A proof-of-concept study of the effect of a novel H_3-receptor antagonist in allergen-induced nasal congestion

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Background: H_1-receptor inverse agonists are used effectively for treating several symptoms of allergic rhinitis, including nasal itching, rhinorrhea, and sneezing, although most agents are not very effective in treating nasal congestion.
Objective: This study evaluated the relative efficacy of a novel selective H_3-receptor antagonist, JNJ-39220675, in preventing nasal congestion induced by exposing participants with ragweed allergy to ragweed allergen in an environmental exposure chamber model.
Methods: In this single-dose, patient-blind, double-dummy, placebo- and active-controlled, phase IIa cross-over study, 53 participants were randomized to JNJ-39220675 plus placebo, placebo plus pseudoephedrine, or only placebo. The primary efficacy assessment was change in nasal patency assessed by measuring the minimal cross-sectional area of the nasal cavity by using acoustic rhinometry. Secondary assessment included total nasal symptom scores (TNSSs) over the 8-hour environmental exposure chamber exposure period.
Results: Smaller decreases in minimal cross-sectional area were observed after JNJ-39220675 (least square mean difference, −0.126; $P = .06$) and pseudoephedrine (least square mean difference, −0.195; $P = .004$) treatment compared with placebo. The means for the baseline-adjusted area under the curve of TNSSs were significantly smaller for JNJ-39220675 ($P = .0003$) and pseudoephedrine ($P = .04$) versus placebo. JNJ-39220675 was significantly effective in treating all 4 individual symptoms ($P \leq .05$ for all scores) compared with placebo, whereas pseudoephedrine only showed a trend for improvement in individual symptom scores of the TNSS. Insomnia was the most frequent adverse event (17.3%) associated with JNJ-39220675 treatment.
Conclusion: Prophylactic treatment with the H_3-antagonist JNJ-39220675 relieved allergen-induced nasal congestion by using standard nasal symptom scoring; however, in contrast to pseudoephedrine, it only showed a trend for increasing nasal patency by using objective measures. (J Allergy Clin Immunol 2013;132:838-46.)

Key words: *Allergic rhinitis, acoustic rhinometry, environmental exposure chamber, H_3-receptor antagonist, JNJ-39220675, pseudoephedrine, total nasal symptom scores*

Allergic rhinitis is the most common chronic atopic disease[1] and is associated with considerable cost and comorbidity.[2] Although a variety of mediators are implicated in the pathogenesis of the allergic reaction, histamine is shown to play a central role, and many of the early symptoms of allergic rhinitis are mediated by the action of histamine at the H_1-receptor site.[3] Inverse agonists of the H_1-receptor are hence used effectively as first-line treatment for many of the hallmark symptoms of seasonal allergic rhinitis (SAR), including nasal itching, rhinorrhea, and sneezing; however, they are not very effective in treating nasal congestion.[4]

Although H_1- and H_2-receptors are well-known targets for many drugs used clinically, newer histamine receptors, including the H_3-receptor, have recently been described.[5] The H_3-receptors are presynaptic autoreceptors present on histamine neurons controlling the stimulated release of histamine and presynaptic heteroreceptors on non–histamine-containing neurons, with the greatest densities found in the central nervous system (CNS).[6-9] H_3-receptors are predominantly expressed in the brain[10] and are also localized in the nasal mucosa.[11] Earlier *in vitro* experiments with isolated human turbinate mucosa have shown that the H_3-receptor agonist R-α-methylhistamine inhibited neurogenic sympathetic vasoconstriction, whereas clobenpropit, a selective H_3-receptor antagonist, blocked this effect, probably by reducing norepinephrine release from sympathetic nerve terminals in the nasal mucosa.[12]

Exploratory studies done earlier with H_3-antagonists have shown mixed results in human allergic rhinitis models. The compounds used in these studies were either dual H_1- and H_3-antagonists or were studied in combination with an H_1-antagonist and hence inconclusive regarding the specific contribution of the H_3-antagonism.[13]

JNJ-39220675, also known as (4-cyclobutyl-[1,4]diazepam-1-yl)-(6-[4-flurophenoxy]-pyridin-3-yl)-methanone, is a novel and selective H_3-receptor antagonist (inhibition constant, 1.4 nmol/L),[14-16] which does not have any significant affinity for the H_1-receptor (data on file, Janssen Research & Development). It has been shown to occupy up to 90% of H_3-receptors in the brain after subcutaneous and oral administration in rats and after intravenous and oral administration in anesthetized baboons.[14-16] After subcutaneous

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Abbreviations used
AcR: Acoustic rhinometry
AUC: Area under the curve
CNS: Central nervous system
ECP: Eosinophil cationic protein
EEC: Environmental exposure chamber
LSM: Least square mean
MCA: Minimal cross-sectional area
SAR: Seasonal allergic rhinitis
TEAE: Treatment-emergent adverse event
TNSS: Total nasal symptom score

administration, JNJ-39220675 is shown to significantly increase histamine levels in the frontal cortex and wake duration in rats.[14]

In this exploratory study we evaluated the relative efficacy of JNJ-39220675 in preventing nasal congestion induced by exposure of participants with ragweed allergy to ragweed allergen in an environmental exposure chamber (EEC) model by using acoustic rhinometry (AcR), an objective method to assess nasal patency,[17] as well as traditional subjective symptom measures. The effect of JNJ-39220675 on T_H2 cytokines and other biomarkers was also explored.

METHODS

Study population

Men and women aged 18 to 65 years (inclusive) with a body mass index of between 18 and 32 kg/m^2 (inclusive) and a body weight of 50 kg or greater and in good health were enrolled. Participants were required to have a clinical history of SAR with a seasonal onset and offset of nasal allergy symptoms during each of the last 2 ragweed allergy seasons and a positive skin prick test response to ragweed allergen (defined as a wheal diameter ≥3 mm larger than that elicited by the negative control) or a positive intradermal skin test response to ragweed allergen (defined as a wheal ≥7 mm larger than that elicited by the negative control) within 12 months before screening.

The study protocol was approved by the institutional review board (IRB Services, Aurora, Canada), and the study was conducted in accordance with the ethical principles originating in the Declaration of Helsinki and in accordance with the International Conference on Harmonisation Good Clinical Practice guidelines and applicable regulatory requirements and in compliance with the respective protocols. All participants provided written informed consent before participation.

Study design

In this phase IIa, single-dose, patient-blind, double-dummy, placebo- and active-controlled 3-way crossover study, participants were randomized to one of 6 predetermined treatment sequences: ABC, BCA, CAB, ACB, BAC, or CBA (treatment A, 1 mL of 10 mg/mL JNJ-39220675 oral solution plus placebo tablet; treatment B, 1 mL of placebo oral solution plus 60-mg pseudoephedrine tablet; and treatment C, 1 mL of placebo oral solution plus placebo tablet). Each treatment period consisted of 1 EEC session with a minimum 6-day washout period (see Fig E1 in this article's Online Repository at www.jacionline.org). During each EEC treatment session, participants were exposed to airborne ragweed for 8 hours with a mean pollen concentration of approximately 3500 particles/m^3 (SD, 500 particles/m^3). The 10-mg dose of JNJ-39220675 was the highest dose studied in women in a single, ascending-dose, phase I study in which doses of up to 50 mg were studied in men; the 10-mg dose was well tolerated by both men and women and was therefore selected for this study (data on file, Janssen Research & Development).

