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Original article

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Effect of fexofenadine hydrochloride on allergic rhinitis aggravated by air pollutants

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Take-home message

This is the first randomised, double-blind, large study to demonstrate the beneficial effect of a histamine H1-receptor antagonist by reducing ragweed pollen-induced seasonal allergic rhinitis symptoms aggravated by controlled exposure to air pollutants.

Plain language summary

Allergic rhinitis is an inflammation of the inside of the nose that occurs in response to air allergens, such as pollen or dust. Allergic rhinitis is usually accompanied by itching of the throat, nasal congestion, watery eyes and sneezing. Fexofenadine hydrochloride is a medicine that relieves the symptoms of allergic rhinitis by blocking the effects of histamine, a natural substance that is released by the body's immune system during inflammation. In the last decades, air pollution has been associated with an increased incidence of allergic rhinitis worldwide. Diesel exhaust particles originate from diesel motor emission and are a major component of air pollution. They can also interact with allergens and worsen the allergic reaction. This study evaluated the effect of DEP air pollutant on the symptoms of allergic rhinitis in adults who are allergic to ragweed. A total of 257 participants were exposed to ragweed mixed with diesel exhaust particles; of these, 253 were randomised: some were given fexofenadine, and others were given placebo (a substance that doesn't have a therapeutic effect). Their allergic rhinitis symptoms (runny nose, sneezing, itchy nose) were scored up to 12 hours. The study showed that the allergic symptoms were worse if ragweed was mixed with diesel exhaust particles compared with ragweed exposure alone. Participants who received fexofenadine had lower symptom scores (sum of all allergic rhinitis symptoms) than those who received placebo. Fexofenadine was well-tolerated with few adverse effects. This was the first large study to demonstrate the beneficial effect of a histamine-blocking medicine in reducing the symptoms of allergic rhinitis aggravated by air pollutants.

Abstract

In recent decades, seasonal allergic rhinitis (SAR) prevalence has increased and recent studies have shown that air pollutants, such as diesel exhaust particles (DEP), can increase inflammatory and allergic biomarkers. The aim of this study was to investigate the effects of DEP on SAR symptoms induced by ragweed and to evaluate the efficacy and safety of fexofenadine HCl 180 mg vs. placebo.

This Phase 3, single-centre, sequential, parallel-group, double-blind, randomised study (NCT03664882) was conducted in an environmental exposure unit (EEU) during sequential exposures: Period 1 (ragweed pollen alone), Period 2 (ragweed pollen + DEP), and Period 3 (ragweed pollen + DEP + single-dose fexofenadine HCl 180 mg or placebo). Efficacy and safety were evaluated in Period 3. Primary endpoints were the area-under-the-curve of Total Nasal Symptom Score (TNSS) from baseline to hour 12 ($AUC_{0–12}$) during Period 1 and Period 2; and the AUC of the TNSS from hour 2 to 12 ($AUC_{2–12}$) during Period 3.

251/257 evaluable subjects were included in the modified intent-to-treat population. Least squares (LS)-mean difference (95% confidence interval [CI]) for TNSS Log AUC_{0-12} in Period 2 vs Period 1 was 0.13 (0.081, 0.182; p<0.0001). LS-mean difference in TNSS Log AUC_{2-12} for fexofenadine HCl vs placebo during Period 3 was −0.24 (−0.425, −0.047, p=0.0148). One fexofenadine HCl-related AE was observed.

SAR symptoms evoked by ragweed were aggravated by DEP. Fexofenadine HCl 180 mg was effective in relieving pollen-induced, air pollution-aggravated allergic rhinitis symptoms.

Keywords

Allergic rhinitis, air pollution, environmental exposure unit, allergen challenge, fexofenadine hydrochloride, Diesel Exhaust Particles (DEP)

Main text

Introduction

Seasonal allergic rhinitis (SAR) is caused by a type I immunoglobulin (Ig) E-mediated hypersensitivity reaction to allergens, such as pollens (e.g. ragweed), which provokes characteristic symptoms including sneezing, rhinorrhoea, nasal obstruction, itching of the throat, and watery eyes.[1] Worldwide, AR affects between 10–30% of the population and an overall increase in prevalence has been shown in recent studies.[2] Epidemiological studies have identified environmental risk factors, such as air pollutant exposure and climate change, as potentially associated with this trend.[3, 4]

Diesel exhaust particles (DEP) comprise a carbon core with adsorbed organic compounds;[5, 6] their small size and large surface area allow them to infiltrate airway epithelial cells, leading to inflammation and cytotoxicity.[7] Of note, DEP have been implicated in exacerbating long-distance spread of viral infection, for example during the coronavirus 2019 infectious disease pandemic.[8] Additionally, they can interact with allergens to enhance allergen-induced responses, up to 50 times more than allergens alone.[9]

Fexofenadine hydrochloride (HCl) is a second-generation, selective histamine H1-receptor antagonist, approved to relieve SAR symptoms in adults, and children,[10] and is marketed in approximately 100 countries worldwide, including the US, Japan and Europe. Depending on the country, the approved single daily dose is either 120 mg or 180 mg (Allegra®/Telfast®; Sanofi).[11, 12]

This study (NCT03664882) was a prospective, sequential and parallel-group, double-blind, single-dose, placebo-controlled, randomised clinical investigation of the effects of DEP on SAR ragweed-induced symptoms and evaluating the efficacy and safety of fexofenadine HCl 180 mg versus placebo in alleviating such pollution-aggravated symptoms.

Methods

Study population

Eligible subjects were 18–65 years with a 2-year history of SAR provoked by ragweed; a positive skin prick test to ragweed (defined as a wheal diameter ≥3 mm larger than the control one); a self-reported history of SAR symptoms aggravated by pollen or air pollutants exposure; and at least one documented Total Nasal Symptom Score (TNSS) ≥3 during the first 3 h pollen exposure in Period 1. At the Investigator's discretion, subjects who had

significant allergy to perennial allergens that could not be avoided during the study were excluded. Other major exclusion criteria are listed in the Supplementary Material. Regulatory approval was obtained from Health Canada and ethical clearance was provided by the Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (HSREB). The trial was conducted in accordance with the Declaration of Helsinki, the International Council for Harmonisation guidelines for Good Clinical Practice, and all applicable regulations. Written informed consent was obtained prior to study enrolment.

