Petasol butenoate complex (Ze 339) relieves allergic rhinitis–induced nasal obstruction more effectively than desloratadine

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Background: Allergic rhinitis symptoms of itching, sneezing, rhinorrhea, and nasal obstruction significantly decrease patients' quality of life. Compared with histamine and leukotriene receptor antagonists, the petasol butenoate complex Ze 339 displays pharmacologically distinct properties. *In vitro* it inhibits the biosynthesis of leukotrienes and mediator release from activated eosinophils.

Objective: This study aimed to assess the efficacy and mode of action of Ze 339, desloratadine, and placebo on allergic rhinitis symptoms, nasal airflow, and local mediator levels after unilateral nasal allergen provocation.

Methods: In this double-blind, randomized, crossover study 18 subjects with allergic rhinitis to grass pollen received Ze 339, desloratadine, and placebo for 5 days before nasal allergen challenge with grass pollen extract. Rhinomanometry, symptom assessment, and local inflammatory mediator measurement were performed during the 24 hours after allergen challenge.

Results: With Ze 339, the patient's time to recovery (5.4 ± 1.6 hours) from nasal obstruction after allergen challenge (time for return to 90% of baseline value ± SEM) was significantly shorter than with placebo (9.1 ± 2.3 hours, $P = .035$) and desloratadine (10.7 ± 2.5 hours, $P = .022$). Likewise, Ze 339's standardized symptom assessment for nasal obstruction (3.2 ± 1.3 hours) showed significantly faster relief (time for return to baseline value ± SEM compared with placebo, 8.3 ± 2.4 hours; $P = .027$) and desloratadine (4.5 ± 1.2 hours, $P = .030$). One interesting finding was that Ze 339 significantly reduced IL-8 and leukotriene B_4 levels in nasal secretions before challenge.

Conclusion: When compared with desloratadine and placebo, Ze 339 shows better efficacy in relieving nasal obstruction symptoms and inhibiting critical components of the chemokine network and as such represents a novel symptomatic and possible prophylactic treatment for allergic rhinitis. (J Allergy Clin Immunol 2011;127:1515-21.)

Key words: *Randomized controlled trial, allergic rhinitis, nasal obstruction, rhinomanometry, IL-8, leukotriene B_4, histamine, Ze 339, petasol butenoate complex,* Petasites hybridus*, desloratadine, nasal allergen challenge*

With its average prevalence of 25%, allergic rhinitis, or hay fever, is the most common atopic disease in the industrialized world.[1] Symptoms of itching, sneezing, rhinorrhea, and nasal obstruction significantly decrease quality of life and increase the risk of having asthma.[2-4] The effective therapeutic management of allergic rhinitis and, in particular, nasal obstruction remains a critical issue.

With allergen exposure, nasal obstruction occurs within minutes and lasts for hours throughout the late-phase response. Mast cells and eosinophils immediately release inflammatory mediators (eg, histamine and arachidonic acid metabolites), whereas sensory nerve endings release neurogenic peptides (eg, substance P). These mediators cause vasodilatation and plasma exudation, resulting in nasal mucosal edema.[5-7]

Mediator release (eg, histamine and leukotriene B_4 [LTB_4]) promotes vasodilatation but also induces the expression of proinflammatory and chemotactic cytokines, such as IL-8, from epithelial cells.[8] Both the combined secretion and *de novo* expression of chemokines, including IL-8 or CCL-5 (RANTES), promote even further recruitment of leukocytes into the nasal mucosa, and this cascade represents a critical step in the allergic late-phase response. Ideally, a symptomatic treatment would block both early- and late-phase responses to prevent nasal obstruction. For most patients, nasal obstruction represents the dominant symptom, causing discomfort and having a negative effect on both quality of life and work productivity.[1-5,9]

Current allergic rhinitis treatment is based on 3 approaches: allergen avoidance, specific immunotherapy, and pharmacotherapy, in which common drugs include histamine H1 receptor antagonists. Second-generation antihistamines provide good symptom control and anti-inflammatory properties, and recent

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Abbreviations used
AOC: Area over the curve
AUC: Area under the curve
LTB_4: Leukotriene B_4
PGD_2: Prostaglandin D_2
RTB: Return to baseline
VAS: Visual analog scale

data report a statistically significant relief of nasal blockage.[7,9] Nevertheless, physicians' treatment decisions are based primarily on their experiences with allergic patients,[10] and antihistamine treatment for nasal congestion is discussed controversially.

Topical steroids act through generic anti-inflammatory mechanisms and provide good control of all nasal symptoms but face patients' reservations to steroids. One meta-analysis reports that the use of leukotriene receptor antagonists for allergic rhinitis is not more effective than the use of antihistamines or topical nasal steroids.[11] Alternate treatment options that restore nasal airflow without the side effects of sympathomimetic topical nasal decongestants are thus needed.

Ze 339 is a carbon dioxide extract derived from the leaves of a special variety (Petzell) of *Petasites hybridus* registered at the European Community Plant Variety Office. Currently, Ze 339 is available by prescription in Switzerland and elsewhere for treating allergic rhinitis. *In vitro* studies show that Ze 339 blocks degranulation in activated immune cell populations and also inhibits leukotriene biosynthesis.[12,13] Previous clinical trials indicated that the efficacy of Ze 339 was similar to that of cetirizine and fexofenadine during the peak season in patients with allergic rhinitis.[14,15] However, proof of concept in a defined allergic model and mechanistic insight into the drug's mode of action were lacking. The aim of this study was therefore to assess the ability of Ze 339 to relieve nasal congestion in an allergic rhinitis model investigated outside of the pollen season and to compare the results with those seen after placebo and a commonly used antihistamine. The second objective was to generate a hypothesis regarding the mode of action by analyzing the expression of inflammatory mediators in nasal secretions.

METHODS

Study population

At one site (the Department of Otorhinolaryngology, Heinrich Heine University Hospital, Dusseldorf, Germany), we recruited 18 otherwise healthy adult volunteers with at least a 2-year medical history of moderate-to-severe allergic rhinitis to grass pollen. Patients with further sensitizations were only included if there were no exposures during the study. Consequently, none of the included patients had nasal symptoms before grass pollen allergen exposure. Subjects were included when the skin prick test response to grass pollen allergens was positive, showing a raised wheal of at least 3 mm in diameter when compared with that seen after application of a negative control (Allergopharma Grass Pollen mixture; Allergopharma, Reinbek, Germany). Additionally, grass pollen–specific IgE levels were 0.7 to 3.5 IU/mL, corresponding to a RAST class of 2 (Pharmacia CAP, Uppsala, Sweden) or greater. Exclusion criteria included asthma with an FEV_1 of less than 80% of predicted value, other types of rhinitis, sinusitis, current or concomitant antiallergic or anti-inflammatory drug use, and pregnancy. Each participant provided written informed consent before entering the study.

