ELSEVIER

European Journal of Pharmacology 278 (1995) 161–166

ejp

Effect of azelastine on airway hyperresponsiveness mediated by stimulated macrophages

Jun Tamaoki *, Hisashi Takemura, Etsuko Tagaya, Atsushi Sakai, Isao Yamawaki, Kimio Konno

First Department of Medicine, Tokyo Women's Medical College, Tokyo 162, Japan

Received 5 December 1994; revised 13 February 1995; accepted 17 February 1995

Abstract

The effect of the anti-allergic drug azelastine, 4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1*H*-azepine-4-yl)-1-(2*H*)-phthalazione), on airway hyperresponsiveness induced by immunologically stimulated pulmonary alveolar macrophages was investigated in canine bronchial segments under isometric conditions in vitro. Macrophages stimulated with anti-dinitrophenyl immunoglobulin E (IgE) antibody and dinitrophenyl-human serum albumin potentiated the contractile responses to electrical field stimulation at all frequencies, an effect that was abolished by azelastine (3×10^{-5} M). In contrast, azelastine had no effect on the potentiation of the contractile responses to electrical stimulation by U46619, a thromboxane A_2 mimetic. The IgE-mediated release of thromboxane A_2 from macrophages was inhibited by azelastine in a concentration-dependent fashion, the maximal decrease and the concentration required to produce a half-maximal effect being $84 \pm 6\%$ ($P < 0.001$) and 16 μM, respectively. These results suggest that azelastine may attenuate macrophage-induced parasympathetic airway hyperresponsiveness through an inhibition of the release of thromboxane A_2.

Keywords: Macrophage; Thromboxane; Azelastine; Airway hyperresponsiveness

1. Introduction

Pulmonary alveolar macrophages reside on top of epithelial cells not only in the alveoli and small airways but also in the central airways including main bronchi and lobar bronchi (Eschenbacher and Gravelyn, 1987). Because of this strategic location and the presence of functional receptors on the cells for binding of immunoglobulin E (IgE) (Spiegelberg, 1984), macrophages have been proposed to play a role in the induction of allergic reactions by encountering inhaled allergens (Fels and Cohn, 1986). We previously showed that macrophages stimulated with calcium ionophore A23187 (Tamaoki et al., 1987b) or IgE immune complexes (Tamaoki et al., 1991) can augment parasympathetic nerve-mediated contraction of airway smooth muscle probably through a release of thromboxane A_2, thereby causing airway hyperresponsiveness that is a characteristic feature of asthma.

Azelastine, 4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1*H*-azepine-4-yl)-1-(2*H*)-phthalazione, is an orally effective and long-lasting anti-allergic drug that prevents allergic bronchospasm by inhibiting the release of histamine from mast cells (Chand et al., 1985) and leukotriene synthesis in the lung (Katayama et al., 1987). In addition, this drug has recently been shown to possess anti-inflammatory actions, such as suppressions of superoxide generation by leukocytes (Taniguchi and Takanaka, 1985), sulfur dioxide-induced impairment of airway ciliary motility (Tamaoki et al., 1993) and passive Arthus reaction-mediated vascular leakage in the skin (Tanigawa et al., 1981). These observations led us to determine whether azelastine protects against macrophage-mediated airway hyperresponsiveness. Therefore, we studied the effect of azelastine on the increased cholinergic neurotransmission induced by immunologically stimulated macrophages in canine isolated bronchial segments under isometric conditions in vitro.

* Corresponding author. First Department of Medicine, Tokyo Women's Medical College, 8-1 Kawada-Cho, Shinjuku, Tokyo 162, Japan. Tel. 81-3-3353-8111, fax 81-3-5379-5457.

