

RESEARCH

Open Access

Effects of a dual CCR3 and H₁-antagonist on symptoms and eosinophilic inflammation in allergic rhinitis

Lennart Greiff^{1*}, Cecilia Ahlström-Emanuelsson¹, Ash Bahl², Thomas Bengtsson³, Kerstin Dahlström³, Jonas Erjefält⁴, Henrik Widegren¹, Morgan Andersson¹

Abstract

Background: The CC-chemokine receptor-3 (CCR3) has emerged as a target molecule for pharmacological intervention in allergic inflammation.

Objective: To examine whether a dual CCR3 and H₁-receptor antagonist (AZD3778) affects allergic inflammation and symptoms in allergic rhinitis.

Methods: Patients with seasonal allergic rhinitis were subjected to three seven days' allergen challenge series. Treatment with AZD3778 was given in a placebo and antihistamine-controlled design. Symptoms and nasal peak inspiratory flow (PIF) were monitored in the morning, ten minutes post challenge, and in the evening. Nasal lavages were carried out at the end of each challenge series and α_2 -macroglobulin, ECP, and tryptase were monitored as indices of allergic inflammation.

Results: Plasma levels of AZD3778 were stable throughout the treatment series. AZD3778 and the antihistamine (loratadine) reduced rhinitis symptoms recorded ten minutes post challenge during this period. AZD3778, but not the anti-histamine, also improved nasal PIF ten minutes post challenge. Furthermore, scores for morning and evening nasal symptoms from the last five days of the allergen challenge series showed statistically significant reductions for AZD3778, but not for loratadine. ECP was reduced by AZD3778, but not by loratadine.

Conclusions: AZD3778 exerts anti-eosinophil and symptom-reducing effects in allergic rhinitis and part of this effect can likely be attributed to CCR3-antagonism. The present data are of interest with regard to the potential use of AZD3778 in allergic rhinitis and to the relative importance of eosinophil actions to the symptomatology of allergic rhinitis.

Trial registration: EudraCT No: 2005-002805-21.

Background

The CC-chemokine receptor-3 (CCR3) is a transmembrane protein that constitutes one of the receptors for CC-chemokines. It is localized to cells of key importance to allergic inflammation including dendritic cells, Th2-lymphocytes, eosinophils, basophils, and mast cells as well as to epithelial, smooth muscle, and neural cells [1-7]. Chemokines interacting with CCR3 include eotaxin-1, 2, and 3, MCP-4, and RANTES [8]. Stimulation of the receptor produces chemotaxis and cellular

activation [8], and experiments in CCR3 knock-out mice and such involving CCR3-neutralizing antibodies have demonstrated the importance of the CCR3-pathway to eosinophil activity *in vivo* [9-12]. CC-chemokines are increased in allergic airway conditions [10,13-16]. Accordingly, CCR3 may be a treatment target in allergic rhinitis and asthma. Recently, this possibility was substantiated by a report on symptom-reducing effects of topical anti-sense therapy directed towards CCR3 in asthma [17], although that particular study did not discriminate between an effect on CCR3 and an effect mediated through the common β -chain of the IL-3, IL-5, and GM-CSF receptors.

* Correspondence: lennart.greiff@skane.se

¹Department of ORL, Head & Neck Surgery, Lund University Hospital, Lund, Sweden

A series of experimental studies have shown that small molecular weight CCR3-antagonists can reduce allergic inflammation *in vivo*. Wegmann *et al.*, in a study involving ovalbumin-sensitized mice repeatedly challenged with ovalbumin to produce inflammation, showed that a CCR3-antagonist reduced BAL and tissue eosinophilia and that this effect was associated with normalization of airway reactivity and prevention of goblet cell hyperplasia [18]. Das *et al.* reported that two separate CCR3-antagonists reduced eotaxin-elicited and allergen-induced eosinophil recruitment to bronchial airways in an experimental murine model [19]. Nakamura *et al.*, in a mouse model of allergic conjunctivitis, demonstrated that a highly selective CCR3-antagonist attenuated early and late phase symptoms [20], suggesting a symptom-reducing potential in allergic conditions, and that the effect was associated with mast cell stabilization. Additional observations on effects of CCR3-antagonists comprise prevention of immediate and late-phase allergic skin reactions to allergen in a mouse allergy model [21], inhibition of eosinophil infiltration into the airways of monkeys following segmental bronchial provocation with eotaxin [22], and dose-dependent reduction of eosinophil recruitment into the lungs in an animal model of allergic airway inflammation [23]. The examples above highlight the anti-allergic potential of CCR3-antagonism. However, observations on effects of CCR3-antagonists on allergic inflammation in man are lacking. Hence, explorations of CCR3-inhibition in man are highly warranted.

AZD3778 is a novel small molecular weight dual CCR3 and histamine H₁-receptor antagonist. It has been characterized by MDS Pharma Services (Taipei, Taiwan: <http://discovery.mdsp.com>) in a battery of 226 assays covering a diverse range of enzyme, transporter, and receptor targets (not including CCR3). The only significant activity detected (defined as >50% inhibition) was against the guinea-pig H₁-receptor with a K_i of 54 nM. AZD3778 also has been shown to inhibit the binding of a specific CCR3 radioligand, ¹²⁵I-eotaxin, to the human CCR3-receptor expressed on CHO-cells with a pIC₅₀ of 8.1 ± 0.1 (mean ± SEM, equivalent to an IC₅₀ of 8 nM.) The functional potency of AZD3778 has been assessed in test systems in which a response mediated by human CCR3 could be evoked using human eotaxin 1 or 2. For example, AZD3778 inhibited chemotaxis of eosinophils. From *in vitro* experiments on whole blood, where plasma protein binding would reduce the free fraction of AZD3778, the A₂ (the concentration of antagonist required to produce a two-fold shift of the agonist response) for CCR3 was 200 nM. AZD3778 also inhibited the binding of a specific H₁-radioligand, ³H-pyrilamin, to the human H₁-receptor expressed on CHO-cells with an IC₅₀ of 40 nM. In human HeLa-cells expressing

the human H₁-receptor, AZD3778 inhibited histamine-induced calcium flux with an IC₅₀ of 63 nM (expected A₂ in whole blood of 1000 nM). Taken together, the above observations indicate that AZD3778 has CCR3 and H₁-antagonistic properties.