This study was conducted in the fall and winter months after the local ragweed season had concluded. Participants attended a 3-hour screening EEC visit to ensure that they would have an adequate symptomatic response to ragweed exposure in the EEC. Participants who had a decrease of 10% or greater in the minimal cross-sectional area (MCA) of either nostril after EEC exposure compared with their pre-EEC MCA and who had a minimum total nasal symptom score (TNSS) of 6 or more of 12, including a score of 2 or greater for congestion, on 1 or more diary cards during the EEC screening period were randomized to treatment period 1 (treatment A, B, or C) after a 6-day washout period (see Fig E1).

During each treatment period, participants fasted for approximately 8 hours before receiving the study drug and were given a light snack 2 hours after study drug administration. Study drug was administered in a blinded manner approximately 2 hours before entering the EEC. Participants recorded nasal symptom scores and underwent AcR assessment before receiving the study drug. On entering the EEC, participants underwent AcR assessments every hour throughout the 8-hour ragweed allergen exposure period and were asked to assess symptoms every 30 minutes throughout the 8-hour period.

Nasal lavage

Nasal lavage specimens were obtained from all participants before and after the screening EEC visit and after each treatment period EEC visit. A 10-mL syringe with a nasal "olive" (Crest Tech, Toronto, Ontario, Ontario) on the hub was used to perform the procedure. Under the instruction and supervision of trained EEC personnel, each participant instilled approximately 5 mL of saline solution into their nasal cavities through the left nostril from a forward-flexed neck position (60° from the upright position) and withdrew the fluid. The procedure was repeated twice and completed within 1 minute. The lavage fluid collected was centrifuged (1500 rpm for 10 minutes at 4°C), and the supernatant obtained was then stored at −80°C for cytokine analysis.

Cytokine analysis

Nasal lavage aliquots were concentrated 10-fold and lyophilized to obtain a final volume of 50 μL. Total protein levels were determined by using the Pierce Bicinchoninic Acid Protein Assay (Thermo Fisher Scientific, Uppsala, Sweden). Human albumin (Bethyl Laboratories, Montgomery, Tex) and human eosinophil cationic protein (ECP; MBL, Nagoya, Japan) levels were measured by using ELISA. Levels of human cytokines, including IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12 (p70), IL-13, TNF-α, and IFN-γ, were measured by using a multiplex immunoassay.

Efficacy assessments

Efficacy assessments included AcR and nasal symptom scores.

AcR

Changes in nasal patency were assessed by using AcR to determine the MCA of the nasal cavity. All measurements were done by blinded operators trained in the use of the acoustic rhinometer (Rhinoscan; Interacoustics, Assens, Denmark), and the same operator and the same equipment were used for each measurement to ensure consistency. The MCA was measured along the nasal passage from 0 cm (at the nares) to 5.5 cm. For each nostril, the MCA1 (0-2.2 cm) and MCA2 (2.2-5.5 cm) values were measured simultaneously. Four measures (the left MCA1, right MCA1, left MCA2, and right MCA2 values) were captured to determine the average MCA. Each set of 4 measurements were repeated thrice to obtain 12 data points. The minimum value from the 3 MCA averages was reported as the MCA across both nostrils.

Safety

The safety assessments included monitoring treatment-emergent adverse events (TEAEs), physical examinations, vital signs, electrocardiographic results, and laboratory parameters.

Statistical analysis

The efficacy analyses were based on the intent-to-treat population, which included all participants who received 1 or more doses of the study medication

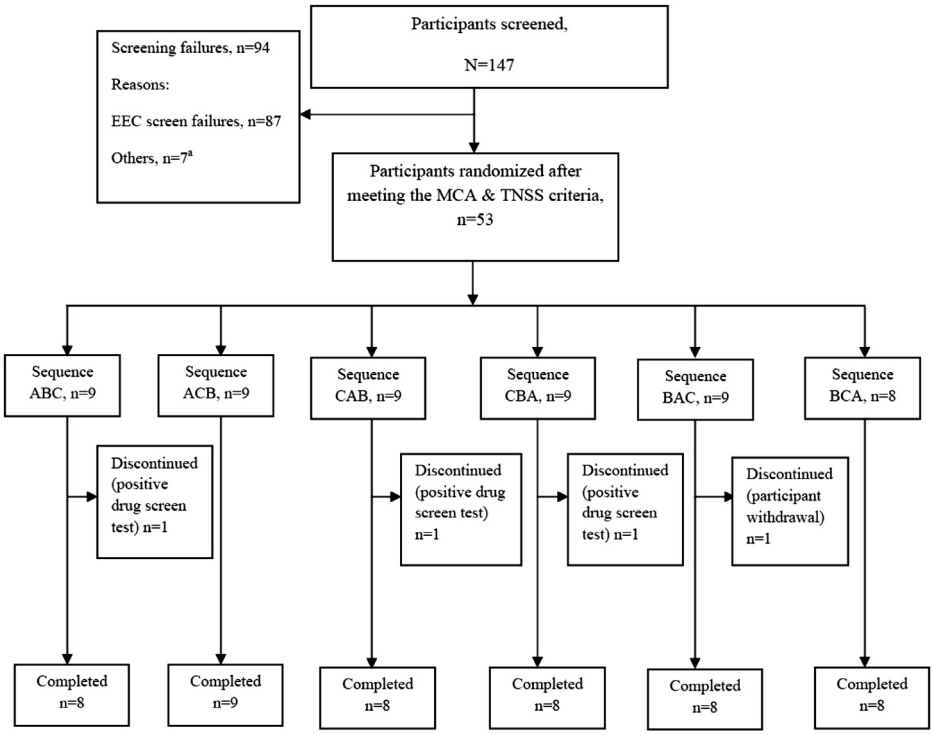


FIG 1. Participant accounting. *Treatment A,* 1 mL × 10 mg/mL JNJ-39220675 oral solution plus 1× placebo tablet; *treatment B,* 1 mL of placebo oral solution plus 1× 60-mg pseudoephedrine tablet; and *treatment C,* 1 mL of placebo oral solution plus 1× placebo tablet. [a]Other screen failures include patients who were withdrawn in error (n = 2), withdrawn from the study (n = 2), prohibited medication use (n = 1), lost to follow-up (n = 1), and unable to have blood drawn for assessments (n = 1). Participants randomized into the study should pass the EEC screening and must have had a decrease of 10% or greater in the MCA in either nostril, as assessed by using AcR before exposure (before entering the EEC) and after exposure (measured in the chamber) and must have a minimum TNSS of 6 of 12, including a score of at least 2 for congestion, on at least 1 diary card before dosing.

and had 1 or more postbaseline AcR efficacy assessments; baseline was defined as the predose (approximately 2 hours before EEC entry) assessment for each treatment period. The primary efficacy variable was the baseline adjusted area under the curve (AUC) of the MCA assessed by means of AcR for active treatments versus placebo. The secondary efficacy variables included (1) the baseline-adjusted AUC of TNSSs for active treatments versus placebo and between active treatments and (2) the change from baseline by time point in TNSSs and MCAs for active treatments versus placebo and between active treatments. Analysis of covariance was used to compare each active group with the placebo group. *Post hoc* exploratory analyses included the baseline-adjusted AUC of individual nasal symptom scores for active treatments versus placebo. The safety analysis set included all participants who received 1 or more doses of study drug.