Elsevier Science B.V.
SSDI 0014-2999(95)00114-X

2. Materials and methods

2.1. Preparation of macrophages

Mongrel dogs of either sex (19–27 kg) were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and bronchoalveolar lavage was carried out using 20×50 ml cold phosphate-buffered saline. The lavage fluid was filtered through four layers of gauze and centrifuged for 20 min at $400 \times g$. The cell pellets were washed 3 times with Ca^{2+}-Mg^{2+}-free Hank's balanced salt solution. Macrophages were separated from epithelial cells by density-gradient centrifugation (Shaw and Anderson, 1984) and resuspended in Krebs-Henseleit (KH) solution of the following composition (mM): NaCl, 118; KCl, 5.9; $CaCl_2$, 2.5; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; and glucose, 5.6, to produce a cell count of 5×10^7/ml. Approximately 96% of the cells in this preparation were macrophages as determined morphologically by Wright-Giemsa staining, and viability was greater than 90% as assessed by Trypan blue exclusion.

2.2. Preparation of bronchial segments

After the completion of bronchoalveolar lavage, dogs were euthanized by intravenous sodium pentobarbital (60 mg/kg), and their whole pulmonary lobes were rapidly removed and immersed in oxygenated KH solution. Rings of lobar or segmental bronchi were dissected free of loose connective tissue and mounted in glass chambers filled with 14 ml of KH solution maintained at 37° C and continuously aerated with a mixture of 95% O_2-5% CO_2 to obtain a pH of 7.4, a PCO_2 of 38 Torr, and a PO_2 of > 500 Torr. The outer diameters of the tissues used in our studies ranged from 3 to 7 mm. The lower end of the bronchial ring was attached to a glass hook at the base of the organ chamber by a loop of silk thread, and the upper end was attached in the same manner to a strain gauges (TB-652T, Nihon Kohden, Tokyo, Japan) for continuous recording of isometric tension by a pen recorder (WT-685G, Nihon Kohden). Each organ chamber was fitted with two rectangular platinum electrodes (3×30 mm) placed alongside the tissue for transmural electrical field stimulation, biphasic pulse, pulse width 0.5 ms, supramaximal voltage of 20 V for 20 s. The tissues were allowed to equilibrate in the bath for 60 min while they were washed with KH solution every 15 min. Because separate experiments on the relationship between resting tension and active tension of canine bronchial rings showed maximal responses to electrical field stimulation with approximately 4 g of resting tension, the resting tension was adjusted to 4 g in all tissues, and a contractile response was measured as the difference between peak tension developed and resting tension. The β-adrenoceptor antagonist propranolol (10^{-6} M) was present in the chamber throughout the experiments to avoid a β-adrenergic component of neurally mediated contraction of canine airway smooth muscle. Under this condition, contractile responses to electrical field stimulation was abolished by either tetrodotoxin (10^{-6} M) or atropine (10^{-6} M), implying that the responses were produced by acetylcholine released from the cholinergic nerve endings.

2.3. Contractile responses

After incubating suspensions of macrophages for 20 min at 37° C with affinity-purified mouse monoclonal hybridoma anti-dinitrophenyl IgE antibody (final IgE concentration 10 μg/ml), macrophages were added to organ chambers containing bronchial segments in the absence and presence of azelastine (3×10^{-5} M), and dinitrophenyl-human serum albumin was then added. This procedure did not alter the resting tension of bronchial segments.

To assess whether IgE immune complexes affect contractile responses of bronchial smooth muscle to electrical field stimulation, we first measured responses at increasing impulse frequencies (1–40 Hz), added macrophages (10^7 cells/ml) and dinitrophenyl-human serum albumin (100 ng/ml) consecutively, and 5 min later the measurements were repeated. To determine whether azelastine exerts its effect through alteration in the release of thromboxane A_2 from macrophages or the sensitivity of smooth muscle cells to thromboxane A_2, we examined the effect of the thromboxane A_2 mimetic 11α,9α-epoxymethano-prostaglandin H_2 (U46619, 2×10^{-9} M) (Coleman et al., 1981) on the electrical stimulation-induced contraction in the absence and presence of azelastine (3×10^{-5} M) with the same time sequence for the experiment with macrophages. The concentration of U46619 was chosen based on our previous study (Tamaoki et al., 1987a) that thromboxane A_2 at concentrations insufficient to alter the resting tension of airway smooth muscle can enhance cholinergic neurotransmission. To characterize the frequency-response curves, the stimulus frequency of electrical stimulation required to produce 50% of the maximal contraction (ES_{50}) was determined from the data using linear regression analysis.