In the present study, involving patients with allergic rhinitis examined outside the pollen season in an allergen challenge model [24,25], we examined whether treatment with AZD3778 affected symptoms and signs of allergic rhinitis. Accordingly, we monitored rhinitis symptoms and nasal lavage fluid levels of ECP, reflecting eosinophil activity, as well as tryptase and α₂-macroglobulin, reflecting mast cell activity and plasma exudation, respectively. We have previously shown that nasal lavage recovery of inflammatory mediators can be improved by utilization of histamine-challenges. In this regard, histamine produces a plasma exudation response that facilitates their luminal entry [25]. In the present study, we aimed at using this experimental tool, but since AZD3778 exerts H₁-antagonistic effects we chose to induce plasma exudation through nasal challenge with bradykinin [26]. Finally, reflecting the H₁-antagonistic property of AZD3778, we compared the effect of this drug to an anti-histamine (loratadine). We report on symptom-reducing and anti-eosinophil effects that likely can be attributed to CCR3-antagonism.

Methods

Study design

The study was of a randomized, double blind, placebo-controlled (double-dummy), and three-way crossover design. It comprised three ten days' treatment periods carried out in the pollen-free autumn/winter months separated by at least two weeks washout-periods. The treatments were AZD3778, loratadine, and placebo. After three days of treatment, a series of seven individualized, once-daily allergen challenges commenced while the treatment continued. Nasal symptoms and peak inspiratory flow (PIF) were recorded. In addition, at the end of each treatment and allergen challenge series, nasal lavages were carried out. Tryptase, ECP, and α₂-macroglobulin were measured as indices of mast cell activity, eosinophil activity, and plasma exudation, respectively. The study was approved by the Regional Ethics Committee, Lund, and the Swedish Medical Product Agency. It was conducted according to the Declaration of Helsinki and in compliance with Good Clinical Practice. Informed consent was obtained.

Subjects

Forty-six patients were enrolled and subjected to physical examination and skin prick test. Inclusion criteria: Men and post-menopausal or surgically sterile women, seasonal allergic rhinitis for at least two years, positive

skin prick test to birch or grass pollen allergen, asymptomatic condition outside the pollen season, need for treatment at seasonal allergen exposure, and a positive response to nasal allergen challenge (see the titration procedure below). Exclusion criteria: Any relevant disease including perennial allergic rhinitis, asthma, clinically relevant structural nasal abnormalities, upper respiratory tract infection within two weeks prior to the start of the study, use of topical corticosteroids within four weeks prior to the study and use of antihistamines within one week, and immunotherapy. Of the 46 subjects enrolled, 38 were randomized and received at least one dose of a study drug. Of those randomized, 37 were analyzed for efficacy. There were six withdrawals from the study: One due to an adverse event and five because of lack of compliance. Of the 38 subject allocated to treatment, all were men. The average age was 25 years (range 20-51) and average BMI was 24 kg/m² (range 20-29). The median time since diagnosis of allergic rhinitis was 14 years (range 2-33).

Treatment

AZD3778 (AstraZeneca, Lund, Sweden) was administered as an oral suspension (300 mg twice daily). Loratadin Biochemie (Sandoz, Helsingborg, Sweden) was given as a tablet (10 mg once daily). Placebo was a suspension to match AZD3778 (AstraZeneca, Lund, Sweden) and a tablet to match Loratadin Biochemie (AstraZeneca, Charnwood, UK). Each treatment period comprised ten days and compliance was assured by supervising administration on the first treatment day and thereafter by control of daily diary recordings of intake of study medication. Allergen challenges (see below) were administered during the last seven days of each treatment period. On the first day of treatment as well as on days eight, nine, and ten, pharmacokinetic samples (plasma) were collected within two to five hours after drug administration. Also, such samples were obtained before drug administration on days eight, nine, and ten.

Allergen challenge model

In order to establish individually tolerable, repeatable, yet symptom-producing allergen challenge-doses, a nasal titration procedure was performed [24,25]. Increasing doses of allergen were administered at 10 min intervals using a spray-device delivering 100 µL per actuation. One puff was administered into each nostril resulting in effective doses of 100, 300, 1,000, and 3,000 SQ units per nasal cavity (Aquagen, ALK-Abelló, Horsholm, Denmark). This scheme was followed until the subject responded with at least 5 sneezes or recorded a symptom score of 2 or more on a scale from 0 to 3 for either nasal secretion or nasal blockage. The dose that produced this effect was chosen for the allergen challenge

series and was given once daily for seven days. The allergen challenge was administered two to five hours after the morning dose of the study drug.

Clinical evaluation

The subjects scored nasal symptoms every morning and evening, starting three days prior to each treatment and continuing until day six after the last treatment day. The scores were entered into diary cards and each registration reflected the preceding twelve hours. Nasal secretion and blockage, respectively, as well as the most severe of the symptoms sneezing and itching were each scored on a four-grade scale where 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms. The scores were added to a daily total nasal symptom score (TNSS), with separate scores for morning and evening observations. Morning and evening scores, respectively, from the 5th, 6th, and 7th allergen-challenge day were added and divided by three resulting in a mean TNSS based on *three days'* observations (range 0-9). In addition, morning and evening scores, respectively, from the 3rd through 7th allergen-challenge day were added and divided by five resulting in a mean TNSS based on *five days'* observations (range 0-9). Nasal symptoms were also scored immediately prior to and 10 min post allergen challenge: Secretion and blockage were scored as described above, whereas the number of sneezes were counted and transformed into a sneezing score by the investigators: 0 sneezes = 0, 1-4 sneezes = 1, 5-9 sneezes = 2, and 10 or more sneezes = 3. The scores were added to a daily post challenge TNSS, and a mean TNSS for post challenge symptom scores calculated from observations made on the 5th through 7th challenge day as described above. The subjects recorded nasal PIF in the morning, immediately prior to and 10 min post allergen challenge, and in the evening using a flow-meter (Clement-Clarke, Harlow, U. K.) equipped with a facial mask. Mean nasal PIF, for morning observations, observations immediately prior to and 10 min post challenge, and evening observations, respectively, from the last three days of each allergen challenge series were calculated.

