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Original article

Therapeutic effect of bilastine in Japanese cedar pollinosis using an artificial exposure chamber (OHIO Chamber)

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ARTICLE INFO

Article history:

Received 13 May 2016

Received in revised form

6 June 2016

Accepted 16 June 2016

Available online xxx

Keywords:

Bilastine

Fexofenadine

H₁-antihistamine

OHIO Chamber

Therapeutic effect

Abbreviations:

ADR, adverse drug reaction; AE, adverse event; ANCOVA, analysis of covariance; ANOVA, analysis of variance; AR, allergic rhinitis; CI, confidence interval; EEC, environmental exposure chamber; ECG, 12-lead electrocardiogram; FAS, full analysis set; FDA, the US Food and Drug Administration; FEX, fexofenadine hydrochloride; MeDRA, Medical Dictionary for Regulatory Activities; PMDA, the Pharmaceutical and Medical Devices Agency; PPS, per protocol set; QOL, quality of life; SAR, seasonal allergic rhinitis; SAS, statistical analysis software; SD, standard deviation; SE, standard error; SP, safety population; TNSS, total nasal symptom score; VAS, visual analog scale; WO, washout

ABSTRACT

Background: Environmental exposure chambers have been used to expose subjects to aeroallergens to investigate the efficacy of prophylactic treatment with symptomatic agents in Japan. We first examined the therapeutic effect of bilastine (BIL), a novel non-sedative second-generation H₁-antihistamine, in subjects with Japanese cedar pollinosis using an artificial exposure chamber (OHIO Chamber).

Methods: This was a randomized, double-blind, four-way crossover, placebo- and active-controlled phase II study (trial registration number JapicCTI-132213). Subjects were exposed to cedar pollen (8000 grains/m³) for 2 h on Day –1 and 4 h each on Day 1 and 2. BIL 10 or 20 mg, placebo, or fexofenadine hydrochloride (FEX) 60 mg was administered orally 1 h after the start of pollen exposure on Day 1. Placebo or FEX was administered 12 h after the first dosing. The primary efficacy endpoint was the sum of total nasal symptom score (TNSS) from 0 to 3 h after the Day 1 dosing.

Results: We enrolled 136 subjects and the sum of TNSS on Day 1 of the three active treatments was significantly lower than that of placebo and was maintained up to 26 h after the first dosing (Day 2). The sum of TNSS or sneezing score on Day 1 after BIL 20 mg was more significantly decreased than after FEX. Moreover, BIL showed a faster onset of action than FEX.

Conclusions: We demonstrated the efficacy, rapid onset, and long duration of action of BIL in subjects with Japanese cedar pollinosis exposed to cedar pollen using the OHIO Chamber.

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Introduction

Japanese cedar (JC) pollinosis is a seasonal allergic rhinitis (SAR), which is unique to Japan, and the causative allergen (JC pollen) is dispersed usually between February and April although the pollen count varies every year. According to a recent epidemiological

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Peer review under responsibility of Japanese Society of Allergy.

<http://dx.doi.org/10.1016/j.alit.2016.06.009>

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survey, the average national morbidity rate of JC pollinosis was 26.5% in 2008.¹

Environmental exposure chambers (EECs) have been used to assess the efficacy and onset and duration of action of anti-allergic drugs in subjects with seasonal or perennial allergic rhinitis exposed to aeroallergens.^{2,3} Efficacy assessment using the EEC is approved and accepted by the US Food and Drug Administration (FDA),⁴ the European Medicines Agency⁵ and the Japanese Pharmaceutical and Medical Devices Agency (PMDA). While there is no universally adopted guideline on the clinical development of new drugs for allergic rhinitis, the PMDA has accepted clinical studies using EEC for the new drug application.

We built an artificial allergen exposure chamber (OHIO Chamber) in the center of Tokyo in September, 2008,⁶ and have used it to conduct basic and clinical studies in subjects with JC pollinosis exposed to JC pollen.^{6–10} There are three allergen exposure facilities in Japan (Wakayama,¹¹ Tokyo,⁶ Chiba¹²), which currently conduct clinical studies in JC pollinosis. In addition, to the best of our knowledge, most of the studies conducted assessed prophylactic treatments, except for two clinical studies that evaluated the therapeutic effect of the H₁-antihistamine agents, cetirizine hydrochloride¹¹ and bepotastine besilate.¹³ Alternatively, numerous overseas clinical studies using the EEC have been conducted to evaluate the efficacy, as well as onset and duration of treatment with study drugs.^{2,3,14}

Bilastine (BIL) is a novel non-sedating second-generation H₁-antihistamine, which has been approved for the symptomatic treatment of allergic rhinitis and urticaria in numerous countries (Europe, Central and South America, and Africa), but not Japan.¹⁵ The overseas clinical studies in subjects with SAR demonstrated that BIL 20 mg administered once daily for 2 weeks exerted an efficacy that was comparable to that of cetirizine¹⁶ and desloratadine.¹⁷

We conducted a single-center, randomized, double-blind, four-way crossover, placebo-, and active-controlled phase II study to evaluate the efficacy of therapeutic treatment with BIL in Japanese subjects with cedar pollinosis by using the OHIO Chamber.

Methods

Subjects

The study subjects were 20–60-year-old patients with JC pollinosis presenting with a ≥ 2 -year history of symptoms, who had received medical treatment including oral H₁-antihistamines during the JC pollen season, and had a positive CAP-radioallergosorbent test (RAST) class ≥ 2 of serum specific immunoglobulin E (IgE) to JC pollen. In addition, subjects meeting any of the following conditions were excluded:

- Diagnosed with or had a history of severe systemic disease (such as liver, renal, blood, and cardiac disease, neurological disorder, or malignant tumor).
- Nasal disorder, which may affect the efficacy of the study drugs (e.g., vasomotor, drug-induced, and hypertrophic rhinitis; deviated nasal septum, sinusitis, and nasal polyp)
- Underwent nasal surgery to improve nasal symptoms or underwent specific or nonspecific immunotherapy within 5 years before the start of the study.
- Used any of the following drugs before the start of the study: steroid injection (180 days), other study drug (90 days), oral or topical steroid, drugs with P-glycoprotein (P-gp) inhibitory

action (30 days), antihistamines, or leukotriene receptor antagonists (14 days).

- Pregnant or lactating women, and women who wish to become pregnant during the study period.
- Symptoms of upper respiratory infections within 14 days prior to first dosing.
- Concurrent bronchial asthma.
- History of allergic reaction to FEX.
- Determined ineligible to participate in the study by the investigator.

Study design

This study was a phase II study, performed outside of the JC pollen season in subjects with asymptomatic JC pollinosis, using the OHIO Chamber as described elsewhere.^{6–8} The study comprised three periods, the screening, treatment, and follow-up periods. The screening period was conducted in a single-blind manner for subjects while the treatment period was a randomized, double-blind, placebo- and active-controlled, four-way crossover study (Fig. 1A).

After obtaining informed consent from the subjects, those who met the inclusion criteria and did not violate the exclusion criteria were asked to re-visit the institution during the screening period to undergo exposure to JC pollen (8000 grains/m³) for 4 h. The subjects were orally administered the placebo 1 h after the start of exposure to assess the nasal symptom score and exclude any placebo effect.

The total nasal symptom score (TNSS) was used as an index to evaluate the subjects for eligibility to transition from the screening to the treatment period. The TNSS represents the scores of the subjective symptoms of rhinorrhea, sneezing, nasal obstruction, and nasal itching assessed on a 5-point scale (0–4). The inclusion criteria were set as follows:

- TNSS ≥ 2 , 1 h after the start of pollen exposure (maximum 16).
- A sum of TNSS ≥ 36 at specific time points after the placebo administration to 3 h (12 time points, maximum 192).

