

Pharmacodynamic and pharmacokinetic studies with azelastine in the guinea pig: evidence for preferential distribution into the lung

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The correlation between the antiasthma activity of azelastine and the concentrations of azelastine and its major metabolite, desmethylazelastine, in the blood and lung were investigated in guinea pigs. Blood and lung tissue samples collected at 15 min after aeroallergen (ovalbumin, 0.5 mg/ml, 30 s, 15 psi) challenge, i.e., 2–1/4 h after oral administration of the drug, were analyzed for azelastine and its desmethyl metabolite by specific HPLC assay. Azelastine afforded protection against immediate allergic responses (IAR, characterized by gasping and bronchospastic convulsions). Based on the reduction of the severity of IAR, the ID_{50} was 0.04 mg/kg. It also delayed the onset of IAR and prevented mortalities. A positive correlation ($r^2 = 0.944$) between the antiallergic activity and the sum concentrations of azelastine and desmethylazelastine in the lung was observed. Preferential uptake of azelastine and desmethylazelastine by the lung was demonstrated by the mean lung/blood ratio of about 22 and 146, respectively. The selective uptake of azelastine and its active metabolite by the lungs could contribute to its antiasthmatic/antiallergic activity in guinea pigs.

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Azelastine HCl (4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1H-azepin-4-yl)-, monohydrochloride) is a novel chemical entity (Fig. 1). In pre-clinical studies it exhibits antiallergic, antiasthmatic and anti-inflammatory activities. It has been reported to inhibit immediate allergic responses in guinea pigs (8, 9, 20, 32), dogs (21), rabbits (22) and man (16, 24). It also inhibits late-phase bronchoalveolar eosinophilia (9) and elevation of microviscosity of mucus in guinea pigs (1), late asthmatic responses in rabbits (22) and airway hyperreactivity in guinea pigs (27), dogs (21), and rabbits (22). Azelastine has also been shown to produce bronchodilatation and to exert β_2 -agonist and theophylline-sparing effects in asthmatics (16). It exerts mucolytic activity in mice (14), rats (7), guinea pigs (1) and dogs (17, 25). In addition, azelastine appears to be preferentially taken up by the lungs in rats and guinea pigs (28, 31).

In the present study possible correlation of anti-allergic activity with both blood and lung drug concentrations (azelastine and its major metabolite desmethylazelastine) was investigated by the con-

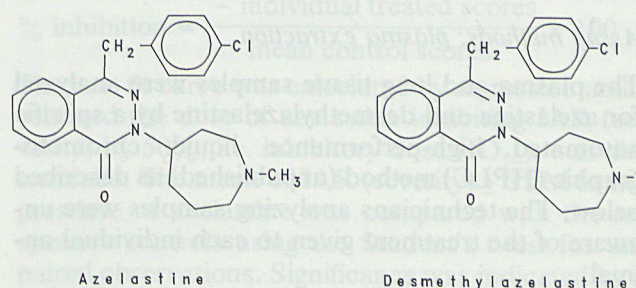


Fig. 1. Chemical structure of azelastine and its pharmacologically active major metabolite, desmethylazelastine.

scious guinea pig model for immediate allergic responses (IAR).

Material and methods

Sensitization

Guinea pigs were sensitized by an intraperitoneal injection of 10 μ g ovalbumin (OA) mixed in 100 mg aluminum hydroxide, $Al(OH)_3$ in 0.5 ml of physio-

logical saline 14–21 days prior to the aeroallergen challenge. This procedure of sensitization produces both IgG and IgE antibodies (4).

Aeroallergen challenge

Guinea pigs were marked for identification and weighed. Azelastine HCl was dissolved in 1% acacia in distilled water. The drug or vehicle (1% acacia in distilled H₂O; 5 ml/kg) was administered by gavage. Two hours later each animal was placed in a 2-liter glass chamber and exposed to a fine fog of ovalbumin solution (0.5 mg/ml in physiologic saline containing 2% silicone-based antifoam B emulsion (Sigma); 9.8 ml physiologic saline + 0.2 ml of antifoam B emulsion + 5 mg OA) for a period of 30 s.