Sample size determination

With 8 participants in each of the 6 treatment sequences, the study was expected to have approximately 80% power with an overall 1-sided significance level of .05 to detect a 0.8-unit difference in the MCA between each of 2 comparisons with placebo. After adjusting for a rate of 10% for participants who might discontinue before providing postbaseline efficacy assessments, approximately 9 participants were required per treatment sequence (ie, 54 participants in total).

RESULTS

Participant disposition

The study was conducted from November 10, 2008, to February 8, 2009, at Cetero Research, Mississauga, Ontario, Canada. Of the 147 participants screened, 53 were randomized to one of 6 treatment sequences (Fig 1) and included in the safety and intent-to-treat analysis. The majority (72%) were men and 49% were white, with a mean age of 42 (SD, 12) years (Table I). Four participants discontinued the study (Fig 1).

Efficacy

Carryover effects of active treatments on MCA and nasal symptom scores in periods 2 and 3 were found to be insignificant (see Tables E1-E3 in this article's Online Repository at www.jacionline.org for more information).

Primary efficacy

The means for the baseline-adjusted AUC of the MCA were larger (less of a decrease in nasal patency) for JNJ-39220675 versus placebo with a least square mean (LSM) difference of −0.126, which showed a trend toward preventing the decrease in nasal patency ($P = .06$) with allergen exposure. The mean baseline-adjusted AUC for pseudoephedrine versus placebo was statistically significant (LSM difference, −0.195; $P = .004$), confirming assay sensitivity (Table II).

Secondary efficacy: TNSSs

The means for the baseline-adjusted AUCs of TNSSs were significantly smaller (less of an increase in symptom severity) for

TABLE I. Participants' demographics

	Placebo (n = 51)	JNJ-39220675 (n = 52)	Pseudoephedrine (n = 51)	Total population (n = 53)
Sex, no. (%)				
Men	36 (71)	37 (71)	37 (73)	38 (72)
Women	15 (29)	15 (29)	14 (28)	15 (28)
Race, no. (%)				
White	24 (47)	25 (48)	25 (49)	26 (49)
Black or African heritage	8 (16)	8 (15)	7 (14)	8 (15)
Native Indian/Alaskan	1 (2)	1 (2)	1 (2)	1 (2)
Asian	11 (22)	11 (21)	11 (22)	11 (21)
Other	7 (14)	7 (14)	7 (14)	7 (13)
Age (y), mean (SD)	42 (11.9)	42 (11.9)	42 (12.0)	42 (11.8)
Height (m), mean (SD)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)	1.7 (0.1)
Weight (kg), mean (SD)	72.6 (11.1)	73.0 (11.2)	72.9 (11.3)	72.9 (11.1)
BMI (kg/m^2), mean (SD)	25.1 (2.8)	25.1 (2.8)	25.1 (2.8)	25.2 (2.7)

BMI, Body mass index.

TABLE II. Baseline-adjusted AUC of the MCA and TNSS for all treatments (intent-to-treat population)

Treatment	Placebo (n = 51)	JNJ-39220675 (n = 52)	Pseudoephedrine (n = 51)
AUC of MCA			
Mean (SD)	−0.68 (0.80)	−0.50 (0.81)	−0.55 (0.84)
Median	−0.66	−0.47	−0.44
LSM	−0.67	−0.55	−0.48
LSM difference		−0.126	−0.195
95% CI		−0.258 to 0.006	−0.328 to −0.063
P value		.0602	.0043
AUC of TNSS			
Mean (SD)	45.72 (24.71)	36.54 (24.32)	43.20 (26.20)
Median	42.00	38.63	44.25
LSM	45.68	37.07	40.98
LSM difference		8.604	4.699
95% CI		4.104 to 13.104	0.155 to 9.243
P value		.0003	.0428

JNJ-39220675 (LSM difference, 8.604; $P = .0003$) and for pseudoephedrine (LSM difference, 4.699; $P = .04$; Table II) versus placebo.

JNJ-3922675 showed significant improvement in the mean change from baseline versus placebo at multiple time points (Fig 2, *A*). In the EEC a separation from the placebo response was seen beginning at 0.5 hours, and the difference was significant throughout at all time points (8 hours, $P < .05$), except the 3.5-hour and 4-hour time points ($P > .05$). Pseudoephedrine showed a statistically significant difference versus placebo only at the 0.5-hour ($P = .003$) and 4.5-hour ($P = .021$) time points.

Exploratory efficacy results

Nasal congestion. JNJ-39220675 showed a statistically significant difference at 8 of 16 time points versus placebo, whereas pseudoephedrine showed a significant difference at 1 time point (Fig 2, *D*). Furthermore, pseudoephedrine was slower to separate from placebo (not until the 3-hour time point). The baseline-adjusted AUC revealed a statistically significant treatment effect compared with placebo for JNJ-39220675 ($P = .007$) but only a trend for pseudoephedrine ($P = .064$).

Runny nose. JNJ-39220675 showed a statistically significant difference at 4 of 16 time points versus placebo, whereas pseudoephedrine versus placebo showed a significant difference at 2 time points (Fig 2, *B*). The baseline-adjusted AUC revealed a statistically significant treatment effect compared with placebo for JNJ-39220675 ($P = .016$) but only a trend for pseudoephedrine ($P = .072$).

Itchy nose. JNJ-39220675 showed a statistically significant difference at 8 of 16 time points versus placebo, whereas pseudoephedrine versus placebo did not show a significant difference at any time point (Fig 2, *C*). The baseline-adjusted AUC analysis revealed a statistically significant treatment effect compared with placebo for JNJ-39220675 ($P = .007$) but not for pseudoephedrine ($P = .464$).

Sneezing. JNJ-39220675 showed a statistically significant difference versus placebo at 12 of 16 time points, whereas pseudoephedrine showed a significant difference at 4 of 16 time points (Fig 2, *E*). The baseline-adjusted AUC revealed a statistically significant treatment effect compared with placebo for JNJ-39220675 ($P < .0001$) but only a trend for pseudoephedrine ($P = .067$).

Biomarkers

Among the 13 proteins measured from nasal lavage specimens, only ECP, the T_H2 cytokines (IL-4, IL-5, and IL-13), and serum albumin levels were significantly increased during EEC ragweed exposure in patients with placebo treatment in period 1, with the greatest increase seen in ECP and IL-5 levels, followed by IL-13, albumin, and IL-4 levels (Fig 3 and Table III). Increases in the levels of these cytokines during EEC ragweed exposure in the placebo group in periods 2 and 3 were not as robust as in period 1, suggesting a carryover effect of active treatments on these biomarkers (see Fig E2 in this article's Online Repository at www.jacionline.org). Therefore the effect of the active treatments on levels of selected proteins increased during EEC ragweed exposure was analyzed by comparing only period 1 with baseline (prescreening EEC). Both JNJ-39220675 and pseudoephedrine were associated with less increase in levels of these cytokines during EEC ragweed exposure versus placebo (Table IV and see Fig E3 in this article's Online Repository at www.jacionline.org); pseudoephedrine showed a more reduced increase in cytokine levels compared with JNJ-39220675.