The exclusion criteria were set as follows:

- Total amount of rhinorrhea ≤ 2 g during pollen exposure (4 h).
- A decrease of ≥ 2 in TNSS from baseline (just before placebo administration) at any time point during the 1 h after the placebo administration.

Subjects deemed eligible during the screening exposure were rolled over to the treatment period, which was comprised of four groups. The subjects were randomly assigned to one of the four groups and the treatment period consisted of four 3-day periods that ran consecutively (Fig. 1B). A washout period of ≥ 10 days was employed.

On Day –1, the subjects were exposed to pollen for 2 h as the priming exposure. Then, subjects with a mean TNSS ≥ 1 from 1 to 2 h after the start of exposure proceeded to Day 1, during which they were exposed to pollen for 4 h (the following day of the priming exposure). This was followed by the administration of the first study drug 1 h after the start of exposure, and then the secondary drug was administered 12 h after the first dosing. On Day 2, the subjects returned to the OHIO Chamber 22 h after the first dosing and were further exposed to pollen for 4 h. When the subjects could not tolerate the nasal or ocular symptoms after

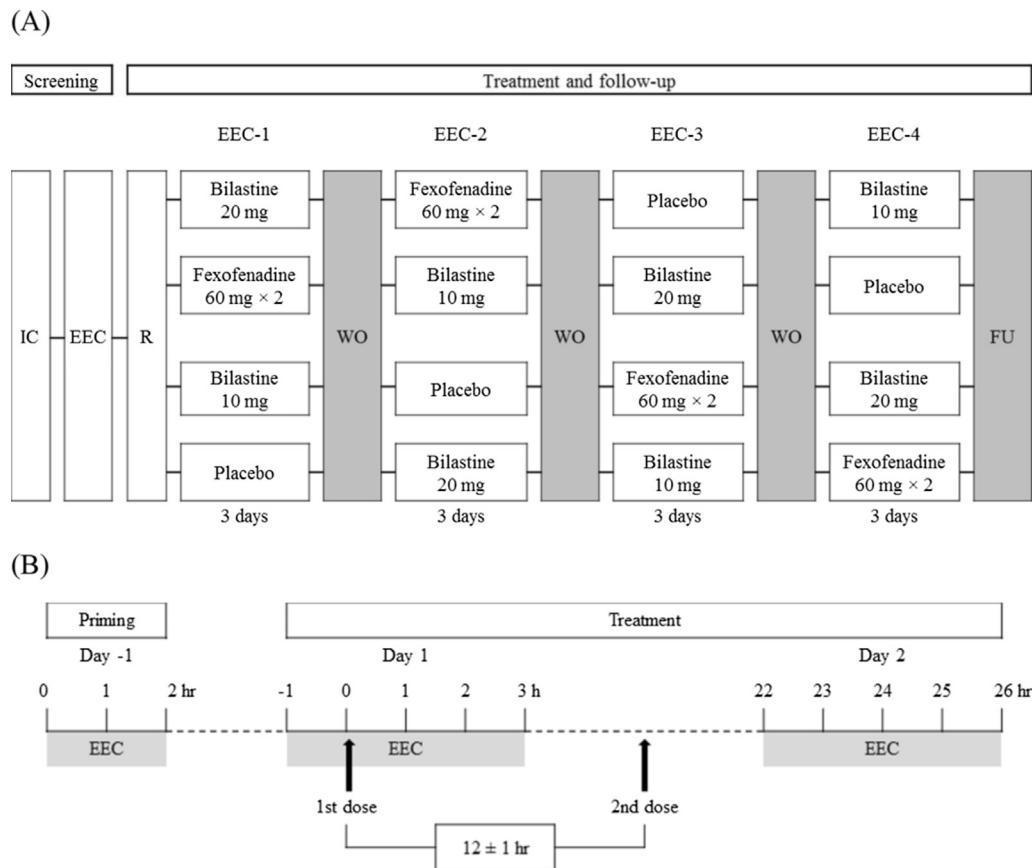


Fig. 1. Study design and schedule of Japanese cedar (JC) pollen exposure. (A) Overall study design was comprised of three periods (screening, treatment, and follow-up). Treatment period was composed of four periods. (B) Each period ran for 3 consecutive days (Day –1, 1, and 2). EEC, environmental exposure chambers; FU, follow up; IC, informed consent; R, randomized; WO, washout.

leaving the OHIO Chamber on Day 2 until the following day, they were allowed to use an α_2 agonist nasal spray or sodium cromoglycate ophthalmic solution.

The study drugs were BIL 20 and 10 mg (BIL 20 and BIL 10, respectively), fexofenadine hydrochloride 60 mg (FEX, Allegra® 60 mg tablet, Sanofi, Tokyo, Japan), and the placebo. Because FEX needs to be orally administered twice daily as the recommended dose in Japan, it was administered twice daily while the placebo was administered as the second dose in groups other than the FEX-treated.

To maintain the blindness, the study drugs were encapsulated in opaque oral capsules to ensure the contents were indistinguishable and were randomly assigned to subjects by using a set assignment order. The encapsulated study drugs were supplied by Taiho Pharmaceutical.

The subjects were assessed for nasal symptoms (rhinorrhea, sneezing, and nasal obstruction and itching) and ocular symptoms (itchy eyes and ocular tearing) using a 5-point scale (0, no symptoms; 1, mild; 2, slightly severe; 3, severe; and 4, very severe). This assessment method was used in our validation and clinical studies.^{6–8} During the pollen exposure, the subjects self-assessed their nasal and ocular symptoms every 15 min.

The TNSS, total ocular symptom score (TOSS), and total symptom score (TSS) were presented as the sum of the scores of four nasal, two ocular, and a combination of four nasal and two ocular symptoms, respectively. In addition, the number of sneezes was recorded every 15 min during the exposure. Furthermore, the subjects blew their noses with pre-weighed tissue papers, which were subsequently weighed and the difference in weight before

and after was recorded as the nasal secretion. No carry-over effect was anticipated because we allowed for a sufficient washout interval between the treatment periods.

Efficacy evaluation

The primary objective was to compare the efficacy of BIL against that of the placebo during the treatment period. The primary endpoint was the sum of TNSS at 0–3 h after the first study drug administration (Day 1).

The secondary efficacy endpoints were the sum of TNSS and nasal and ocular symptoms 22–26 h after the first study drug dosing (Day 2), and the onset of action on TNSS and nasal symptoms, which was defined as the time point when the first significant difference from placebo was observed.⁴ The subjects comprehensively assessed the severity of symptoms using a 10-cm visual analog scale (VAS), and the level of treatment satisfaction such as efficacy and safety at the end of the exposure Day 2 of each period compared with that at the end of the screening exposure. The 5-point scale used for this assessment was as follows; 1, very dissatisfied; 2, dissatisfied; 3, normal; 4, satisfied; and 5, very satisfied. The nasal secretion was examined every 60 min after the start of exposure as well as the dose–response of BIL.

Evaluation of safety

All the signs, symptoms, and disorders observed in the subjects were considered adverse events (AEs), and the safety was evaluated

including abnormalities in laboratory measurements, vital signs, and the 12-lead electrocardiogram (ECG).