2.4. Release of thromboxane A_2

Among arachidonic acid metabolites released from macrophages, thromboxane A_2 is known to potentiate electrical field stimulation-induced contraction of canine airway (Munoz et al., 1986; Tamaoki et al., 1987b). Thus, to test whether azelastine actually inhibited thromboxane A_2 release, aliquots (100 μl) taken from the medium in the organ chamber before and 5 min

after IgE challenge were analyzed in duplicate for thromboxane B_2, a stable metabolite of thromboxane A_2, by radioimmunoassay (New England Nuclear, Boston, MA) according to the method by Jose and coworkers (Jose et al., 1976). Our preliminary studies showed that bronchial rings applied with IgE immune complexes in the absence of macrophages did not cause accumulation of thromboxane B_2. Thus, thromboxane B_2 in the medium is derived presumably from macrophages. We then examined the effect of various concentrations of azelastine (10^{-6} to 3×10^{-4} M) on the release of thromboxane A_2 from macrophages in response to IgE challenge.

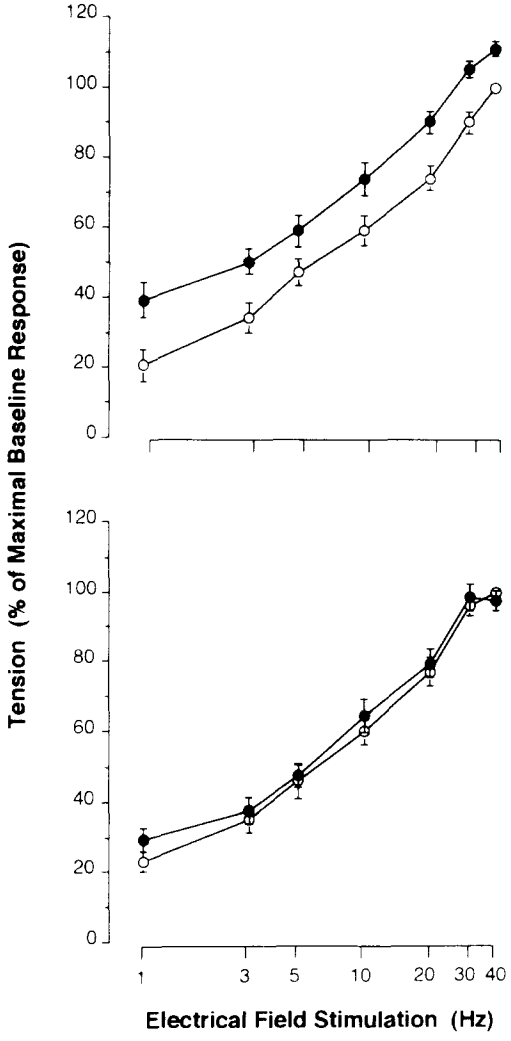

Fig. 1. Effect of pulmonary alveolar macrophages on contractile responses of canine bronchial segments to electrical field stimulation. After obtaining baseline responses (open circles), macrophages stimulated with monoclonal anti-dinitrophenyl IgE antibody were added to the chamber (10^7 cells/ml) in the absence (upper panel) or presence of azelastine (3×10^{-5} M, lower panel). Then dinitrophenyl-human serum albumin was added and 5 min later the measurements were repeated (closed circles). Data are expressed as percentage of maximal baseline responses. Each point represents mean ± S.E.; $n = 8$.