Safety

Of the 41 TEAEs reported, 37 were reported in 16 participants receiving JNJ-39220675, 3 in 2 participants receiving

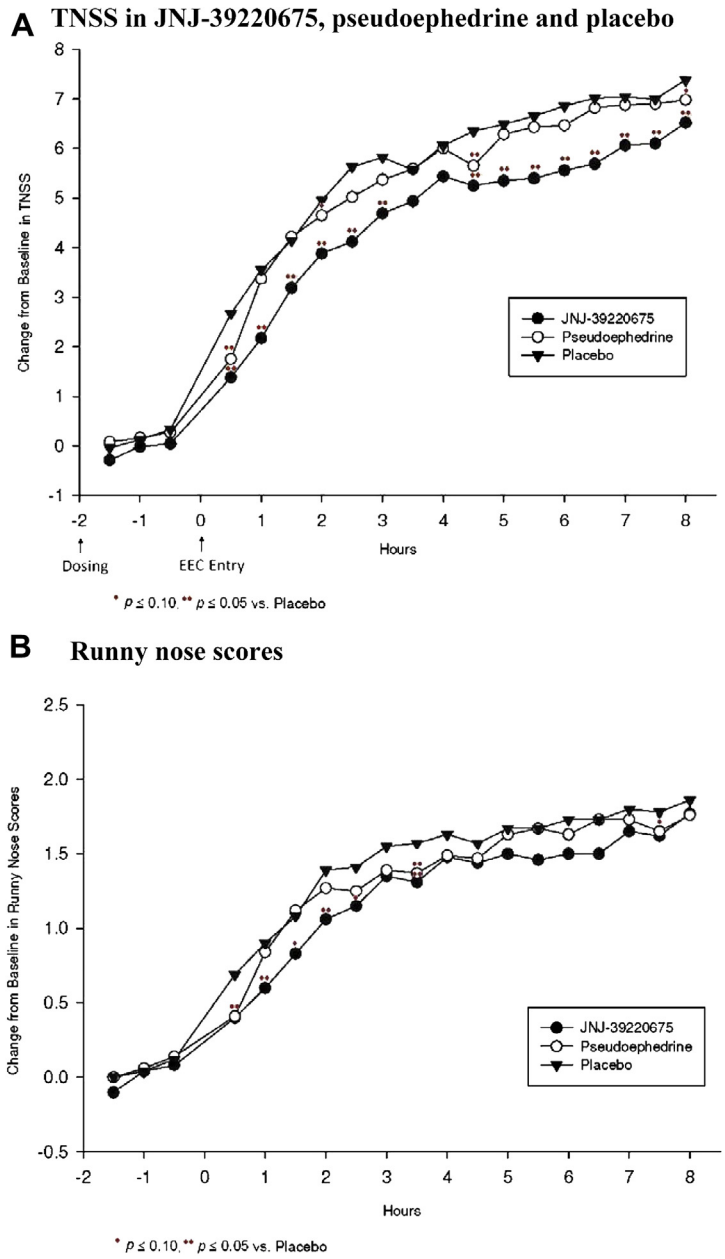
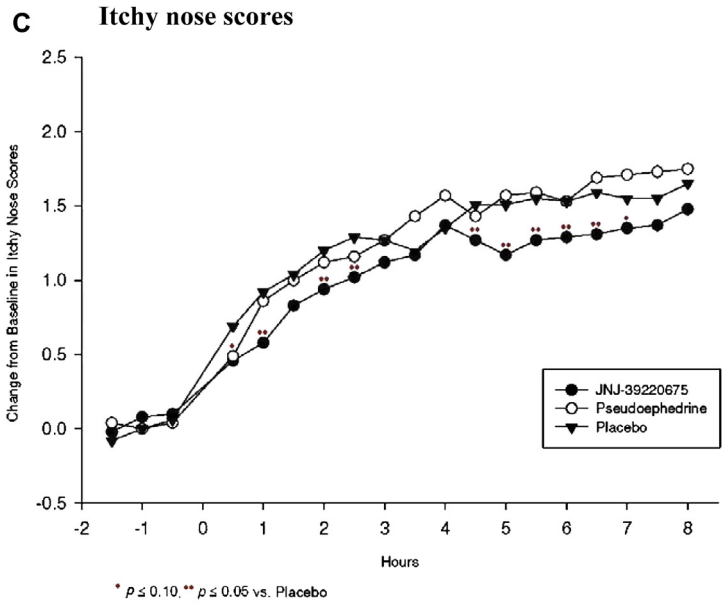


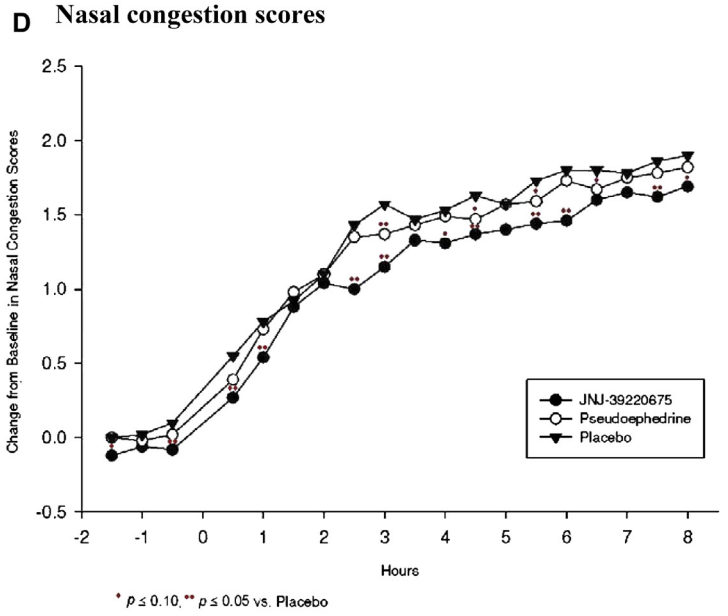
FIG 2. The mean changes from baseline in TNSSs and individual nasal symptom scores (intent-to-treat population) in the JNJ-39220675, pseudoephedrine, and placebo groups. Note: The severity of nasal symptoms (runny nose, itchy nose, nasal congestion, and sneezing) were recorded every 30 minutes while in the EEC on a 4-point scale (0, absent; 1, mild; 2, moderate; and 3, severe). **A,** TNSSs. **B,** Runny nose scores. **C,** Itchy nose scores. **D,** Nasal congestion scores. **E,** Sneezing scores.

pseudoephedrine, and 1 in a participant receiving placebo. Most TEAEs reported were experienced by less than 2% (1/53) of participants; TEAEs reported by greater than 2% of participants were insomnia (17.3% [9/53]), nausea (13.5% [7/53]), headache (9.6% [5/53]), and dizziness (3.8% [2/53]). The majority of the adverse events were mild in severity (75% for JNJ-39220675 and 100% for pseudoephedrine). There were no serious adverse events, deaths, and discontinuations caused by adverse events. There were no clinically significant changes observed in laboratory values, vital signs, and electrocardiographic assessments.

DISCUSSION

Both JNJ-39220675 and pseudoephedrine resulted in a smaller decrease in the MCA (less of a decrease in nasal patency or less congestion) versus placebo in participants with ragweed allergy exposed to ragweed in an EEC. A statistically significant difference was observed with pseudoephedrine versus placebo in reducing the baseline-adjusted MCA. JNJ-39220675 showed a strong trend in the same direction ($P = .06$). On the basis of these data, the hypothesis that H_3-receptor inhibition would result in vasoconstriction and an increase in nasal patency was not proved, but the concept appears to have been supported.