Statistical analysis

The objective of this study was to detect a difference in the primary efficacy endpoint between BIL 20 and placebo for per protocol set (PPS) population. The full analysis set (FAS) included all randomized subjects who completed the study without protocol deviations or violations of the inclusion, exhibiting exclusion criteria, and who had a baseline and 13 assessments of the primary efficacy endpoint and received, at least, one dose of the study medication. The PPS included all the FAS subjects who completed the study without major protocol violations or deviations from the main efficacy and safety variables, as well as the administration regimen. The safety population (SP) included all randomized subjects who had received at least one dose of the study medication.

For the primary efficacy endpoint, 108 subjects were required for a power of 90% at a two-sided significance level of 5% to detect a 20-point difference between BIL 20 and placebo in TNSS with a standard deviation (SD) of 45, based on a previous overseas study.¹⁸ Assuming that approximately 20% of the PPS population might withdraw from this study, 130 subjects were required.

For the primary efficacy endpoint, the four-period crossover study was analyzed using a linear mixed effect model with the PPS population. The model had baseline, treatment, sequence, and period as the fixed effects and a random intercept for each subject. Similar analyses were carried out on both the main efficacy variables and the primary efficacy endpoint. In addition, supportive analyses for the FAS population were included.

When the superiority of BIL 20 over placebo was confirmed for the primary and secondary endpoints, the efficacy of BIL was exploratively examined against that of FEX. The onset of action, nasal secretion, and VAS were analyzed by using a pairwise Student *t*-test. For subject's satisfaction with the treatment, the results of those with a score of 4 or 5 were summarized, and an intergroup comparison was performed using Fisher's exact test.

The safety analyses were performed on the SP data while the qualitative and quantitative variables were summarized using a frequency distribution and the mean $\pm$ SD or standard error (SE) and 95% confidence interval (95% CI), respectively.

All the statistical analyses were performed using the statistical analysis software (SAS) version 9.2 (SAS Institute, Cary, NC, USA). All reported *P*-values were two-sided, and a *P* < 0.05 was considered statistically significant. The statistical analysis was conducted at Mediscience Planning Inc., (Tokyo, Japan).

Ethical approval and clinical trial registration

This study was conducted in the OHIO Chamber managed by the Tokyo Research Center of Clinical Pharmacology, according to the Good Clinical Practice (GCP) and Declaration of Helsinki guidelines. The protocol and informed consent were reviewed and approved by the Institutional Review Board of Shinanozaka Clinic. All the subjects submitted written informed consent before the start of the study, which was registered at the Japan Pharmaceutical Information Center (JAPIC, JapicCTI-132213).

Results

Subjects

Of the 391 subjects who submitted informed consent, 136 were randomly assigned to the treatment groups in the treatment period. Most dropout subjects did not meet the inclusion criteria

during the screening exposure, or they exhibited any of the exclusion criteria.

All the 136 subjects assigned to the treatment groups were included in the SP and FAS while 126 of them were included in the PPS for the efficacy evaluation. Ten subjects were excluded from the PPS, out of which nine had missing efficacy data on Day 1 and 2 including discontinuation (five subjects), and medication time noncompliance was noted for one subject. Table 1 shows the subject demographics and characteristics at baseline in the PPS.

Efficacy

Primary endpoint: sum of TNSS on Day 1

The mean $\pm$ SD of the sum of TNSS 0–3 h after administration of the study drugs on Day 1 for the BIL 20, BIL 10, FEX, and placebo was 67.7 ± 28.4 , 69.7 ± 26.7 , 73.3 ± 26.0 , and 85.0 ± 31.1 , respectively. The estimated mean of the difference in the sum of TNSS between BIL 20 and placebo was 16.2 (95% CI, 12.3–20.1), showing a significant difference compared with placebo (*P* < 0.001, Table 2A). Similarly, the significant differences in the sum of TNSS between BIL 10 or FEX and placebo were 14.5 (10.7–18.4) and 12.0 (8.1–15.8), respectively (*P* < 0.001). When the effect of BIL 20 on the sum of TNSS was exploratory compared to that of FEX, the difference was 4.2 (0.4–8.1), which was significant (*P* = 0.032; Table 2A). Alternatively, no significant difference existed between BIL 10 and FEX while the results of the FAS were similar to those of the PPS (data not shown).

Sum of TNSS on Day 2

The mean $\pm$ SD of the sum of TNSS 22–26 h after the administration of BIL 20, BIL 10, FEX, and placebo was 84.4 ± 33.0 , 82.8 ± 34.0 , 84.6 ± 36.3 , and 109.5 ± 41.6 , respectively. The

Table 1

Subject demographic and characteristics at baseline in per protocol set (PPS).

Variable	PPS population (n = 126)
Sex n (%)	
Female subjects	82 (65.1%)
Male subjects	44 (34.9%)
Age (years)	
Mean $\pm$ SD	40.7 $\pm$ 10.6
Range	20–59
BMI (kg/m ²)	
Mean $\pm$ SD	21.65 $\pm$ 3.20
Range	15.8–31.2
Sum of TNSS _{75–240 min} at screening	
Mean $\pm$ SD	65.1 $\pm$ 23.2
Range	36–157
Sum of TOSS _{75–240 min} at screening	
Mean $\pm$ SD	17.8 $\pm$ 17.7
Range	0–91
Sum of TSS _{75–240 min} at screening	
Mean $\pm$ SD	82.9 $\pm$ 36.8
Range	36–248
Sum of amount of nasal secretion at screening (g)	
Mean $\pm$ SD	12.20 $\pm$ 10.17
Range	2.1–48.2
Serum levels of JC pollen-IgE	
Class 0	0 (0.0)
Class 1	0 (0.0)
Class 2	7 (5.6)
Class 3	42 (33.3)
Class 4	52 (41.3)
Class 5	13 (10.3)
Class 6	12 (9.5)

BMI, body mass index; IgE, immunoglobulin E; JC, Japanese Cedar; SD, standard deviation; TNSS, total nasal symptom score; TOSS, total ocular symptom score; TSS, total symptom score.

Table 2

Sum of total nasal symptom score (TNSS) in per protocol set (PPS).

Day 1	Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
(A) Primary efficacy endpoint: sum of TNSS at 0–3 h after first study drug dosing on Day 1				
Mean ± SD	85.0 ± 31.1	69.7 ± 26.7	67.7 ± 28.4	73.3 ± 26.0
Estimated	84.7	70.2	68.5	72.7
Difference (95% CI)	–	14.5 (10.7–18.4)	16.2 (12.3–20.1)	12.0 (8.1–15.8)
P-value*	–	<0.001	<0.001	<0.001
Difference (95% CI)	–	2.6 (–1.3–6.4)	4.2 (0.4–8.1)	–
P-value**	–	0.194	0.032	–
Difference (95% CI)	–	–	1.7 (–2.2–5.5)	–
P-value***	–	–	0.395	–
Day 2	Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
(B) Secondary efficacy endpoint: sum of TNSS at 22–26 h after first study drug on Day 2				
Mean ± SD	109.5 ± 41.6	82.8 ± 34.0	84.4 ± 33.0	84.6 ± 36.3
Estimated	109.5	83.1	85.0	84.2
Difference (95% CI)	–	26.4 (20.4–32.4)	24.5 (18.5–30.5)	25.3 (19.4–31.3)
P-value*	–	<0.001	<0.001	<0.001
Difference (95% CI)	–	1.1 (–4.9–7.0)	–0.8 (–6.8–5.2)	–
P-value**	–	0.730	0.789	–
Difference (95% CI)	–	–	–1.9 (–7.9–4.1)	–
P-value***	–	–	0.540	–

Analysis of a linear mixed effect model with the PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. * vs. placebo, ** vs. fexofenadine, and *** vs. bilastine 10 mg.

estimated differences from placebo in the sum of TNSS for BIL 20, BIL 10, and FEX was 24.5 (18.5–30.5), 26.4 (20.4–32.4), and 25.3 (19.4–31.3), respectively, and all the active drugs showed a significant difference compared with placebo ($P < 0.001$, Table 2B). Moreover, there was no significant difference between the drugs.