Study design

This phase 3, single-centre (Kingston Health Sciences Centre – KGH Site, Canada), sequential and parallel-group, double-blind, single-dose, placebo-controlled, randomised study evaluated the efficacy and safety of fexofenadine HCl 180 mg, compared with placebo. The study was conducted outside of the typical ragweed pollen season in the locale of the EEU. Details of the EEU methodology are available in the Supplementary Material. **Figure 1** schematically depicts the study design. At Visit 1 ragweed sensitivity was determined via skin testing. Additionally, skin prick tests were performed to assess sensitivity to other common allergens (dog, cat, Alternaria, D. pteronyssinus, D. farinae, grass, trees). During Visit 2 (Period 1), eligible subjects were exposed to ragweed pollen alone in the EEU and TNSS was self-assessed every 30 minutes during the first 3 h of exposure (in the EEU). Subjects were then discharged and recorded hourly TNSS scores for the following 9 hours; those who reported at least one TNSS score of ≥3 during the first 3 h of Visit 2 were eligible for Visit 3 (Period 2). In Period 2 eligible subjects were exposed to ragweed + DEP in the EEU for 3 h and then discharged. The timing of TNSS assessments was identical to that in Period 1. At Visit 4 (Period 3) subjects were randomised 1:1 to receive either fexofenadine HCl 180 mg or placebo. Subjects were exposed to ragweed + DEP for 3 h and study medication was administered at hour 2 (H2). TNSS was rated every 30 minutes during the first 3 h of ragweed + DEP exposure and then hourly up to hour 12. Each challenge period was separated by at least a two-week washout. Diary collection and safety follow-up occurred during Visit 5.

Outcomes

The primary objective was to demonstrate the aggravation of the SAR symptoms caused by DEP exposure. The second primary objective was to evaluate the efficacy of fexofenadine HCl in alleviating symptoms aggravated by DEP presence. The first primary endpoint was the area-under-the-curve (AUC) for TNSS from baseline (H0) to H12 (TNSS $AUC_{0\text{-}12}$) in Periods 1 and 2. Tested sequentially, the second primary endpoint was AUC for TNSS during Period 3 from time of study medication administration (H2) to H12 (TNSS $AUC_{2\text{–}12}$).

Of note, the primary and second primary endpoints were independent and therefore not comparable. Secondary efficacy endpoints in Period 3 were sequentially evaluated as follows: Total Symptom Score (TSS) AUC_{2-12}; each individual symptom score AUC_{2-12}; TSS and individual symptom score by time point from H2 to H12. All adverse events (AEs) were recorded.

Subjects scored AR symptom severity on a diary card using a 0–3 scale adapted from Food and Drug Administration (FDA)-recommended definitions
(0 = None, symptom is completely absent; 1 = Mild, symptom is present, but not bothersome; 2 = Moderate, symptom is bothersome, but tolerable; 3 = Severe, symptom is hard to tolerate, I would like to have a treatment).[13, 14]

Eight symptoms were rated: rhinorrhoea, sneezing, nasal itching, nasal congestion, itchy eyes, watery eyes, red or burning eyes, and itching of the ears or palate or throat. TNSS was calculated as the sum of rhinorrhoea, sneezing, and nasal itching scores.[13] TSS was calculated as the sum of all eight symptoms.

The safety analysis defined pre-treatment AEs as those that developed, worsened, or became serious from the signed informed consent date up to study drug administration. Treatment-emergent adverse events (TEAE) were any adverse events recorded from study drug intake up to 3 following days.

Randomisation and masking

Evaluable subjects completing Period 1 and 2 and returning for Period 3 were randomly assigned 1:1 to fexofenadine HCl or placebo according to a double-blind, permuted block schedule prepared by Sanofi. Fexofenadine HCl 180 mg and matching film-coated placebo tablets were individually packaged by pharmacy personnel otherwise not involved with the study. This enabled subjects and all study personnel to be blinded to treatment assignment.

Statistical analysis

The Evaluable Population (EP), the population analysed for the first primary endpoint, comprised all subjects who participated in Periods 1 and 2; had a TNSS recorded at H0; and at ≥1 subsequent time points for both periods. The intent-to-treat (ITT) population consisted of all subjects randomised during Period 3. The modified ITT (mITT) population comprised all subjects receiving study medication who recorded TNSS at H2 (before study drug intake) and at ≥1 subsequent time points. The second primary endpoint and all efficacy secondary endpoints were analysed using the mITT population. The safety population included all randomised subjects who received study medication.

Assuming an effect size of 0.25 for the aggravation of SAR symptoms in the presence of DEP, 260 evaluable subjects were required to provide 98% power for the first primary endpoint. Assuming 23% of subjects would be deemed ineligible or non-evaluable, approximately 340 subjects were needed to qualify for Visit 2 (Period 1). A mITT population of approximately 240 subjects was required to detect an effect size of 0.37 for fexofenadine HCl 180 mg with 81% power for the second primary endpoint. A 0.5 change in TNSS when 4 individual symptoms (rhinorrhoea, nasal congestion, nasal itching, and sneezing) are measured is commonly defined as the minimum clinically important difference. [15] Because fexofenadine lacks α-adrenergic vasoconstrictor activity, nasal congestion was not a TNSS score component; therefore, in this analysis an adjusted difference of 0.65 was deemed clinically important (rather than 0.5).

A hierarchical procedure was used to control for type I error and handle multiple comparisons (details available in the Supplementary Material). The first sequential primary analysis, TNSS (AUC_{0-12}) compared between Periods 1 and 2, employed a two-sided test controlled for a 5% type I error rate using a mixed model for repeated measures (MMRM). The MMRM was adjusted for baseline TNSS (measured at H0) and pollen counts (at subject level) for Periods 1 and 2, with period as a fixed categorical effect. The second primary analysis, TNSS (AUC_{2-12}) in Period 3 compared between treatment groups, employed a two-sided test controlled for a 5% type I error rate using an analysis of covariance (ANCOVA) with treatment group as a fixed categorical effect and baseline TNSS (measured at H2) as covariate. If required, log transformation of the primary endpoints was performed to improve normality of the distributions. All secondary efficacy endpoints based on AUC_{2-12} were analysed using an ANCOVA with treatment group as fixed categorical effect and the respective baseline score (H2) as covariate. TNSS, TSS, and individual symptom score analysis at each time point used a MMRM, with treatment group, time point, and treatment-group-by-time-point interaction as fixed effects and the respective baseline score (H2) as covariate. Secondary endpoints were analysed sequentially to maintain a 5% Type I error. The safety analysis was descriptive and conducted on the safety population.