The aerosol was produced by forcing compressed air at a pressure of 15 pounds per square inch through a Wright's nebulizer containing 10 ml of ovalbumin solution.

The animals were observed for a period of 14 min for induction of IAR (characterized by gasping, bronchospastic convulsions and death). The following parameters were recorded: (i) time of onset of IAR (s), (ii) severity of IAR and (iii) time of death, if any. The IAR severity was scored as: 0 – no symptoms (protected); 3 – mild symptoms; 6 – severe symptoms (gasping and convulsions); 9 – death.

Fifteen min after aeroallergen challenge, the animals were killed by CO₂ exposure, and blood samples, as well as lung tissues samples, were collected. If death occurred before 15 min, blood and lung samples were collected immediately.

Assay methods: plasma extraction

The plasma and lung tissue samples were analyzed for azelastine and desmethylazelastine by a specific automated high-performance liquid chromatographic (HPLC) method (unpublished) as described below. The technicians analyzing samples were unaware of the treatment given to each individual animal.

One ml of plasma was fortified with 100 µl of internal standard (IS) solution (15 ng in 0.2% v/v HOAc), or 100 µl of a solution of IS plus azelastine and desmethylazelastine for daily calibration standards, and the sample was incubated for 30 min at room temperature.

A total of 200 µl 1 N NaOH and 9.0 ml of 95:5 hexane:n-octanol was added and the samples were extracted by rotation for 1 h (RD-250 with RD-10 head, 50 rpm, Glas-Col, Terre Haute, IN, USA). The phases were separated by centrifugation and 8.0 ml of the organic layer was transferred to another tube. The organic phase was back-extracted by vig-

orous vortexing with 125 µl of 0.2% HOAc. The sample was centrifuged, and the aqueous phase was transferred to autosampler vials for HPLC analysis under the following conditions. Column: Shandon Hypersil CPS, 5 µm, 2 × 250 mm (Keystone Scientific, Bellefonte, PA, USA); mobile phase: 0.009 M triethylammonium phosphate, pH 3.00; acetonitrile (50:50); flow rate: 0.45 ml/min at 40°C; run time: 11 min; injection volume: 90 µl; fluorescence detection: 215 nm excitation and 360 nm cutoff emission filter. Range 0.01; 5 s rise time.

Lung extraction

A 10% w/w homogenate of the entire lung was made in water. One gram of homogenate (0.100 g of tissue) was spiked with 100 µl of IS solution (1.00 µg in 0.2% v/v HOAc), or 100 µl of a solution of IS plus azelastine and desmethylazelastine for daily calibration standards. One hundred µl of 10 N NaOH and 9.0 ml of 95:5 hexane:n-octanol were added, and the samples were extracted by vigorous vortexing for 1 min. The phases were separated by centrifugation and 8.0 ml of the organic layer was transferred to another tube. The organic phase was back-extracted by vigorous vortexing with 125 µl of 0.2% HOAc. The aqueous phase was transferred to autosampler vials for HPLC analysis under the following conditions. Column: Shandon Hypersil CPS, 5 µm, 2 × 250 mm (Keystone Scientific, Bellefonte, PA, USA);

Table 1. Effect of azelastine on the severity of aeroallergen (ovalbumin 0.05%, 0.5 mg/ml, 30 s., 15 psi)-induced immediate allergic responses (IAR) and the duration of onset of IAR in actively sensitized, conscious guinea pigs

Oral dose mg/kg	Time of onset of IAR (sec)	Severity of IAR	% Inhibition [†]
0	153 ± 17 (9)	7.0 ± 0.7	0
0.030	174 ± 20 (8) > 14 min (1)	5.3 ± 1.0	20 ± 11
0.050	204 ± 39 (5) > 14 min (1)	3.0 ± 1.1*	60 ± 14
0.075	289 ± 32 (8) > 14 min (1)	3.7 ± 0.7*	47 ± 10
0.100	408 ± 98 (4) > 14 min (5)	1.3 ± 0.5*	81 ± 8
0.300	335 ± 24 (3) > 14 min (6)	1.0 ± 0.5*	86 ± 7
1.000	523 ± 21 (3) > 14 min (6)	1.0 ± 0.5*	86 ± 7
3.000	> 14 min (9)	0 ± 0*	100 ± 0

Values are means ± SEM. Numbers in parentheses represent the number of guinea pigs. * $P < 0.05$ as compared to controls; [†] % reduction in the severity of IAR; ID₅₀ (95% CL) = 0.04 (0.02–0.09) mg/kg, $r = 0.74$.