Nasal lavages and bradykinin challenges

A 2.5 min nasal lavage with isotonic saline was carried out at the first study visit prior to any treatment and prior to any allergen challenge (*baseline observation*). Nasal lavages were also obtained twice on day seven of each allergen challenge series. Accordingly, a 2.5 min saline lavage was carried out first and this lavage was separated from the allergen challenge on this particular day by at least 1 hour. It was followed by two 30 sec lavages carried out in order to remove remaining solutes on the nasal mucosal surface. (These lavages were

discarded.) Fifteen minutes later a nasal spray challenge with bradykinin was performed (in order to improve the recovery of inflammatory mediators [25].) and followed, an additional 15 min later, by a final 2.5 min saline lavage. Nasal lavages were carried out using a pool-device containing 15 ml of isotonic saline [27]. The lavage fluids were kept in the right nasal cavity at all occasions. The recovered lavage fluid was centrifuged and the supernatant was homogenized, prepared in aliquots, and frozen (-30°C). The bradykinin challenge (100 µg) was carried out using a spray-device delivering 100 µL per actuation.

Analysis

α₂-Macroglobulin was measured using a radioimmunoassay sensitive to 7.8 ng/mL. The intra- and inter-assay coefficients of variation are between 3.8-6.0% and 3.1-7.2%, respectively [25]. ECP was measured using a fluoroimmunoassay (Pharmacia-Diagnostics, Uppsala, Sweden) with a sensitivity of 2.0 ng/mL. Tryptase was measured using a radioimmunoassay with detection limit of 0.5 ng/mL (Pharmacia-Diagnostics, Uppsala, Sweden). α₂-Macroglobulin and ECP were analyzed in the lavage fluids as they were, whereas lavage fluids samples were concentrated five times before the analysis of tryptase.

Statistics

All hypothesis-testing was done using two-sided alternatives at a 5% significance level. The comparison between AZD3778 and placebo was primary and comparisons versus loratadine secondary. Accordingly, no adjustments for multiplicity were applied. The full analysis set was used for all analyses, i.e., all subjects with evaluable data collected from at least two treatment periods. In total 37 of the 38 randomized subjects could be evaluated for efficacy. Change in period means from baseline (i.e., mean over the last three days of the run-in/wash-out period that preceded the treatment period) to treatment in symptom scores and nasal PIF recorded in the diary were compared between treatment groups using an analysis of variance model with subject, period, and treatment as fixed factors and using baseline as a covariate. Period means for symptoms and nasal PIF recorded post-challenge, and variables from the nasal lavages, were compared between treatment groups using analysis of variance models with subject, period and treatment as fixed factors.

Results

Pharmacokinetic observations

Plasma levels of AZD3778 were stable late into the treatment series (Table 1). The minimum level recorded was 0.3 µmol/L and a total of six pre-dose samples were

Table 1 Summary statistics on plasma concentrations of AZD3778 (µmol/L).

Variable	n	Gmean	CV	Min	Median	Max
Day 1, post-dose	34	5.49	38.5	2.45	5.83	10.40
Day 8, pre-dose	24	2.51	98.0	0.29	3.41	6.27
Day 8, post dose	34	7.77	44.4	3.14	7.95	20.10
Day 9, pre-dose	26	2.21	106.4	0.32	2.79	8.13
Day 9, post dose	34	7.55	36.0	3.15	7.51	15.60
Day 10, pre-dose	34	2.53	71.0	0.28	2.95	7.84
Day 10, post dose	34	7.19	36.5	3.29	7.34	13.50

Plasma levels of AZD3778 were stable during the last three days of the treatment series.

below the 3 × A₂ level of 0.6 µmol/L over treatment days 8, 9, and 10. The median trough plasma levels of AZD3778 for days 8, 9, and 10 were 3.41, 2.79, and 2.95 µmol/L, respectively.

Post challenge symptom scores

During the last *three days* of the allergen challenge series, AZD3778 and loratadine reduced post-challenge mean TNSS (p < 0.001 and 0.001, respectively) (Tables 2 and 3). In addition, AZD3778, but not loratadine, reduced post-challenge nasal blockage (p = 0.008, c.f. placebo): mean scores for nasal blockage was 1.41, 1.52, and 1.65 for AZD3778, loratadine, and placebo, respectively.

Morning and evening symptom scores

The allergen challenge-induced change in mean TNSS from baseline was lowest in the AZD3778 group during last *three days* of the allergen challenge series, but treatment comparisons did not reach statistically significant differences for either AZD3778 or loratadine compared with placebo: mean TNSS in the morning were 1.66, 1.98, and 2.00 for AZD3778, loratadine, and placebo, respectively. Corresponding values for TNSS in the evening were 1.59, 1.86, and 1.72. Similarly, there were no

Table 2 Period means and ranges for pre- and 10 min post-allergen challenge mean TNSS and mean nasal PIF over the last three days of the allergen challenge series.

Variable	Treatment	n	Pre challenge		10 min post challenge	
			Mean	Range	Mean	Range
TNSS	AZD3778	35	1.25	0, 3	4.11	0.7, 7.7
	Loratadine	35	1.64	0, 8	3.85	1, 8
	Placebo	34	1.56	0, 5.5	5.57	1.7, 9
Nasal PIF	AZD3778	35	179	76.7, 247	125	50, 200
	Loratadine	35	165	50, 243	114	30, 210
	Placebo	34	177	100, 257	108	57, 177

Table 3 Treatment comparisons for 10 min post challenge mean TNSS and mean nasal PIF over the last three days of the allergen challenge series.

