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**Effect of azelastine, montelukast, and their combination on
allergen-induced bronchoconstriction in asthma**

*Kai Richter MD¹, Lars Grönke MD¹, Stanislaw Janicki¹,
Joachim Maus MD², Rudolf A. Jörres PhD³, Helgo Magnussen MD¹*

¹Pulmonary Research Institute at Hospital Großhansdorf, Germany

²MEDA Pharma GmbH & Co. KG, Bad Homburg, Germany

³Occupational and Environmental Medicine, LMU, Munich, Germany

Running head: Azelastine, montelukast and allergen responses

2nd revised version

Correspondence to:

Rudolf A. Jörres, PhD

Institute and Outpatient Clinic for Occupational and Environmental Medicine

Ludwig-Maximilians-University Munich

Ziemssenstr. 1

D-80336 Munich

Germany

Telephone: +49 89 5160-2466 or -2436

Facsimile: +49 89 5160-3957 or -4445

e-mail: rudolf.joerres@med.uni-muenchen.de

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ABSTRACT

Objectives: Histamine and cysteinyl leukotrienes play an important role in early (EAR) and late (LAR) allergen reactions. Although protection by anti-histamines and anti-leukotrienes has been studied extensively, little is known about the effect of their combination. We, therefore, assessed the effect of clinically recommended doses of azelastine and montelukast alone and in combination on EAR and LAR.

Methods: Seventeen patients (mean age 31y, 14m/3f) with asthma and proven EAR and LAR received an oral dose of 4 mg azelastine twice daily, or 10 mg montelukast once daily, or both for one week, in a double-blind, double-dummy, cross-over fashion. FEV₁ was measured after single-dose allergen challenges during EAR (0-2 hours) and LAR (2-9 hours).

Results: Azelastine, montelukast and their combination protected against both EAR and LAR ($p < 0.004$, each) by 46 % and 43 %, 76 % and 59 %, and 89 % and 78 %, respectively. Azelastine was not as effective during EAR but equally effective to montelukast during LAR. The combination was superior to each drug alone during both EAR and LAR ($p < 0.05$, each).

Conclusion: The combination of azelastine and montelukast in clinically recommended doses has a greater effect in suppressing early and late allergen reactions than each drug alone.

(Word Count of Abstract: 200)

Key words: Anti-histamine, leukotriene receptor antagonist, bronchial allergen response, early phase response, late phase response, bronchoprotection

INTRODUCTION

In patients with allergic asthma the lung function response to an inhaled allergen often shows a typical time course comprising an early allergen reaction (EAR) occurring within 30 minutes, a late allergen reaction (LAR) delayed by several hours, and often both, i.e. a dual reaction [1,2]. These responses are quite common, with a prevalence of 9-53, 7-50, and 18-84 %, respectively [1]. The LAR is considered particularly important, as it is associated with prolonged inflammation involving eosinophils, lymphocytes, mast cells and basophiles [3,4]. Based on this it is believed that blocking of allergen responses is beneficial for both the clinical state and the course of the asthmatic disease.

In vitro and *in vivo* studies have shown that histamine and cysteinyl leukotrienes play a major role during EAR and LAR [5-7] in patients with asthma and rhinitis. As a consequence, anti-histamines are capable of providing protection against allergen inhalation-induced bronchoconstriction, particularly during the EAR. In particular, the H₁-receptor antagonist azelastine has been shown to be effective against allergen inhalation, in addition to other bronchoconstrictor stimuli [8-12].

Similarly, anti-leukotrienes, such as zafirlukast and montelukast, afford protection against allergen-induced bronchoconstriction [13-16]. As the protection is not complete, ranging from 50 to 75%, the question is raised whether the combination with an anti-histamine can further attenuate allergen responses. Indeed, concurrent treatment with zafirlukast and the anti-histamine loratadine was capable of enhancing the protection against allergen-induced bronchoconstriction, compared to the individual agents [17]. However, the dose of the anti-leukotriene employed in this study was four-times greater than that recommended for clinical use.

