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Effect of azelastine on bronchoconstriction induced by histamine and leukotriene C4 in patients with extrinsic asthma

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ABSTRACT Azelastine, a new oral agent with antiallergic and antihistamine properties, has been shown to inhibit the effect of histamine and leukotriene (LT) in vitro, though not a specific leukotriene receptor antagonist. The effect of both a single dose (8·8 mg) and 14 days' treatment (8·8 mg twice daily) with azelastine on bronchoconstriction induced by LTC_4 and histamine has been examined in 10 patients with mild asthma in a placebo controlled, double blind, crossover study. LTC_4 and histamine were inhaled in doubling concentrations from a dosimeter and the results expressed as the cumulative dose (PD) producing a 20% fall in FEV_1 ($PD_{20}FEV_1$) and 35% fall in specific airways conductance (PD_{35}sGaw). The single dose of azelastine produced a significantly greater FEV_1 and sGaw values than placebo at 3 hours, but this bronchodilator effect was not present after 14 days of treatment. Azelastine was an effective H_1 antagonist; after a single dose and 14 days' treatment with placebo the geometric mean $PD_{20}FEV_1$ histamine values (μmol) were 0·52 (95% confidence interval 0·14–1·83) and 0·54 (0·12–2·38), compared with 22·9 (11·5–38·3) and 15·2 (6·47–35·6) after azelastine ($p < 0·01$ for both). LTC_4 was on average 1000 times more potent than histamine in inducing bronchoconstriction. Azelastine did not inhibit the effect of inhaled LTC_4; the geometric mean $PD_{20}FEV_1$ LTC_4 (nmol) after a single dose and 14 days' treatment was 0·60 and 0·59 with placebo compared with 0·65 and 0·75 with azelastine. The PD_{35}sGaw LTC_4 was also unchanged at 0·66 and 0·73 for placebo compared with 0·83 and 0·74 for azelastine. Thus prolonged blockade of H_1 receptors did not attenuate the response to LTC_4, suggesting that histamine and LTC_4 act on bronchial smooth muscle through different receptors. Four patients complained of drowsiness while taking azelastine but only one who was taking placebo and three patients complained of a bitter, metallic taste while taking azelastine.

Airway hyperresponsiveness to specific and non-specific stimuli is characteristic of bronchial asthma, though the mechanisms are unclear. It has been suggested that the sulphidopeptide leukotrienes (LT), derived from membrane arachidonic acid, may play a part in airway hyperresponsiveness in asthmatic patients.[1–5] LTC_4 and LTD_4 are released in vitro and in vivo after allergen challenge[6,7] and both are extremely potent bronchoconstrictors in man. Inhaled LTE_4 has been reported to enhance airway responsiveness to inhaled histamine in patients with asthma.[8]

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Azelastine hydrochloride is a phthalazione derivative (4-(*p*-chlorobenzyl)-2-(hexahydro-1H-azepin-4yl)-(2H) phthalazione) with prolonged antiallergic and antihistamine activity after oral administration.[9–11] It inhibits release of mediators from mast cells in response to antigen, calcium ionophore, concanavalin A, and compound 48/80 and in this respect is from 100 to 1000 times more potent than sodium cromoglycate, theophylline, ketotifen, astemizole, and verapamil.[10] It also inhibits the synthesis and release of leukotrienes from the rat peritoneal mast cell and is reported to modify leukotriene induced bronchoconstriction in guinea pigs.[11,12] Recently azelastine has been shown to attenuate the early bronchoconstrictor response to allergen in asthmatic patients.[13] The effect of azelastine on leukotriene induced bronchoconstriction in patients with asthma has not been studied

previously. We have examined the effect of a single dose (8·8 mg) and of two weeks' treatment (8·8 mg twice daily) with azelastine on the resting bronchomotor tone and histamine and LTC_4 induced bronchoconstriction in patients with mild extrinsic asthma in a double blind, placebo controlled, cross-over study.

Methods

We studied 10 patients (five of them women), mean age 32 (range 22–40) years, with mild extrinsic asthma and positive responses to skinprick tests with common inhaled allergens. None of the patients was taking oral corticosteroids, theophyllines, sodium cromoglycate, or antihistamine or anticholinergic drugs. Inhaled β agonists were stopped at least 12 hours before the test. The study was approved by the hospital ethics committee and informed written consent was obtained from each subject.