Variable	Treatment	Mean difference ^a	95% CI	P-value
TNSS	AZD3778 vs. placebo	-1.56	-2.03, -1.09	<0.001
	Loratadine vs. placebo	-1.87	-2.34, -1.4	<0.001
	AZD3778 vs. loratadine	0.312	-0.149, 0.773	0.181
Nasal PIF	AZD3778 vs. placebo	16.8	5.73, 27.9	0.004
	Loratadine vs. placebo	8.67	-2.43, 19.8	0.124
	AZD3778 vs. loratadine	8.14	-2.83, 19.1	0.143

^aAdjusted mean difference from ANOVA.

AZD3778 as well as loratadine reduced post challenge TNSS. In addition, AZD3778 improved post challenge nasal PIF.

differences between the treatments regarding morning and evening nasal PIF (data not shown).

In contrast to the three-day observation period, mean TNSS of the last *five days* of the allergen challenge series showed statistically significant reductions in mean TNSS differences between AZD3778 and placebo for morning as well as evening observations ($p = 0.023$ and 0.022 , respectively) (Tables 4 and 5). In contrast, no such effects were observed for loratadine.

Lavage fluid observations

In the placebo run, nasal lavage fluid levels of tryptase, ECP, and α_2 -macroglobulin were elevated in saline lavage fluids collected late into the allergen challenge series (*c.f.* baseline observation prior to the challenge series) (Figure 1). Also, compared with the baseline as well as with the saline lavage carried out late into the allergen challenge series, bradykinin produced increased levels of α_2 -macroglobulin (Figure 2).

Whereas loratadine failed to affect the lavage fluid indices that were increased by the repeated allergen

Table 4 Period means and ranges for TNSS from the diary cards: Period means over five days.

Variable	Treatment	n	Baseline period		Treatment period	
			Mean	Range	Mean	Range
TNSS -morning	AZD3778	35	0.581	0, 2	1.6	0.2, 4.67
	Loratadine	35	0.829	0, 4	1.88	0, 7.2
	Placebo	35	0.695	0, 3.33	2	0, 8
TNSS -evening	AZD3778	35	0.486	0, 2	1.48	0, 4.33
	Loratadine	35	0.743	0, 4	1.85	0, 8.6
	Placebo	34	0.657	0, 3.33	1.88	0, 5.6

Table 5 Treatment comparisons for TNSS from the diary cards: Period means over five days.

Variable	Treatment	Mean difference ^a	95% CI	P-value
TNSS -morning	AZD3778 vs. placebo	-0.459	-0.85, -0.067	0.023
	Loratadine vs. placebo	-0.174	-0.569, 0.22	0.381
	AZD3778 vs. loratadine	-0.285	-0.685, 0.115	0.159
TNSS -evening	AZD3778 vs. placebo	-0.562	-1.04, -0.083	0.022
	Loratadine vs. placebo	-0.172	-0.649, 0.305	0.474
	AZD3778 vs. loratadine	-0.39	-0.868, 0.088	0.108

AZD3778 reduced TNSS in the morning as well as in the evening.

^aAdjusted mean difference from ANOVA.

challenges, AZD3778 reduced the levels of ECP compared with placebo (Figure 3). For the lavage obtained pre-bradykinin challenge, the numerical reduction in ECP failed to reach statistical significance (Figure 3), whereas the reduction was statistically significant for the post-bradykinin observation ($p = 0.038$) (Figure 3). Also, focusing on post-bradykinin observations, AZD3778 reduced the levels of ECP compared with loratadine ($p = 0.012$) (Figure 3). Neither AZD3778 nor loratadine reduced the levels of tryptase and α_2 -macroglobulin (Figure 3).

Discussion

The present study demonstrates that a dual CCR3 and histamine H_1 -receptor antagonist (AZD3778) can exert anti-eosinophil and symptom-reducing effects in a condition in man characterised by allergic airway inflammation. Our data are of interest with regard to the pharmacology of CCR3-antagonism, the relative importance of eosinophil actions to the symptomatology of allergic rhinitis, and the potential use of AZD3778 in the treatment of allergic rhinitis.

Preceding *in vitro* studies performed by MDS Pharma Services indicated that AZD3778 had a whole blood A_2 at the CCR3-receptor of $0.2 \mu\text{mol/L}$. Furthermore, with regard to antagonism of the histamine H_1 -receptor, a whole blood exposure of $1.0 \mu\text{mol/L}$ was equivalent to A_2 . In the present study, plasma levels of AZD3778 were stable late into the treatment series. At trough, the plasma concentration of AZD3778 suggested that exposures were close to those required for 24-hour antagonism of the CCR3 and H_1 -receptors.

It is difficult to compare treatment-effects in allergic rhinitis during the pollen season, reflecting uncertainties regarding onset and intensity of natural allergen