Study medication

The medications investigated (Ze 339, placebo, and desloratadine) were dispensed in a double-blind, double-dummy manner by using blinded vials. Ze 339 was provided as film-coated tablets containing 20 mg of a carbon dioxide extract from leaves of a registered *Petasites hybridus* (Petzell) variety. The active comparator, desloratadine (Aerius [Schering-Plough Canada, Inc, Kirkland, Quebec, Canada] or Clarinex [Merck & Co, Inc, Whitehouse Station, NJ]), a nonsedative, long-acting, and selective H1-receptor antagonist and common allergic rhinitis treatment, was filled in hard gelatin capsules. Corresponding placebos, manufactured in accordance with current good manufacturing practice and European Medicines Agency Good Medical Practice Annex 13 by Max Zeller Söhne AG, Romanshorn, Switzerland, were identical in color, shape, and appearance to the investigational preparations. Desloratadine was taken once in the morning, whereas Ze 339 was taken twice daily (morning and evening). A double-dummy approach was chosen with the crossover design; the daily treatment contained 1 tablet and 1 capsule in the morning and 1 tablet in the evening (either placebo or active drug). Drug accountability was checked after each treatment sequence before nasal allergen challenge. Max Zeller Söhne AG (sponsor) commissioned the generation of the randomization sequence. The study was unblinded after database closure.

Study design

This exploratory, monocentric phase II trial was performed between January and April (ie, not during the grass pollen season). A prospective, randomized, double-blind, double-dummy, 3-arm crossover design (Latin square) was chosen. Every subject randomly received each treatment for 5 days (Fig 1). Patients were randomly allocated to 6 different treatment sequences (n = 3 per sequence). The following sequences represent the 6 possible treatment allocations: (A, B, C), (A, C, B), (B, A, C), (B, C, A), (C, A, B), and (C, B, A), with A referring to Ze 339, B referring to placebo, and C referring to desloratadine. There were no dropouts during the study.

On the fifth day (after the morning dose), a unilateral nasal allergen challenge was performed with a nasal spray application. Primary and secondary end points were assessed during the 24 hours after nasal allergen challenge. At least 10 days (5 days of treatment and at least 5 days of washout) elapsed between each nasal allergen challenge. Pollen exposure was followed by using the regional pollen count service. Continuous monitoring, including vital signs and adverse event documentation, was undertaken throughout the study. Routine laboratory tests were assessed before and after the study. Blinded experimental laboratory tests were performed in the Allergy and Clinical Immunology Section, Imperial College London. Both clinical and laboratory study data were monitored by the independent University Hospital Clinical Research Coordination Center in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice and the Declaration of Helsinki. The trial was approved by the Ethics Committee, University of Düsseldorf, and registered at http://www.ClinicalTrial.gov (NCT00862225).

Allergen challenge

On the day of provocation (ie, treatment day 5), the respiratory tract was examined. Next, nasal lavage with 0.9% NaCl solution in a 10-mL syringe was used to rinse the provocation side before clinical assessments. Subsequently, subjective symptoms were recorded through visual analog scales (VASs), rhinomanometry was used to measure nasal obstruction, and the baseline collection of nasal secretions was performed with adsorbent discs. A solvent provocation was performed to exclude unspecific hyperreactivity of the nasal mucosa, and clinical assessments were repeated, thereby providing baseline values for clinical parameters. Then 2 puffs of a grass pollen solution (25,000 BU/mL, Allergopharma) were applied into 1 nostril.

Clinical assessments

The primary outcome parameter was resolution of nasal obstruction, which was defined as restitution of nasal airflow in a time-dependent manner subsequent to unilateral nasal allergen challenge. At baseline and then 15 minutes and 1, 2, 3, 4, 5, 6, 7, and 24 hours after baseline, anterior rhinomanometry (Rhinotest, 2000 plus; EVG GmbH, Böhl-Iggelheim, Germany) was performed, with the nasal airflow being measured in cubic centimeters per second (150 Pa) at ambient room conditions. For each subject

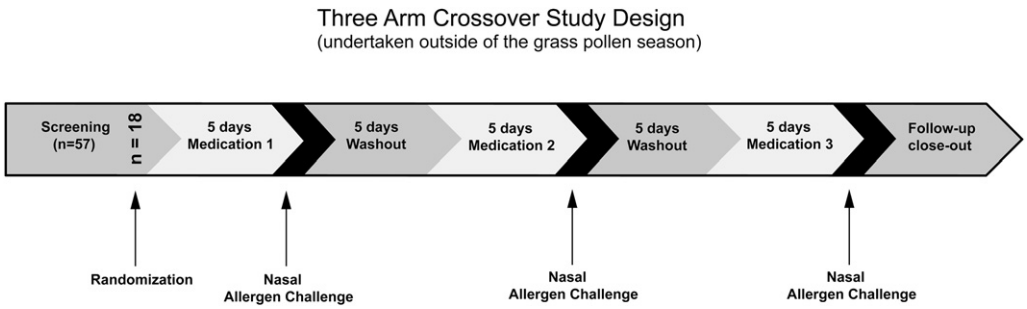

FIG 1. Study design. Ze 339, desloratadine, and placebo were applied in a randomized 3-way crossover design. Medication was taken for 5 days, and allergen challenge was performed on the fifth day of treatment.

TABLE I. Demographic data of enrolled subjects

Subjects, no.	18
Male sex, no. (%)	7 (38.9)
Female sex, no. (%)	11 (61.1)
Age (y), mean (minimum-maximum)	29.3 (21-51)
Weight (kg), mean (minimum-maximum)	71.8 (50-96)
Height (cm), mean (minimum-maximum)	173.2 (160-185)

and provocation, the percentage flow reduction at a transnasal pressure difference of 150 Pa was calculated as follows:

$$\Delta V\ [\%] = [(Vpre - Vpost)/Vpre] * 100\%.$$

At the same time points, secondary clinical end points were assessed: nasal secretions were obtained (with adsorbent discs), and the global nasal assessment (sneezing, itching, nasal obstruction, and rhinorrhea) was scored by patients using a 0- to 10-point VAS.