Results

Disposition and demographic characteristics

Overall, 375 subjects were screened, and 266 (70.9%) met the eligibility criteria, of which 257 (96.6%) were included in the EP (**Figure 2**). The first subject was enrolled in November 2018 and the last subject completed the study in January 2019. Five eligible subjects discontinued before entering Period 2. Four subjects were excluded from the EP due to the absence of baseline TNSS in Period 1 or Period 2, leaving 257 subjects. In total, 13 eligible

subjects discontinued the study before entering Period 3 due to: scheduling conflicts (five), AEs (two), meeting an exclusion criterion (two), withdrawal of consent (one), poor compliance to protocol (one), EEU challenge intolerance (one), and withdrawal from study (one). Of the 253 subjects in Period 3, 127 were randomised to fexofenadine HCl 180 mg and 126 to placebo. The ITT and safety populations consisted of 253 subjects, and the mITT population of 251 subjects due to missing baseline TNSS scores for two individuals. Baseline demographics and characteristics for the ITT and mITT populations are shown in **Table 1A** and **1B**, respectively. Mean (standard error [SE]) age was 40.8 (0.78) and 40.7 (0.79) years in the Evaluable and mITT populations, respectively. In both populations, the majority of subjects were female and non-smokers. The mean (SD) values of common perennial allergens using the skin prick test were all significantly lower compared to ragweed in the EP (ragweed, 12.4 [6.2] mm; D. pteronyssinus, 5.2 [5.5] mm; D. farinae, 4.8 [5.7] mm; Alternaria, 1.8 [2.8] mm; full results from skin prick test are reported in the Supplementary Material, Table 1). Similarly, no notable differences were observed between fexofenadine HCl and placebo group with regards to the skin prick results. In addition, individuals were asymptomatic before challenge test at each period. Mean (SE) EEU baseline pollen exposure (grains/m^3) was comparable between all three periods: 3272.59 (129.48; Period 1), 3296.97 (144.21; Period 2) and 3374.64 (107.61; Period 3). Mean (SE) EEU DEP exposure (ng/m^3) was also similar between periods: 640.3 (22.49; Period 2) and 643.7 (10.28; Period 3). In the included population, skin prick test responses to other allergens resulted in a wheal diameter smaller than 3 mm compared to control (vehicle alone); therefore, they are not presented. All subjects received study medication in Period 3 and were included in the safety population (N=253).

$AUC_{0\text{-}12}$ of the TNSS during Period 1 and 2

Mean (SE) AUC_{0-12} of the TNSS was higher in Period 2, compared with Period 1 (41.22 [1.16] and 36.25 [1.05], respectively; **Figure 3**). The LS-mean difference (SE; 95% CI) for Log $AUC_{0\text{-}12}$ of the TNSS was statistically significant between the two periods (0.13 [0.03]; 95% CI: 0.081 to 0.182; p<0.0001), demonstrating an aggravation of pollen-induced SAR symptoms in the presence of DEP versus pollen alone. Beginning 30 minutes following ragweed + DEP challenge, and continuing at all subsequent time points, TNSS scores were higher in Period 2 than in Period 1.

Effect of fexofenadine HCl versus placebo on TNSS, TSS and individual symptom scores following pollen + DEP challenge

The second sequential primary endpoint, mean (SE) AUC_{2-12} of the TNSS in Period 3, was lower among fexofenadine HCl-treated subjects than placebo-treated subjects (18.53 [1.22]

vs 26.34 [1.49], respectively; **Figure 4**). The LS-mean difference (SE; 95% CI) for Log AUC_{2-12} of the TNSS between treatment groups was −0.24 (0.096, 95% CI: −0.425 to −0.047; p=0.0148). AUC_{2-12} of the TNSS at all time-points was consistently lower in fexofenadine HCl-treated versus placebo-treated subjects (**Figure 5**).

Mean AUC_{2-12} of the TSS in Period 3 was lower in the fexofenadine HCl-treated group, versus placebo (35.16 vs 47.96, respectively). The LS-mean difference (SE; 95% CI) for Log TSS AUC_{2-12} between groups was not statistically significant: −0.18 (0.10; 95% CI; −0.369 to 0.015; p=0.0711). Due to the sequential testing procedure used to maintain a 5% type I error rate throughout the multiple secondary endpoints, analyses of subsequent pre-specified secondary endpoints after TSS (Log AUC_{2-12}) in Period 3 were descriptive only. At H2.5, and all subsequent time points in Period 3, mean TSS in fexofenadine HCl-treated subjects was lower than among placebo-treated subjects: the LS-mean differences between groups ranged from −0.6 to −1.4.

Mean AUC_{2-12} for all individual symptom scores during Period 3 was lower in fexofenadine HCl-treated subjects than in the placebo group. Mean TNSS at H2.5 (30 minutes after drug administration) and all subsequent time points during Period 3 was lower in the fexofenadine HCl group than the placebo group with LS-mean differences between treatments ranging from −0.4 to −0.8. Mean AUC_{2-12} of all eight individual symptom scores in Period 3 was numerically lower in the fexofenadine HCl group, versus placebo (**Figure 6**). Proportional mean symptom reduction with fexofenadine versus placebo, was: rhinorrhoea (28.8%); sneezing (39.2%); nasal itching (23.0%); nasal congestion (24.8%); itchy eyes (23.0%); watery eyes (27.5%); red or burning eyes (24.8%); and ear, palate, or throat itching (18.6%); all data were cumulative, from treatment intake up to 10 hours post treatment.