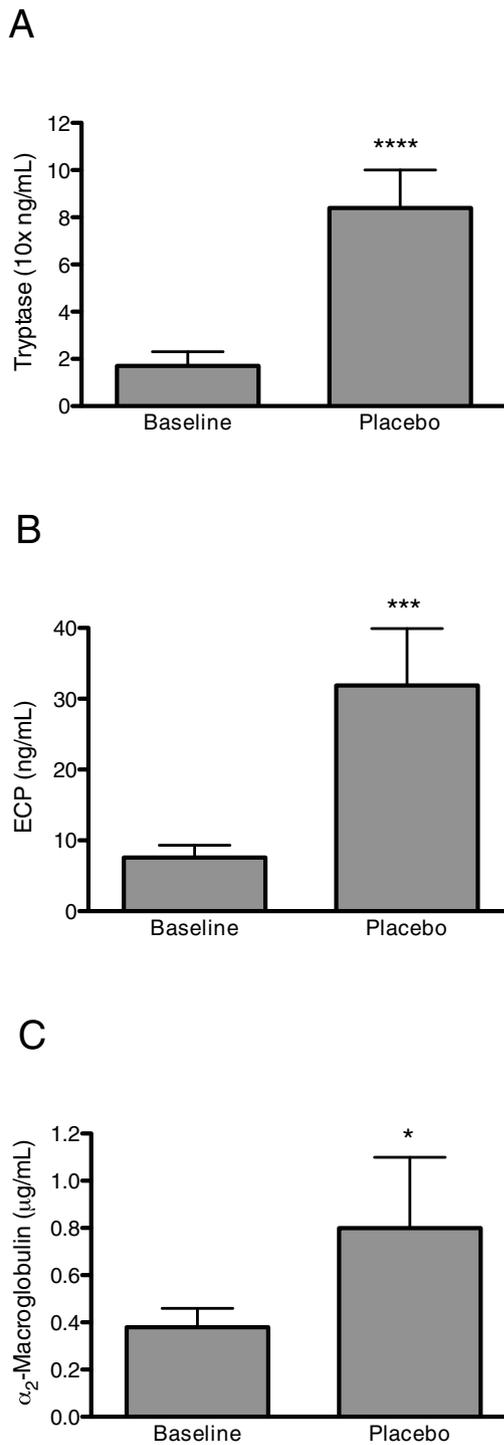


Figure 1 A. Tryptase (A), ECP (B), and α_2 -macroglobulin (C) in lavages obtained at baseline (before allergen challenge) and in corresponding lavages late into the allergen challenge series (prior to bradykinin challenge) in the placebo run (median values with interquartile ranges). The allergen challenge series produced an inflammatory response characterized by increased mast cell and eosinophil activity as well as by plasma exudation. (*Denotes $p < 0.05$, ***denotes $p < 0.001$, and ****denotes $p < 0.0001$.)

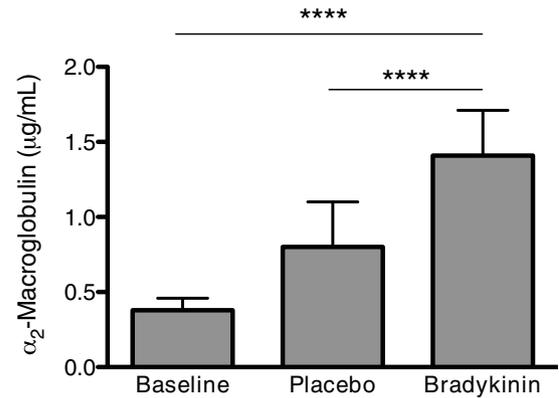
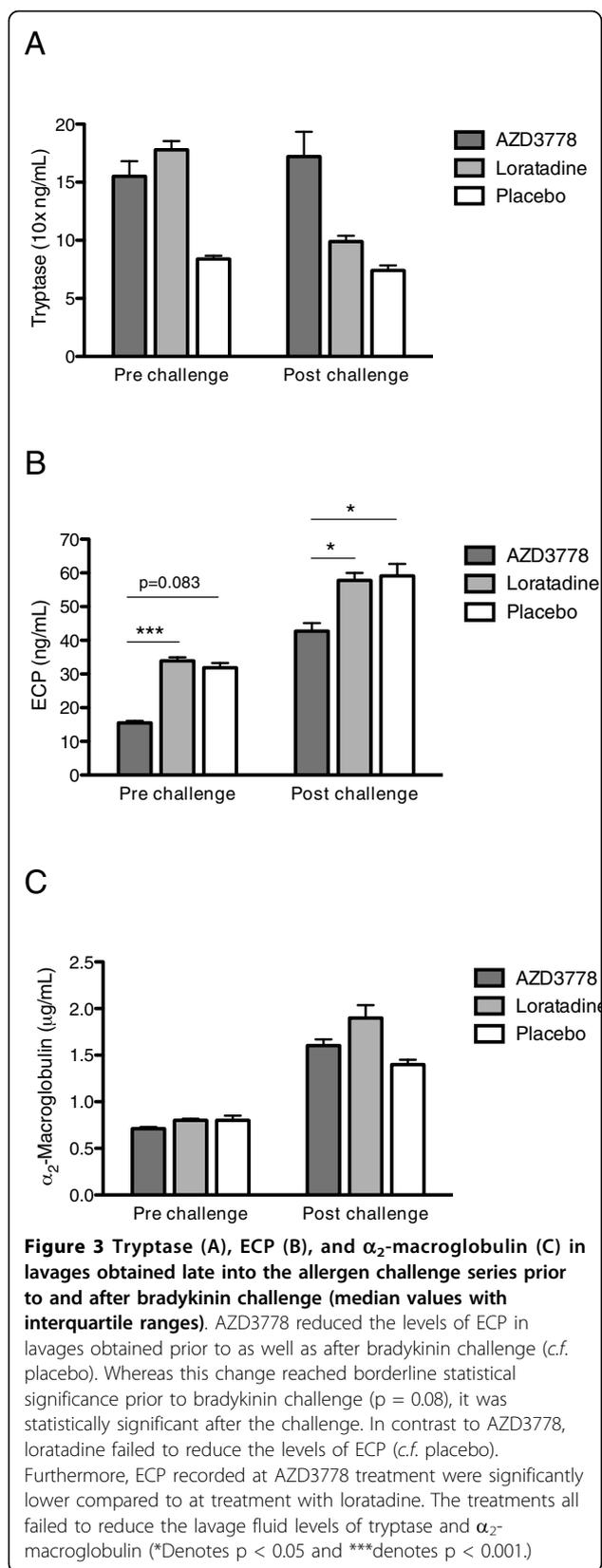


Figure 2 α_2 -Macroglobulin in lavages obtained at baseline and late into the allergen challenge series prior to and after bradykinin challenge in the placebo run (median values with interquartile ranges). Bradykinin produced plasma exudation, a process that may facilitate luminal entry of tissue solutes including tryptase and ECP. (****Denotes $p < 0.0001$.)

exposure. Accordingly, it is impossible to perform accurate studies of crossover design and when resorting to parallel group studies these are hampered by inter-individual differences in allergen sensitivity. In order to overcome these problems, we have introduced a model where nasal challenges with individualized doses of allergen are given for seven consecutive days to create a repeatable artificial pollen season characterized by development of rhinitis symptoms and allergic inflammation [24,25]. In the present study, this model was used and around-the-clock rhinitis symptoms were produced by the allergen-challenges in the placebo-run. Notably, symptom scores reached at placebo treatment were very similar to those recorded previously in the model.