Sum of TOSS, TSS, total 3 nasal symptom score (T3NSS), and individual symptom scores on Day 1 and 2

All the active drugs showed a significant difference compared to placebo in the sum of TOSS, total 3 nasal symptom score (T3NSS: rhinorrhea, sneezing, and nasal itching), TSS, and for each nasal or ocular symptom (rhinorrhea, sneezing, nasal obstruction, nasal itching, itchy eyes, and ocular tearing) on Day 1 and 2 using a mixed-effects model ($P \leq 0.001$ each, Supplementary Table 1).

The exploratory comparison of the efficacy of the above variables between BIL and FEX revealed a significant improvement in the sum of T3NSS and sneezing score for BIL 20 on Day 1 ($P = 0.036$ and 0.011, respectively) and itchy eyes for BIL 10 on Day 2 ($P = 0.044$) was observed (Supplementary Table 1).

Dose–response relationship

There was no statistically significant difference in any of the efficacy variables in the direct comparison of BIL 20 and BIL 10. In addition, the dose–response of BIL on primary and secondary efficacy endpoints was exploratory investigated by using the contrast test using contrast coefficients (1, 0, –1), (2, –1, –1), and (1, 1, –2) for the treatment groups (placebo, BIL 10, and BIL 20), and no clear dose–response relationship was observed (data not shown).

Onset of action and duration

The time-course of the mean TNSS after pollen exposure is shown in Figure 2 and values for BIL 20, BIL 10, FEX, and placebo before pollen exposure on Day 1 were 2.6 ± 2.48 , 2.7 ± 2.03 , 2.5 ± 2.37 , and 2.6 ± 2.36 , respectively. After the start of pollen exposure, the TNSS of each group rapidly increased time-dependently. The TNSS at baseline (just before the first study drug dosing) was 6.4 ± 2.8 , 6.5 ± 2.9 , 6.6 ± 2.8 , and 6.6 ± 2.8 for BIL 20, BIL 10, FEX and placebo, respectively. There was no significant difference between the drugs before pollen exposure and at baseline.

After administration of placebo, the mean TNSS was maintained at 6–7 points during the pollen exposure on Day 1 and then it gradually decreased until the start of the second pollen exposure (at 22 h), after which it rapidly increased and reached a plateau 1 h after the second pollen exposure on Day 2.

A significant decrease in TNSS following the active drug treatments was observed from approximately 1 h and was maintained for up to 26 h after the first drug dosing compared with placebo (Fig. 2, and Supplementary Table 2). The onset of action for TNSS was 45 min for BIL 20 (versus [vs.] placebo, $P = 0.019$), and 60 min for BIL 10 and FEX (vs. placebo, $P = 0.002$ and $P = 0.037$, respectively, Table 3 and Supplementary Table 2). All the active drugs significantly suppressed the increase in TNSS for up to 26 h after the first drug dose compared to placebo (Fig. 2 and Supplementary Table 2).

Figure 3 shows the time-course of each mean nasal symptom score (rhinorrhea, sneezing, nasal obstruction, and nasal itching) after pollen exposure on Day 1 and 2. The change in each nasal symptom score showed a similar pattern with that of the TNSS. The rhinorrhea, nasal obstruction, and itching score were the main components of TNSS based on the comparison with the change in mean score after pollen exposure. The mean nasal symptom scores

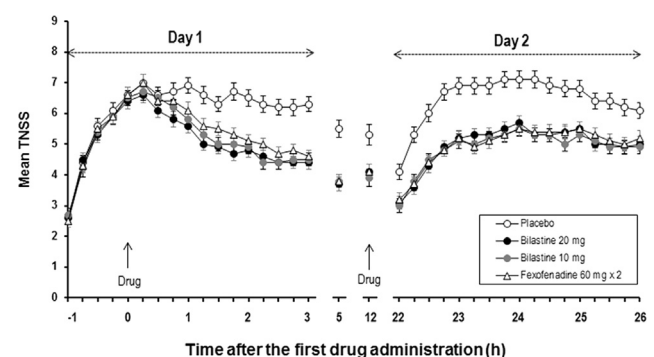


Fig. 2. Time course of total nasal symptom score (TNSS) assessed every 15 min during pollen exposure on Day 1 and 2 in per protocol set (PPS). Data are mean ± standard error (SE, $n = 126$). Subjects were exposed to JC pollen for 4 h; first study drug was orally administered 1 h after start of exposure. Second study drug was administered 12 h after first drug dose. On Day 2, subjects returned to OHIO Chamber 22 h after first dose and were further exposed to JC pollen for 4 h. Statistical analyses are shown in Supplementary Table 2. JC, Japanese cedar.

Table 3

Onset of action for total nasal symptom score (TNSS) and each nasal symptom score in per protocol set (PPS).

(min)	TNSS (n = 126)	Rhinorrhea (n = 126)	Sneezing (n = 126)	Nasal obstruction (n = 126)	Nasal itching (n = 126)
Bilastine 10 mg	60	60	60	105	60
Bilastine 20 mg	45	60	45	60	60
Fexofenadine 60	60	75	60	180	75

Onset of action was first time point after first study drug dosing when drug demonstrated a significant difference from placebo (Student *t*-test).

at baseline were not significantly different between the drugs. All of the active drugs significantly decreased each nasal symptom on Day 1 and 2 compared with placebo, similar to their effects on the TNSS (Supplementary Table 2). The onset of action for BIL 20 on each nasal symptom was within 60 min. Compared with FEX, BIL 20 had a rapid onset of action against all the nasal symptoms (Table 3).

Symptom severity using 10-cm VAS

The mean change in the VAS from baseline every 1 h during the 4-h pollen exposure is shown in Figure 4. The mean $\pm$ SD of the VAS at baseline for BIL 20, BIL 10, FEX, and placebo was 39.6 ± 21.1 , 40.5 ± 20.5 , 41.3 ± 20.6 , and 42.3 ± 21.3 , respectively. The mean VAS of the placebo group was not largely changed from the baseline during pollen exposure on Day 1, and it partly decreased before the

second pollen exposure (at 22 h), after which it increase during the second pollen exposure on Day 2. On the other hand, the VAS of the active drugs decreased and increased time-dependently on Day 1 and 2, respectively. The change in the VAS from the baseline for BIL 20 was more significantly decreased than that of placebo from 1 to 26 h after the first drug dose, except at 22 h. The BIL 10 and FEX also significantly improved the VAS from 2 to 26 h after the first drug dose (except at 22 h for BIL 10) compared with placebo. There was no significant difference between the active drugs.

Nasal secretion

Figure 5 shows the mean nasal secretion every 1 h during the pollen exposure, and all the active drugs showed a significant attenuation from 2 to 26 h after the first drug dose compared to placebo. There was no significant difference between the active drugs.

Subject treatment satisfaction

The percentage (ratio) of subjects who responded that they were satisfied or very satisfied with the treatment was 44.4% (56/126), 41.3% (52/126), 40.5% (51/126), and 14.3% (18/126) for BIL 20, BIL 10, FEX, and placebo, respectively. All the active drugs showed a significant difference compared with placebo ($P < 0.001$ each).