Measurements of mediators in nasal secretions

Nasal secretions were collected with endonasal cellulose adsorbent discs (10 mm in diameter, 1.2-mm thickness, punched out from Shandon Filter Cards; Thermo Scientific, Waltham, Mass) placed in the anterior septal region for 45 seconds. Secretion weights were assessed immediately afterward. Each collection disc was eluted in 1000 μL of 0.9% NaCl solution for 1 hour at 4°C. The disc was then removed, and the eluate was stored at −80°C until assayed. The collected nasal secretions were assessed for a panel of proinflammatory cytokines, chemokines, prostaglandin D_2 (PGD_2), LTB_4, and histamine. Expression levels were adjusted to secretion weights for concentrations per milligram of secretion and as total detected protein per time point, with values representing the total amounts recovered by the individual collection discs, as previously described.[16]

Cytokine concentrations in nasal secretions were analyzed on a Bio-Plex Suspension Array System (Bio-Rad Laboratories, Munich, Germany) that permits the simultaneous detection of multiple cytokines in a single well of a 96-well microplate. As shown in Table E1 (available in this article's Online Repository at www.jacionline.org), the concentrations of 27 cytokines were profiled in samples by using a Luminex 100 ISTM (Luminex Corp, Austin, Tex) with a detection limit of less than 0.5 pg/mL. Histamine levels were measured with a commercially available enzyme immunoassay kit (Immunotech, Marseille, France) with an analytic sensitivity of 0.5 nmol/L. LTB_4 and PGD_2 levels were determined by using an enzyme immunoassay kit (Cayman Chemicals Company, Ann Arbor, Mich). The sample concentrations were calculated according to the manufacturer's instructions; detection limits for LTB_4 and PGD_2 were 13 pg/mL and 200 pg/mL, respectively.

Statistical analysis

The primary end point (course over time of nasal obstruction relief measured by rhinomanometry in cubic centimeters per second) was assessed by (1) the time to return to 90% of the measured baseline value of the nasal flow (return to baseline [RTB] 90% in hours), as calculated by using a linear interpolation between the first time point at greater than 90% and the last time point at less than 90%; (2) area over the curve (AOC) of the relative change (as a percentage) from baseline to 3 hours (AOC 0-3h [as percentage times hour]);

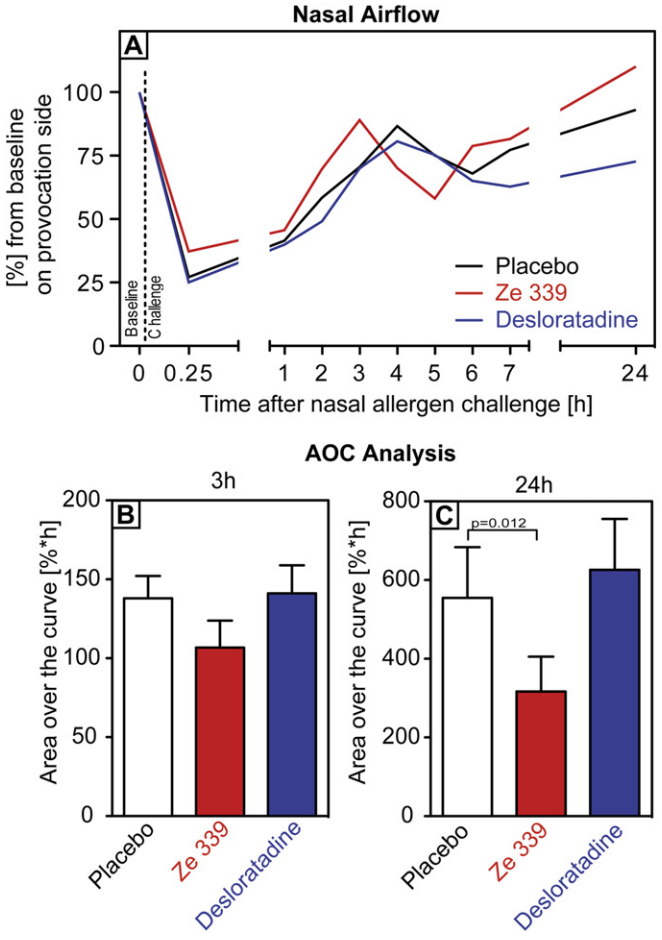

FIG 2. Determination of nasal flow by means of anterior rhinomanometry after baseline measurement at time point 0 and nasal allergen challenge. **A**, Median nasal air flow over time (relative change from baseline). **B**, AOC 3 hours after nasal allergen challenge (means ± SEMs). **C**, AOC 24 hours after nasal allergen challenge (means ± SEMs). Significance was reached for the reduction in AOC at 0 to 24 hours for Ze 339 compared with placebo (mean ± SEM: 316.8 ± 88.5 vs 555.0 ± 128.8; $P = .012$, Wilcoxon signed-rank test).

TABLE II. Outcome of end points

	Placebo (mean ± SEM)	Ze 339 (mean ± SEM)	Desloratadine (mean ± SEM)	Placebo/Ze 339	Placebo/ desloratadine	Ze 339/ desloratadine
Rhinomanometry						
Time to return to 90% baseline (h)	9.1 ± 2.3	5.4 ± 1.6	10.7 ± 2.5	$P = .035$*	$P = .758$	$P = .022$*
AOC 3 h (%*h)	138.1 ± 14.1	106.8 ± 17.0	141.1 ± 17.8	$P = .094$	$P = .744$	$P = .071$
AOC 24 h (%*h)	555.0 ± 128.8	316.8 ± 88.5	626.0 ± 129.1	$P = .012$*	$P = .811$	$P = .071$
VAS nasal obstruction: RTB (h)	8.3 ± 2.4	3.2 ± 1.3	4.5 ± 1.3	$P = .027$*	$P = .678$	$P = .030$*
AUC 3 h (%*h)	6.8 ± 1.1	6.4 ± 1.1	5.8 ± 0.6	$P = .811$	$P = .647$	$P = .983$
AUC 24 h (%*h)	20.2 ± 3.7	15.5 ± 2.6	14.1 ± 2.4	$P = .215$	$P = .372$	$P = .913$
VAS sneezing: RTB (h)	1.1 ± 0.1	1.3 ± 0.3	0.7 ± 0.2	$P = .380$	$P = .134$	$P = .059$
Nasal mediators before challenge (per mg nasal secretion in pg/mL)						
IL-8	52.9 ± 18.9	8.5 ± 1.7	50.1 ± 20.3	$P = .044$*	$P = .760$	$P = .025$*
LTB_4	57.5 ± 26.8	9.3 ± 3.7	44.6 ± 14.0	$P = .036$*	$P = .582$	$P = .014$*
IP10	443.7 ± 129.0	178.6 ± 87.1	289.5 ± 76.1	$P = .063$	$P = .251$	$P = .341$
RANTES	0.22 ± 0.05	1.2 ± 0.98	0.4 ± 0.14	$P = .759$	$P = .840$	$P = .678$
PGD_2	69.7 ± 13.1	42.4 ± 8.1	79.6 ± 18.3	$P = .09$	$P = .682$	$P = .076$
Histamine	1.5 ± 0.8	2.0 ± 1.2	2.3 ± 1.3	$P = .301$	$P = .794$	$P = .127$
Eotaxin	1.3 ± 0.4	1.1 ± 0.3	1.4 ± 0.3	$P = .810$	$P = .367$	$P = .371$