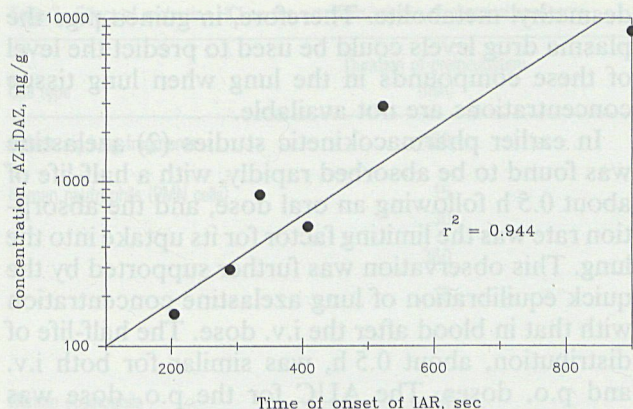


Fig. 2. Sum of azelastine and desmethylazelastine in lung vs mean times prior to onset of IAR. Each point represents mean of 9 observations.

mobile phase: water:acetonitrile:triethylamine:phosphoric acid (500:500:0.4:0.2); flow rate: 0.60 ml/min at 60°C; run time: 12 min; injection volume: 90 µl; fluorescence detection: 215 nm excitation and 360 nm cutoff emission filter, range 0.01; 5 s rise time.

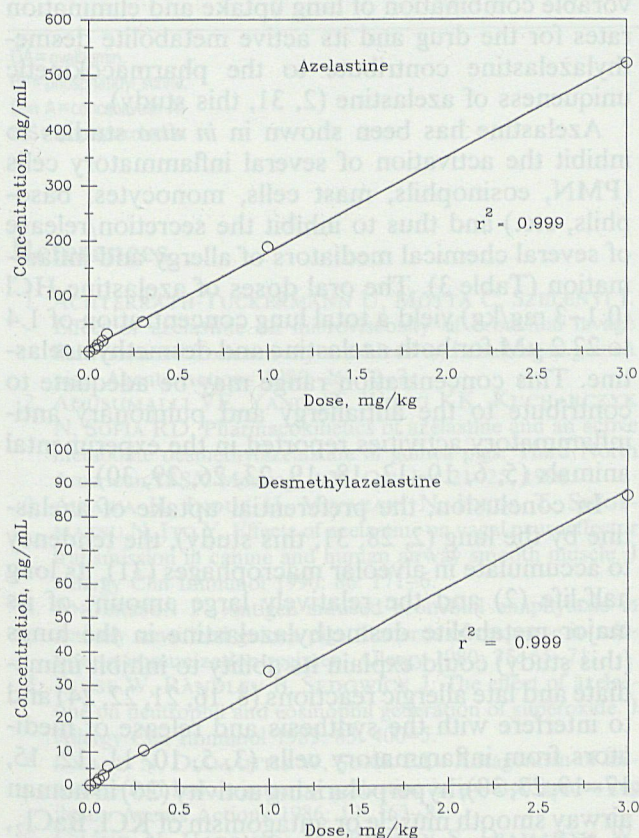


Fig. 3. Mean azelastine and desmethylazelastine plasma concentrations vs azelastine HCl dose. Each point represents mean of 9 observations.