As it is not known whether the enhanced protection also occurs with the doses that are clinically used, the aim of our study was to investigate the effect of a combination of daily recommended

doses of montelukast (10 mg) and azelastine (8 mg) on allergen-induced bronchoconstriction in patients with allergic asthma.

PATIENTS AND METHODS

Patients

Seventeen patients with a history of allergic asthma and a forced expiratory volume in 1 second (FEV_1) ≥ 75 % predicted [18] completed the study (14m/3f; mean (range) age, 31 (22-54) years; FEV_1 , 4.05 (2.84-5.62) L). All patients showed airway hyperresponsiveness to inhaled methacholine as indicated by a provocative concentration, $PC_{20}FEV_1$, of < 8 mg/mL (maximum 4.4 mg/mL). Additionally, at screening all patients had to demonstrate decreases in FEV_1 of ≥ 20 % and ≥ 15 %, respectively, during EAR and LAR and decreases in FEV_1 of ≥ 20 % and ≥ 10 % during EAR and LAR at a second screening challenge. Thirtythree patients were screened for this study. Sixteen of them did not meet the criteria for EAR and LAR at screening and were not randomized. The allergic status of each individual was confirmed by a skin-prick test comprising a panel of 21 allergens (ALK, Hamburg, Germany). Patients receiving treatment for asthma, other than short-acting β_2 -agonists, were excluded. Patients were also required to be nonsmokers and not to suffer from an acute respiratory tract infection within the last three weeks prior to inclusion. The study was approved by the local Ethics Committee and all patients gave their written informed consent.

Study design

This was a randomized, double-blind, cross-over study involving a screening and a treatment phase. The screening phase comprised three visits, during which the patient's history of asthma was confirmed, skin prick test and radio-allergo-sorbent test (RAST) were performed, and lung function, as well as $PC_{20}FEV_1$ of methacholine, $PD_{20}FEV_1$ of allergen and LAR were assessed. At screening allergen provocation was repeated after ≥ 2 weeks, to confirm reproducibility of EAR and LAR.

Two or more weeks later the cross-over treatment phase comprising three treatment periods per patient was started. Patients were randomly assigned to receive either 4 mg azelastine twice daily plus montelukast placebo once daily, or 10 mg montelukast once daily plus azelastine placebo twice daily, or 4 mg azelastine twice daily plus 10 mg montelukast once daily. After treatment for one week (day 1-7), allergen provocation was repeated two hours after the last intake of study medication in the morning of day 8. This cycle of a two-week wash-out period, followed by treatment for one week and subsequent allergen challenge, was then repeated twice for a total of three treatment periods per patient.

Study medication

The study was performed in a double-blind design. For this purpose the commercially available tablets for montelukast and azelastine were covered with an inert gelatin capsule. The placebo capsules contained only inert material. Due to this procedure all capsules appeared identical to the investigator and patients. Study medication was provided by Asta Medica, Frankfurt, Germany.

Pulmonary function tests and inhalation challenges

Spirometry was performed according to the ATS recommendations [19]. Baseline FEV₁ was measured at least three times, until reproducibility within 5 %, and the highest value was selected. Provocation with inhaled methacholine was performed following a standard protocol [20] and the results were expressed as concentration causing a 20 % fall in FEV₁ (PC₂₀FEV₁).

Dependent on the findings of prick and RAST tests, patients were challenged with individual allergen preparations (ALK, Hamburg, Germany), each of which was used over the course of the entire study. Allergen content of lyophilised, purified allergen extract is expressed in SQ-units correlating with the amount of the specific major allergen. During the second screening visit, allergen responsiveness was determined using increasing doses of allergen, whereby FEV₁ was measured up to 10 minutes following each inhalation. After achieving a 20 % drop in FEV₁,

measurements were repeated at 15 minutes intervals for up to 2 hours, to determine the EAR, and at 30 minutes intervals thereafter for up to 9 hours after the challenge, to determine the LAR. If the FEV₁ dropped by more than 10 % during the LAR, measurements were performed every 15 minutes until recovery was achieved. The cumulative dose of allergen which led to a 20 % fall in FEV₁ (PD₂₀FEV₁) was calculated for each patient individually and was used as a single dose in all subsequent allergen challenges. Details on allergen type used for the individual patient and EAR and LAR at screening are given in table 1.