Using the mean over the last three days of the allergen challenge series, based on previous evaluations in the present model in which symptoms gradually increased over time [24,25], AZD3778 reduced post-challenge TNSS compared with placebo. In contrast, no statistically significant effects were observed for morning and evening symptoms when focusing on this period. By these characteristics, which were of the same profile and magnitude as for anti-histamines: loratadine (this study) and cetirizine [28], it might be suggested that AZD3778 acted through H_1 -antagonism or that CCR3-antagonism resulted in the same effect-profile as that of anti-histamines. However, AZD3778 also reduced post-challenge nasal blockage and improved post-challenge nasal PIF. Moreover, when focusing on the last five days of the allergen challenge series (which was valid since an effect plateau was reached already at allergen challenge day three), AZD3778 exerted statistically significant reducing effects also on morning and evening symptoms. These latter effects, which were not observed for loratadine,



also suggest that CCR3-antagonism *per se* has specific anti-rhinitis effects. Accordingly, CCR3-antagonists may have a potential as a treatment for allergic rhinitis. From a therapeutic point of view, the possibility that AZD3778 exerts CCR3 as well as H_1 -antagonistic effects is attractive. However, compared with previous observations in the present model [24,25], the effect of AZD3778 will likely be inferior that of a topical corticosteroid. Further studies are warranted to evaluate AZD3778 and other CCR3-antagonists in allergic rhinitis.

In the present placebo run, nasal lavage fluid levels of ECP, tryptase, and α_2 -macroglobulin increased during the allergen challenge series compared with baseline data. This was in agreement with previous observations in the present model and indicated a development of allergic airway inflammation [25]. Whereas loratadine failed to affect these indices, AZD3778 reduced the levels of ECP compared with placebo. For the pre-bradykinin challenge lavage, the numerical 37% reduction in ECP failed to reach statistical significance, whereas the reduction was significant for the post-bradykinin observation. (Plasma exudation events acutely produced by histamine is known to improve the recovery of tryptase and ECP [25]: bradykinin was employed in the present study to achieve this and its exudative effect was confirmed.) The effect of AZD3778 likely reflected CCR3-antagonism and not H_1 -antagonism, since it was not observed for loratadine. In contrast to its effects on ECP, AZD3778 failed to affect the levels of tryptase, which disagreed with the observation by Nakamura *et al.* on mast cell stabilizing effects of a CCR3-antagonist in a mouse model [20]. Differences between test systems, species, and the employed antagonists might account for the discrepancy. AZD3778 as well as loratadine also failed to reduce the allergen challenge induced plasma exudation (α_2 -macroglobulin). Taken together, our findings suggest that AZD3778 exerts a selective anti-eosinophil effect. The observation is in keeping with previous findings on such effects by CCR3 antagonism in experimental models [9-12,17-23], and extends them to include allergic rhinitis. While reducing eosinophil activity in allergic rhinitis, the effect profile of AZD3778 is different from what is expected for a topical corticosteroid, which reduces all the employed lavage fluid indices in the present model [25].

In the present study, AZD3778 exerted a moderate anti-eosinophilic and symptom-reducing effect in allergic rhinitis. We might not conclude that the effect on nasal symptoms was secondary to anti-eosinophilic actions associated with CCR3-antagonism. However, the findings suggest that part of the symptomatology of allergic rhinitis depends on eosinophil activity. Indirectly, this is in agreement with observations on seasonal

allergic rhinitis as a condition featuring particularly intense eosinophil activity. For instance, eosinophil degranulation (assessed by transmission electron microscopy) is much greater in on-going allergic rhinitis than in asthma [29]. Based on the present findings, and observations by Erjefält *et al.* [29], it can be suggested that interventions aiming at reducing eosinophil activity in airway inflammation may better be evaluated in allergic rhinitis than in asthma. Such intervention may include IL-5 receptor antagonists, despite the fact that trials with IL-5 active drugs (in asthma) have been disappointing [30-32]. A specific area in which it may be of interest to explore effects of anti-eosinophil active drugs, notably CCR3-antagonists, is the interaction between allergic/eosinophilic inflammation and the common cold. For example, a series of observations indicate the importance of rhinovirus infections to exacerbations of asthma [33], and experimental inoculation studies suggest that CC-chemokines and eosinophils may be involved in this interaction [34,35].

Conclusion

In summary, we conclude that AZD3778 exerts moderately anti-eosinophilic and symptom-reducing effects in allergic rhinitis. This effect can in part be attributed to CCR3-antagonism.

List of abbreviations used

BMI: Body mass index; CCR3: CC-chemokine receptor-3; ECP: Eosinophil cationic protein; GM-CSF: Granulocyte-macrophage colony-stimulating factor; IL: Interleukin; MCP-4: Monocyte chemoactive protein-4; PIF: Peak inspiratory flow; RANTES: Regulated on Activation, Normal T-cell Expressed, and Secreted; SQ-unit: Standard quantity unit; TNSS: Total nasal symptoms score.

Acknowledgements

We thank L. Glantz-Larsson and C. Cervin-Hoberg for assistance.