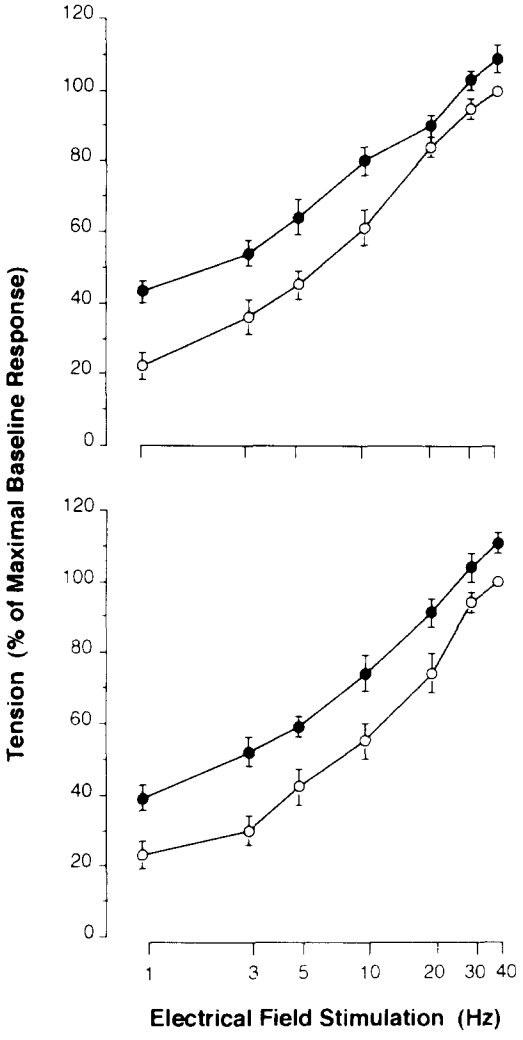

Fig. 2. Effect of U46619 on contractile responses to electrical field stimulation. After obtaining baseline responses (open circles), U46619 (2×10^{-9} M) was added to the chamber in the absence (upper panel) or presence of azelastine (3×10^{-5} M, lower panel), and 5 min later the measurements were repeated (closed circles). Data are expressed as percentage of maximal baseline responses. Each point represents mean ± S.E.; $n = 9$.

2.5. Drugs

The following drugs were used: azelastine (Eisai Co., Tokyo), anti-dinitrophenyl IgE antibody, dinitrophenyl-human serum albumin, propranolol hydrochloride (Sigma Chemical Co., St. Louis, MO), U46619 (Cayman Chemical Co., Ann Arbor, MI).

2.6. Statistics

All data are expressed as means ± S.E. Statistical analysis was performed by ANOVA or Games-Howell multiple comparison test, and a P value of less than 0.05 was considered significant.

3. Results

Macrophages stimulated with anti-dinitrophenyl IgE antibody and dinitrophenyl-human serum albumin had no effect on the resting tone of canine bronchial segments, but potentiated the contractile responses to electrical stimulation at all stimulus frequencies (Fig. 1). Thus, the frequency-response curves were displaced to the left, so that the ES_{50} values decreased from 5.3 ± 1.0 to 2.7 ± 0.5 Hz ($P < 0.01$, $n = 8$). In contrast, macrophages similarly stimulated with IgE immune complexes did not alter electrical stimulation-induced contraction in the presence of azelastine (3×10^{-5} M), the ES_{50} values before and after challenge being 5.7 ± 0.9 and 5.4 ± 1.1 Hz, respectively ($n = 8$). Application of U46619 (2×10^{-9} M) likewise increased the contractile responses to electrical stimulation, causing a decrease in the ES_{50} values from 6.0 ± 1.1 to 2.3 ± 0.4 Hz ($P < 0.001$, $n = 9$), an effect that was not altered by azelastine (Fig. 2).

Incubation of macrophages with anti-dinitrophenyl IgE antibody alone had no effect on the release of thromboxane B_2, but incubation with the antibody followed by dinitrophenyl-human serum albumin increased the thromboxane B_2 release from 56 ± 9 to 507 ± 72 pg/10^6 cells min ($P < 0.001$, $n = 9$). In the presence of azelastine (3×10^{-5} M), stimulated macrophages released thromboxane B_2 at 143 ± 35 pg/10^6 cells min ($n = 9$), which was significantly less than the values in the absence of azelastine ($P < 0.01$) (Fig. 3). Azelastine inhibited IgE-mediated release of thromboxane B_2 from macrophages in a concentration-dependent fashion, the maximal decrease and the concentration required to produce a half-maximal effect (EC_{50}) being $84 \pm 6\%$ ($P < 0.001$, $n = 6$) and 16 μM, respectively (Fig. 4).