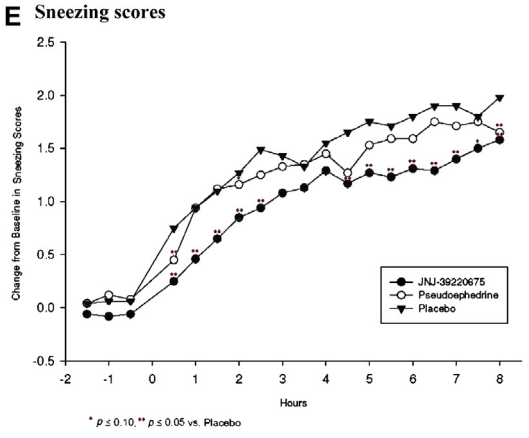


FIG 2. *(Continued)*

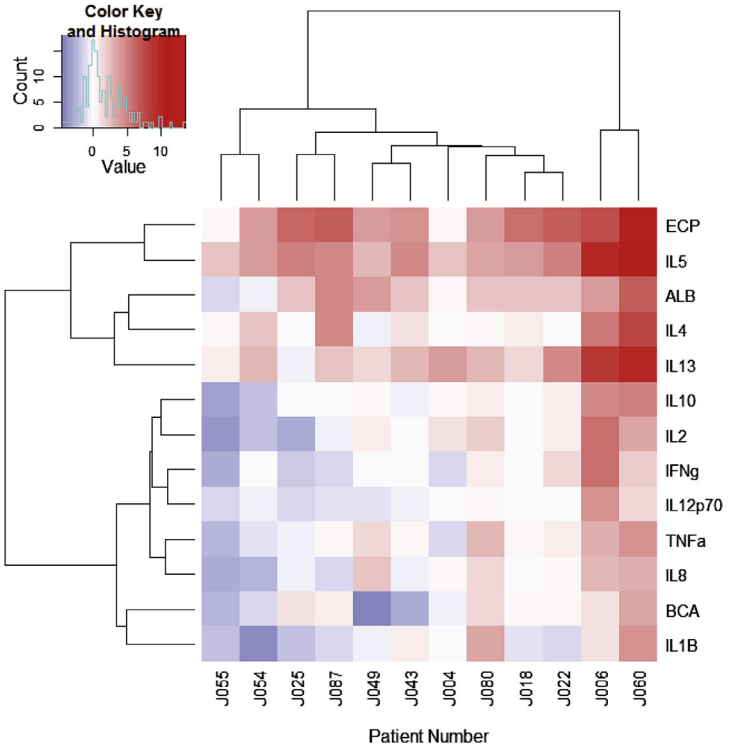


FIG 3. Effect of EEC ragweed exposure on protein concentrations obtained from nasal lavage specimens of participants in the placebo group in period 1. Heat map of the $\log_2$ ratios of 13 proteins tested between post-EEC samples in period 1 and corresponding samples of prescreening EEC (baseline) from 12 patients of the placebo group. Each *row* represents 1 tested protein, and each *column* represents 1 patient. *Red* and *blue* indicate increases and decreases from baseline values, respectively. Hierarchic clustering on the proteins illustrates 2 major groups of proteins that exhibit distinctive expression patterns across patients.

JNJ-39220675 was effective in showing a smaller increase in the subjective symptom response for the composite of nasal symptoms, the TNSS, a commonly used primary outcome measure in allergic rhinitis clinical trials (AUC analysis, $P = .0003$; mean change from baseline analysis, 14/16 time points with statistically significant P values). The effect of pseudoephedrine was marginal (AUC analysis, $P = .04$; mean change from baseline analysis, 2/16 time points with statistically significant P values). Exploratory analyses suggest that the TNSS effect with JNJ-39220675 was primarily due to a strong effect on sneezing and lesser effects on pruritus and congestion, whereas the marginal pseudoephedrine effect was likely to be due to effects on sneezing, congestion, and rhinorrhea.

These are the first data with selective H_3-antagonist monotherapy in patients with allergic rhinitis. Previous studies have tested either a combination of a selective H_3-antagonist with a conventional H_1-antihistamine or used compounds with combined H_3- and H_1-antagonistic activity.[13] One such combined H_1-H_3 compound, GSK-835726, did not show a more pronounced reduction in nasal congestion than the H_1-compound cetirizine,[18] whereas another similar H_1-H_3 combined compound reduced H_1-related symptoms, such as itching, sneezing, and rhinorrhea, but not nasal congestion.[18] Another selective H_3-antagonist, PF-03654746, in combination with an H_1-antihistamine, fexofenadine, reduced allergen-induced nasal symptoms, including nasal congestion, itch, rhinorrhea, and sneezing, in a dose-related manner.[19] The effects of this combination were more pronounced than the combination of fexofenadate with 120 mg of pseudoephedrine. There was no significant effect of any of the treatments on MCA or nasal volume.

The effect of JNJ-39220675 on other symptoms of allergic rhinitis, such as sneezing, nasal pruritus, and, to a lesser extent, rhinorrhea, was somewhat unexpected. It is known that CNS mechanisms are involved in the sneezing and pruritus associated with allergic rhinitis.[20,21] In fact, H_3 receptors have been shown to be expressed in the caudal spinal trigeminal nucleus of rhesus monkeys, which is where nasal sensory afferents terminate, although their contribution to nasal allergy and symptoms is currently unknown.[22] Selective H_3-antagonists increase the release of histamine from histamine neurons in the CNS, resulting in increased wakefulness, the opposite of what H_1-antihistamines with central activity tend to do.[23] Selective H_3-antagonists have also been shown to increase CNS levels of norepinephrine and acetylcholine.[23] The mechanism by which the central effects of JNJ-39220675 might have contributed to reduction of sneezing and pruritus observed in this study remains to be determined.

Our results indicate that H_3-antagonism can reduce allergen-induced nasal congestion by at least a 60-mg dose of pseudoephedrine as assessed by using AcR. Although AcR is not a typical outcome measurement in SAR clinical trials, it has been demonstrated that AcR is a reliable technique to objectively assess nasal congestion; in a previous study AcR was able to confirm the expected greater clinical benefit of the combination of oxymetazoline and fluticasone compared with oxymetazoline alone.[24] In the present study there was a significantly smaller decrease in nasal patency in the EEC with pseudoephedrine (active comparator)

TABLE III. Effect of EEC ragweed exposure on protein concentrations in nasal lavage fluid of patients with placebo treatment in period 1 (n = 12)

Protein	Ratio from baseline	Raw *P* value	Adjusted *P* value
Albumin	5.7	.0021	.0085
Total protein	0.9	.88	.88
ECP	39.3	.00025	.0016
IL-1β	0.9	.85	.88
IL-2	1.4	.58	.8
IL-4	3.7	.031	.079
IL-5	31.5	.00006	.0008
IL-8	1.2	.57	.8
IL-10	1.5	.38	.7
IL-12 (p70)	1.1	.76	.88
IL-13	10.6	.0026	.0085
IFN-γ	1.3	.61	.8
TNF-α	1.7	.19	.41

Adjusted *P* values are calculated by using the Benjamini-Hochberg method.

TABLE IV. Effect of active treatments on protein concentration of nasal lavage fluid from patients in period 1

Protein	JNJ-39220675 vs placebo	Pseudoephedrine vs placebo
Albumin	$R = 0.60$, $P = .40$	$R = 0.32$, $P = .068$
ECP	$R = 0.12$, $P = .016$	$R = 0.05$, $P = .0009$
IL-4	$R = 0.52$, $P = .20$	$R = 0.32$, $P = .028$
IL-5	$R = 0.24$, $P = .07$	$R = 0.11$, $P = .006$
IL-13	$R = 0.17$, $P = .017$	$R = 0.11$, $P = .004$

R, Ratio between active treatment and placebo.

compared with placebo, which is similar to an earlier study[25]; it thus proved itself as a good control and comparator for this study. However, in the PF-03654746 study contradictory findings were observed; the expected reduction of nasal patency by using AcR was not detected for the pseudoephedrine and fexofenadine combination,[19] a finding that the authors suggested might reflect variability inherent in the AcR method and that also might be due to differences in the method of AcR collection and analysis compared with those used in our study.