Patients received either 8·8 mg of azelastine twice daily or identical placebo for 14 days with a washout period of 14 days between treatments. Treatment was given double blind and in random order. The full blood count and serum urea and electrolyte concentrations were determined and liver function tests were carried out on their entry to the study and at the end of each treatment period. Airway response was assessed by measuring FEV_1 with a dry wedge spirometer (Vitalograph, Buckingham) and specific airways conductance (sGaw) with a constant volume body plethysmograph (Fenyves and Gut, Basel, Switzerland). The best of three attempts was recorded for FEV_1 and the mean of eight satisfactory manoeuvres for sGaw by on line data acquisition.[14]

Histamine inhalation challenge was carried out in seven patients three hours after medication on the first and the 14th day of each treatment period. After pretreatment and post-treatment baseline FEV_1 had been recorded patients inhaled 10 breaths of phosphate buffered saline (control) from a Mefar 120 nebuliser (Mefar, Elettromedicali, Brescia, Italy) with a dosimeter set at a constant delivery time (1·0 s) and pressure (25 lb/in^2, 172 kPa). The patient breathed from functional residual capacity to total lung capacity with a breath hold time of three seconds between inhalations. Patients proceeded to histamine challenge if the change in FEV_1 after inhalation of buffered saline was less than 5%. Each subject inhaled 10 breaths of histamine diphosphate dissolved in phosphate buffered saline in doubling concentrations (from 0·018 to 39·4 μmol) until the FEV_1 had fallen more than 20% below the lowest FEV_1 value after inhalation of buffered saline (control). The results, expressed as the cumulative dose producing a 20% fall in the FEV_1 ($PD_{20}FEV_1$), were obtained from the log dose-response curves.

LTC_4 challenge was performed one hour after the histamine inhalation challenge (four hours after treatment) and when FEV_1 readings had returned to within 5% of post-treatment baseline values. LTC_4 (Miles Laboratories, Slough) was stored at -70°C in sealed ampoules until it was used, and appropriate dilution was made freshly with phosphate buffered saline (pH 7·4). The dilutions were kept in ice until immediately before they were placed in the nebuliser. LTC_4 was inhaled in doubling concentrations (from 0·025 to 3·2 nmol), and FEV_1 and sGaw were measured five, seven, 10, 15, and 20 minutes later.

$PD_{20}FEV_1$ and PD_{35}sGaw (cumulative dose producing a 35% fall in sGaw) were obtained from log dose-response curves. The changes in FEV_1 and sGaw at each time after placebo and after azelastine were compared by analysis of variance. Log $PD_{20}FEV_1$ and PD_{35}sGaw were compared by analysis of variance and Student's t test.

Results

The subjects' mean (SEM) FEV_1 was 87% (3·3%) predicted on entry to the study. There was no significant difference in the mean pretreatment baseline FEV_1 and sGaw values on the four study days. FEV_1 and sGaw were unchanged after a single dose and after 14 days' treatment with placebo (table). After the single dose of azelastine mean FEV_1 was 9·6% greater than after placebo (2·91 *v* 2·61 l) and mean sGaw 20% greater (1·4 *v* 0·95 s^{-1} kPa^{-1}) at 3 hours, both changes being significant ($p < 0·05$). After 14 days' azelastine, however, there was no significant difference in mean FEV_1 and sGaw values before and after azelastine. The drug was a potent H_1 receptor antagonist in the airways (table, fig 1). After a single dose and 14 days' treatment with placebo the geometric mean $PD_{20}FEV_1$ (95% confidence interval) for histamine was 0·52 (0·14–1·83) and 0·54 (0·12–2·38) μmol. After a single dose of azelastine the geometric mean $PD_{20}FEV_1$ was 22·9 (11·55–38·30) μmol, a 45 fold increase over placebo values ($p < 0·01$). After 14 days' treatment with azelastine the geometric mean $PD_{20}FEV_1$ was 15·2 μmol (6·47–35·6), a 28 fold increase over placebo values ($p < 0·01$). The difference in inhibition after a single dose and 14 days' treatment was not significant.

In seven patients who underwent LTC_4 and histamine challenges, LTC_4 was about 1000 times more potent than histamine. Azelastine had no effect on LTC_4 induced bronchoconstriction (table, fig 2). The geometric mean $PD_{20}FEV_1$ for LTC_4 was 0·60 (95% confidence interval 0·19–1·97) and 0·59 (0·22–1·54) nmol after a single dose and 14 days' treatment with placebo respectively, compared with 0·65 (0·25–1·70) and 0·75 (0·46–1·22) nmol after a single dose and 14

Effect of a single dose and of 14 days' treatment with azelastine and placebo on baseline respiratory function and the responses to inhalation challenge in patients with asthma