Safety evaluation

One subject withdrew in Period 2 due to chest discomfort and dyspnoea. There were no discontinuations due to an AE in Periods 1 or 3. No subject experienced a TEAE leading to study discontinuation. The proportion of subjects reporting a TEAE was higher in the placebo group (19/126 [15.1%]), compared with the fexofenadine HCl group (16/127 [12.6%]). One (0.8%) fexofenadine HCl-treated subject experienced a TEAE (dry mouth). The most frequently reported TEAEs were seasonal allergies (fexofenadine HCl, N=6 [4.7%]; placebo, N=7 [5.6%]) and upper respiratory tract infection (fexofenadine HCl, N=2 [1.6%]; placebo, N=2 [1.6%]; **Table 2**; additional details are available in the Supplementary Material).

Discussion

This study demonstrated that a 3 h exposure to DEP and ragweed pollen significantly increased SAR symptom severity over a 12 h observation period. Furthermore, a single dose of fexofenadine HCl 180 mg significantly decreased all analysed symptoms compared with placebo. A limit to the comprehensive assessment of fexofenadine HCl efficacy could be the lack of comparison with placebo in the presence of pollen alone. However, the objective of the study was not to demonstrate the efficacy of fexofenadine HCl, which has been well documented in previous clinical trials[16, 17] showing significant decreases in ragweed-induced allergic symptoms in an EEU model.[18]

The symptomatic effects of DEP were seen at the first post-exposure measurement (H0.5). Symptom aggravation occurred during the initial 3 h exposure to ragweed + DEP and persisted during the 9 h following the subject's departure from the EEU. The extended effect of DEP observed several hours after the exposure period suggests that even relatively short exposures to an air pollutant could have a significant impact on SAR symptoms during the late phase of the allergic reaction,[19] consistent with prior studies.[20] A study of 18 atopic individuals found increased allergen-induced inflammation of the lower respiratory tract following diesel exhaust exposure.[21] A co-exposure to allergens and diesel exhaust augments inflammatory cells and TH2-related cytokines, while lowering host defence peptides and altering DNA methylation and protein responses in the lungs.[22-24] Diaz-Sanchez et al. (1997) documented a significant increase in IgG4 in nasal lavage samples from ragweed-sensitised individuals after ragweed + DEP exposure versus ragweed alone, which was believed to be mediated via an increase in ragweed-specific IgE.[25] These investigators also found that exposing dust mite-allergic subjects to DEP increased mean symptom scores and decreased, by approximately 80%, the inducing dose of dust mite allergen, versus no DEP exposure.

Recently the exposure to pollutants and climate change has been linked to AR symptom exacerbation.[26] In an 11-country survey the majority of participants attributed climate changes (81.1%) and pollutants (51.2%) as contributors to their AR symptoms.[27] In another survey conducted in 4 regions including Europe, 71.15% of participants attributed the increasing prevalence of AR to "increased exposure to allergens, irritants and pollutants"[28] Another recent study showed a harmful effect of air pollution (particularly ozone) on AR control, especially during grass pollen season. Positive associations were found between air pollutants (ozone and particulate matter 2.5 μm) and AR symptoms. Differences between pollen seasons were also found, suggesting an interaction between air pollution and pollen exposure (e.g., the dose-dependent deleterious effects of pollen exposure were magnified

by air pollutant exposure).[29] While a growing body of evidence supports a link between allergic diseases and air pollution,[3, 30] other studies show mixed results.[31, 32]Molter et al (2015) found no significant association between asthma prevalence and exposure to selected air pollutants in a meta-analysis of five European birth cohorts. [32] Similarly, Burte et al. (2018) found no association between long-term air pollution exposure and the incidence of self-reported rhinitis among the same European cohorts analysed by Molter et al (2015).[31] Reasons for these heterogeneous observations may include differences in study design, exposure assessment, air pollutants included, and air pollution monitoring site locations. Ultimately, air pollution has an important role in the development and prevalence of allergic symptoms, but its precise influences have not been fully established.[2, 26, 33, 34]

The EEU is designed to replicate effectively an outdoor environment while removing those variables that may affect allergy research. The EEU allows for allergen specificity; control over antigen exposure level; temperature; and air quality.[35] Although a high degree of concordance has been reported for allergic symptoms induced in the EEU and those experienced through natural exposure,[35] the results of this study are limited to the controlled environment of analysis and additional real-world evidence is needed.

Pharmacological options for the treatment of AR are well-established, but additional research is needed on the efficacy of conventional pharmacotherapy in patients exposed to air pollution.[26] A review of the studies investigating AR symptoms aggravated by air pollutants showed that fexofenadine HCl is the only AR medication with demonstrated efficacy and tolerability for the management of DEP-aggravated symptoms.[26, 30] DEP exposure has been shown to trigger numerous pro-inflammatory signalling pathways other than histamine-mediated ones.[30] DEP increases circulating neutrophils, eosinophils, and cytokines, and induces the expression of adhesion molecules as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) which are critical for T cell activation.[9,21] Additionally, an exacerbation of the inflammatory response may result from the generation of reactive oxygen species and oxidant overload.[26] Noteworthy, it has been demonstrated that fexofenadine has additional anti-inflammatory properties besides the anti-histaminergic activity.[36] Fexofenadine decreases cytokine levels, including ICAM-1 and VCAM-1 among others; inhibits eosinophil adherence and chemotaxis; inhibits cyclo-oxygenase 2; and reduces the production of leukotrienes and prostaglandins.[36] These additional effects of fexofenadine could further contribute to the improvement of allergic rhinitis symptoms aggravated by the inflammatory response induced by DEP; however, more research is needed to demonstrate this hypothesis.

In summary, DEP exposure can significantly exacerbate ragweed-induced SAR symptoms and fexofenadine HCl 180 mg is an effective and well-tolerated treatment to alleviate these pollution-aggravated symptoms.

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Author contribution

PP was instrumental in analysing the data and performing the statistical analysis. MMA and SF designed the study. MMA and AKE contributed to the protocol preparation, review of SAP and results interpretation. CC contributed to study design and interpretation of results. All authors have participated in the development of this publication and have provided their approval for its submission.

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Declaration of interests

AKE (over her lifetime) has participated in advisory boards for ALK, Abello, AstraZeneca, Aralez, Bausch + Health, Circassia Ltd, GlaxoSmithKline, Johnson & Jhonson, Merck, Mylan, Novartis, Nuvo, Pediapharm Pfizer and Sanofi-Genzyme, and has been a speaker for ALK, Abello, AstraZeneca, Aralez, Boehringer-Ingelheim, Meda, Medesus, Merck, Mylan, Novartis, Pediapharm, Pfizer and Takeda. Her institution has received research grants from ALK, Abello, AstraZeneca, Bayer LLC, Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Merck, Novartis, Pfizer, and Regeneron.

