miwa M, Hasegawa H *et al.* Characterization and localization of thromboxane A₂ receptor in human and guinea-pig nasal mucosa using radiolabeled (+)-S-145. *Br. J. Pharmacol.* 1998; **124**: 795–803.
- 3 Doyle WJ, Boehm S, Skoner DP. Physiologic responses to intranasal dose-response challenges with histamine, methacholine, bradykinin, and prostaglandin in adult volunteers with and without nasal allergy. *J. Allergy Clin. Immunol.* 1990; **86**: 924–35.
- 4 Yasui K, Asanuma F, Furue Y, Arimura A. Involvement of thromboxane A₂ in antigen-induced nasal blockage in guinea pigs. *Int. Arch. Allergy Immunol.* 1997; **112**: 400–5.
- 5 Narita S, Asakura K, Kataura A. Effects of thromboxane A₂ receptor antagonist (Bay u 3405) on nasal symptoms after antigen challenge in sensitized guinea pigs. *Int. Arch. Allergy Immunol.* 1996; **109**: 161–6.
- 6 Terada N, Yamakoshi T, Hasegawa M *et al.* The effect of ramatroban (Bay u 3405), a thromboxane A₂ receptor antagonist, on nasal cavity volume and minimum cross-sectional area and nasal mucosal hemodynamics after nasal mucosal allergen challenge in patients with perennial allergic rhinitis. *Acta Otolaryngol. Suppl.* 1998; **537**: 32–7.

- 7 Ishikawa T, Oyama M, Okuda M *et al.* Clinical guideline for allergic rhinitis in Japan: Perennial and seasonal allergic rhinitis. In: Okuda M (ed.). *Establishment Committee of the Clinical Guideline for Allergic Rhinitis*, 3rd edn. Tokyo: Life Science Medica, 1999; 20–1 (in Japanese).
- 8 Tadokoro K. Basic studies and clinical application of histamine radioimmunoassay kit. *Jpn. J. Med. Pharm. Sci.* 1987; **18**: 131–40.
- 9 Kawano K. A simple, rapid and simultaneous extraction of thromboxane B₂, 6-keto-prostaglandin F_{1α} and prostaglandin E₂. *Jpn. J. Inflammation* 1987; **7**: 511–15.
- 10 Powell WS. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins* 1980; **20**: 947–57.
- 11 Powell WS. Rapid extraction of arachidonic acid metabolites from biological samples using octadecylsilyl silica. *Methods in Enzymology* 86. New York: Academic Press, 1982; 467–77.
- 12 Baba S, Unno T, Takasaka T *et al.* A comparison of the efficacy and safety between ramatroban and terfenadine on perennial allergic rhinitis. *Pract. Otorhinolaryngol. Suppl.* 1996; **87**: 1–32.
- 13 Terada N, Yamakoshi T, Hasegawa M *et al.* Effect of a thromboxane A₂ 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. *Allergol. Int.* 1998; **47**: 59–67.
- 14 Suzuki H, Imai T, Ohkubo K *et al.* Efficacy of ramatroban in Japanese cedar pollinosis. *Otorhinolaryngology* 2002; **45**: 517–26.