Data analysis

Mean values and standard deviations (SD) or standard errors of mean (SEM) were computed for all relevant variables. The primary efficacy variable used was the maximal percentage fall in FEV₁ compared to baseline during 0-2 hours after allergen challenge (EAR) as well as 2-9 hours after allergen challenge (LAR). Statistical significance was assumed for $p < 0.05$. Data were analyzed using Friedman's nonparametric ANOVA, whereby EAR and LAR after single-dose provocation at screening were compared with the respective responses after treatment. In case of significant treatment effects, differences between treatments were assessed by the Wilcoxon matched-pairs signed-ranks test.

RESULTS

Bronchial provocation with allergen led to a dual EAR and LAR in all patients, which was highly reproducible in terms of the fall in FEV₁. Mean (range) EAR at the two screening visits was 33.7 (20.4-57.1) and 30.3. (19.8-49.9) %, and mean (range) LAR was 29.2 (14.7-59.7) and 29.4 (13.1-66.9) % (Table 1).

Early allergen reaction

All three treatments significantly attenuated allergen-induced EAR, as indicated by the maximal fall of FEV₁ compared with that observed during the second screening challenge ($p < 0.001$; Table 2, Figure 1). The combination of azelastine and montelukast achieved about 89 % protection, montelukast 75 %, and azelastine 46 %. According to pair-wise comparisons, the combination of azelastine and montelukast was superior to both azelastine (mean \pm SEM, $\Delta = 12.5 \pm 1.6$ %; $p < 0.001$) and montelukast ($\Delta = 3.8 \pm 1.1$ %; $p = 0.003$) alone, and montelukast was superior to azelastine ($\Delta = 8.7 \pm 1.8$ %; $p < 0.001$).

Late allergen reaction

All three treatments also protected against allergen-induced LAR, compared with the screening visit ($p < 0.004$; Table 1, Figure 1). The level of protection ranked in the order of the combination of azelastine and montelukast (78 %), montelukast (59 %), and azelastine (43 %). Pair-wise comparisons confirmed that the combination of azelastine and montelukast was superior to both azelastine ($\Delta = 10.3 \pm 2.8$ %; $p < 0.001$) and montelukast ($\Delta = 5.5 \pm 2.0$ %; $p = 0.017$) alone, whereas the efficacy of azelastine and montelukast was not significantly different ($\Delta = 4.8 \pm 2.3$ %; $p = 0.080$).

DISCUSSION

The present study demonstrated significant protection against allergen-induced early and late phase reactions after treatment for one week with clinically recommended oral doses of azelastine and montelukast, either alone or in combination. The combined treatment was superior to the single treatments. Furthermore, montelukast was more effective than azelastine in diminishing the early, but not the late phase response.

Regarding the effects of montelukast or azelastine as single drugs, our findings are in accordance with previous results. In dose-finding experiments, 4-8 mg azelastine were found to protect against EAR after allergen challenge [8,9]. Other studies found azelastine to attenuate bronchoconstriction by 24-37 % during EAR and by 36-100 % during LAR [10,11]. Similarly, montelukast, when given alone, is known to protect against allergen-induced responses in asthma [13-16]. For example, pre-treatment with 10 mg montelukast, given 36 and 12 hours before and 12 hours after provocation with house dust mite, significantly reduced EAR and LAR between 0-3 and 3-8 hours, respectively [16].

At present, one other study is available which has investigated the effect of combined treatment with an anti-histamine and an anti-leukotriene on EAR and LAR in asthma [17]. Treatment was performed for one week with zafirlukast 80 mg twice daily, loratadine 10 mg twice daily, or a combination of the two. The combination led to about 75 % protection during both EAR and LAR, compared to 25 and 40 %, respectively, by loratadine, and 62 and 55 % by zafirlukast alone.

