Agents Actions 38 (1993)

0065-4299/93/040165-06 $ 1.50+0.20/0

Mucolytic activity of azelastine in mice and rats

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Abstract

The mucolytic activity of azelastine, an antiallergic/antiasthmatic drug, in mice and rats was investigated. The oral administration of test compounds 30 min before phenol red i.p. injection stimulated dye secretion in the trachea of mice. The resulting oral ED_{50}'s (mg/kg) were: azelastine, 0.16; salbutamol, 2.5; *N*-acetylcysteine, 61.8; *S*-Carboxymethyl-*l*-cysteine, < 100; bromhexine, > 100; and potassium iodide, ~ 200. In rats, several drugs stimulated secretion of fluorescein sodium (FINa) in the tracheobronchial lumen. The resulting oral ED_{50}'s (mg/kg) were: azelastine, 0.33; terbutaline, 0.3; salbutamol, 0.89; and *S*-carboxymethyl-*l*-cysteine, 56.8. Terfenadine and diphenhydramine (1–10 mg/kg, p.o.) did not stimulate tracheal secretion in rats and mice. The mucolytic activity of azelastine may contribute to its overall effectiveness, including antitussive activity in asthmatics. Finally, this activity seems to be dissociated from its H_1-histamine receptor blocking activity.

Introduction

Azelastine has been reported to produce bronchodilation, to inhibit early and late asthmatic responses and exercise-induced asthma, and to reduce the use of β_2-bronchodilators and theophylline in asthmatics [1–3]. In animal studies, it has been demonstrated to inhibit immediate allergic responses in the lungs of rats [4], guinea pigs [5–7], rabbits [8], and dogs [9]. Furthermore, azelastine also inhibits late asthmatic responses in rabbits [8], allergic bronchial eosinophilia in guinea pigs [10], and bronchial hyperresponsiveness in guinea pigs [11], rabbits [8], and dogs [9]. Azelastine also exerts anti-inflammatory [10, 15, 16] and immunomodulatory [12–14] activities.

Excessive respiratory secretions and mucus plugs are obvious problems and may contribute to the pathogenesis and mortalities in asthma and in chronic obstructive respiratory diseases [17, 18]. Azelastine lowers the viscosity of tenacious mucus during late-phase asthmatic responses in guinea pigs [19]. It also inhibits aeroallergen-induced decline in tracheal mucus viscoelasticity and improves mucus clearance by coughing and ciliary action in Ascaris-sensitive dogs [20]. In this study, the mucolytic activity of azelastine was evaluated and compared with known mucolytic agents (*N*-acetylcysteine, *S*-carboxymethyl-*l*-cysteine and bromhexine), selected histamine H_1-receptor antagonists and β_2-bronchodilators in mice and rats.

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Methods

Materials

The following drugs and chemicals were used in this study: azelastine hydrochloride (Wallace Labora-

tories, Cranbury, NJ), terbutaline sulphate (Astra Pharmaceutical,Worcester, MA), salbutamol sulphate, phenol red, gum arabic, potassium iodide, bromhexine HCl, *N*-acetylcysteine, fluorescein sodium salt, and *S*-carboxymethyl-*l*-cysteine (Sigma Chemical Co., St. Louis, MO), sodium hydroxide (Matheson Coleman & Bell, Norwood, OH), terbutaline sulfate (ASTA Pharm. Products, Worcester, MA), terfenadine (Merrell-Dow Res. Center, Cincinnati, OH), and diphenhydramine (Parke-Davis, Detroit, MI).

Evaluation of mucolytic activity in mice

Tracheal secretion in mice, expressed by the amount of phenol red dye secreted into the lumen of the trachea, was measured according to the method of Engler and Szelenyi [21]. Male mice were randomly placed into appropriate treatment groups. Each compound was tested at a minimum of three different doses. Each dose was tested in at least five animals. Drugs or vehicle (1% acacia) were administered orally (0.1 ml/10 g body weight) 30 min prior to intraperitoneal injection of phenol red (500 mg/kg; 5% solution in saline, 0.1 ml/10 g body weight). Thirty minutes after phenol red injection, the animals were sacrificed by exposure to CO_2. The whole trachea was carefully excised and washed in 1.0 ml saline for a period of 30 min. Subsequently, 0.1 ml of 1 *M* NaOH was added to the tracheal washings to stabilize the pH of the lavage fluid. The amount of phenol red was measured photometrically at 546 nm in a Model 690 Sequoia-Turner spectrophotometer or Beckman Model 25 spectrophotometer.