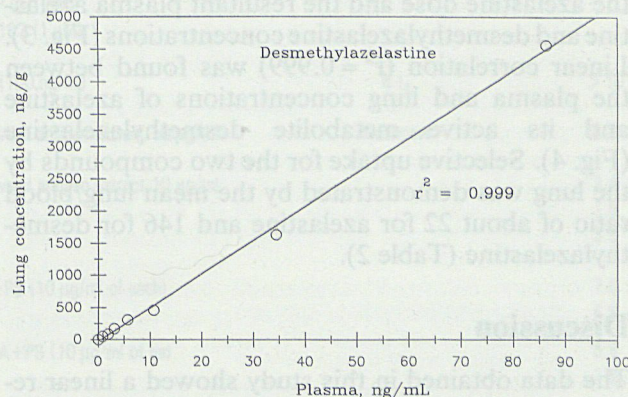
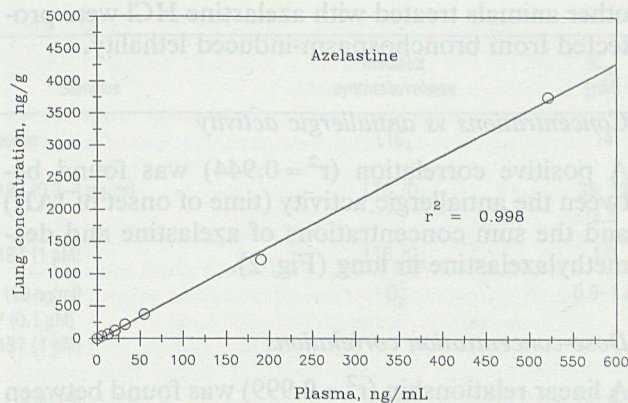


Fig. 4. Mean azelastine and desmethylazelastine concentrations in plasma vs lung. Each point represents mean of 9 observations.

Calculations

The percent inhibition of IAR was calculated based on scoring of the severity as follows:

$$\% \text{ inhibition} = \frac{\text{mean control scores} - \text{individual treated scores}}{\text{mean control scores}} \times 100$$

The best fit curve was calculated by regression analysis and the dose of azelastine inhibiting IAR (severity) by 50%, i.e. inhibitory dose (ID_{50}), was determined. The mean of IAR (severity) $\pm$ SEM in the presence of azelastine was compared with the respective controls using the Student's t-test for unpaired observations. Significance was indicated by a *P* value of 0.05 or less.

Results

The oral administration of azelastine 2 h before aeroallergen challenge (ovalbumin 0.5 mg/ml, 30 s, 15 psi) afforded protection against IAR, yielding an ID_{50} of 40 µg/kg, and delayed the onset of IAR (Table 1).

Five control animals (vehicle-treated) out of nine died within 14 min (56% mortality). Three of the nine animals treated with the lowest dose of azelastine HCl (0.03 mg/kg) died (33% mortality). All

other animals treated with azelastine HCl were protected from bronchospasm-induced lethality.

Concentrations vs antiallergic activity

A positive correlation ($r^2 = 0.944$) was found between the antiallergic activity (time of onset of IAR) and the sum concentrations of azelastine and desmethylazelastine in lung (Fig. 2).

Dose-concentration correlations

A linear relationship ($r^2 = 0.999$) was found between the azelastine dose and the resultant plasma azelastine and desmethylazelastine concentrations (Fig. 3). Linear correlation ($r^2 = 0.999$) was found between the plasma and lung concentrations of azelastine and its active metabolite desmethylazelastine (Fig. 4). Selective uptake for the two compounds by the lung was demonstrated by the mean lung/blood ratio of about 22 for azelastine and 146 for desmethylazelastine (Table 2).

Discussion

The data obtained in this study showed a linear relationship between the pharmacological activity (reduction in severity of IAR and delay in time prior to onset of symptoms) and the sum concentration of azelastine and desmethylazelastine in the lung. The calculated ID_{50} s of azelastine to afford protection against immediate allergic responses were 40 $\mu\text{g/kg}$ (this study) and 75 $\mu\text{g/kg}$ (32) and 50 $\mu\text{g/kg}$ (the increase in lung resistance) (8). These observations demonstrate that this drug is highly effective in inhibiting early asthmatic responses in guinea pigs.

Further, the data obtained in this study demonstrated an excellent linear correlation between the plasma and lung concentrations of azelastine and its

desmethyl metabolite. Therefore, in guinea pigs the plasma drug levels could be used to predict the level of these compounds in the lung when lung tissue concentrations are not available.