All values are presented as means ± SEMs.
IP10, Chemokine (C-X-C motif) ligand 10; *RANTES*, chemokine (C-C motif) ligand 5.
*Statistical significance.

and (3) AOC of the relative change (as a percentage) from baseline to 24 hours (AOC 0-24h [percentage times hour]). AOC was calculated by using the linear trapezoidal rule with the relative flow values at measurement time points. AOC was chosen because the responses to allergen provocation resulted in decreased nasal flow values compared with baseline. AOC and the area under the curve (AUC) are closely related and interconvertible (see Fig E1 and the Methods section in this article's Online Repository at www.jacionline.org):

$$\text{AOC} = [\text{Baseline flow} * (T_{3h\ or\ 24h} - T_{0h})] - \text{AUC},$$

where the term *Baseline flow* $*(T_{3h\ or\ 24h} - T_{0h})$ represents the rectangle under the baseline (baseline was set to 100%).

As secondary end points, we used the following: (1) deviation from baseline of numeric scores obtained by means of VAS and (2) cytokine and chemokine levels in nasal secretions over the observation period.

For rhinomanometric parameters (AOC 0-3h, AOC 0-24h, and RTB90%), a nonparametric test (the Wilcoxon signed-rank test) was performed in a conservative approach. However, because AOC 0-3h and log AOC 0-24h data showed a normal distribution, these parameters were additionally evaluated by using linear mixed models to estimate potential carryover and period effects (see the Methods section in this article's Online Repository at www.jacionline.org). Carryover and period effects for RTB90% (not normally distributed) were assessed by means of (distribution-independent) Cox regression analysis. Statistical testing for cytokine and chemokine levels was performed after logarithmic transformation of the values to obtain a Gaussian approximation, which was the basis for using the Student *t* test. All hypothesis tests were conducted as pairwise tests, uncorrected 2-sided tests; a *P* value of .05 was considered significant.

Sample size estimations were not performed. Because of the complex design of this exploratory study, sample size was chosen on the basis of practical considerations. Therefore this study was not designed to have sufficient power, and the results of statistical testing have to be interpreted as descriptive, explorative, and hypothesis generating rather than as confirmatory. No correction for multiple testing has been applied. The statistical analysis was performed with SPSS 15.0 (SPSS, Inc, Chicago, Ill) and R version 2.9.0 software.

RESULTS

Study population

In total, 57 subjects were screened. Eighteen (7 male and 11 female subjects) patients with moderate-to-severe allergic rhinitis caused by grass pollen sensitization with no clinically significant cosensitization who were otherwise healthy were enrolled and randomized into the study. Patients' demographics are shown in Table I. The remaining 39 patients could not be enrolled because of exclusion criteria. Most of these patients (n = 36) showed clinically significant cosensitization and were therefore not included. All randomized participants completed all treatments and assessments.

Safety

Treatment with Ze 339, desloratadine, and placebo and all clinical interventions were well tolerated. No serious adverse events occurred. No significant changes in the safety laboratory assessments occurred, particularly with respect to hepatic parameters. All adverse events reported were mild in nature. There were 6 adverse events with Ze 339 treatment: headache, dysgeusia, urticaria, procedural pain, head pressure, and nose bleed. There were 5 adverse events with desloratadine: fatigue, dizziness, tiredness, nausea, and sneezing. Finally, there were 8 adverse events with placebo: vomiting, loose stools, toothache, nose swelling, dizziness, nausea, headache, and Hashimoto thyroiditis. The occurrence of Hashimoto thyroiditis with placebo was assessed as unlikely to be related to the investigation because the morphologic changes in the thyroid gland were not considered a recent development and therefore were assessed as being preexisting.

Primary outcome

Nasal airflow (in cubic centimeters per second), as determined by means of rhinomanometry, was measured directly before and up to 24 hours after unilateral nasal allergen challenge. Time-dependent profiles of the median percentage change in nasal airflow are displayed in Fig 2, *A*, and show clear differences between the treatments. After nasal allergen challenge, a biphasic reaction of symptoms was observed, primarily in nasal airflow, which were identified as early- and late-phase responses in all

treatment groups. Early response was detected at the time point 15 minutes after provocation, and the late-phase response was detected approximately 5 to 6 hours after provocation. Calculations from 0 to 3 hours and from 0 to 24 hours revealed a reduced AOC for Ze 339 (ie, an improved nasal airflow over time) compared with values seen with both placebo and desloratadine (Fig 2, *B* and *C*, and Table II). Significance was reached for the reduction in the 0- to 24-hour AOC for Ze 339 compared with placebo (mean ± SEM: 316.8 ± 88.5 vs 555.0 ± 128.8; $P = .012$, Wilcoxon signed-rank test).

Recovery of nasal airflow after allergen challenge was defined as the time (in hours) needed to return to 90% of the baseline value of the nasal flow (RTB90%). Ze 339 provided faster recovery of nasal airflow than desloratadine after allergen challenge, as shown by means of Cox regression ($P = .046$; Fig 3, *A*). Neither period nor carryover effects were detected by means of Cox regression (RTB90%) or linear mixed-effects models (AOC 0-3h and log AOC 0-24h). The mean values, as determined by means of rhinomanometry, showed faster return to 90% of the baseline value for Ze 339 compared with placebo (mean ± SEM: 5.4 ± 1.6 vs 9.1 ± 2.3 hours, $P = .035$) and desloratadine (5.4 ± 1.6 vs 10.7 ± 2.5 hours; $P = .022$, Wilcoxon signed-rank test; Fig 3, *B*, and Table II).

Secondary clinical end points

Consistent with rhinomanometry, the subjective assessment of nasal obstruction by the patient using the VAS showed a significantly shorter time to RTB (in hours) with Ze 339 than with placebo (mean ± SEM: 3.2 ± 1.3 vs 8.3 ± 2.4 hours, $P = .027$) or desloratadine (3.2 ± 1.3 vs 4.5 ± 1.2 hours; $P = .030$, Wilcoxon signed-rank test; Fig 3, *C*, and Table II). In Fig E2 (available in this article's Online Repository at www.jacionline.org), the time courses for sneezing and nasal obstruction, as evaluated by the patients' VAS scores, are shown, whereas corresponding AUC values are displayed in Table II. These VAS data were further correlated with the rhinomanometric data. In patients treated with Ze 339, the correlation of the objective, airflow measurements (log AOC 0-3h of rhinomanometry), and subjective data (log AUC 0-3h of the VAS nasal obstruction) showed a trend but did not reach statistical significance ($P = .06$, Pearson correlation; $R = 0.45$).