Author details

¹Department of ORL, Head & Neck Surgery, Lund University Hospital, Lund, Sweden. ²New Opportunities, AstraZeneca R&D, Charnwood, UK. ³Clinical Information Science, AstraZeneca R&D, Lund, Sweden. ⁴Department of Experimental Medical Science, Lund University, Lund, Sweden.

Authors' contributions

All authors participated in the design of the study. LG, CAE, HW, and MA carried out the clinical experiments. LG and JE were responsible for the analyses of the biological samples. TB performed the statistical analysis. All authors were involved in the interpretation of the results. LG, AB, and MA drafted the manuscript. All authors read and approved the manuscript.

Competing interests

Lennart Greiff has consulting agreements with AstraZeneca, CC10 Sweden, and Orexo. Jonas Erjefält has consulting agreements with AstraZeneca, GSK, and Orexo. Morgan Andersson has a consulting agreement with ALK-Abellö. Ash Bahl, Thomas Bengtsson, and Kerstin Dahlström are employees of AstraZeneca. Cecilia Ahlström-Emanuelsson and Henrik Widegren have no relationships to declare. The study was supported in parts by grants from

the Swedish Research Council, Skåne County Council, Lund University, and AstraZeneca.

Received: 19 October 2009

Accepted: 9 February 2010 Published: 9 February 2010

References

1. Ponath PD, Qin S, Post TW, Wang J, Wu L, Gerard NP, Newman W, Gerard C, Mackay CR: **Molecular cloning and characterization of a human eotaxin receptor expressed selectively on eosinophils.** *J Exp Med* 1996, **183**:2437-2448.
2. Sallusto F, Mackay CR, Lanzavecchia A: **Selective expression of the eotaxin receptor CCR3 by human T-helper 2 cells.** *Science* 1997, **227**:2005-2007.
3. Uguccioni M, Mackay CR, Ochenberger B, Loetscher P, Rhis S, LaRosa GJ, Rao P, Ponath PD, Baggiolini M, Dahinden CA: **High expression of the chemokine receptor CCR3 in human blood basophils. Role in activation by eotaxin, MCP-4, and other chemokines.** *J Clin Invest* 1997, **100**:1137-1143.
4. Ochi H, Hirani WM, Yuan Q, Friend DS, Austen KF, Boyce JA: **T helper cell type 2 cytokine-mediated comitogenic responses and CCR3 expression during differentiation of human mast cells in vitro.** *J Exp Med* 1999, **190**:267-280.
5. Joubert P, Lajoie-Kadoch S, Labonte I, Gounni AS, Maghni K, Wellemans V, Chakir J, Laviolette M, Hamid Q, Lamkhoue B: **CCR3 expression and function in asthmatic airway smooth muscle cells.** *J Immunol* 2005, **175**:2702-2708.
6. Beck LA, Tancowny B, Brummet ME, Asaki SY, Curry SL, Penno MB, Foster M, Bahl A, Stellato : **Functional analysis of the chemokine receptor CCR3 on airway epithelial cells.** *J Immunol* 2006, **177**:3344-3354.
7. Fryer AD, Stein LH, Nie Z, Curtis DE, Evans CM, Hodgson ST, Jose PJ, Belmonte KE, Fitch E, Jacoby DB: **Neuronal eotaxin and the effects of CCR3 antagonist on airway hyperreactivity and M2 receptor dysfunction.** *J Clin Invest* 2006, **116**:228-236.
8. Elsner J, Escher SE, Forssmann U: **Chemokine receptor antagonists: a novel therapeutic approach in allergic diseases.** *Allergy* 2004, **59**:1243-1258.
9. Sabroe I, Conroy DM, Gerard NP, Li Y, Collins PD, Post TW, Jose PJ, Williams TJ, Gerard CJ, Ponath PD: **Cloning and characterization of the guinea pig eosinophil eotaxin receptor, C-C chemokine receptor-3: blockade using a monoclonal antibody in vivo.** *J Immunol* 1998, **161**:6139-6147.
10. Terada N, Hamano N, Kim WJ, Hirai K, Nakajima T, Yamada H, Kawasaki H, Yamashita T, Kishi H, Nomura T, Numata T, Yoshie O, Konno A: **The kinetics of allergen-induced eotaxin level in nasal lavage fluid: its key role in eosinophil recruitment in nasal mucosa.** *Am J Respir Crit Care Med* 2001, **164**:575-579.
11. Ma W, Bryce PJ, Humbles AA, Laouini D, Yalcindag A, Alenius H, Friend DS, Oettgen HC, Gerard C, Geha RS: **CCR3 is essential for skin eosinophilia and airway hyperresponsiveness in a murine model of allergic skin inflammation.** *J Clin Invest* 2002, **109**:621-628.
12. Humbles AA, Lu B, Friend DS, Okinaga S, Lora J, Al-Garawi A, Martin TR, Gerard NP, Gerard C: **The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness.** *Proc Natl Acad Sci USA* 2002, **99**:1479-1484.
13. Rajalulasingam K, Hamis Q, O'Brien F, Shotman E, Jose PJ, Williams TJ, Jacobson M, Barkans J, Durham SR: **RANTES in human allergen-induced rhinitis: cellular source and relation to tissue eosinophilia.** *Am J Respir Crit Care Med* 1997, **155**:696-703.
14. Ying S, Meng Q, Zeibecoglou K, Robinson DS, Macfarlane A, Humbert M, Kay AB: **Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and non-atopic (intrinsic) asthmatics.** *J Immunol* 1999, **163**:6321-6329.
15. Greiff L, Petersen H, Mattsson E, Andersson M, Erjefält JS, Linden M, Svensson C, Persson CG: **Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment.** *Clin Exp Allergy* 2001, **31**:1321-1327.
16. Lilly CM, Nakamura H, Belostotsky OI, Haley KJ, Garcia-Zepeda EA, Luster AD, Israel E: **Eotaxin expression after segmental allergen challenge in subjects with atopic asthma.** *Am J Respir Crit Care Med* 2001, **163**:1669-1675.