	Placebo		*Azelastine*	
	Day 1	*Day 14*	*Day 1*	*Day 14*
Baseline respiratory function (mean (SEM))				
FEV_1 (l): n = 10				
Before drug	2·79 (0·16)	2·70 (0·16)	2·61 (0·18)	2·71 (0·16)
After drug	2·75 (0·17)	2·76 (0·14)	2·91 (0·21)	2·79 (0·14)
p value	NS	NS	<0·05	NS
sGaw (s^{-1} kPa^{-1}): n = 10				
Before drug	0·81 (0·13)	0·84 (0·10)	0·95 (0·16)	0·93 (0·13)
After drug	0·85 (0·16)	0·86 (0·14)	1·14 (0·16)	0·97 (0·16)
p value	NS	NS	<0·05	NS
Responses to inhalation challenges (geometric mean (95% confidence interval))				
LTC_4 (nmol): n = 10				
$PD_{20}FEV_1$	0·60 (0·19 to 1·97)	0·59 (0·22 to 1·54)	0·65 (0·25 to 1·70)	0·75 (0·46 to 1·22)
PD_{35}sGaw	0·66 (0·27 to 1·65)	0·73 (0·39 to 1·40)	0·83 (0·32 to 2·14)	0·74 (0·42 to 1·33)
p value*			NS	NS
Histamine (μmol): n = 7:				
$PD_{20}FEV_1$	0·52 (0·14 to 1·83)	0·54 (0·12 to 2·38)	22·9 (11·55 to 38·31)	15·2 (6·47 to 35·6)
p value*			<0·01	<0·01

*In the comparison with placebo.
sGaw, specific airways conductance; LTC_4, leukotriene C_4; $PD_{20}FEV_1$, provocative dose causing a 20% fall in FEV_1; PD_{35}, provocative dose causing a 35% fall in sGaw.

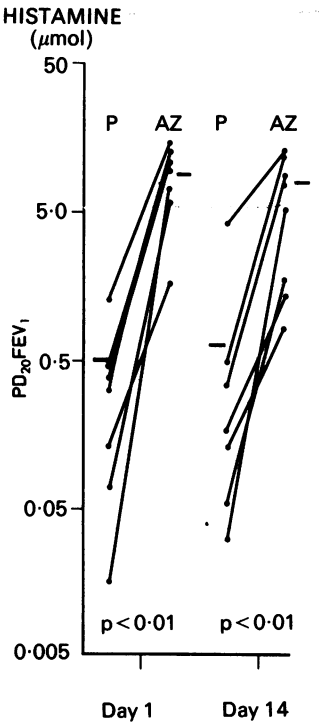

Fig 1 *Individual and geometric mean (—) $PD_{20}FEV_1$ histamine values after treatment with placebo (P) and azelastine (AZ). $PD_{20}FEV_1$—provocative dose causing a 20% fall in FEV_1.*

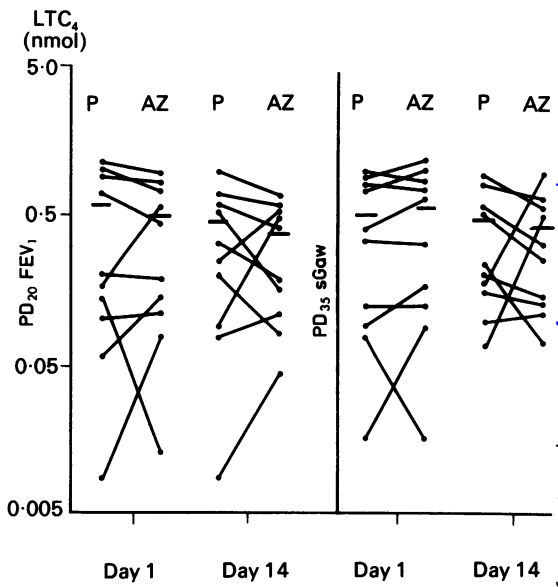

Fig 2 *Individual and geometric mean (—) $PD_{20}FEV_1$ and PD_{35}sGaw leukotriene C_4 values after treatment with placebo (P) and azelastine (AZ). $PD_{20}FEV_1$—provocative dose causing a 20% fall in FEV_1; PD_{35}sGaw—provocative dose causing a 35% fall in specific airways conductance.*

days' treatment with azelastine. There was no significant period effect (analysis of variance).

Four patients taking azelastine complained of drowsiness and three patients complained of a bitter

metallic taste. One patient taking placebo noted drowsiness. There were no significant changes in haematological and biochemical indices after azelastine treatment.