As expected, desloratadine showed enhanced performance for control of sneezing; however, this did not reach statistical significance when compared with Ze 339 (mean ± SEM: 0.7 ± 0.2 vs 1.3 ± 0.3 hours; $P = .059$, Wilcoxon signed-rank test; see Fig E2, *A*, and Table II).

Cytokine and chemokine expression profiles

Treatment with Ze 339 led to reduced local mediator expression in initial nasal secretions before the nasal allergen challenge (results are expressed in picograms per milliliter of protein per milligram of nasal secretion; all data were tested with the paired *t* test). IL-8 expression was reduced 6-fold in comparison with that seen after placebo (mean ± SEM: 8.5 ± 1.7 vs 52.9 ± 18.9 pg/mL, $P = .044$) and also in comparison with that seen after desloratadine (8.5 ± 1.7 vs 50.1 ± 20.3 pg/mL, $P = .025$; Fig 4, *A*, and Table II). The same pattern was seen with LTB_4: levels with Ze 339 were 6 times lower than those with placebo (mean ± SEM: 9.3 ± 3.7 vs 57.5 ± 26.8 pg/mL, $P = .036$) and almost 5 times lower than those with desloratadine (9.3 ± 3.7 vs 44.6 ± 14.0 pg/mL, $P = .014$; Fig 4, *B*, and Table II). To check plausibility and to assess whether levels of inflammatory mediators are related to improved nasal airflow, we performed a correlation analysis (Pearson correlation) between rhinomanometric data and the expression of IL-8 at baseline, yielding significant correlations: log AOC at 0 to 3 hours versus log IL-8 ($P = .04$, $R = 0.29$) and log AOC at 0 to 24 hours versus log IL-8 ($P = .03$, $R = 0.30$).

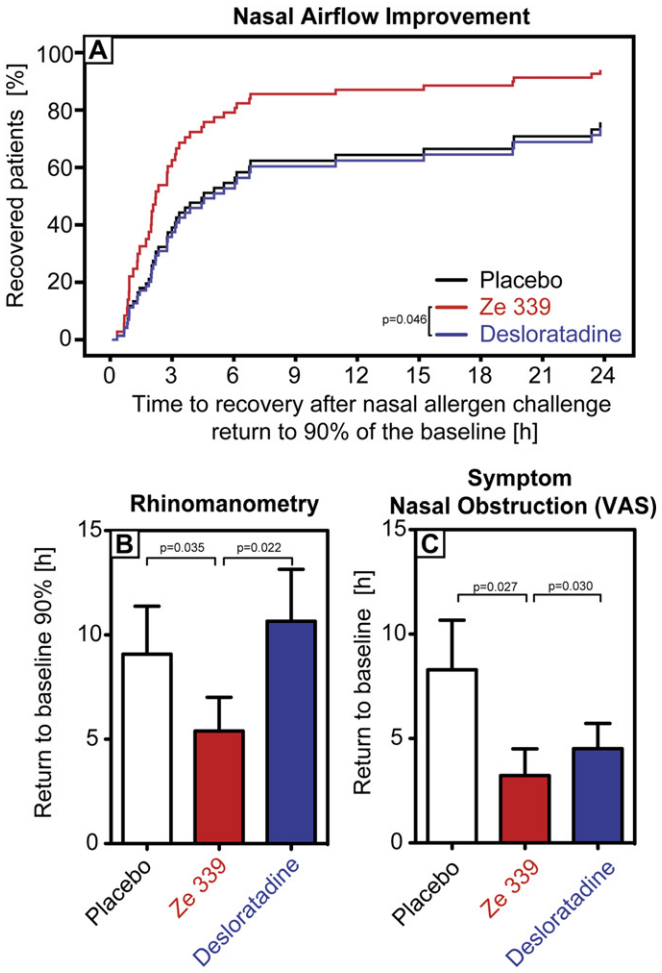

FIG 3. Recovery of nasal obstruction. **A**, Cox regression model of the time to return to 90% of nasal air flow after nasal allergen challenge assessed by means of rhinomanometry (Ze 339, 2.46 hours; desloratadine, 3.94 hours [medians], $P = .046$). **B**, Time to return to 90% of basal flow (means ± SEMs assessed by means of rhinomanometry: Ze 339, 5.4 ± 1.6 hours; placebo, 9.1 ± 2.3 hours; desloratadine, 10.7 ± 2.5 hours; Wilcoxon signed-rank test). **C**, Time to RTB value of the symptom of nasal obstruction (means ± SEMs assessed by means of VAS: Ze 339, 3.2 ± 1.3 hours; placebo, 8.3 ± 2.4 hours; desloratadine, 4.5 ± 1.2 hours; Wilcoxon signed-rank test).

Mediator kinetics after nasal challenge

The allergen challenge induced a marked early-phase response in all treatment groups. The late-phase response was depicted by LTB_4 and histamine but not by IL-8 (see Fig E3 in this article's Online Repository at www.jacionline.org). The secretion of PGD_2 in the early phase, calculated as the AUC of secretion over time in the first 3 hours after provocation, was significantly lower during treatment with Ze 339 when compared with that