CC declares no conflict of interests.

MMA and **SF** are employees of Sanofi.

PP is a paid consultant of Sanofi.

Data sharing statement

Qualified researchers may request access to patient-level data and related documents (including, e.g., the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and dataset specifications). Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at https://www.clinicalstudydatarequest.com.

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Figures and tables

Figures

Figure 1. Schematic of study design

DEP=diesel exhaust particles, H=hour, V=visit. Note: V5 on D35 could be accomplished via a telephone contact

Figure 2. Subjects' disposition

ITT=intent-to-treat, mITT=modified intent-to-treat

Figure 3. Mean (SE) TNSS $AUC_{0\text{-}12}$ in Periods 1 and 2 (EP)

*p-value was obtained using a mixed model for repeated measures (MMRM) on log transformed values of TNSS AUC (H0-H12) plus 0.1, adjusted on baseline TNSS (H0) for each period (1 and 2) and on pollen counts at each EEU session, with period as a fixed categorical effect.

Abbreviations: AUC=area under the curve, DEP=diesel exhaust particles, EP=evaluable population, SE= standard error, TNSS=total nasal symptom score

Figure 4. Mean (SE) TNSS $AUC_{2\text{-}12}$ in Period 3 (mITT population)

*p-value was obtained using analysis of covariance (ANCOVA) of Log-transformed values of TNSS $AUC_{2\text{-}12}$ plus 0.1, with treatment group as a fixed categorical effect and baseline TNSS (H+2) as covariate.

Abbreviations: AUC= area under the curve, mITT= modified intent-to-treat, SE= standard error, TNSS= total nasal symptom score.

Figure 5. Mean (SE) TNSS $AUC_{2\text{-}12}$ by time point in Period 3 (mITT population)

Abbreviations: AUC= area under the curve, mITT= modified intent-to-treat, SE= standard error, TNSS= total nasal symptom score.

Figure 6. Mean (SE) $AUC_{2–12}$ of individual symptom scores after pollen + DEP exposure (mITT population)

DEP= diesel exhaust particles, mITT= modified intent-to-treat; SE, standard error.

Tables

Table 1. Subject demographics and baseline characteristics **A.** EP and **B.** mITT

A	**EP N=257**
Mean age, years (SE)	40.8 (0.78)
Sex, N (%)	
Male	90 (35.0)
Female	167 (65.0)
Smoking status	
Never smoked	173 (67.3)
Quit smoking	52 (20.2)
Currently smokes	32 (12.5)
Allergic medical history, N (%)	
Seasonal allergic rhinitis	257 (100.0)
Perennial rhinitis	183 (71.2)
Mean allergen wheal diameter, mm (SE)	
Control	0.5 (0.06)
Ragweed	12.4 (0.4)
Baseline TNSS, mean (SE)	
Period 1 (H0)[a]	0.5 (0.05)
95% CI	(0.40–0.62)
Median	0
Q1;Q3	0.0;1.0
Min;Max	0;5

Period 2 (H0)[a]	0.5 (0.06)
95% CI for the mean	(0.39–0.61)
Median	0
Q1;Q3	0.0;1.0
Min;Max	0;5

Percentages are calculated from non-missing data. [a]For Period 1 and Period 2, the baseline is defined as the value at H0 (start of challenge).

CI=confidence interval, EP=evaluable population, HCl=hydrochloride, SE=standard error.

B

	mITT population		
	Placebo N=125	**Fexofenadine HCl 180 mg N=126**	**All N=251**
Mean age, years (SE)	41.5 (1.12)	40.0 (1.12)	40.7 (0.79)
Sex, N (%)			
Male	37 (29.6)	49 (38.9)	86 (34.3)
Female	88 (70.4)	77 (61.1)	165 (65.7)
Smoking status, N (%)			
Never smoked	77 (61.6)	92 (73.0)	169 (67.3)
Quit smoking	30 (24.0)	22 (17.5)	52 (20.7)
Currently smokes	18 (14.4)	12 (9.5)	30 (12.0)
Allergic medical history, N (%)			
Seasonal allergic rhinitis	125 (100.0)	126 (100.0)	251 (100.0)
Perennial rhinitis	85 (68.0)	92 (73.0)	177 (70.5)
Mean allergen wheal diameter, mm (SE)			

Control	0.5 (0.09)	0.4 (0.07)	0.5 (0.06)
Ragweed	11.9 (0.47)	12.9 (0.62)	12.4 (0.39)
Mean TNSS (SE),			
Period 3 (H2) [a]	6.2 (0.20)	5.6 (0.18)	–
95% CI for the mean	(5.77–6.55)	(5.27–6.00)	–
Median	6.0	6.0	–
Q1;Q3	5.0;8.0	4.0;7.0	–
Min;Max	0;9	0;9	–

Percentages are calculated from non-missing data. [a]Period 3 baseline is defined as the last available value after challenge and before treatment administration.

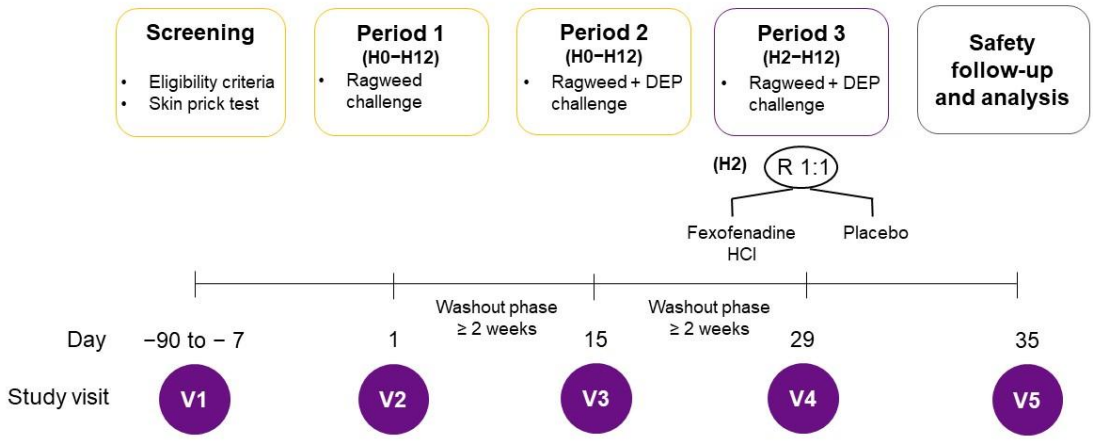
CI=confidence interval, HCl=hydrochloride, SE=standard error; mITT=modified intent-to-treat.