The reason why the effect of JNJ-39220675 was greater than the effect of pseudoephedrine on the subjective individual nasal congestion score is not clear. It might be that the more pronounced effect of JNJ-39220675 on the other subjective assessments could have benefited the subjective scoring of congestion.

In the present study analysis of the change from baseline in the absolute MCA for either nostril at each time point was measured and analyzed separately. The rationale for this analytic approach is based on known variability in the location of nasal congestion over time such that the effect of nasal congestion cycles from nostril to nostril and longitudinally shifts along the nasal turbinates and nasal pathway. Furthermore, the patient's perception of nasal congestion is likely set by the MCA for either nostril at any time point. This was demonstrated earlier by Salapatek et al,[26] who found a good correlation between nasal congestion scores and AcR assessments when patients were congested after a 3-hour EEC session or when patients were effectively treated (nasal patency increased) with a nasal corticosteroid spray before testing.

In terms of testing the hypothesis that an H_3-receptor antagonist might have beneficial effects on allergen-induced nasal congestion, the study design had both strengths and weaknesses. The EEC model offered a more controlled clinical study environment compared with a field study because confounding variables, such as unpredictable and variable pollen levels and varying weather conditions, were eliminated. Furthermore, the model allowed for examination of the efficacy of the treatments used in this study in multiple participants simultaneously within a single center and consistently over 3 periods of study. The cross-over design minimized variance because each participant was able to act as his or her own control subject, and the 6-treatment sequence study design allowed every treatment to be preceded and succeeded an equal number of times, making it fully variance balanced in comparison with a 3-treatment sequence study design. The double-dummy study design preserved the single blinding because both the active tablet and active liquid were blinded with the matching placebo. The minimum 6-day washout period between 2 treatments allowed study medications to be eliminated from the systemic circulation and allergy symptoms to subside. However, not incorporating multiple dose levels of JNJ-39220675 into the study was a significant drawback of the study design. Given the trend for a reduction of nasal congestion by AcR, as seen in this study, a higher dose might have resulted in a significant effect.

Five proteins, namely ECP, IL-4, IL-5, IL-13, and serum albumin, were significantly induced in nasal lavage fluid in response to EEC ragweed exposure in participants undergoing placebo treatment. This is consistent with previous findings of increased levels of nasal proteins, namely ECP, IL-5, IL-13, and albumin, after allergen challenge.[27-29] The increase in levels of T_H2 cytokines and ECP in response to EEC ragweed exposure in nasal lavage specimens could be reduced by both JNJ-39220675 and pseudoephedrine. However, the accompanying reduction in albumin levels seen with both compounds suggests that this is due to vasoconstriction with an accompanying reduction in the size of the turbinates and is not an anti-inflammatory effect.

The most common side effect in this study, insomnia, is consistent with the fact that JNJ-39220675 has central activity. If a rigorous dose-finding study should indicate that doses of less than than 10 mg have a similar effect on nasal congestion, it might be possible to reduce this side effect.

In conclusion, prophylactic treatment with the selective H_3-antagonist JNJ-39220675 (10 mg, single-dose) relieved allergen-induced nasal congestion by using standard nasal symptom scoring; however, in contrast to pseudoephedrine (60 mg), it only showed a trend for increasing nasal patency by using the objective measures. No significant anti-inflammatory effect of the compound was demonstrated.

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Clinical implications: Despite reducing nasal congestion, the H_3-receptor antagonist did not significantly attenuate nasal patency reduction in patients with allergen-induced allergic rhinitis, whereas pseudoephedrine did, suggesting that H_3-receptor antagonists have limited potential for treating nasal congestion in patients with allergic rhinitis.

REFERENCES

1. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. J Allergy Clin Immunol 2001;108(1 suppl):S2-8.
2. Reed SD, Lee TA, McCrory DC. The economic burden of allergic rhinitis: a critical evaluation of the literature. Pharmacoeconomics 2004;22:345-61.
3. Xie H, He SH. Roles of histamine and its receptors in allergic and inflammatory bowel diseases. World J Gastroenterol 2005;11:2851-7.
4. Simons FE, Simons KJ. Histamine and H1-antihistamines: celebrating a century of progress. J Allergy Clin Immunol 2011;128:1139-50.e4.
5. Thurmond RL, Gelfand EW, Dunford PJ. The role of histamine H1 and H4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov 2008;7:41-53.
6. Leurs R, Bakker RA, Timmerman H, de Esch IJ. The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat Rev Drug Discov 2005;4:107-20.
7. Letavic MA, Barbier AJ, Dvorak CA, Carruthers NI. Recent medicinal chemistry of the histamine H3 receptor. Prog Med Chem 2006;44:181-206.
8. Bonaventure P, Letavic M, Dugovic C, Wilson S, Aluisio L, Pudiak C, et al. Histamine H3 receptor antagonists: from target identification to drug leads. Biochem Pharmacol 2007;73:1084-96.
9. Celanire S, Wijtmans M, Talaga P, Leurs R, de Esch IJ. Keynote review: histamine H3 receptor antagonists reach out for the clinic. Drug Discov Today 2005;10: 1613-27.
10. Arrang JM, Garbarg M, Schwartz JC. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 1983;302:832-7.
11. Nakaya M, Takeuchi N, Kondo K. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. Ann Otol Rhinol Laryngol 2004; 113:552-7.
12. Varty LM, Gustafson E, Laverty M, Hey JA. Activation of histamine H3 receptors in human nasal mucosa inhibits sympathetic vasoconstriction. Eur J Pharmacol 2004;484:83-9.
13. Kuhne S, Wijtmans M, Lim HD, Leurs R, de Esch IJ. Several down, a few to go: histamine H3 receptor ligands making the final push towards the market? Expert Opin Investig Drugs 2011;20:1629-48.
14. Letavic MA, Aluisio L, Atack JR, Bonaventure P, Carruthers NI, Dugovic C, et al. Pre-clinical characterization of aryloxypyridine amides as histamine H3 receptor antagonists: identification of candidates for clinical development. Bioorg Med Chem Lett 2010;20:4210-4.
15. Logan J, Carruthers NI, Letavic MA, Sands S, Jiang X, Shea C, et al. Blockade of the brain histamine H3 receptor by JNJ-39220675: preclinical PET studies with [(1)(1)C]GSK189254 in anesthetized baboon. Psychopharmacology (Berl) 2012; 223:447-55.
16. Galici R, Rezvani AH, Aluisio L, Lord B, Levin ED, Fraser I, et al. JNJ-39220675, a novel selective histamine H3 receptor antagonist, reduces the abuse-related effects of alcohol in rats. Psychopharmacology (Berl) 2011;214:829-41.
17. Nathan RA, Eccles R, Howarth PH, Steinsvag SK, Togias A. Objective monitoring of nasal patency and nasal physiology in rhinitis. J Allergy Clin Immunol 2005; 115(suppl 1):S442-59.
18. Daley-Yates P, Ambery C, Sweeney L, Watson J, Oliver A, McQuade B. The efficacy and tolerability of two novel H1/H3 receptor antagonists in seasonal allergic rhinitis. Int Arch Allergy Immunol 2012;158:84-98.
19. Stokes JR, Romero FA Jr, Allan RJ, Phillips PG, Hackman F, Misfeldt J, et al. The effects of an H3 receptor antagonist (PF-03654746) with fexofenadine on reducing allergic rhinitis symptoms. J Allergy Clin Immunol 2012;129: 409-12, e1-2.
20. Sarin S, Undem B, Sanico A, Togias A. The role of the nervous system in rhinitis. J Allergy Clin Immunol 2006;118:999-1016.
21. Undem BJ, Kajekar R, Hunter DD, Myers AC. Neural integration and allergic disease. J Allergy Clin Immunol 2000;106(5 suppl):S213-20.
22. Sekizawa S, Bechtold AG, Tham RC, Kott KS, Hyde DM, Joad JP, et al. House-dust mite allergen and ozone exposure decreases histamine H3 receptors in the brainstem respiratory nuclei. Toxicol Appl Pharmacol 2010;247:204-10.
23. Esbenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD, Brioni JD. The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. Br J Pharmacol 2008;154:1166-81.
24. Baroody FM, Brown D, Gavanescu L, DeTineo M, Naclerio RM. Oxymetazoline adds to the effectiveness of fluticasone furoate in the treatment of perennial allergic rhinitis. J Allergy Clin Immunol 2011;127:927-34.
25. Latte J, Taverner D, Slobodian P, Shakib S. A randomized, double-blind, placebo-controlled trial of pseudoephedrine in coryza. Clin Exp Pharmacol Physiol 2004; 31:429-32.
26. Salapatek AM, Patel P, Gopalan G, Varghese ST. Mometasone furoate nasal spray provides early, continuing relief of nasal congestion and improves nasal patency in allergic patients. Am J Rhinol Allergy 2010;24:433-8.
27. Greiff L, Petersen H, Mattsson E, Andersson M, Erjefalt JS, Linden M, et al. Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment. Clin Exp Allergy 2001;31:1321-7.
28. Saengpanich S, Assanasen P, deTineo M, Haney L, Naclerio RM, Baroody FM. Effects of intranasal azelastine on the response to nasal allergen challenge. Laryngoscope 2002;112:47-52.
29. Erin EM, Leaker BR, Zacharasiewicz AS, Higgins LA, Williams TJ, Boyce MJ, et al. Single dose topical corticosteroid inhibits IL-5 and IL-13 in nasal lavage following grass pollen challenge. Allergy 2005;60:1524-9.