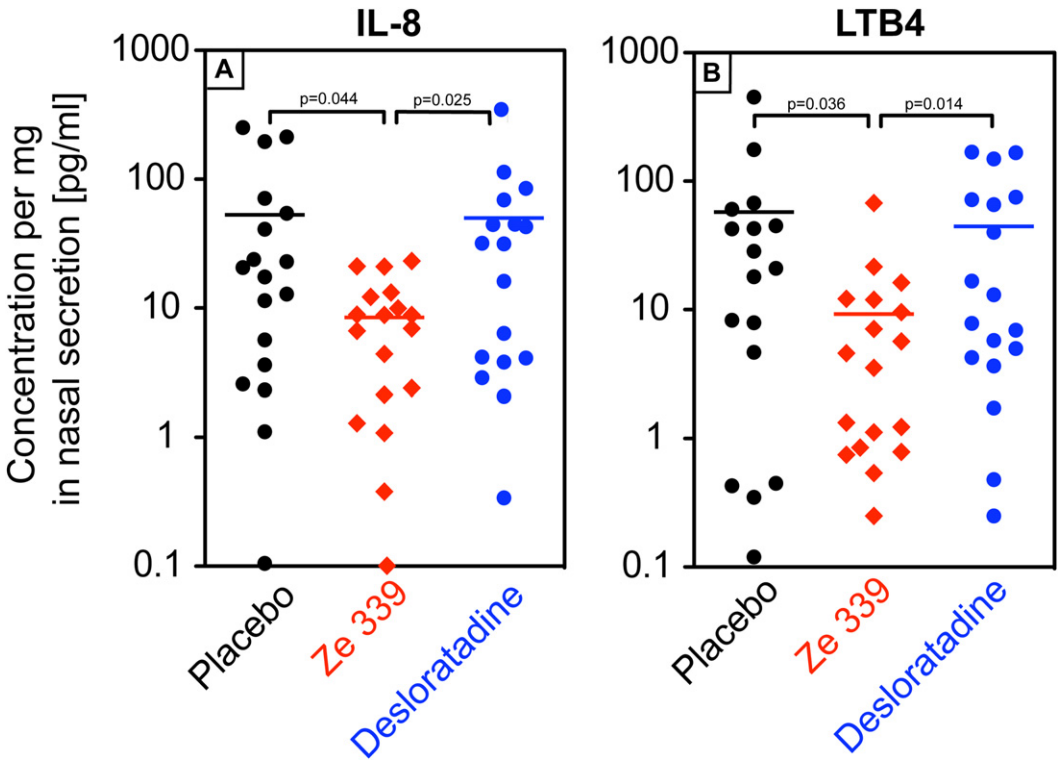

FIG 4. Concentrations in picograms per milliliter per milligram of nasal secretion) of IL-8 **(A)** and LTB_4 **(B)** in nasal secretions. Individual values of all patients (n = 18) are displayed as data points, and mean levels are indicated as *bars*. Levels were determined on the fifth day of the treatment before allergen challenge. IL-8: Ze 339, 8.5 ± 1.7 pg/mL; placebo, 52.9 ± 18.9 pg/mL; desloratadine, 50.1 ± 20.3 pg/mL. LTB_4: Ze 339, 9.3 ± 3.7 pg/mL; placebo, 57.5 ± 26.8 pg/mL; desloratadine, 44.6 ± 14.0 pg/mL. *P* values were determined by using the paired *t* test.

seen during desloratadine treatment (979.1 ± 149.8 vs 1421 ± 164.1 pg/mL; $P = .0077$, paired *t* test) but did not reach significance compared with values seen with placebo. The AUC of histamine peaked in the late phase in both active treatment groups compared with that seen in the placebo group (see Fig E3, *C* and *D*, and see Table E2).

DISCUSSION

This randomized, double-blind, placebo-controlled trial shows that Ze 339 is effective in relieving allergen-induced nasal obstruction, the dominant symptom of allergic rhinitis, through a generic mechanism involving LTB_4 and IL-8.

The efficacy and safety of Ze 339 for the treatment of seasonal allergic rhinitis has been demonstrated in several controlled clinical trials[14,15] and again confirmed by this study. A reference method was used to quantify nasal obstruction for assessing the drug's efficacy in the treatment of allergic rhinitis.[17,18] The relief from nasal obstruction on allergen challenge was significantly faster with Ze 339 compared with desloratadine or placebo, as consistently demonstrated by means of both rhinomanometry and the patients' assessments with VAS symptom scores. In this study desloratadine did not improve nasal congestion, thereby contradicting other clinical studies performed in patients with seasonal hay fever,[19] despite evidence that it might relieve nasal congestion in patients with perennial allergic rhinitis.[20] It is unclear whether the effect on nasal congestion detected in earlier studies is due to a longer treatment period in patients with perennial allergic rhinitis, the nature of the allergen, or the sample size in this study. Nonetheless, in this study desloratadine did exert the expected palliative effect on sneezing but did not show any influence on the chemokine network. The discovery of mediators that might act as Ze 339 targets was an essential focus and a secondary end point in the study design. Among 30 inflammatory mediators investigated, reduced levels of LTB_4 and IL-8 were measured in nasal secretions obtained before allergen challenge during treatment with Ze 339. These results might suggest that patients, although not complaining about symptoms before nasal provocation, could exhibit nasal or allergic symptoms at a subclinical level. Nevertheless, normal values for these mediators are not available. Furthermore, it has to be taken into account that these results were obtained after nasal lavage with 0.9% NaCl solution.

It has been previously suggested that Ze 339 exhibits a dual mode of action by inhibiting LT synthesis (inhibitory concentration of 50%, <24 μg/mL) in platelet-activating factor or complement factor C5a–stimulated granulocytes and by blocking degranulation in activated mast cells and eosinophils.[12] LTB_4 is synthesized from arachidonic acid in mast cells and eosinophils and has important stimulatory effects on mast cell progenitors in bone marrow.[21] Leukotrienes are among the most potent disease mediators in patients with upper airway disease, including allergic rhinitis, and contribute substantially to aspirin-sensitive asthma and the associated chronic rhinosinusitis and nasal polyps.[22] The inhibitory effect of Ze 339 on IL-8 might depend

on reduced LTB_4 release because it has been previously shown that LTB_4 can induce IL-8 expression in epithelial cells. IL-8 binds to the chemokine receptor CXCR1 and recruits mainly neutrophils but also eosinophils, macrophages, and T cells.[23] IL-8 and monocyte chemotactic and activating factor (monocyte chemoattractant protein 1) are essentially involved in inflammatory and immune reactions.[24] Indeed, nasal challenge with recombinant IL-8 induces a significant neutrophilic infiltration in the nasal mucosa in atopic and nonatopic patients.[25]

Importantly, Ze 339 reduced the expression of LTB_4 and IL-8 after the pretreatment period before allergen challenge and might thus exert a prophylactic effect on allergen-induced nasal obstruction. Consistent with its lacking effect on nasal congestion, the histamine receptor antagonist desloratadine did not reduce IL-8 secretion, whereas an earlier pilot study with 30 patients showed that another histamine receptor antagonist, levocetirizine, reduced IL-8 and IL-4 secretions.[19] However, the latter study focused on seasonal effects and therefore was not timed, as was this study, and the effects are thus probably of an indirect nature. In line with these results, the nasal secretion levels of histamine and PGD_2 over 24 hours on allergen challenge might provide a mechanistic layout for the faster relief of nasal congestion. After treatment with Ze 339, the AUC of PGD_2 in the early-phase response was clearly reduced in comparison with that after treatment with desloratadine, whereas surprisingly, the AUC of histamine peaked in both active treatment groups in terms of the late-phase response.

Therefore, Ze 339 represents a symptomatic treatment with clearly different properties than those of antihistamines. A further potential local application of the study drug is being evaluated in an animal model and shows reduced inflammation in the airways with an ovalbumin challenge model.[26]

In conclusion, this exploratory trial shows that treatment with Ze 339 is superior to both desloratadine and placebo in improving the dominant symptom of nasal obstruction in a seasonal allergic rhinitis model by inhibiting critical elements of the leukotriene and chemokine network. Because of the important role of these mediators in recruiting inflammatory cells to the allergen stimulation site, it can be speculated that a prophylactic treatment with Ze 339 might be effective in counteracting allergic inflammation in the upper airways, whereas specific and adaptive immune responses remain intact.