In earlier pharmacokinetic studies (2) azelastine was found to be absorbed rapidly, with a half-life of about 0.5 h following an oral dose, and the absorption rate was the limiting factor for its uptake into the lung. This observation was further supported by the quick equilibration of lung azelastine concentration with that in blood after the i.v. dose. The half-life of distribution, about 0.5 h, was similar for both i.v. and p.o. doses. The AUC for the p.o. dose was about five times less than that for the i.v. dose, which indicated an extensive first-pass metabolism of azelastine in the guinea pig. The clearance after the p.o. dose was two to three times faster than that after the i.v. dose. The rate of elimination of azelastine from the lung was one-fourth of its uptake. Desmethylazelastine was formed at a slow rate which appeared to be the rate-limiting factor for its uptake by the lung. The rate of elimination of desmethylazelastine from the lung was about one-half of its uptake. The volume of distribution values suggested that azelastine and desmethylazelastine were widely distributed in the body. The clearance of azelastine was faster than that of desmethylazelastine. The favorable combination of lung uptake and elimination rates for the drug and its active metabolite desmethylazelastine contribute to the pharmacokinetic uniqueness of azelastine (2, 31, this study).

Azelastine has been shown in *in vitro* studies to inhibit the activation of several inflammatory cells (PMN, eosinophils, mast cells, monocytes, basophils, etc.) and thus to inhibit the secretion/release of several chemical mediators of allergy and inflammation (Table 3). The oral doses of azelastine HCl (0.1–3 mg/kg) yield a total lung concentration of 1.4 to 22.2 μM for both azelastine and desmethylazelastine. This concentration range may be adequate to contribute to the antiallergy and pulmonary anti-inflammatory activities reported in the experimental animals (5, 6, 10–13, 18, 19, 23, 26, 29, 30).

In conclusion, the preferential uptake of azelastine by the lung (2, 28, 31, this study), the tendency to accumulate in alveolar macrophages (31), its long half-life (2) and the relatively large amount of its major metabolite desmethylazelastine in the lungs (this study) could explain its ability to inhibit immediate and late allergic reactions (8, 16, 21, 22, 24) and to interfere with the synthesis and release of mediators from inflammatory cells (3, 5, 10, 11, 12, 15, 17–19, 23, 30), hyperpolarizing activity (26) in human airway smooth muscle or antagonism of KCl, BaCl₂, Ca²⁺, TEA, and other mediators in smooth muscles (6, unpublished observations).

Table 2. Pharmacokinetic parameters of azelastine and its metabolite desmethylazelastine after a single oral administration of 1.0 mg/kg azelastine hydrochloride in guinea pigs

Parameter	Azelastine		Desmethylazelastine	
	Blood	Lung	Blood	Lung
C _{max} (ng/ml) ^a	119	1778	17.6	2863
t _{max} (h)	1.0–2.0	1.0	3.0–6.0	4.0–6.0
t _{1/2} (h)	0.19	0.22	0.25	0.39
AUC (ng·h/ml) ^a	401	8017	180	28502
Lung-blood ratio ^b	22		146	
t _{1/2} (h)	2.99	2.25	4.24	3.90
MRT (h)	3.58	4.09	8.75	8.72
CL (l/h)	0.825	c	0.213	c

^a For lung, the unit in grams (g) instead of milliliter (ml).

^b Mean value calculated from all time points.

^c Not applicable.

Table 3. Effect of azelastine HCl on the synthesis/release of chemical mediators

Cell type	Duration of preincubation (min)	Stimulus	Mediator synthesis/release	IC ₅₀ (μM)
Guinea pig lung fragments	120	Ovalbumin	LTC ₄	14
Human neutrophils (PMN cells)	15	A23187 (0.3–1 μg/ml)	LTC ₄ /D ₄	36; 33
	30			2.3
	360	A23187 (1 μM)	LTB ₄ /C ₄	1.0
	15	PMA (10 ng/ml) FMLP (0.1 μM) A23187 (1 μM)	O ₂ ⁻	0.9–1.6
Human eosinophils	15	PMA (10 ng/ml) FMLP (0.1 μM) A23187 (1 μM)	O ₂ ⁻	1.7–3.0
Human monocyte	10	LPS (100 ng/ml)	IL1 _β	> 10
Human basophils	10	Ragweed antigen extract 10 μg/ml	Histamine	3.3
Rabbit basophils	0	Ragweed antigen extract 10 μg/ml		2.4
	10			1.9
	30			3.5
Rat peritoneal mast cells	0	OA+PS (10 μg/ml of each)		7.6
	10			4.8
	0	Con A+PS (10 μg/ml of ea)		6.8
	10			1.9
	0	A23187 (0.1 μg/ml)		7.7
	10			5.0

OA=ovalbumin.