The amount of phenol red secreted in the tracheal lumen, expressed in μg/10 g body weight/h, was determined by the formula of Graziani and Cazzulani [22] :

$$S = s\frac{V}{W} \times 10$$

where

S = phenol red secreted per 10 g of body weight per hour (μg/10 g/h),
s = concentration of the phenol red contained in 1.0 ml of tracheal lavage as determined from the standard curve of phenol red (0.5, 1, 5, and 10 μg/ml),
V = volume of the tracheal lavage (1.0 ml),
W = body weight of the mice expressed in grams.

The percentage enhancement in tracheal secretion of phenol red by each dose of a drug was determined by using the following formula:

$$\text{percentage enhancement} = \frac{T-C}{C} \times 100$$

where

T = tracheal secretion of phenol red (μg/10 g/h) in an individual drug-treated mouse each day,
C = mean tracheal secretion of phenol red (μg/10 g/h) in vehicle-treated group.

Evaluation of mucolytic activity in rats

Tracheobronchial secretion in rats, expressed by the amount of fluorescein sodium (FINa) dye excreted into the respiratory tract, was measured by modification of methods published earlier [23, 24]. Azelastine, terfenadine and *S*-Carboxymethyl-*l*-cysteine were suspended in 1% acacia, and salbutamol, terbutaline, and diphenhydramine were dissolved in distilled water. The drugs were administered orally (1 ml/100 g body weight) 30 min before intravenous injection of FINa (300 mg/kg, 0.1 ml/100 g body weight) in fasted rats. One hour after drug administration, rats were sacrificed with CO_2 exposure; an incision was made and the trachea was immediately exposed. An infant feeding tube was gently inserted into the trachea (about 30 mm deep). Five milliliters of 5% $NaHCO_3$ solution (37 °C) was injected into the respiratory tract and immediately aspirated while gently massaging the thoracic area. The tracheobronchial lavage fluid (4.9 ± 0.1 ml) was centrifuged (3500 × *g* for 10 min) and the supernatant was transferred to STP B & L tubes. The fluorescence intensity of the supernatant was measured with a Sequoia-Turner spectrophotometer (Model 690) set at a wavelength of 510 nm (emission).

The effect of the drugs on the tracheobronchial secretion of FINa was determined by the following formula [22]:

$$S = s\frac{V}{W} \times 100$$

where

S = FINa secreted per 100 g of body weight (μg/100 g/h),
s = intensity of emission by the FINa contained in 4.9 ± 0.1 ml of tracheobronchial lavage fluid as

determined from the standard curve of FINa (0.3, 3, 30 and 300 μg/ml),
V = volume of the tracheobronchial lavage fluid (4.9 ± 0.1 ml),
W = body weight of the rats expressed in grams.

The results were expressed as mean ± SEM. Statistical significance was evaluated using the t-test for independent means, where $p < 0.05$ is considered to be significant as compared to the appropriate vehicle "control" group.
The percentage enhancement of the tracheobronchial secretion of FINa by each dose of a drug was determined by the following formula:

$$\text{percentage enhancement} = \frac{T - C}{C} \times 100$$

where

T = the amount of FINa excreted in the respiratory tract (μg/100 g/h) in an individual drug-treated animal.
C = the mean amount of FINa excreted in the respiratory tract (μg/100 g/h) in vehicle-treated group.

The line of best fit was calculated from the dose–response curve of each drug and the ED_{50} (mg/kg), i. e. the dose enhancing tracheal secretion of dye by 50% over the control level, was determined and 95% confidence limits were also calculated.

Results

Mucolytic effects in mice

Azelastine (0.03–1 mg/kg), salbutamol, *N*-acetylcysteine, bromhexine, and potassium iodide stimulated the secretion of phenol red dye in the trachea of mice. The resulting oral ED_{50}'s (mg/kg p.o., 30 min) were as follows: azelastine 0.16; salbutamol, 2.5; *N*-acetylcysteine, 61.8; and potassium iodide, ~200. Bromhexine and *S*-carboxymethyl-*l*-cysteine (100 mg/kg p.o., 30 min) significantly stimulated tracheal secretion, whereas terfenadine (0.3–10 mg/kg) and diphenhydramine (1–10 mg/kg p.o., 30 min) did not influence phenol red secretion in mice (Table 1).