Safety

No serious AEs were reported in this study while AEs occurred in 31 subjects, which consisted of eight subjects each in the BIL 10, BIL

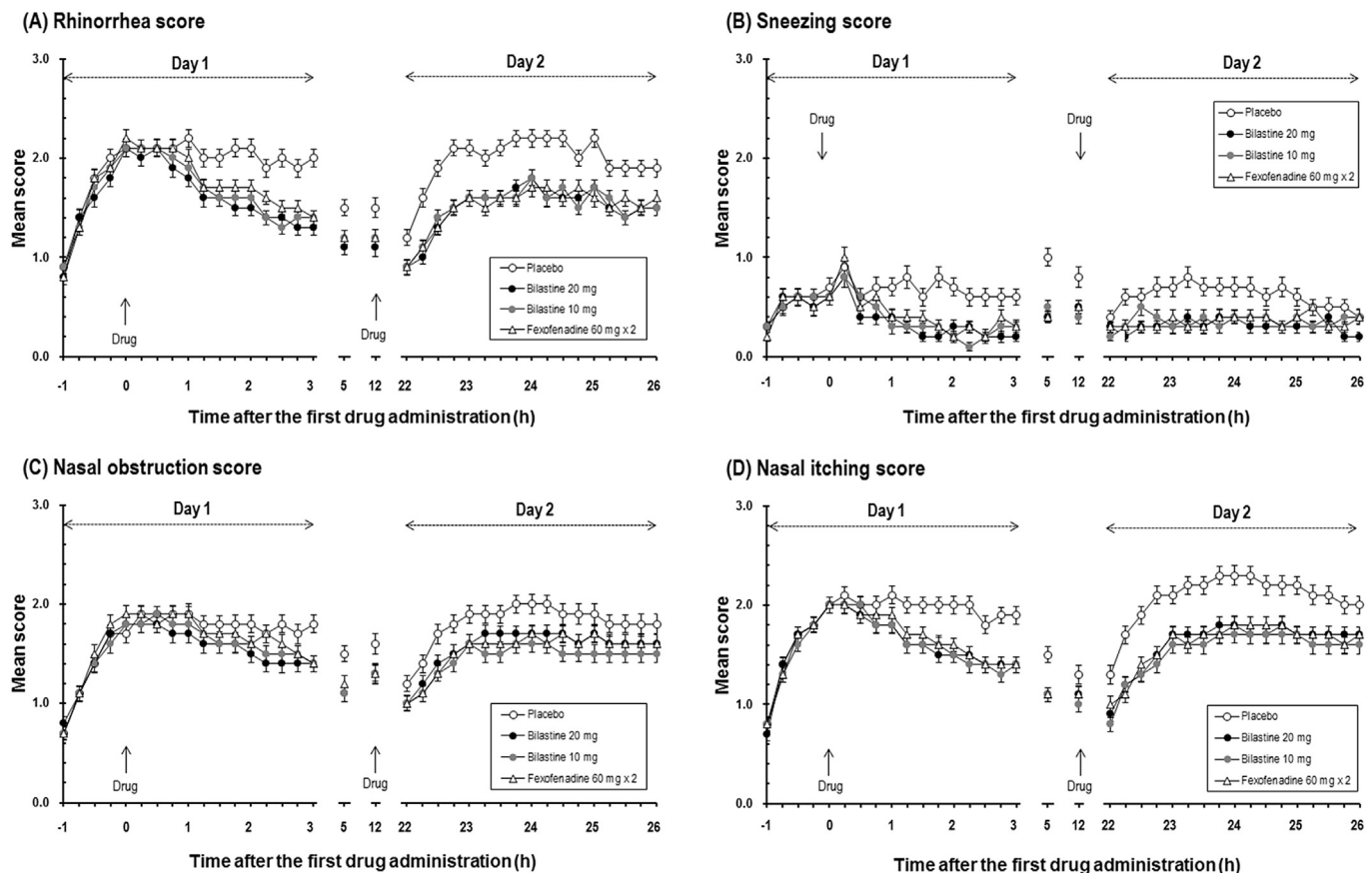


Fig. 3. Time course of nasal symptoms. (A) Rhinorrhea, (B) sneezing, (C) nasal obstruction, and (D) nasal itching assessed every 15 min during JC pollen exposure on Day 1 and 2 in per protocol set (PPS). Data are mean $\pm$ standard error (SE, $n = 126$). Procedures of exposure and study drug administration are same as for Figure 2. Statistical analyses are shown in Supplementary Table 2. JC, Japanese cedar.

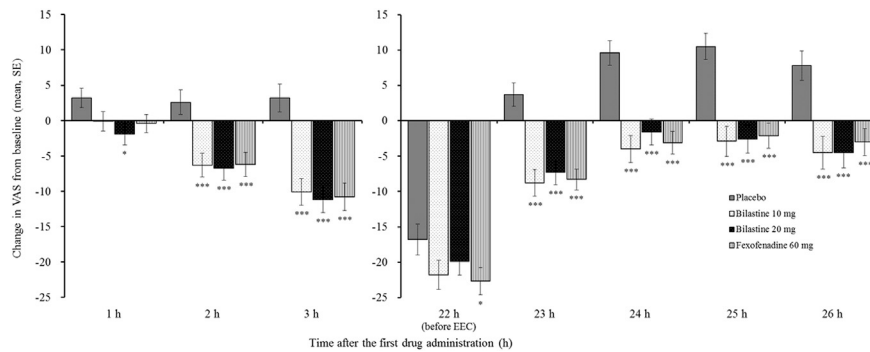


Fig. 4. Change in visual analog scale (VAS) score from baseline every 1 h during Japanese cedar (JC) pollen exposure on Day 1 and 2 in per protocol set (PPS). Data are mean $\pm$ standard error (SE, $n = 126$). * $P < 0.05$, *** $P < 0.001$ for bilastine (BIL) or fexofenadine (FEX) versus placebo (Student's t -test). EEC, environmental exposure chamber; VAS, visual analog scale. No significant difference observed between active drugs (Student's t -test).

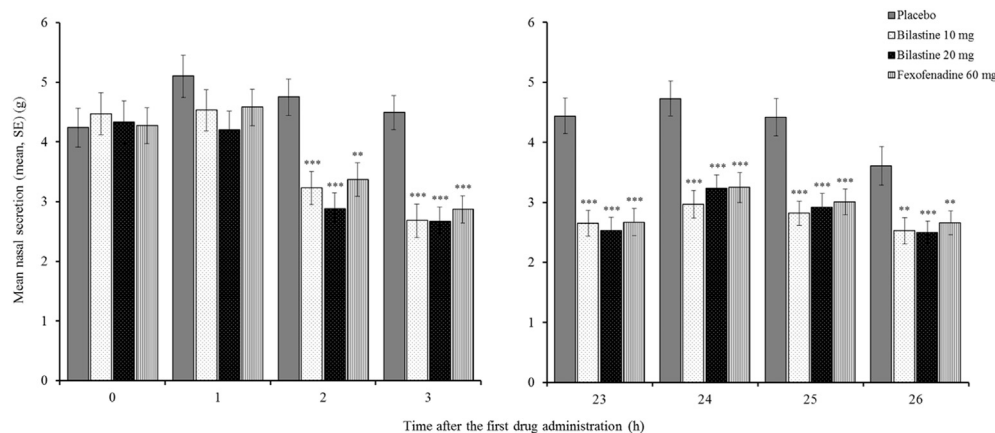


Fig. 5. Amount of nasal secretion every hour during Japanese cedar (JC) pollen exposure on Day 1 and 2 in per protocol set (PPS). Data are mean $\pm$ standard error (SE, $n = 126$). ** $P < 0.01$ and *** $P < 0.001$ for bilastine (BIL) or fexofenadine (FEX) versus placebo (Student's t -test). No significant difference observed between active drugs (Student's t -test).