PS=phosphatidyl serine.

Con A=concanavalin A.

LPS=lipopolysaccharide.

References

1. ACHTERRATH-TUCKERMANN U, MOTTA C, SZELENYI I. Effect of azelastine on microviscosity of bronchial lavage fluid obtained from actively sensitized and challenged guinea pigs. *Agents Actions* 1989; 26: 70–2.
2. ADUSUMALLI VE, YANG JT, WONG KK, KUCHARCZYK N, SOFIA RD. Pharmacokinetics of azelastine and an active metabolite desmethylazelastine in guinea pigs. Third North American ISSX Meeting, A20, October 21–25, 1990.
3. AIZAWA H, INOUE H, MIYAZAKI N, IDEDA T, SHIGEMATSU N, ITO Y. Effects of azelastine on vagal neuroeffector transmission in canine and human airway smooth muscle. *J Allergy Clin Immunol* 1990; 86: 171–6.
4. ANDERSSON P. Antigen-induced bronchial anaphylaxis in actively sensitized guinea pigs. Patterns of response in relation to immunization regimen. *Allergy* 1980; 35: 65–71.
5. BUSSE W, RANDLEV B, SEDGWICK J. The effect of azelastine on neutrophil and eosinophil generation of superoxide. *J Allergy Clin Immunol* 1989; 83: 400–5.
6. CHAND N, DIAMANTIS W, SOFIA RD. Antagonism of histamine and leukotrienes by azelastine in isolated guinea pig ileum. *Agents Actions* 1986; 19: 163–8.
7. CHAND N, HARRISON JE, ROONEY S, DIAMANTIS W, SOFIA RD. Effect of azelastine on tracheobronchial secretion in rats: fluorescein sodium test as a measure of mucolytic activity. *J Allergy Clin Immunol* 1990; 85: A288.
8. CHAND N, NOLAN K, SOFIA RD, DIAMANTIS W. Changes in aeroallergen-induced pulmonary mechanics in actively sensitized guinea pig: inhibition by azelastine. *Ann Allergy* 1990; 64: 151–4.
9. CHAND N, NOLAN K, DIAMANTIS W, SOFIA RD. Inhibition of aeroallergen-induced immediate asthmatic response and late-phase bronchoalveolar eosinophilia by azelastine in actively sensitized guinea pigs. *Am Rev Respir Dis* 1990; 141: A176.
10. CHAND N, PILLAR J, DIAMANTIS W, PERHACH JL, SOFIA RD. Inhibition of calcium ionophore (A23187)-stimulated histamine release from rat peritoneal mast cells by azelastine. Implications for its mode of action. *Eur J Pharmacol* 1983; 96: 227–33.
11. CHAND N, PILLAR J, DIAMANTIS W, SOFIA RD. Inhibition of allergic histamine release from rat peritoneal mast cells by azelastine. *Int Arch Allergy Appl Immunol* 1988; 86: 256–60.
12. CHAND N, PILLAR J, DIAMANTIS W, SOFIA RD. Inhibition of allergic histamine release by azelastine and selected anti-allergic drugs from rabbit leukocytes. *Int Arch Allergy Appl Immunol* 1985; 77: 451–5.
13. CHAND N, PILLAR J, NOLAN K, DIAMANTIS W, SOFIA RD. Inhibition of allergic and nonallergic leukotriene C₄ formation and histamine secretion by azelastine: implication for its mechanism of action. *Int Arch Allergy Appl Immunol* 1989; 90: 67–70.