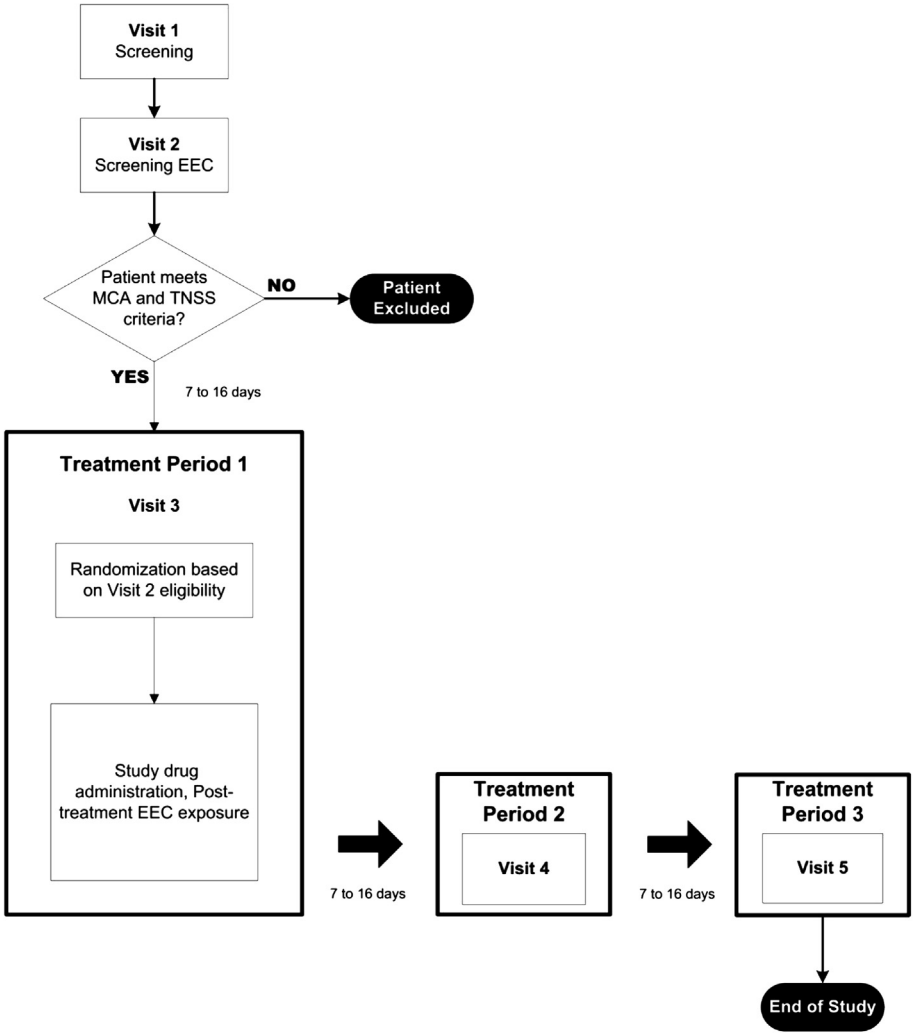

FIG E1. Study design.