20, and FEX, and seven in the placebo group. Of these AEs, thirst (one subject in the BIL 10) and abnormal liver function test, headache, and somnolence (in one subject each in the FEX group) were determined to be adverse drug reaction (ADRs). However, they were all mild and clinically insignificant. One subject (BIL 10 treatment, Period 2) who reported nasopharyngitis was withdrawn from the study. The investigator considered this AE to have been caused by a transient infection and was deemed unrelated to study drug.

Discussion

The aim of this study was to compare the efficacy of therapeutic treatment with BIL and placebo, and to assess the onset and duration of action of BIL on JC pollinosis symptoms in Japanese subjects exposed JC pollen using the OHIO Chamber. Therefore, we adopted a study design that incorporated a consecutive 3-day (Day -1, Day 1 and Day 2) JC pollen exposure in the present study (Fig. 1B). The 2-h JC pollen exposure on Day -1 was performed to induce a priming effect. Yuki A *et al.*¹⁹ reported that with the 3-consecutive-day challenge (50,000 grains/mm³ for 2 h), the severity of symptoms was seen to increase proportionally with the number of days of challenge in patients with JC pollinosis. In addition, 1-h priming pollen exposure (8000 grains/mm³) of the previous day increased the sensitivity of the nasal mucosa and subsequent 3-h exposure induced more severe allergic symptoms on the next day in patients with JC pollinosis.²⁰ We examined the effect of a 2-h priming JC pollen exposure on the development of

nasal symptoms following consecutive JC pollen exposure in subjects with JC pollinosis before the conduct of this study. As the results, we obtained the same results indicating that the priming 2-h exposure facilitated the occurrence of the nasal symptoms, and increased the nasal symptom score following pollen exposure on Day 1 (unpublished data). To investigate the efficacy, onset, and duration of action of BIL, the JC pollen exposure was performed for 4 h on Day 1 and 2, followed by the oral administration of the study drug 1 h after the pollen exposure on Day 1. Moreover, to compare the efficacies of BIL and FEX, FEX or placebo was re-administered orally 12 h after the first drug dose (approximately 10 h before the pollen exposure on Day 2). The duration of pollen exposure and timing of the first study drug dosing was determined for the following reasons. (i) The TNSS achieved a plateau 90–120 min after the start of the exposure during our initial experience in the implementation of the EEC studies without the priming exposure.^{6–8} (ii) The manifestation of the nasal symptoms on Day 1 could be prompted to occur earlier by performing the priming exposure on Day -1. (iii) The onset of action of BIL and FEX was expected to occur approximately 60 min after dosing.^{18,21} (iv) The efficacy assessment of study drugs has usually been performed following a 3-h pollen exposure in our previous EEC studies.^{9,10,13} (v) In addition, to ensure the completion of the study by the subjects, we needed to mitigate the associated inconveniences as much as possible.

The results of the present study clearly demonstrated that treatment with a single 10 or 20 mg dose of BIL significantly suppressed the sum of TNSS at 0–3 h, as well as the sum of TNSS at

22–26 h after the first dose compared with placebo (Table 2). In addition, both BIL 10 and BIL 20 improved the sum of TOSS, T3NSS, TSS, each nasal or ocular symptom score, the VAS, and nasal secretion on Day 1 and 2 compared with placebo (Fig. 3–5).

FEX was orally administered twice a day according to the Japanese dose regimen in the present study. Therefore, the therapeutic effects and onset of action of FEX on Day 1 following the administration of a single 60 mg dose, and its duration of action on Day 2 twice daily dosing with 60 mg were investigated. The results revealed that FEX also suppressed the sum of TNSS, TOSS, T3NSS, TSS, each nasal or ocular symptom score, the VAS, and nasal secretion on Day 1 and 2, similar to BIL. Furthermore, there was no significant difference in the sum of TNSS on Day 2 among the active drugs (Table 2B). The changes in TNSS, each nasal symptom score, the VAS, and nasal secretion also showed a similar tendency with the active drugs on Day 2 (Fig. 2–5), suggesting that the duration of action of a single 10 or 20 mg dose of BIL was comparable to that of a twice daily 60 mg dose of FEX. On the other hand, the following differences were observed in the inhibitory effects of BIL and FEX. (i) The inhibitory effect of BIL 20 on the sum of TNSS, T3NSS, and sneezing score on Day 1 was significantly greater than that of FEX. (ii) The sum of itchy eye score of BIL 10 on Day 2 was more significantly decreased than that of FEX. (iii) The onset of action for TNSS and each nasal symptom score of BIL 10, BIL 20, and FEX was 60–105, 45–60, and 65–180 min, respectively. These results suggest that the inhibitory effect of BIL 20 was relatively greater than that of FEX was, and the onset of action of BIL 20 was faster than that of FEX and BIL 10. Furthermore, these results were consistent with that of a previous study, which reported that the antihistamine activity of a single dose of BIL 20 was faster than that of BIL 10 in the histamine prick test in healthy Japanese subjects (submitted for publication).

This study did not entirely clarify if the complete dose–response of BIL is within the dose range of 10–20 mg. However, BIL 20 showed a faster onset of action than BIL 10 or FEX did, and the sum of T3NSS and sneezing scores of BIL 20 on Day 1 were significantly lower than those of FEX but not BIL 10 were. Therefore, it could be concluded that BIL 20 is more clinically useful than BIL 10 is, and should be selected as the candidate dose level for pivotal studies in patients with allergic rhinitis. All the medications investigated were safe and well tolerated in this study population.

Our results were consistent with those of a previous overseas EEC study,¹⁸ which reported that BIL 20 had a rapid onset of action and a long duration of action (1 h and greater than 26 h, respectively). Some differences were observed between the study design of this present study and that of the overseas study. These differences were in the pollen type used (this study vs. the overseas, JC vs. glass pollen), the duration of exposure (Day 1 and 2, 4 h each vs. Day 1 and 2, 6 and 4 h, respectively), the study drug administration time (1 vs. 2 h after the start of exposure), and the study drug dosing times (twice vs. once). The mean of the sum of TNSS in the overseas study was higher than that of this study; however, compared to the placebo, the percentage reduction of BIL 20 on Day 1 and 2 was –20.4 and –22.9% (this study) vs. –16.7 and –21.8% (overseas study). This comparison indicates that the efficacy of BIL 20 on the JC pollinosis was comparable to its effects on grass pollen allergy in Caucasians subjects.

This study has some limitations, which are worth mentioning. (i) This EEC study may have resulted in the short duration, the restricted study design and the manner of execution. The onset of action of the active drugs appeared shorter than that observed in phase III trials conducted during the pollen season.⁴ Therefore, it would be necessary to assess the onset of action of BIL in phase III trials that reflect the real life setting. (ii) We set the priming exposure on Day –1 to induce the priming effect. However, the

priming effects in this study may differ from those obtainable in a natural environment. The reactivity of patients to the JC allergen could have been increased by repeated low-level JC pollen exposure before the start of JC pollen dispersal, which would induce the priming effect.²² In fact, the pollen dispersal concentration (8000 grains/m³) in the chamber, which is comparable to that obtainable when a large quantity of JC pollen is dispersed, was needed to elicit the mild to moderate nasal symptoms in our EEC studies.⁸ Therefore, differences in the pathophysiological characteristic may have existed between the EEC and natural environment study after the priming effect. (iii) Based on the protocol used, the duration of this study was relatively short and, therefore, it may not be suited nor intended to explore extended long-term efficacy or safety of BIL.