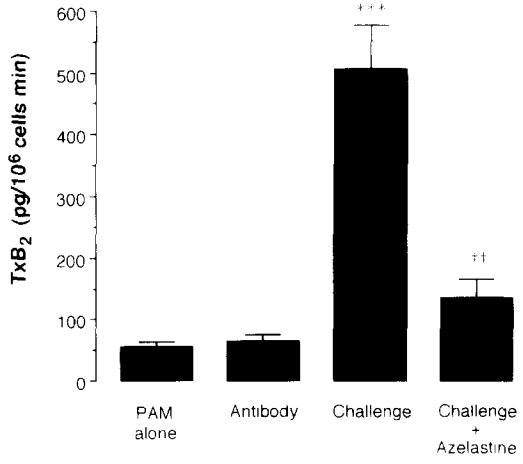

Fig. 3. Release of thromboxane B_2 from pulmonary alveolar macrophages. Macrophages were exposed to monoclonal anti-dinitrophenyl IgE antibody (Antibody) or antibody plus dinitrophenyl-human serum albumin (Challenge) in the absence and presence of azelastine (3×10^{-5} M). Values are expressed as means ± S.E.; $n = 9$ for each column. $^{***}P < 0.001$, significantly different from values for macrophages alone. $^{++}P < 0.01$, significantly different from values for challenge.

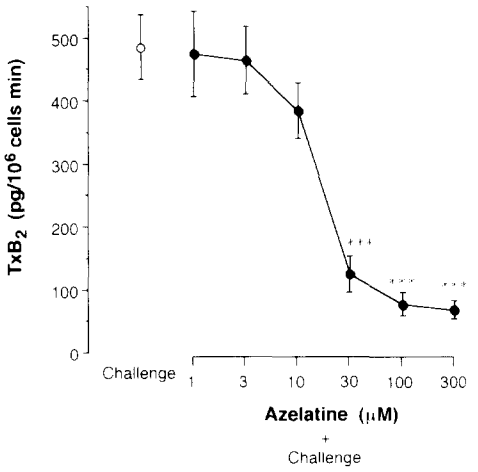

Fig. 4. Concentration-dependent effect of azelastine on the release of thromboxane B_2 from pulmonary alveolar macrophages stimulated with anti-dinitrophenyl IgE antibody and dinitrophenyl-human serum albumin (Challenge). Values are expressed as means ± S.E.; $n = 6$ for each point. $^{***}P < 0.001$, significantly different from values for challenge.

4. Discussion

Our in vitro studies demonstrate that azelastine, an anti-allergic drug, attenuates macrophage-mediated airway hyperresponsiveness through an inhibition of thromboxane A_2 release from macrophages and the resultant reduction in the increased cholinergic neuroeffector transmission in bronchial smooth muscle.

The exposure of canine bronchial segments to macrophages incubated with monoclonal anti-dinitrophenyl IgE antibody and the subsequent application of dinitrophenyl-human serum albumin increased the contractile responses to electrical field stimulation. Previous studies have shown that immunologically activated macrophages potentiate the contractile responses to electrical stimulation without affecting those to exogenously applied acetylcholine (Tamaoki et al., 1991) and that this potentiating effect can be abolished by the specific thromboxane A_2 receptor antagonist SQ29548 (Ogletree et al., 1985). Our present experiment also showed that macrophages released thromboxane A_2 in response to consecutive application of monoclonal anti-dinitrophenyl IgE antibody and dinitrophenyl-human serum albumin. Thus, the effect of macrophages on electrical stimulation-induced contraction observed in the present study may be accounted for by the thromboxane A_2-mediated enhancement of

exocytotic release of acetylcholine from postganglionic cholinergic nerve fibers in airway smooth muscle.