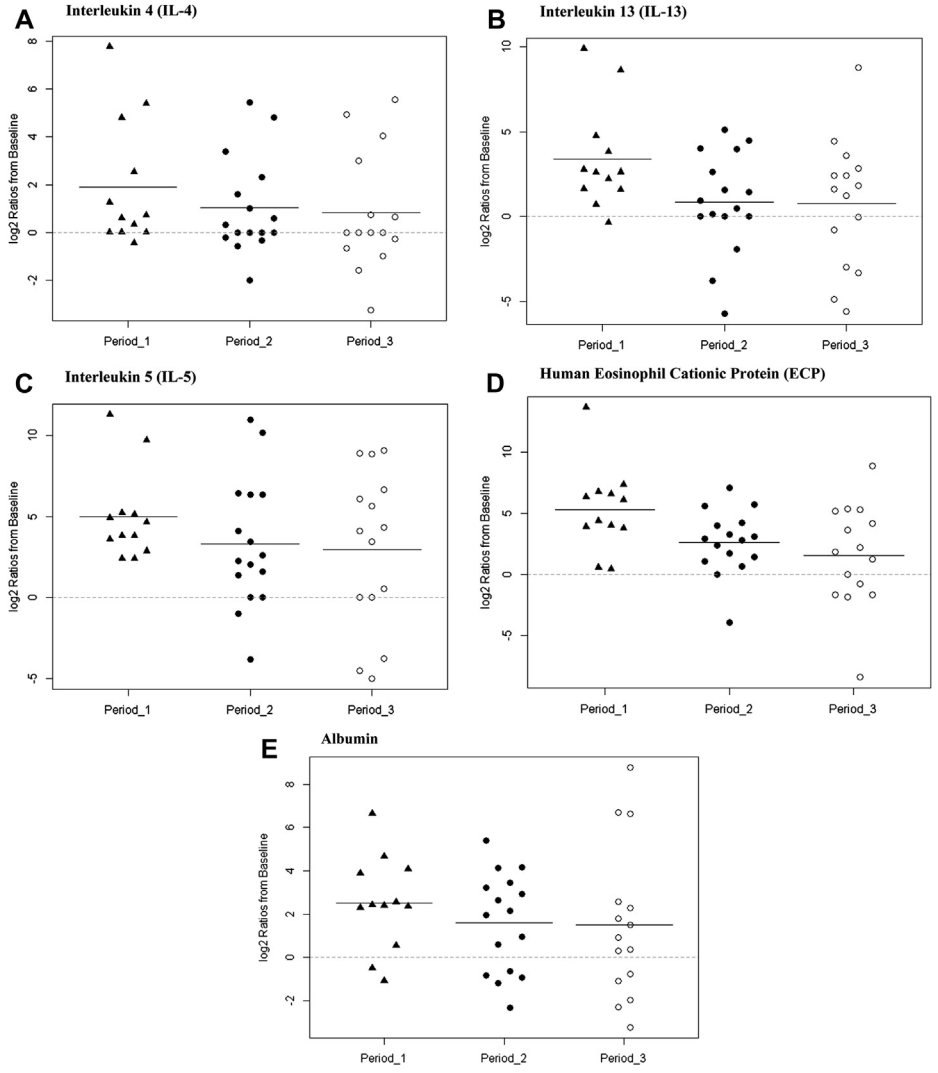

FIG E2. Cytokine and ECP levels in response to EEC ragweed exposure in the placebo group for each treatment period. **A,** IL-4. **B,** IL-5. **C,** IL-13. **D,** Human ECP. **E,** Albumin.

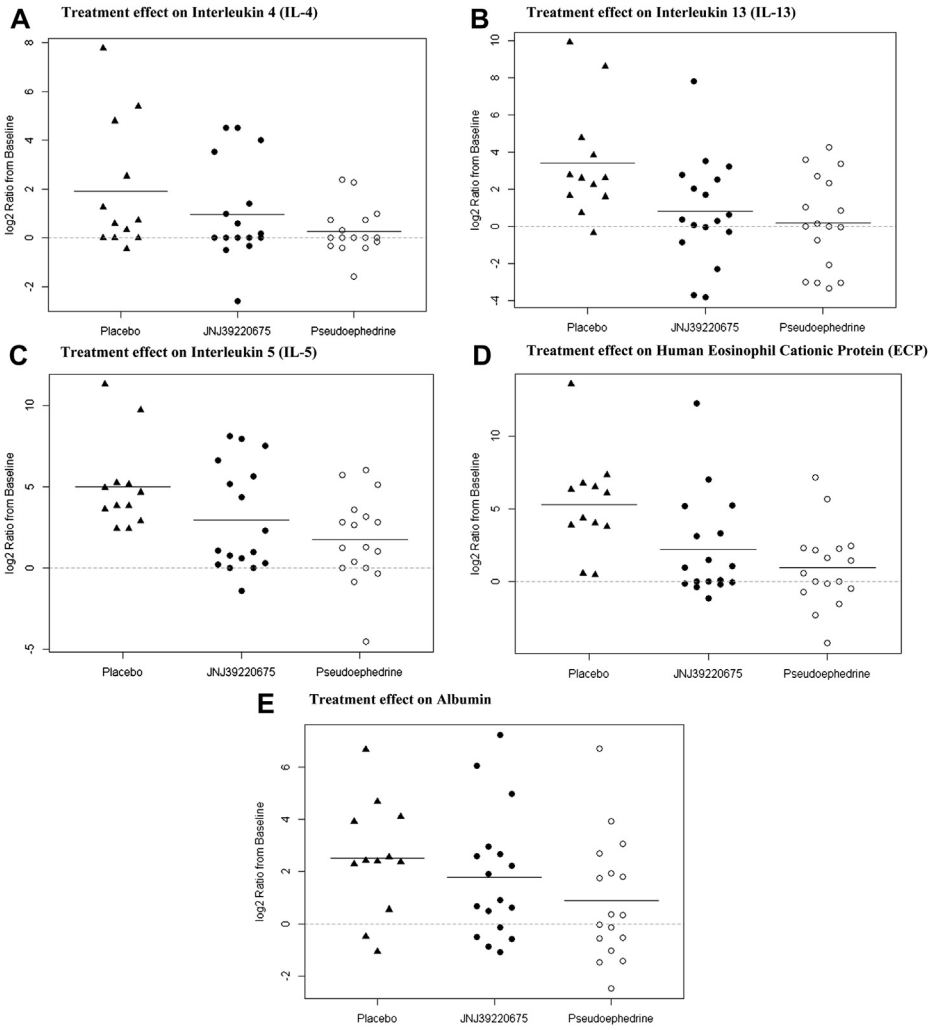

FIG E3. Effect of JNJ39220675 and pseudoephedrine on cytokine and ECP levels in response to EEC ragweed exposure during period 1. **A,** Treatment effect on IL-4. **B,** Treatment effect on IL-5. **C,** Treatment effect on IL-13. **D,** Treatment effect on human ECP. **E,** Treatment effect on albumin.

TABLE E1. Carryover effect analysis

	JNJ-39220675	Pseudoephedrine
MCA	$P = .5206$	$P = .3204$
TNSS	$P = .1139$	$P = .0576$

Carryover effects for active treatments in periods 2 and 3 were tested by adding terms for first-order carryover effects in periods 2 and 3 to the original analysis of covariance model used for the primary efficacy analysis, as specified in the protocol. This additional analysis showed that the carryover effects for treatment with JNJ-39220675 or pseudoephedrine were not statistically significant. Thus it is concluded that carryover is not an issue.

TABLE E2. Efficacy analysis: analysis of covariance results of the baseline-adjusted AUC of the MCA for the active treatment versus placebo groups (completer population)

				Difference of active vs placebo			
Treatment	**No.**	**LSM**	**SE LSM**	**LSMs**	**SE LSMs**	**95% CI**	***P* value**
Overall	147						.0150
Placebo	49	−0.673	0.085				
JNJ-39220675	49	−0.550	0.085	0.122	0.064	−0.005 to 0.250	.0604
Pseudoephedrine	49	−0.484	0.085	0.188	0.064	0.060 to 0.316	.0043

Note: The table is based on the linear mixed model, with sequence treatment and period as fixed effects, subject nested within the sequence as a random effect, and baseline as a covariate. A baseline and treatment interaction was found not to be statistically significant ($P > .05$), and hence it was removed from the final model.

TABLE E3. Analysis of covariance results of the baseline-adjusted AUC of the TNSS (completer population)

				Difference of active vs placebo			
Treatment	**No.**	**LSM**	**SE LSMs**	**LSM**	**SE LSM**	**95% CI**	***P* value, treatment**
Overall	147						.0024
Placebo	49	45.676	3.439				
JNJ-39220675	49	37.073	3.434	−8.675	2.412	−13.466 to −3.885	.0005
Pseudoephedrine	49	40.978	3.448	−4.348	2.417	−9.149 to 0.453	.0753

Note: The table is based on the linear mixed model, with sequence treatment and period as fixed effects, subject nested within sequence as a random effect, and baseline as a covariate. A baseline and treatment interaction was found not to be statistically significant ($P > .05$), and hence it was removed from the final model.