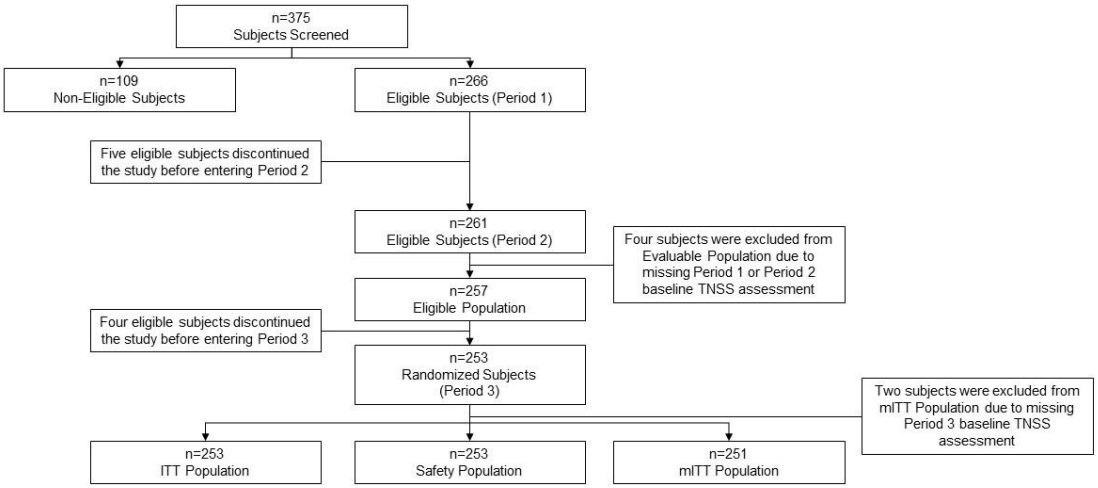
Table 2. Incidence of treatment-emergent adverse events by primary system organ class and preferred term at an incidence of ≥2% (safety population) [a]

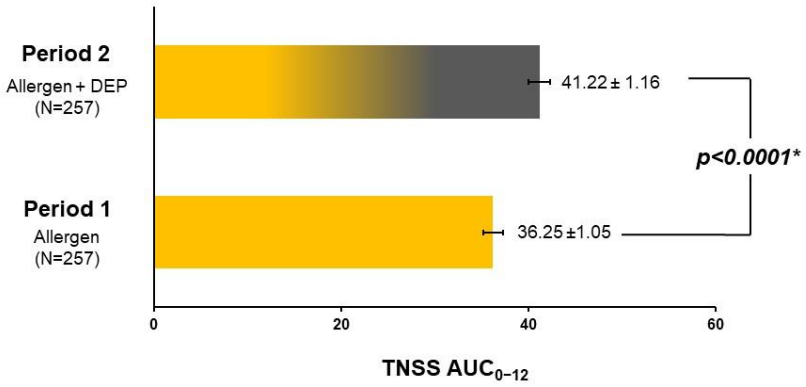
Primary system organ class (preferred term)	Placebo, N (%) N=126	Fexofenadine HCl, N (%) N=127
Any class	19 (15.1)	16 (12.6)
Infections and infestations	3 (2.4)	2 (1.6)
Upper respiratory tract infection	2 (1.6)	2 (1.6)
Gastroenteritis	1 (0.8)	0 (0.0)
Immune system disorders	7 (5.6)	6 (4.7)
Seasonal allergic rhinitis	7 (5.6)	6 (4.7)
Respiratory, thoracic and mediastinal disorders	5 (4.0)	2 (1.6)
Nasal dryness	0 (0.0)	2 (1.6)
Cough	1 (0.8)	0 (0.0)
Nasal congestion	1 (0.8)	0 (0.0)
Nasal pruritus	1 (0.8)	0 (0.0)
Rhinorrhoea	1 (0.8)	0 (0.0)
Sneezing	1 (0.8)	0 (0.0)
Upper-airway cough syndrome	1 (0.8)	0 (0.0)
Gastrointestinal disorders	0 (0.0)	3 (2.4)
Dry mouth	0 (0.0)	1 (0.8)
Enlarged uvula	0 (0.0)	1 (0.8)
Nausea	0 (0.0)	1 (0.8)

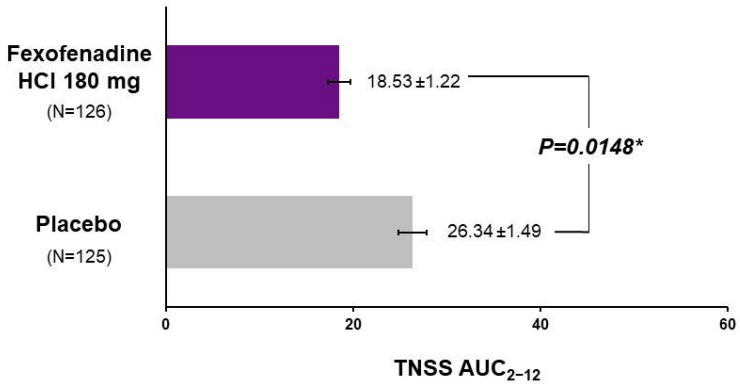
[a] Subjects may have experienced more than one type of AE within any primary system organ class