Azelastine attenuated the increase in the contractile responses to electrical stimulation produced by macrophages, suggesting that this drug may have exerted its effect by inhibiting the IgE-mediated release of thromboxane A_2 from macrophages or by decreasing the sensitivity of airway smooth muscle cells to thromboxane A_2. However, the latter possibility seems unlikely because, in contrast to the experiment with macrophages, the potentiation of the contractile responses produced by exogenously applied stable thromboxane A_2 mimetic U46619 (Coleman et al., 1981) was not altered by azelastine. Therefore, azelastine may possess an inhibitory effect on thromboxane A_2 release. This notion was confirmed by the finding that the release of thromboxane A_2 from immunologically activated macrophages was decreased by azelastine in a concentration-dependent manner. However, another possibility that azelastine could have increased the breakdown of thromboxane A_2 cannot be ruled out.

Several clinical trials have shown that the anti-allergic drug azelastine is effective in the treatment of mild and moderate asthma (Perhach et al., 1989). The efficacy of this drug may be associated mainly with the inhibition of histamine release from mast cells. Additionally, azelastine has been reported to inhibit prostaglandin E_2 release from peritoneal macrophages (Nakamura et al., 1988), leukotriene release from lung fragments (Katayama et al., 1987), and superoxide generation by macrophages (Kurosawa et al., 1991). These inhibitory actions on the mediator release may be derived from common mechanisms such as the inhibition of Ca^{2+} influx of the cells and the elevation of intracellular cyclic AMP levels followed by stabilization of cell membrane (Akagi et al., 1983; Nakamura et al., 1988).

Among several cells contributing individually and in concert to the development and maintenance of airway hyperresponsiveness, interest has been focused on macrophages as one of the potential candidates for the following reasons: (1) macrophages have functional Fc receptors for binding of IgE (Spiegelberg, 1984); (2) stimulation of macrophages by IgE and specific allergens, or IgE and anti-IgE, elicits the synthesis and release of a variety of biochemically different substances (Balter et al., 1988); and (3) some of these mediators have a putative role in asthmatic symptoms by modulating humoral and cellular components of airway inflammation (Henderson, 1987). We have recently shown that immunologically stimulated macrophages increase cholinergic neuro-effector transmission in airway smooth muscle through a release of thromboxane A_2 (Tamaoki et al., 1991). It has been reported that blood concentration of azelastine reaches approximately 2×10^{-5} M when it was administered intravenously at 1 mg/kg (Tatsumi et al., 1980). We found that the EC_{50} value of this drug in inhibiting the release of thromboxane A_2 was 16 μM. Therefore, we speculate that clinical dose of azelastine might be beneficial in the treatment of airway hyperresponsiveness associated with macrophages.

Acknowledgements

The authors thank Masayuki Shino and Yoshimi Sugimura for their technical assistance. We are grateful to Eisai Co. for providing us with azelastine. This work was supported in part by Grant for Scientific Research No. 04670476 from the Ministry of Education, Science and Culture, Japan.

References

Akagi, M., M. Mio, K. Tanaka and S. Kiniwa, 1983, Mechanism of histamine release inhibition induced by azelastine, Pharmacometrics 26, 191.

Balter, M.S., W.L. Eschenbacher and M. Peters-Golden, 1988, Arachidonic acid metabolism in cultured alveolar macrophages from normal, atopic, and asthmatic subjects, Am. Rev. Respir. Dis. 138, 1134.

Chand, N., J. Pillar, W. Diamantis and R.D. Sofia RD, 1985, Inhibition of IgE-mediated allergic histamine release by azelastine and selected antiallergic drugs, Agents Actions 16, 318.

Coleman, R.A., P.P.A. Humphrey, I. Kennedy, G.P. Levy and P. Lumley, 1981, Comparison of the actions of U-46619, a prostaglandin H_2 analogue with those of prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations, Br. J. Pharmacol. 73, 773.

Eschenbacher, W.L. and T.R. Gravelyn, 1987, A technique for isolated airway segment lavage, Chest 92, 105.

Fels, A.O.S. and Z.A. Cohn, 1986, The alveolar macrophage, J. Appl. Physiol. 60, 353.

Henderson, W.R., Jr., 1987, Eicosanoids and lung inflammation, Am. Rev. Respir. Dis. 135, 1176.