Mucolytic effects in rats

Azelastine, salbutamol, terbutaline and *S*-carboxymethyl-*l*-cysteine exerted dose-dependent augmentation of tracheobronchial secretion of FINa. The oral ED_{50}'s (mg/kg p.o.) were as follows: azelastine, 0.33; terbutaline, 0.3, salbutamol, 0.89; and *S*-carboxymethyl-*l* -cysteine, 56.8. Terfenadine and diphenhydramine (1 and 10 mg/kg) did not exert any significant effect on tracheobronchial secretion of FINa (Table 2).

Discussion

The data obtained in this study showed that azelastine stimulated tracheal secretion of fluorescein sodium in rats and of phenol red in mice. These effects are characteristic of mucolytic activity. Terfenadine (a new H_1-receptor antagonist) and diphenhydramine (a traditional H_1-histamine receptor blocker) did not exert any mucolytic activity in mice and rats. Thus, mucolytic activity of azelastine, a drug preferentially distributed to the lungs [5], is probably related to properties other than H_1-histamine receptor blockade in the airways. Furthermore, the long-lasting antiallergic (anti-PCA) activity [25], the inhibition of superoxide generation (O_2^-) in human neutrophils and eosinophils [26], nonspecific anti-inflammatory activities in mice [15], and inhibition of bronchial hyperreactivity in rabbits [8] have all been shown to be dissociated from its antihistaminic activities.
In rats and mice, azelastine stimulates serous secretion in the airway (this study). In guinea-pig asthma models, it normalized allergen-induced increase in microviscosity by increasing phospholipid content and by reducing protein content and the protein to phospholipid ratio [19]. It stimulates mucociliary clearance in normal rabbits [27].
Orally administered azelastine (3 mg/kg) prevented aeroallergen-induced decrease in airway transepithelial potential difference, antigen-elevated viscoelasticity of tracheal mucus and improved mucus clearability by ciliary and cough mechanisms in ascaris-sensitive dogs. Azelastine also stabilizes canine tracheal epithelium [20].
Leukotrienes have been proposed as important mediators of defective mucociliary clearance mechanisms. Therefore, the ability of azelastine to inhibit leukotriene generation [28] may be responsible for the improvement in mucociliary clearance in atopic dogs, and for the mucolytic activity in guinea-pig asthma model.
A functional defect in the tracheobronchial secretory function and in the mucociliary clearance mechanism may play an important role in the morbidity and mortality of chronic obstructive

Table 1
Effect of azelastine, salbutamol, potassium iodide, bromhexine, *N*-acetylcysteine, *S*-carboxymethyl-*l*-cysteine, terfenadine, and diphenhydramine on phenol red secretion in the trachea of mice.

Drug	Dose (mg/kg) p.o.	Phenol red secretion (μg/10 g/h)		Percentage enhancement	ED^{a}_{50} (mg/kg) (95% C.L)
		Control	Treated		
Azelastine	0.03		0.77 ± 0.12 (6)	25.5 ± 14.9	
	0.1		0.80 ± 0.16 (6)	41.4 ± 21.3	0.16
	0.3	0.62 ± 0.03 (38)	1.10 ± 0.2* (11)	81.2 ± 22.7	(0.02–1.2)
	1.0		0.93 ± 0.1* (11)	54.5 ± 18.4	
Salbutamol	1.0		0.62 ± 0.07 (8)	11.7 ± 6.4	
	3.0		0.87 ± 0.07 (11)	40.6 ± 11.1	2.5
	10.0		1.40 ± 0.2* (8)	133.1 ± 36.2	(1.6–4.0)
Potassium iodide	50.0	0.64 ± 0.40 (12)	0.67 ± 0.07 (11)	5.1 ± 11.2	
	100.0	0.99 ± 0.10 (11)	1.47 ± 0.15* (11)	45.8 ± 14.2	~200
	200.0	0.64 ± 0.40 (12)	0.96 ± 0.10* (11)	49.1 ± 12.2	
Bromhexine	3.0	0.99 ± 0.10 (11)	1.20 ± 0.13 (11)	20.9 ± 12.9	
	30.0	0.71 ± 0.08 (7)	0.67 ± 0.10 (7)	0 ± 0	>100
	100.0	0.64 ± 0.04 (12)	0.90 ± 0.10* (10)	40.6 ± 17.0	
N-acetylcysteine	10.0	0.71 ± 0.08 (7)	0.66 ± 0.06 (8)	2.3 ± 13.2	
	50.0	0.71 ± 0.08 (7)	1.00 ± 0.08* (7)	51.6 ± 10.6	61.8
	200.0	0.74 ± 0.08 (14)	1.30 ± 0.12* (11)	75.7 ± 16.5	(29.3–130.1)
S-carboxymethyl-l-cysteine	50.0	0.65 ± 0.06 (26)	0.62 ± 0.1 (6)	16.2 ± 11.5	
	75.0		0.56 ± 0.08 (10)	7.8 ± 4.7	<100
	100.0		1.50 ± 0.2* (13)	125.2 ± 33.07	
Terfenadine	0.3		0.54 ± 0.01 (6)	0	
	1.0		0.54 ± 0.03 (5)	0	
	3.0	0.64 ± 0.04 (12)	0.71 ± 0.05 (18)	11	Inactive
	10.0		0.66 ± 0.06 (5)	10	
Diphenhydramine	1.0		0.55 ± 0.02 (5)	0	
	3.0		0.70 ± 0.01 (15)	14	Inactive
	10.0		0.54 ± 0.1 (5)	0	