17. Gauvreau GM, Boulet LP, Cockcroft DW, Baatjes A, Cote J, Deschesnes F, Davis B, Strinich T, Howie K, Duong M, Watson RM, Renzi PM, O'Byrne PM: **Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses.** *Am J Respir Crit Care Med* 2008, **177**:952-958.
18. Wegmann M, Goggel R, Sel S, Erb KJ, Kalkbrenner F, Renz H, Garn H: **Effects of a low molecular weight CCR-3 antagonist on chronic experimental asthma.** *Am J Respir Cell Mol Biol* 2007, **36**:61-67.
19. Das AM, Vaddi KG, Solomon KA, Krauthauser C, Jiang X, McIntyre KW, Yang XX, Wadman E, Welch P, Covington M, Graden D, Yeleswaram K, Trzaskos JM, Newton RC, Mandlekar S, Ko SS, Carter PH, Davies P: **Selective inhibition of eosinophil influx into the lung by small molecule CC chemokine receptor 3 antagonists in mouse models of allergic inflammation.** *J Pharmacol Exp Ther* 2006, **318**:411-417.
20. Nakamura T, Ohbayashi M, Toda M, Hall DA, Horgan CM, Ono SJ: **A specific CCR3 chemokine receptor antagonist inhibits both early and late phase allergic inflammation in the conjunctiva.** *Immunol Res* 2005, **33**:213-221.
21. Suzuki K, Morakata T, Morihira K, Sato I, Takizawa S, Kaneko M, Takahashi K, Shimizu Y: **In vitro and in vivo characterization of a novel CCR3 antagonist, YM-344031.** *Biochem Biophys Res Commun* 2006, **339**:1217-1223.
22. Morakata T, Suzuki K, Masunaga Y, Tagushi K, Morihira K, Sato I, Fujii M, Takizawa S, Torii Y, Yamamoto N, Kaneko M, Yamada T, Takahashi K, Shimizu Y: **A novel, selective, and orally available antagonist for CC chemokine receptor 3.** *J Pharmacol Exp Ther* 2006, **317**:244-250.
23. DeLucca GV, Kim UT, Vargo BJ, Duncia JV, Santella JB, Gardner DS, Zheng C, Liauw A, Wang Z, Emmett G, Wacker DA, Welsh PK, Covington M, Stowell NC, Wadman EA, Das AM, Davies P, Yeleswaram S, Graden GM, Solomon KA, Newton RC, Trainor GL, Decicco CP, Ko SS: **Discovery of CC chemokine receptor-3 (CCR3) antagonists with picomolar potency.** *J Med Chem* 2005, **48**:2194-2211.
24. Ahlström-Emanuelsson V, Persson CG, Svensson C, Andersson M, Hosszu Z, Åkerlund A, Greiff L: **Establishing a model of seasonal allergic rhinitis and demonstrating dose-response to a topical glucocorticosteroid.** *Ann Allergy Asthma Immunol* 2002, **89**:159-165.
25. Ahlström-Emanuelsson C, Andersson A, Persson CG, Thorsson L, Greiff L: **Effects of topical formoterol alone and in combination with budesonide in a pollen season model of allergic rhinitis.** *Respir Med* 2007, **101**:1106-1112.
26. Persson CG, Erjefält JS, Greiff L, Andersson M, Erjefält I, Godfrey RW, Korsgren M, Linden M, Sundler F, Svensson C: **Plasma-derived proteins in airway defence, disease and repair of epithelial injury.** *Eur Respir J* 1998, **11**:958-970.
27. Greiff L, Andersson M, Persson CGA: **Nasal secretions/exudations: Collection and approaches to analysis.** *Methods in molecular medicine: Human airway inflammation* Totowa (NJ): Humana Press; Rogers D, Donnelly L 2001, **56**:61-73.
28. Korsgren M, Andersson M, Borgå O, Larsson L, Aldén-Raboisson M, Malmqvist U, Greiff L: **Clinical efficacy and pharmacokinetic profile of intranasal and oral cetirizine in a pollen season model of allergic rhinitis.** *Ann Allergy Asthma Immunol* 2007, **98**:316-321.
29. Erjefält JS, Greiff L, Andersson M, Ädelroth E, Jeffery PK, Persson CGA: **Degranulation patterns of eosinophil granulocytes as determinants of eosinophil driven disease.** *Thorax* 2001, **51**:159-163.
30. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ: **Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response.** *Lancet* 2000, **356**:2144-2148.
31. Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, Danzig M, Cuss F, Pauwels RA: **Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study.** *Am J Respir Crit Care Med* 2003, **167**:1655-1659.
32. Menzies-Gow A, Flood-Page P, Sehmi R, Burman J, Hamid Q, Robinson DS, Kay AB, Denburg J: **Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics.** *J Allergy Clin Immunol* 2003, **111**:714-719.
33. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, Cunningham A, Robinson BS, Myint SH, Ward ME, Tyrell DA, Holgate ST: **The relationship between upper respiratory tract infections and hospital admissions for asthma: a time-trend analysis.** *Am J Respir Crit Care Med* 1996, **154**:654-660.
34. Greiff L, Andersson M, Andersson E, Linden M, Myint S, Svensson C, Persson CG: **Experimental common colds increases mucosal output of eotaxin in atopic individuals.** *Allergy* 1999, **54**:1204-1208.
35. Teran LM, Seminario MC, Shute JK, Papi A, Compton SJ, Low JL, Gleich GJ, Johnston SL: **RANTES, macrophage-inhibitory protein 1 alpha, and the eosinophil product major basic protein are released into upper respiratory secretions during virus-induced asthma exacerbations in children.** *J Infect Dis* 1999, **179**:677-681.

doi:10.1186/1465-9921-11-17

Cite this article as: Greiff *et al.*: Effects of a dual CCR3 and H₁-antagonist on symptoms and eosinophilic inflammation in allergic rhinitis. *Respiratory Research* 2010 **11**:17.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