Since it is important to predict the efficacy or dose–response of new medications prior to conducting the pivotal study in patients with allergic rhinitis, it might be worth considering using the EEC study to evaluate the efficacy and dose–response of therapeutic or prophylactic treatment with the new medication in Japanese subjects with JC pollinosis. The study design used in the present study might be useful for conducting therapeutic studies using the EEC study in Japanese subjects with JC pollinosis.

In conclusion, this study demonstrated the clinical usefulness of BIL 20 for the treatment of JC pollinosis. Furthermore, BIL 20 showed a rapid onset and long duration of action against nasal symptoms induced by the 3-day consecutive exposure to JC pollen in the OHIO Chamber.

Acknowledgments

The authors gratefully acknowledge Drs. Lourdes Azcárate, Román Valiente, and Luis Labeaga (Faes Farma S.A., Spain) for their scientific review of the manuscript. The authors would also like to thank Satoko Shimizu and all the members who participated in this study at the Tokyo Research Center of Clinical Pharmacology for the construction of the OHIO Chamber as well as managing and coordinating the tests and subject's visiting schedule. We would also like to thank Keika Tsuruya and Yusuke Iida (Taiho Pharmaceutical) who played an overall management role in the study. This study was funded by Taiho Pharmaceutical, Tokyo, Japan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2016.06.009>.

Conflict of interest

MT, AS are employees of Taiho Pharmaceutical. KO received adviser fees from Taiho Pharmaceutical. The rest of the authors have no conflict of interest.

Authors' contributions

KH, the principal investigator, contributed to the conduct of the study, as well as data collection and interpretation. KW contributed to the conduct of the study and data collection. MT, AS contributed to the study concept, design, and conduct, as well as data analysis and interpretation. KH, MT, AS wrote the manuscript. KO, a medical adviser, provided advice on the study design and conduct, as well as data interpretation. All the listed authors were involved in the critical review and revision of the manuscript and approved the final content.

References

- Okubo K, Kurono Y, Fujieda S, Ogino S, Uchio E, Odajima H, et al. Japanese guideline for allergic rhinitis 2014. *Allergol Int* 2014;63:357–75.
- Day JH, Horak F, Briscoe MP, Canonica GW, Fineman SM, Krug N, et al. The role of allergen challenge chambers in the evaluation of anti-allergic medication: an international consensus paper. *Clin Exp Allergy Rev* 2006;6:31–59.
- Krug N, Badorrek P, Hohlfeld JM. Experience with an allergen challenge chamber for clinical trials in allergic rhinitis. *Clin Exp Allergy Rev* 2012;12:14–9.

4. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. *Allergic Rhinitis: Clinical Development Programs for Drug Products*. DRAFT GUIDANCE. 2000.
5. Committee for Medicinal Products for Human Use, European Medicines Agency. *Guideline on the Clinical Development of Medicinal Products for the Treatment of Allergic Rhinconjunctivitis*. EMEA/EWP/2455/02. 2004.
6. Hashiguchi K, Tang H, Fujita T, Tsubaki S, Fujita M, Suematsu K, et al. Preliminary study on Japanese cedar pollinosis in an artificial exposure chamber (OHIO Chamber). *Allergol Int* 2007;**56**:125–30.
7. Hashiguchi K, Tang H, Fujita T, Suematsu K, Tsubaki S, Nagakura H, et al. Pilot study of Japanese cedar pollen exposure using a novel artificial exposure chamber (OHIO Chamber). *Clin Exp Allergy Rev* 2008;**8**:30–6.
8. Hashiguchi K, Tang H, Fujita T, Suematsu K, Tsubaki S, Nagakura H, et al. Validation study of the OHIO Chamber in patients with Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2009;**149**:141–9.
9. Hashiguchi K, Tang H, Fujita T, Suematsu K, Gotoh M, Okubo K. Bepotastine besilate OD tablets suppress nasal symptoms caused by Japanese cedar pollen exposure in an artificial exposure chamber (OHIO Chamber). *Expert Opin Pharmacother* 2009;**10**:523–9.
10. Wakabayashi K, Hashiguchi K, Kanzaki S, Fujioka M, Tanaka N, Kawashima K, et al. Pranlukast dry syrup inhibits symptoms of Japanese cedar pollinosis in children using OHIO chamber. *Allergy Asthma Proc* 2012;**33**:102–9.
11. Enomoto T, Ide T, Ogino S. Construction of an environmental exposure unit and investigation of the effects of cetirizine hydrochloride on symptoms of cedar pollinosis in Japan. *J Invest Allergol Clin Immunol* 2007;**17**:173–81.
12. Yamamoto H, Yonekura S, Sakurai D, Katada K, Inamine A, Hanazawa T, et al. Comparison of nasal steroid with antihistamine in prophylactic treatment against pollinosis using an environmental challenge chamber. *Allergy Asthma Proc* 2012;**33**:397–403.
13. Kanzaki A, Hashiguchi K, Takeshi M, Tsurumi M, Okubo K, Ogawa K. [Artificial exposure chamber (OHIO) trial: inhibitory effect of bepotastine-OD for nasal allergy III (ACTION III study)]. *[J New Rem Clin]* 2012;**61**:2208–15 (in Japanese).
14. North ML, Soliman M, Walker T, Steacy LM, Ellis AK. Controlled allergen challenge facilities and their unique contributions to allergic rhinitis research. *Curr Allergy Asthma Rep* 2015;**15**:11.
15. Ilaxten 20 mg Tablets. Bilastine 20 mg. Summary of Product Characteristics. Available at URL: <http://www.mhra.gov.uk/spc-pil/?prodName=ILAXTEN20MG&subName=&pageID=ThirdLevel&searchTerm=bilastine#retainDisplay> [accessed 27.11.15].
16. Kuna P, Bachert C, Nowacki Z, van Cauwenberge P, Agache I, Fouquert L, et al. Efficacy and safety of bilastine 20 mg compared with cetirizine 10 mg and placebo for the symptomatic treatment of seasonal allergic rhinitis: a randomized, double-blind, parallel-group study. *Clin Exp Allergy* 2009;**39**:1338–47.
17. Bachert C, Kuna P, Sanquer F, Ivan P, Dimitrov V, Gorina MM, et al. Comparison of the efficacy and safety of bilastine 20 mg vs desloratadine 5 mg in seasonal allergic rhinitis patients. *Allergy* 2009;**64**:158–65.
18. Horak F, Ziegelmayer P, Ziegelmayer R, Lemell P. The effects of bilastine compared with cetirizine, fexofenadine, and placebo on allergen-induced nasal and ocular symptoms in patients exposed to aeroallergen in the Vienna Challenge Chamber. *Inflamm Res* 2010;**59**:391–8.
19. Yuki A, Terada T, Ichihara T, Fujii K, Hyo S, Kawata R, et al. Evaluating the effects of testing period on pollinosis symptoms using an allergen challenge chamber. *Allergol Int* 2011;**60**:533–9.
20. Yonekura S, Okamoto Y, Yamamoto H, Sakurai T, Inuma T, Sakurai D, et al. Randomized double-blind study of prophylactic treatment with an antihistamine for seasonal allergic rhinitis. *Int Arch Allergy Immunol* 2013;**162**:71–8.
21. Day JH, Briscoe MP, Welsh A, Smith JN, Clark A, Ellis AK, et al. Onset of action, efficacy, and safety of a single dose of fexofenadine hydrochloride for ragweed allergy using an environmental exposure unit. *Ann Allergy Asthma Immunol* 1997;**79**:533–40.
22. Connell JT. Quantitative intranasal pollen changes. III. The priming effect in allergic rhinitis. *J Allergy* 1969;**43**:33–44.