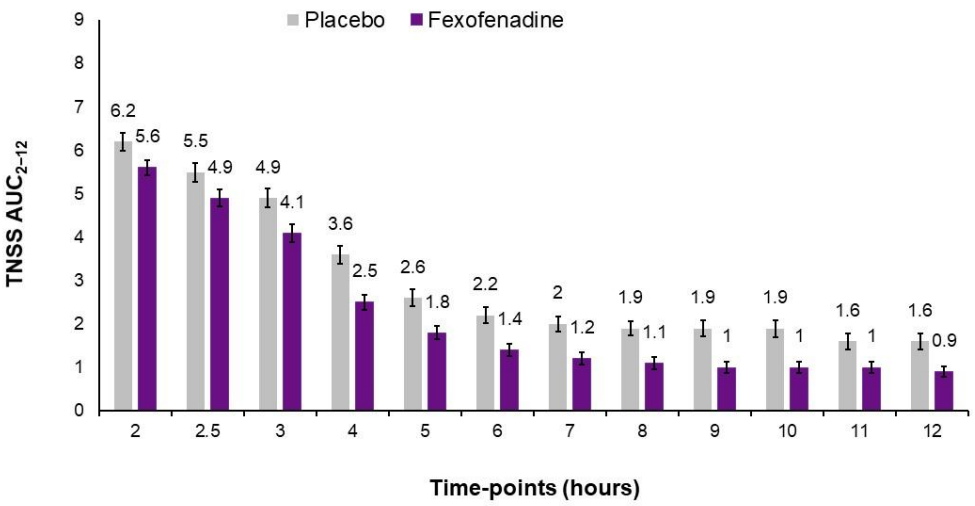
HCl=hydrochloride

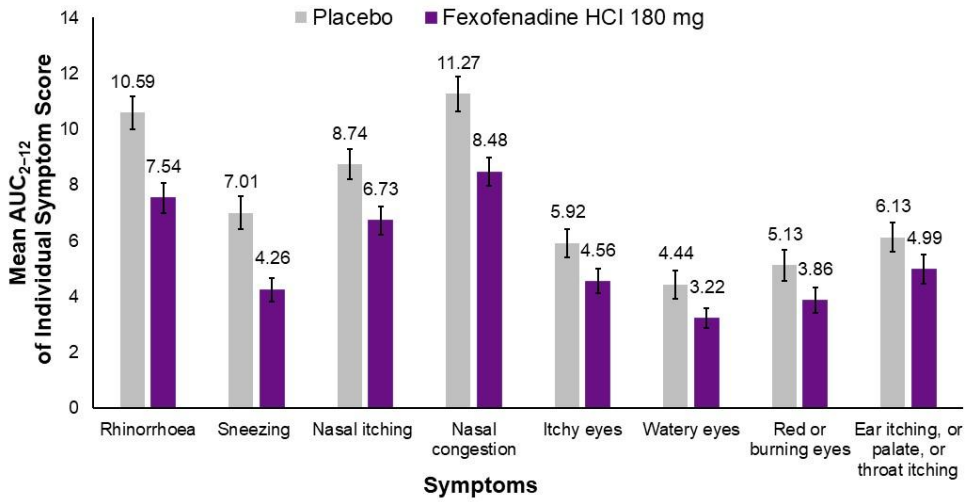

Screening
• Eligibility criteria
• Skin prick test
Period 1
(H0−H12)
• Ragweed challenge
Period 2
(H0−H12)
• Ragweed + DEP challenge
Period 3
(H2−H12)
• Ragweed + DEP challenge
Safety follow-up and analysis
(H2)
R 1:1
Fexofenadine HCl
Placebo
Washout phase ≥ 2 weeks
Washout phase ≥ 2 weeks
Day
−90 to − 7
1
15
29
35
Study visit
V1
V2
V3
V4
V5


n=375
Subjects Screened
n=109
Non-Eligible Subjects
n=266
Eligible Subjects (Period 1)
Five eligible subjects discontinued the study before entering Period 2
n=261
Eligible Subjects (Period 2)
Four subjects were excluded from Evaluable Population due to missing Period 1 or Period 2 baseline TNSS assessment
n=257
Eligible Population
Four eligible subjects discontinued the study before entering Period 3
n=253
Randomized Subjects
(Period 3)
Two subjects were excluded from mITT Population due to missing Period 3 baseline TNSS assessment
n=253
ITT Population
n=253
Safety Population
n=251
mITT Population


Period 2
Allergen + DEP
(N=257)
41.22 ± 1.16
p<0.0001*
Period 1
Allergen
(N=257)
36.25 ±1.05
0
20
40
60
TNSS AUC0–12


Fexofenadine HCl 180 mg
(N=126)
18.53 ±1.22
P=0.0148*
Placebo
(N=125)
26.34 ±1.49
0
20
40
60
TNSS AUC2–12


Placebo
Fexofenadine
TNSS AUC2–12
9
8
7
6
5
4
3
2
1
0
6.2
5.6
5.5
4.9
4.9
4.1
3.6
2.5
2.6
1.8
2.2
1.4
2
1.2
1.9
1.1
1.9
1
1.9
1
1.6
1
1.6
0.9
2
2.5
3
4
5
6
7
8
9
10
11
12
Time-points (hours)


Placebo
Fexofenadine HCl 180 mg
Mean AUC2–12 of Individual Symptom Score
14
12
10
8
6
4
2
0
10.59
7.54
7.01
4.26
8.74
6.73
11.27
8.48
5.92
4.56
4.44
3.22
5.13
3.86
6.13
4.99
Rhinorrhoea
Sneezing
Nasal itching
Nasal congestion
Itchy eyes
Watery eyes
Red or burning eyes
Ear itching, or palate, or throat itching
Symptoms

Supplementary Material

S1. Exclusion criteria

- A history of grade IV anaphylaxis (to any antigen).
- Asthma symptoms or exacerbations requiring regular inhaled steroids for ≥4 weeks in the past 12 months or any oral corticosteroid use/asthma-related hospitalisations.
- Chronic sinusitis.
- Recent (within 30 days) active or suspected systemic infection
- Immunodeficiency.
- Excluded medications included: beta-blockers; tricyclic antidepressants; monoamine oxidase inhibitors; antihistamines or intranasal/inhaled corticosteroids within 7 days of the subsequent EEU challenge; and before enrolment sodium cromoglycate in any form (14 days): systemic corticosteroids or a leukotriene antagonist (30 days); and omalizumab or dupilumab (6 months).
- Consumption of any citrus fruits (grapefruit, orange, etc.) or their juices within 5 days from the study entry.