* $P < 0.05$ as compared to control.
[a] Dose of a drug enhancing tracheal secretion by 50% over the control.

Table 2
Effect of azelastine, salbutamol, terbutaline, terfenadine, diphenhydramine and *S*-carboxymethyl-*l*-cysteine on fluorescein sodium secretion in the tracheobronchial lumen of rats.

Drug	Dose (mg/kg) p.o.	Fluorescein sodium secretion (μg/100 g/h)	Percentage enhancement	ED^{a}_{50} (mg/kg) (95% C.L)
Control	0	1.5 ± 0.07 (25)		
Azelastine	0.1	1.7 ± 0.2 (4)	11.7 ± 11.7	
	0.3	2.0 ± 0.4 (4)	33.3 ± 26.8	0.33
	1.0	2.9 ± 0.6* (6)	97.7 ± 38.1	(0.12–0.94)
Salbutamol	1.0	2.1 ± 0.3 (6)	41.2 ± 19.4	
	3.0	3.1 ± 0.4* (5)	104.0 ± 26.2	0.89
	10.0	3.0 ± 0.6* (6)	105.7 ± 38.2	(0.09–8.4)
Terbutaline	0.1	1.7 ± 0.1 (5)	14.7 ± 6.8	
	0.3	1.7 ± 0.3 (15)	26.7 ± 14.8	0.3
	1.0	3.1 ± 0.7* (6)	112.0 ± 43.2	(0.14–0.66)
Terfenadine	1.0	1.6 ± 0.1 (5)	6.7 ± 6.7	
	10.0	2.1 ± 0.3 (5)	38.6 ± 20.7	>10
Diphenhydramine	1.0	1.6 ± 0.3 (5)	20.0 ± 12.7	
	10.0	2.1 ± 0.3 (4)	11.7 ± 6.8	>10
Control	0	1.3 ± 0.7 (21)		
S-carboxymethyl-	10.0	1.3 ± 0.1 (12)	10.8 ± 5.5	
-*l*-cysteine	30.0	1.9 ± 0.2* (11)	46.1 ± 11.3	
	50.0	1.8 ± 0.1* (12)	40.9 ± 14.8	56.8
	100.0	2.1 ± 0.2* (11)	59.4 ± 15.1	(13.9–234.4)
	300.0	2.4 ± 0.4* (9)	87.1 ± 27.1	

*$P < 0.05$ as compared to respective controls.
[a] Dose of a drug enhancing tracheal secretion by 50% over the control.
Numbers in a parentheses indicate the number of observations.

lung diseases. In fact, viscous mucus secretions may be responsible for part of the airway obstruction [17, 18]. The precise mechanism for the mucolytic activity of azelastine observed in mice and rats (this study), in rabbits [27], guinea pigs [19], and dog [20] is not yet known. However, it is possible that azelastine may stimulate serous gland secretions which are rich in lysozyme, an important enzyme for the liquefaction of mucus [15]. The mucolytic activity of azelastine and stabilization of epithelium may explain its antitussive effects observed in asthmatics [29].

Received 5 October 1992; accepted by A. J. Lewis,
1 December 1992

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