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Clinical implications: The petasol butenoate complex Ze 339 relieves bothersome nasal congestion symptoms more effectively than desloratadine and thus expands the therapeutic options for the symptomatic treatment of allergic rhinitis.

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METHODS

AOC

AOC and AUC are calculated in most of the cases by using the linear trapezoidal rule. Use of AOC seems to be more appropriate when responses (f) to a treatment result in values less than those observed at baseline (see Fig E1). Accordingly, AUC is better used when responses (f) to a treatment result in values greater than those observed at baseline.

In our setting the area of the AOC reflects the degree of nasal obstruction (the decrease of nasal flow compared with baseline). Therefore lower AOC values are indicative for a less impaired nasal flow.

AUC and AOC are interconvertible:

$$\mathrm{AOC} = [\mathrm{f}(\mathrm{Tstart}) * (\mathrm{Tend} - \mathrm{Tstart})] - \mathrm{AUC},$$

where the term *f(Tstart) * (Tend – Tstart)* represents the rectangle under the baseline curve.

Details of the statistical models

The linear mixed-effects models were applied for the primary outcome variables according to the following scheme:

$$\mathrm{y_i} = \mu + \mu_{\mathrm{Ze399}} * \mathrm{Ze339[i]} + \mu_{\mathrm{Deslo}} * \mathrm{Deslo[i]} + \pi_{\mathrm{p2}} * \mathrm{Period2[i]} + \pi_{\mathrm{p3}} * \mathrm{Period3[i]} + \mathrm{a_{P[i]}} + \epsilon_{\mathrm{i}},$$

where y_i is defined as the i-th observation (dependent variable), μ is the mean for placebo in period 1, μ_{Ze339} is the difference between placebo and Ze 339 (fixed effect), μ_{Deslo} is the difference between placebo and desloratadine (fixed effect), *Ze339* is the indicator variable for Ze 339, *Deslo* is the indicator variable for desloratadine, *Period2* is the indicator variable for period 2, *Period3* is the indicator variable for period 3; π_{p2} is the difference between periods 1 and 2 (fixed effect), π_{p3} is the difference between periods 1 and 3 (fixed effect), *P* is patient identification (vector with length 54 and 18 values), a_P is the effect of patient P (random effect; $\sim$ Norm[0, σ_a]), and ϵ_i is the residual ($\sim$ Norm[0, σ]).

The carryover was tested by using a simple linear model on the residuals as follows:

$$\epsilon_{\mathrm{i}} = \lambda + \lambda_{\mathrm{A}} * \mathrm{cA[i]} + \lambda_{\mathrm{B}} * \mathrm{cB[i]} + \lambda_{\mathrm{C}} * \mathrm{cC[i]} + \mathrm{r_i},$$

where *cA* is the indicator variable for the carryover effect of Ze339, *cB* is the indicator variable for the carryover effect of placebo, *cC* is the indicator variable for the carryover effect of desloratadine, λ is the carryover effect for period 1 (expected to be = 0), λ_A is the difference between the carryover effect of period 1 to the carryover effect of Ze339, λ_B is the difference between the carryover effect of period 1 to the carryover effect of placebo, λ_C is the difference between the carryover effect of period 1 to the carryover effect of desloratadine, and r_i is ($\sim$ Norm[0, σ_r]).

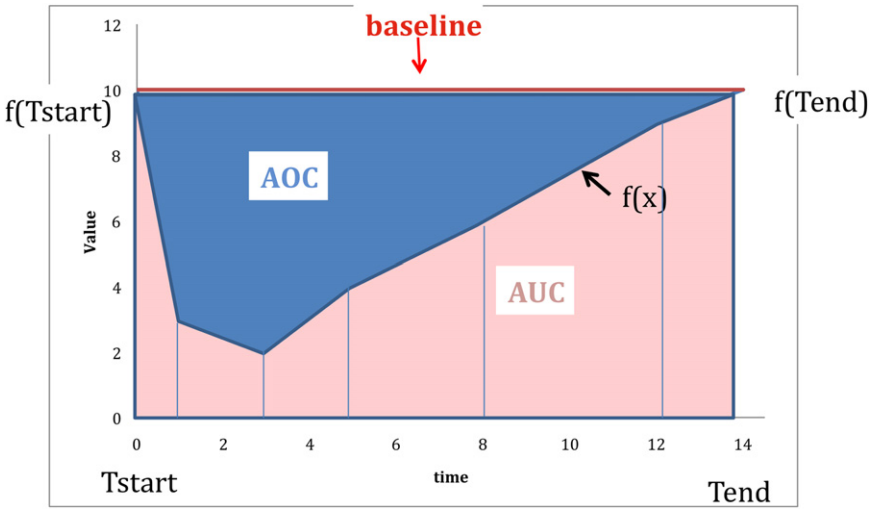

FIG E1. AOCs and AUCs are interconvertible and are calculated in most of the cases by using the linear trapezoidal rule.

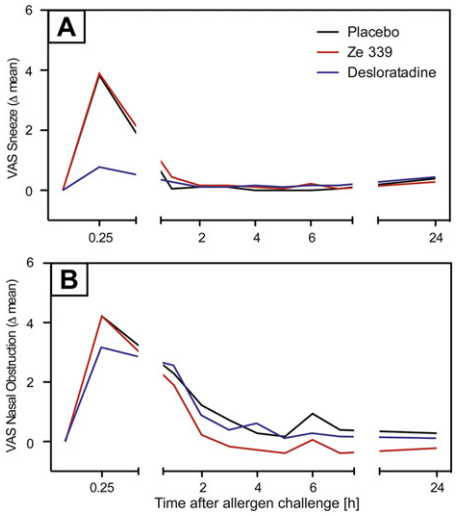

FIG E2. Sneeze symptoms **(A)** and nasal obstruction **(B)** displayed as the difference from baseline values (means assessed as VAS scores).