One starting point for our study was that the dose of zafirlukast as previously used (160 mg daily) was four times greater than the clinically recommended daily dose. The question was whether enhanced protection by combining the two drugs also occurred at doses that are recommended and acceptable for daily treatment. To facilitate the comparison of results, we employed a similar study design. Comparability was further emphasized by the fact that responses in terms of FEV₁ were reproducible in both studies and that there were no carry-over effects. Our results demonstrated that the combined treatment enhanced protection against allergen-induced bronchoconstriction also at doses of the anti-leukotriene that are clinically introduced.

The role of cysteinyl leukotrienes as mediators of allergic responses is well documented [6,21], and studies have demonstrated montelukast to be a potent and selective leukotriene D₄ (cysLT₁) receptor antagonist [22], which is capable of inhibiting a variety of effects, among which

bronchoconstriction, airway smooth muscle hyperresponsiveness and inflammation are particularly important during EAR and LAR in asthma [6,16,21,22].

The same applies for anti-histamines, though their anti-inflammatory effect seems to be less pronounced than that of anti-leukotrienes. In addition to, or as a consequence of their anti-histaminic activity, anti-histamines might also reduce the release of other mediators, including leukotrienes [23,24]. Azelastine shows many of these properties [25-27], among which decreased activation of mast cells coupled with decreased release of cysteinyl leukotrienes seems to be of particular relevance for the present study.

Table 1 demonstrates that azelastine caused a two-fold reduction of the maximal response of FEV₁ during EAR, and montelukast an about four-fold reduction. When both were combined, azelastine attenuated the response relative to montelukast alone again by a factor of about two. A similar pattern of protection emerged during the LAR. These observations do not suggest a true synergism but a multiplicative effect of their combination, indicating an in-series action of both compounds. This conclusion sounds reasonable when considering the mechanisms underlying the action of both drugs. Both interfere with the effects of mediators released within short periods of time after allergen inhalation, and both have similar cell types, such as mast cells, basophils and airway smooth muscle, as primary targets [5-7].

The protection provided by all three types of treatment during LAR was about the same as that observed previously using loratadine and zafirlukast [17], but there seemed to be stronger effects during EAR. This was true particularly for the antihistamines. Probably this stronger effect promoted the significantly greater protection by the combined compared to single drugs in our study. One might argue that with high doses of the drugs involved, potential multiplicative effects are likely to be reduced by plateau-forming of the dose-response curve. Such effects should be most apparent at low doses and would suggest that our results on an additive action are to some extent predictable from existing data. However, the protection achieved by the compounds used,

either alone or in combination, was at least as high as that achieved previously, probably due to the different potency of the compounds used. Compared to zafirlukast and loratadine [17], the stronger protection by azelastine on the EAR was also observed when combined with montelukast. Whether the combination of an anti-leukotriene and an anti-histamine is a realistic therapeutic option in patients with allergic airway diseases, remains, however, to be proven [7,28,29].

In conclusion, our results demonstrate that azelastine, montelukast and their combination can exhibit considerable efficacy in the attenuation of early and late phase responses to allergen in patients with allergic asthma. This was achieved with clinically recommended doses of both preparations, whereby the combination of the two drugs was superior to each drug alone. The findings indicate a multiplicative action of both compounds and are compatible with an additive inhibitory mechanism involving mast cell- and basophil-derived mediators.

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Legend to figure 1

Mean values of the time course of FEV₁ after single-dose allergen inhalation challenge at the second screening challenge and after 1-week treatment with either azelastine or montelukast or the combination of both. The single allergen dose chosen was the cumulative dose which had caused a 20 % fall of FEV₁ in the first screening challenge. Within the first 2 hours after inhalation, lung function was measured every 15 min, and afterwards every 30 min. The early phase response (EAR) was assessed as maximum % fall in FEV₁ between 0 and 120 min after inhalation and the late phase response (LAR) as maximum % fall between 120 and 540 min, relative to baseline. The horizontal line is the common line of reference (baseline) to which curves have been shifted to facilitate the comparison. Absolute baseline values of FEV₁ are given in Table 2.