Jose, P., U. Niederhauser, P.J. Piper, C. Robinson and A.P. Smith, 1976, Degradation of prostaglandin F_{2a} in the human pulmonary circulation, Thorax 31, 713.

Katayama, S., H. Tsunoda, Y. Sakuma, H. Kai, I. Tanaka and K. Katayama, 1987, Effect of azelastine on the release and action of leukotriene C_4 and D_4, Int. Arch. Allergy Appl. Immunol. 83, 284.

Kurosawa, M., T. Ishizuka, S. Kobayashi and M. Nakano, 1991, Effects of antiallergic drugs on superoxide anion generation from activated human alveolar macrophages measured by chemiluminescence method, Arzneim.-Forsch. Drug Res. 41, 47.

Munoz, N.M., T. Shioya, T.M. Murphy, S. Primack, C. Dame, M.F. Sands and A.R. Leff, 1986, Potentiation of vagal contractile response by thromboxane mimetic U-46619, J. Appl. Physiol. 61, 1173.

Nakamura, T., Y. Nishizawa, T. Sato and C. Yamato, 1988, Effect of azelastine on the intracellular Ca^{2+} mobilization in guinea pig peritoneal macrophages, Eur. J. Pharmacol. 148, 35.

Ogletree, M.L., D.N. Harris, R. Greenberg, M.F. Haslanger and M. Nakane, 1985, Pharmacological actions of SQ 29548, a novel selective thromboxane antagonist, J. Pharmacol. Exp. Ther. 234, 435.

Perhach, J., N. Chand, W. Diamantis, R. Sofia and A. Rosenberg, 1989, Azelastine: a novel oral anti-asthma compound with several modes of actions, in: Asthma and Allergy: New Trends and Approaches to Therapy, ed. A.B. Key (Blackwell Scientific Publications, Oxford) p. 236.

Shaw, S.E. and N.V. Anderson, 1984, Isolation and functional analysis of normal canine blood monocytes and resident alveolar macrophages, Am. J. Vet. Res. 45, 87.

Spiegelberg, H.L., 1984, Structure and function of Fc receptors for IgE on lymphocytes, monocytes, and macrophages, Adv. Immunol. 35, 61.

Tamaoki, J., K. Sekizawa, M.L. Osborne, I.F. Ueki, P.D. Graf and J.A. Nadel, 1987a, Platelet aggregation increases cholinergic neurotransmission in canine airway, J. Appl. Physiol. 62, 2246.

Tamaoki, J., K. Sekizawa, I.F. Ueki, P.D. Graf, J.A. Nadel and T.D. Bigby, 1987b, Effect of macrophage stimulation on parasympathetic airway contraction in dogs, Eur. J. Pharmacol. 138, 421.

Tamaoki, J., N. Sakai, T. Kanemura, I. Yamawaki and T. Takizawa, 1991, IgE-dependent activation of alveolar macrophages augments neurally mediated contraction of small airways, Br. J. Pharmacol. 103, 1458.

Tamaoki, J., A. Chiyotani, N. Sakai, K. Takeyama and K. Konno, 1993, Azelastine protects against sulfur dioxide-induced impairment of ciliary motility in human airway epithelium, Thorax 48, 542.

Tanigawa, T., M. Honda and K. Miura, 1981, Effect of azelastine hydrochloride on vascular permeability reaction skin site in guinea pig, Arzneim.-Forsch. Drug Res. 31, 1212.

Taniguchi, K. and K. Takanaka, 1985, Inhibitory effects of various drugs on phorbol myristate acetate and *n*-formyl methionyl leucyl phenylalanine induced O_2^- production in polymorphonuclear leukocytes, Biochem. Pharmacol. 33, 3165.

Tatsumi K., T. Ou, H. Yamada and H. Yoshimura, 1980, Studies on metabolic fate of a new antiallergic agent, azelastine (4-(*p*-chlorobenzyl)-2-[*N*-methylperhydroazepinyl-(4)-l-(2*H*)-phthalazione hydrochloride), Jpn. J. Pharmacol. 30, 37.