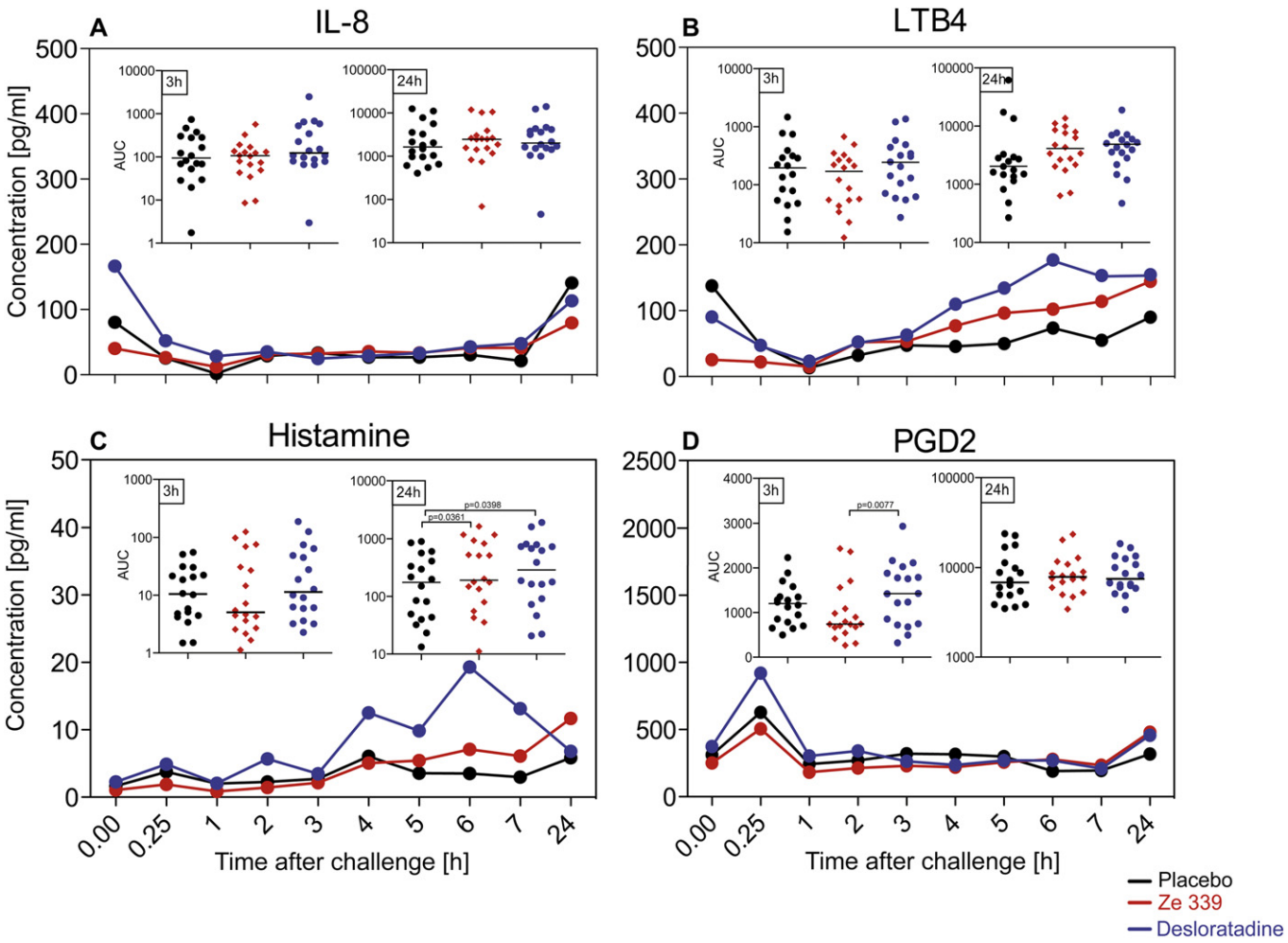

FIG E3. Median concentration (pg/mL) of nasal mediators (**A**, IL-8; **B**, LTB_4; **C**, histamine; and **D**, PGD_2) over 24 hours on allergen challenge (*red*, Ze 339; *blue*, desloratadine; *black*, placebo) and the AUC after 3 and 24 hours (pg/mL * hours) displayed as scatter plots and medians. Significance was reached for the AUC of PGD_2 in the early phase after nasal allergen challenge (mean ± SEM) during treatment with Ze 339 in comparison with desloratadine (979.1 ± 149.8 vs 1421 ± 164.1; $P = .0077$, paired *t* test) and for the AUC of histamine in the late-phase response (mean ± SEM) in both treatment groups in comparison with placebo (Ze 339 vs placebo: 464.9 ± 115.5 vs 272.3 ± 67.4 [$P = .0361$]; desloratadine vs placebo: 513.7 ± 128.5 vs 272.3 ± 67.4 [$P = .0398$, paired *t* test]).

TABLE E1. Mediators measured in nasal secretions

Interleukins	IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17
Eotaxin	CCL-11
Basic fibroblast growth factor	FGF basic
Granulocyte colony-stimulating factor	G-CSF
Granulocyte-macrophage colony-stimulating factor	GM-CSF
Interferon γ	IFN-γ
Chemokine (C-X-C motif) ligand 10	CXCL10 (IP10)
Monocyte chemotactic protein-1	MCP-1
Macrophage inflammatory proteins 1α and 1β	MIP-1α and MIP-1β
Platelet-derived growth factor BB	PDGF-BB
Chemokine (C-C motif) ligand 5	CCL-5 (RANTES)
Tumor necrosis factor α	TNF-α
Vascular endothelial growth factor	VEGF

TABLE E2. Nasal secretion mediator kinetics

	Placebo (mean ± SEM)	Ze 339 (mean ± SEM)	Desloratadine (mean ± SEM)	Placebo/Ze 339	Placebo/desloratadine	Ze 339/desloratadine
Nasal mediator kinetics after challenge						
IL-8						
AUC 3 h	181.2 ± 45.8	130.5 ± 31.2	360.2 ± 135.4	$P = .1040$	$P = .1584$	$P = .1108$
AUC 24 h	3215 ± 867.9	3425 ± 844.4	3560 ± 882.9	$P = .8442$	$P = .6558$	$P = .8920$
LTB_4						
AUC 3 h	296.9 ± 86.7	193.8 ± 42.7	342.8 ± 89.8	$P = .1552$	$P = .6346$	$P = .1180$
AUC 24 h	6639 ± 3424	5205 ± 898.6	5057 ± 949.9	$P = .6503$	$P = .5562$	$P = .8717$
Histamine						
AUC 3 h	16.9 ± 3.9	26.8 ± 9.1	36.6 ± 11.9	$P = .2398$	$P = .0688$	$P = .3338$
AUC 24 h	272.3 ± 67.4	464.9 ± 115.5	513.7 ± 128.5	$P = .0361$*	$P = .0398$*	$P = .6958$
PGD_2						
AUC 3 h	1182 ± 110.1	979.1 ± 149.8	1421 ± 164.1	$P = .2096$	$P = .1021$	$P = .0077$*
AUC 24 h	9465 ± 1553	9300 ± 1236	9100 ± 1016	$P = .9295$	$P = .8125$	$P = .8790$

All values are presented as means ± SEMs (pg/mL * hour).
*Statistical significance.