Discussion

Azelastine in a single dose produced a small but significant increase in FEV_1 and sGaw in the patients in this study. After 14 days' of treatment, however, this bronchodilator effect was attenuated and no significant difference between azelastine and placebo was observed. Our results contrast with the observations of Ollier *et al*,[13] who failed to show any change in mean FEV_1 after single or multiple dose treatment with azelastine; but these workers did show a significant increase in mean sGaw after a single dose of azelastine, and this increase in sGaw was present at three weeks. The difference between our results and those of Ollier *et al*[13] may be related to differences in doses (2·2 mg and 4·4 mg compared with 8·8 mg) and also patient selection. Azelastine is a potent H_1 receptor antagonist and its bronchodilator effect is likely to be due to its airway H_1 receptor blockade. A similar degree of bronchoconstriction has been observed with other H_1 receptor antagonists, such as clemastine, chlorpheniramine, and terfenadine.[15–17] In addition, ketotifen, an antiallergic compound with potent H_1 receptor blocking activity, also produces a small but important amount of bronchodilatation when inhaled.[18] Azelastine shifted the histamine dose-response ($PD_{20}FEV_1$) curve 45 fold to the right after a single dose and 28 fold after 14 days of treatment. Although the mean inhibition of histamine induced bronchoconstriction by azelastine was higher after a single dose than after 14 days of treatment, the difference was not significant. This large effect of azelastine on histamine induced bronchoconstriction confirms that azelastine is a very effective H_1 receptor in blocking activity in human airways.

In contrast to the findings with histamine, the bronchoconstrictor response to LTC_4 was not altered by either a single dose or 14 days' treatment with azelastine. In the present study LTC_4 was about 1000 times more potent than histamine, and this observation is consistent with previous reports.[3 8]

The mechanism of histamine hyperresponsiveness is unclear. Histamine acts on bronchial smooth muscle by interaction with at least two distinct receptors, H_1 and H_2 receptors, and it also increases the rate of firing of bronchial irritant receptors, an effect that can be blocked by atropine.[19] Human airway smooth muscle contracts in vitro in response to histamine, but when H_1 receptors are blocked histamine produces relaxation, an effect attributed to H_2 receptor stimulation as it can be blocked by the H_2 antagonist metiamide.[20 21] Terfenadine and astemizole are specific H_1 receptor antagonists and lack anticholinergic and antiserotonin activity. These drugs have been shown to modify exercise[22 23] and allergen induced bronchoconstriction[24] in patients with asthma. In addition to H_1 receptor antagonism, many antihistamines at high concentrations have the capacity in vitro to stabilise mast cells and ketotifen falls into this class.[24] Ketotifen, however, offers no greater protection against the immediate response to inhaled antigen than can be attributed to its capacity to block histamine.[24]

Astemizole has also been reported to attenuate the early component (2–15 min) of the bronchoconstrictor response to antigen challenge.[25] The protective effect of azelastine in the immediate asthmatic response to allergen inhalation reported by Ollier *et al*[13] can also be explained by its potent H_1 receptor blocking activity.

The time course of the bronchoconstrictor response to leukotrienes and histamine differ in vivo and in vitro in man: leukotrienes have a slow onset of action, which is more prolonged and persistent than that of histamine. After histamine the peak response is reached within 4–8 minutes of inhalation whereas with leukotrienes the response is slower, reaching a peak at 20 minutes.[2 26 27] Recently Arm *et al*[8] have shown that inhaled LTE_4 can enhance histamine responsiveness in asthmatic patients but not in normal subjects. Holroyde *et al*[27] and Barnes *et al*,[28] using the specific leukotriene antagonists FPL 55712, FPL 59257, and L 49923, have shown that the drugs will effectively inhibit LTC_4 and LTD_4 mediated airway responses without modifying histamine responsiveness in normal subjects. H_1 receptor blocking drug[11] did not inhibit leukotriene induced bronchoconstriction and our results with azelastine in this respect are consistent with these observations. Leukotrienes and histamine act independently on the bronchial smooth muscle through specific receptors and studies in animal lung tissues have identified a site specific for LTC_4 and LTD_4.[29] It has been suggested that there may be heterogeneity of leukotriene receptors in view of the very different molar ratios of LTC_4, LTD_4 and LTE_4 required to elicit identical biological effects in different tissues,[30 31] and because the rank order of potency for the leukotrienes in contracting guinea pig tracheal spirals differs from that for contraction of parenchymal strips.[32 33] Drugs may vary in their ability to block responses according to their different receptor affinities.[34] FPL 55712 was found to have a higher affinity for the LTD_4 receptor, which is consistent with its more effective antagonism of the LTD_4 induced contractile response of lung parenchymal strips.[35] The differences between the effects in animals and in patients with asthma of azelastine,[11] sodium cromoglycate, and the calcium channel blocker

verapamil[36] on leukotriene induced bronchoconstriction may be related to species differences and to the lack of a good animal model that can mimic human asthma. Further studies are required to elucidate the role of azelastine and similar compounds in asthma.

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