S2. EEU methodology

The EEU is a specifically engineered room that enables controlled exposure to airborne pollen and DEP. Rotorod® sampling equipment and a microaethalometer measured and tracked ragweed and DEP levels, respectively. A custom-engineered computer delivery system dispersed both airborne compounds via a seated-height, airflow-regulated wall duct, floor-level wall vents, and directional fans.
Ragweed pollen was sourced in the USA (Greer Laboratories, Lenoir, NC), independently tested for fungal and bacterial contamination (Paracel Laboratories Ltd.), and approved for use by a toxicologist. DEP was sourced from NIST (National Institute of Standards & Technology) as “standard reference material 2975” and was supplied with a certificate of analysis and material safety data sheet. Further independent metals content and endotoxin testing was completed (Paracel Laboratories Ltd.) to verify suitability for use. The DEP was resuspended from bulk form using a custom aerosoliser. DEP output through the aerosoliser was adjusted via increasing or decreasing the measured airflow through the main portal while

monitoring the real-time 880nm, black carbon, channel output from the microaethelometer. This output was reflective of the average DEP room concentration. Taken from the NIST COA, subsamples of DEP taken from four separate bulk sources revealed that 50% of the volume was less than 19.4um in size and 10% was less than 5.3um.
Subjects were exposed to a mean target ragweed pollen concentration of 3500 ± 500 grains/m^3 and a mean target DEP concentration equivalent to 0.3 mg of DEP in 300 µL of saline during the three periods.

S3. Hierarchical test procedure

The following hierarchical procedure was used to control the type I error and handle multiple endpoints analysis.

Primary endpoints analysis

First, the first primary endpoint ($AUC_{0\text{-}12}$ of the TNSS compared between Period 1 and Period 2) was tested at a two-sided 5% type I error rate level. If the first primary analysis was significant, the second primary endpoint ($AUC_{2–12}$ of the TNSS in Period 3) was tested at the same two-sided 5% type I error rate level. If the results observed during this first primary analysis did not show that SAR symptoms were significantly aggravated in presence of pollutants, with a two-sided statistical significance of 5%, the analysis of the second primary efficacy endpoint and other secondary efficacy endpoints statistical analyses were planned to be descriptive only, without treatment group comparison.

Secondary endpoints analysis

The secondary efficacy endpoints analyses were planned to be descriptive only if the sequentially-tested comparison for the second primary efficacy endpoint was not significant at the 5%level. Secondary endpoints were analysed sequentially and upon the first non-significant endpoint analysis, all subsequent secondary endpoints were assessed using descriptive statistics. The sequence of testing was as follows:

- $AUC_{2–12}$ of the Total Symptom Score (TSS)
- $AUC_{2–12}$ of individual symptom scores.

    - The sequence of individual symptom analysis was: rhinorrhoea, sneezing, nasal itching, itchy eyes, watery eyes, red or burning eyes and itching of the ears or palate or throat, and nasal congestion.
- TNSS, followed by TSS and then individual symptom scores, each by time point.

Supplementary Table 1. Baseline results of the skin prick test (EP)

Allergen	EP (N=257)
Negative control	
Mean (SD)	0.5 (0.9)
Median	0
Q1;Q3	0.0;0.0
Ragweed	
Mean (SD)	12.4 (6.5)
Median	11.0
Q1;Q3	8.0;15.0
Dog	
Mean (SD)	0.9 (1.7)
Median	0.0
Q1;Q3	0.0;2.0
Cat	
Mean (SD)	3.4 (3.7)
Median	3.0
Q1;Q3	0.0;6.0
D. pteronyssinus	
Mean (SD)	5.2 (5.5)
Median	4.0
Q1;Q3	0.0;9.0
D. farinae	
Mean (SD)	4.8 (5.7)
Median	3.0

Q1;Q3	0.0;9.0
Alternaria	
Mean (SD)	1.8 (2.8)
Median	0.0
Q1;Q3	0.0;3.0
Grass	
Mean (SD)	7.8 (7.5)
Median	7.0
Q1;Q3	0.0;11.0
Trees	
Mean (SD)	5.6 (5.9)
Median	5.0
Q1;Q3	0.0;10.0

EP=evaluable population; SD=standard deviation.

Supplementary Table 2. Incidence of treatment-emergent adverse events by primary system organ class and preferred term (safety population)[a]

Primary system organ class (preferred term)	**Placebo, n (%) N=126**	**Fexofenadine HCl, n (%) N=127**
Any class	19 (15.1)	16 (12.6)
Infections and infestations	3 (2.4)	2 (1.6)
Upper respiratory tract infection	2 (1.6)	2 (1.6)
Gastroenteritis	1 (0.8)	0 (0.0)
Immune system disorders	7 (5.6)	6 (4.7)
Seasonal allergy	7 (5.6)	6 (4.7)
Nervous system disorders	2 (1.6)	2 (1.6)
Headache	1 (0.8)	2 (1.6)
Sinus headache	1 (0.8)	0 (0.0)
Respiratory, thoracic and mediastinal disorders	5 (4.0)	2 (1.6)

Nasal dryness	0 (0.0)	2 (1.6)
Cough	1 (0.8)	0 (0.0)
Nasal congestion	1 (0.8)	0 (0.0)
Nasal pruritus	1 (0.8)	0 (0.0)
Rhinorrhoea	1 (0.8)	0 (0.0)
Sneezing	1 (0.8)	0 (0.0)
Upper-airway cough syndrome	1 (0.8)	0 (0.0)
Gastrointestinal disorders	0 (0.0)	3 (2.4)
Dry mouth	0 (0.0)	1 (0.8)
Enlarged uvula	0 (0.0)	1 (0.8)
Nausea	0 (0.0)	1 (0.8)
Skin and subcutaneous tissue disorders	1 (0.8)	0 (0.0)
Pruritus	1 (0.8)	0 (0.0)
Musculoskeletal and connective tissue disorders	1 (0.8)	0 (0.0)
Back pain	1 (0.8)	0 (0.0)
General disorders and administration site conditions	1 (0.8)	0 (0.0)
Fatigue	1 (0.8)	0 (0.0)
Injury, poisoning and procedural complications	1 (0.8)	1 (0.8)
Muscle strain	1 (0.8)	1 (0.8)

[a] Subjects may have experienced more than one type of AE with each primary system organ class during the study

HCl=hydrochloride