Table 1 – Patients' demographics and early (EAR) and late phase (LAR) responses to allergen upon screening (1st test dose-response, 2nd test single dose)

Patient No.	Sex m/f	Age yrs	Height cm	Weight kg	FEV ₁ % predicted	PC ₂₀ FEV ₁ mg/ml Mch	Type of allergen	PD ₂₀ FEV ₁ SQ-Units	1 st screening test		2 nd screening test	
									EAR	LAR	EAR	LAR
1	m	22	178	77	86	0.09	Der p1	49	20.4	23.0	23.0	13.1
2	m	35	185	83	116	3.75	Bet v1	888	25.8	26.7	20.2	29.6
3	m	32	185	69	97	0.25	Sec c5	35	28.2	14.7	46.4	17.0
4	m	30	169	75	110	0.16	Bet v1	6	39.9	22.0	49.8	32.5
5	f	54	170	67	96	0.99	Bet v1	356	27.5	33.5	19.8	35.3
6	f	35	165	59	135	4.37	Phl p5	263	46.1	25.8	27.2	16.1
7	m	26	178	86	88	0.03	Phl p5	13	26.1	27.0	29.1	26.2
8	m	24	187	88	121	0.88	Sec c5	31	32.7	19.8	32.7	19.3
9	f	32	162	72	99	0.33	Bet v1	14	24.6	18.2	32.9	13.3
10	m	38	172	70	91	0.19	Phl p5	754	34.8	48.6	28.4	52.3
11	m	25	185	90	90	0.73	Der p 1	63	48.9	59.7	30.0	66.9
12	m	26	176	72	87	0.13	Der p 1	13	24.4	36.4	23.0	39.6
13	m	32	170	70	97	4.26	Bet v1	151	25.3	40.0	19.8	32.3
14	m	25	190	83	92	2.13	Bet v1	288	42.9	22.4	29.8	23.6
15	m	29	182	96	99	2.18	Phl p5	19	57.1	27.4	32.1	15.0
16	m	31	186	81	84	0.40	Sec c5	14	25.3	27.7	21.7	13.6
17	m	33	183	68	89	0.37	Bet v1	19	43.6	23.0	49.9	53.2
Mean	-	31	179	76.8	98.7	0.54*	-	56.7*	33.7	29.2	30.3	29.4
SEM	-	1.8	2.1	2.4	3.4	1.42*	-	1.47*	2.6	2.8	2.4	3.9

SEM=standard error of mean, *geometric mean and SEM (to be interpreted as a factor relative to the geometric mean), PC₂₀FEV₁ = provocative concentration of methacholine (Mch) for eliciting a 20 % decrease in FEV₁ versus baseline, PD₂₀FEV₁ = allergen dose for eliciting a 20 % decrease of FEV₁ during EAR. Der p1 = *Dermatophagoide pteronyssinus*, Bet v1 = birch, Sec c5 = rye, Phl p5 = timothy, SQ-Units = standardized allergen content according to the manufacturer (ALK-Scherax, Germany)

Table 2 – Baseline values and early (EAR) and late phase (LAR) responses to allergen at screening and after randomized, three-way, cross-over treatment

	FEV ₁ baseline prior to challenge (L)	EAR 0-120 min after challenge (% fall in FEV ₁)	LAR 120-540 min after challenge (% fall in FEV ₁)
Screening (2 nd challenge)	4.03 ± 0.16	30.3 ± 2.4	29.4 ± 3.9
Azelastine	4.00 ± 0.15	15.9 ± 1.7	16.8 ± 2.3
Montelukast	4.03 ± 0.16	7.2 ± 0.8	12.0 ± 2.2
Azelastine+Montelukast	4.14 ± 0.18	3.4 ± 0.9	6.5 ± 1.9

Mean values ± SEM are given. Each test was performed in n=17 patients according to the cross-over design.

Fig 1