14. DIAMANTIS W, HARRISON JE, CHAND N, ROONEY S, SOFIA RD. Effect of azelastine on tracheal secretion in mice: phenol red test as a measure of mucolytic activity. *J Allergy Clin Immunol* 1990; 85: A288.
15. KAMIKAWA Y. Inhibitory effect of anti-allergy drugs on cholinergic and non-cholinergic neurotransmissions of guinea pig bronchial muscle *in vitro*. *Ann Allergy* 1989; 63: 59-63.
16. KEMP JP. Clinical and therapeutic experiences with azelastine. *J Respir Dis* 1989; 10: S86-S90.
17. KING M, APP EM, RAMIREZ O, RUBIN BK, ZAYAS JG. Effect of infused azelastine on canine tracheal mucus rheology. Transepithelial potential difference and mucus clearability. *J Allergy Clin Immunol* 1990; 85: A288.
18. LITTLE MM, CASALE TB. Azelastine inhibits IgE-mediated human basophil histamine release. *J Allergy Clin Immunol* 1989; 83: 862-5.
19. MATSUMOTO Y. Neutrophil releasability of leukotriene B₄, C₄ and platelet activating factor in patients with bronchial asthma and inhibitory effect of azelastine hydrochloride. *Yokohama Igaku* 1989; 40: 77-88.
20. MCTAVISH D, SORKIN EM. Azelastine: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential. *Drugs* 1989; 38: 778-800.
21. MEILL EA, DENMEAD D, GRAF P, GOLD WM, FRICK OL. Azelastine aerosol attenuates late-phase bronchial hyperresponsiveness in atopic dogs. *J Allergy Clin Immunol* 1990; 85: 198.
22. METZGER WJ, ALI S, MUSTAFA SJ. Inhibition of allergen-induced airway obstruction and airway hyperresponsiveness in an allergic rabbit model by azelastine. *J Allergy Clin Immunol* 1991; 87: 312.
23. NAKAMURA T, NISHIZAWA Y, SATO T, YAMATO C. Effect of azelastine on the intracellular Ca²⁺ mobilization in guinea pig peritoneal macrophages. *Eur J Pharmacol* 1988; 148: 35-41.
24. RAFFERTY P, HOLGATE ST. Azelastine. In: BARNES PJ, THOMSON R, THOMSON NC, eds. *Asthma. Basic mechanisms and clinical management*. New York: Academic Press, 1988: 638.
25. RAMIREZ O, ZAYAS JG, APP EM, RUBIN BK, KING M. Tracheal mucus rheology and clearability in a canine model of asthma: effect of azelastine. *J Allergy Clin Immunol* 1990; 85: A288.
26. RICHARDS IS, MILLER L, SOLOMAN D, KULKARNI A, BROOKS S, SPERELAKIS N. Azelastine and desmethyldazastine suppress acetylcholine-induced contraction and depolarization in human airway smooth muscle. *Eur J Pharmacol* 1990; 186: 331-4.
27. SHINDOH J, SUGIYAMA S, TAKAGI K, SATAKE T, OZAWA T. Laboratory and animal investigations: recovery time course of airway hyperresponsiveness to acetylcholine after ovalbumin challenge in guinea pigs. *Chest* 1991; 99: 1274-9.
28. TATSUMI K, OU T, YAMADA H, YOSHIMURA H. Studies on the metabolic fate of a new antiallergic agent, azelastine (4-(p-chlorobenzyl)-2-[N-methylperhydroazepnyl-(4)]-1-(2H)-phthalazinone hydrochloride). *Jpn J Pharmacol* 1980; 30: 37-48.
29. VARGAFTIG BB. PAF and allergy. *Eur J Pharmacol* 1990; 183: 73-4.
30. WERNER U, SCHMIDT J, SZELENYI I. Influence of azelastine on IL-1 β generation *in vitro* and IL-1 β -induced effect *in vivo*. *Agents Actions* 1991; 32: 243-7.
31. WONG KK, YANG JT, KUCHARCZYK N, SOFIA RD. Tissue distribution and metabolism of [¹⁴C]-azelastine in guinea pigs. Third North American ISSX Meeting, A20, October 21-25, 1990.
32. ZECHEL HJ, BROCK N, LENKE D, ACHTERRATH-TUCKERMANN U. Pharmacological and toxicological properties of azelastine, a novel antiallergic agent. *Arzneimittelforschung* 1981; 31: 1184-93.

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