Supplementary Table 1

Sum of total ocular symptom score (TOSS), total 3 nasal symptom score (T3NSS), total symptom score (TSS), and each nasal or ocular symptom (rhinorrhea, sneezing, nasal obstruction, nasal itching, itchy eyes, and ocular tearing) on Day 1 and 2 in per protocol set (PPS).

(A) TOSS

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	28.6 $\pm$ 21.5	23.2 $\pm$ 20.0	20.8 $\pm$ 17.6	22.8 $\pm$ 19.8
	Estimated	27.5	23.0	22.7	22.4
	Difference (95% CI)	-	4.6 (2.4-6.7)	4.8 (2.6-7.0)	5.1 (3.0-7.3)
	<i>P</i> -value*	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	-0.6 (-2.8-1.6)	-0.4 (-2.5-1.8)	-
	<i>P</i> -value**	-	0.593	0.754	-
	Difference (95% CI)	-		0.2 (-2.0-2.4)	-
Day 2	Mean $\pm$ SD	43.9 $\pm$ 31.4	30.4 $\pm$ 26.9	31.2 $\pm$ 24.2	33.9 $\pm$ 28.7
	Estimated	42.9	30.1	33.2	33.4
	Difference (95% CI)	-	12.8 (9.2-16.5)	9.8 (6.1-13.5)	9.5 (5.9-13.1)
	<i>P</i> -value*	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	3.3 (-0.3-7.0)	0.3 (-3.4-3.9)	-
	<i>P</i> -value**	-	0.075	0.880	-
	Difference (95% CI)	-		-3.0 (-6.7-0.6)	-
	<i>P</i> -value***	-		0.104	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. * vs. placebo, ** vs. fexofenadine, and *** vs. bilastine 10 mg.

(B) T3NSS

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	61.3 $\pm$ 23.4	48.4 $\pm$ 19.5	47.1 $\pm$ 20.5	50.8 $\pm$ 18.3
	Estimated	60.6	48.6	47.7	51.0
	Difference (95% CI)	-	12.0 (8.9-15.0)	12.8 (9.8-15.9)	9.6 (6.5-12.6)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	2.4 (-0.7-5.4)	3.3 (0.2-6.3)	-
	<i>P</i> -value ^{**}	-	0.124	0.036	-
	Difference (95% CI)	-		0.9 (-2.2-3.9)	-
	<i>P</i> -value ^{***}	-		0.572	-
Day 2	Mean $\pm$ SD	79.0 $\pm$ 30.3	58.0 $\pm$ 24.6	57.8 $\pm$ 23.4	58.6 $\pm$ 25.2
	Estimated	78.7	58.3	58.3	58.7
	Difference (95% CI)	-	20.5 (15.9-25.0)	20.4 (15.9-25.0)	20.0 (15.5-24.5)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	0.4 (-4.1-5.0)	0.4 (-4.1-5.0)	-
	<i>P</i> -value ^{**}	-	0.847	0.847	-
	Difference (95% CI)	-		0.0 (-4.5-4.5)	-
	<i>P</i> -value ^{***}	-		0.999	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(C) TSS

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	113.5 $\pm$ 45.5	93.0 $\pm$ 40.0	88.5 $\pm$ 38.9	96.1 $\pm$ 39.8
	Estimated	112.2	93.2	91.1	95.0
	Difference (95% CI)	-	19.1 (14.0-24.1)	21.1 (16.1-26.1)	17.2 (12.2 -22.2)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	1.9 (-3.1-6.9)	3.9 (-1.1-8.9)	-
	<i>P</i> -value ^{**}	-	0.462	0.128	-
	Difference (95% CI)	-		2.0 (-3.0 -7.1)	-
	<i>P</i> -value ^{***}	-		0.428	-
Day 2	Mean $\pm$ SD	153.5 $\pm$ 63.6	113.2 $\pm$ 54.0	115.5 $\pm$ 51.0	118.4 $\pm$ 56.5
	Estimated	152.6	113.4	117.9	117.5
	Difference (95% CI)	-	39.2 (31.0-47.5)	34.7 (26.4-42.9)	35.0 (26.8-43.3)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	4.2 (-4.1-12.4)	-0.4 (-8.6-7.9)	-
	<i>P</i> -value ^{**}	-	0.320	0.927	-
	Difference (95% CI)	-		-4.6 (-12.8-3.7)	-
	<i>P</i> -value ^{***}	-		0.278	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(D) Rhinorrhea score

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	26.6 $\pm$ 10.5	22.1 $\pm$ 9.1	21.5 $\pm$ 9.6	23.3 $\pm$ 9.0
	Estimated	26.6	22.2	22.0	23.0
	Difference (95% CI)	-	4.4 (3.0-5.7)	4.6 (3.3-6.0)	3.6 (2.2 -5.0)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	0.8 (-0.6-2.1)	1.0 (-0.3-2.4)	-
	<i>P</i> -value ^{**}	-	0.270	0.140	-
	Difference (95% CI)	-		0.3 (-1.1-1.6)	-
	<i>P</i> -value ^{***}	-		0.707	-
Day 2	Mean $\pm$ SD	33.4 $\pm$ 13.0	25.7 $\pm$ 11.1	25.4 $\pm$ 11.8	25.5 $\pm$ 11.8
	Estimated	33.5	25.8	25.7	25.3
	Difference (95% CI)	-	7.7 (5.6-9.8)	7.7 (5.6-9.8)	8.1 (6.0-10.2)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	-0.4 (-2.5-1.7)	-0.4 (-2.5-1.7)	-
	<i>P</i> -value ^{**}	-	0.681	0.703	-
	Difference (95% CI)	-		0.0 (-2.1-2.1)	-
	<i>P</i> -value ^{***}	-		0.976	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(E) Sneezing score

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	8.8 $\pm$ 7.0	4.9 $\pm$ 5.2	4.3 $\pm$ 5.3	5.5 $\pm$ 4.5
	Estimated	8.6	5.0	4.4	5.5
	Difference (95% CI)	-	3.7 (2.8-4.5)	4.2 (3.3-5.1)	3.1 (2.2 -3.9)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	0.6 (-0.3-1.4)	1.1 (0.3-2.0)	-
	<i>P</i> -value ^{**}	-	0.179	0.011	-
	Difference (95% CI)	-		0.5 (-0.3-1.4)	-
	<i>P</i> -value ^{***}	-		0.222	-
Day 2	Mean $\pm$ SD	10.4 $\pm$ 7.9	6.1 $\pm$ 5.7	5.2 $\pm$ 4.9	6.0 $\pm$ 5.9
	Estimated	10.3	6.1	5.3	6.0
	Difference (95% CI)	-	4.2 (3.1-5.3)	5.0 (3.9-6.1)	4.3 (3.2-5.4)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	-0.1 (-1.2-1.0)	0.7 (-0.4-1.8)	-
	<i>P</i> -value ^{**}	-	0.839	0.203	-
	Difference (95% CI)	-		0.8 (-0.3-1.9)	-
	<i>P</i> -value ^{***}	-		0.140	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(F) Nasal obstruction score

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	23.7 $\pm$ 11.0	21.4 $\pm$ 10.5	20.6 $\pm$ 10.8	22.4 $\pm$ 10.8
	Estimated	24.2	21.6	20.7	21.6
	Difference (95% CI)	-	2.7 (1.4-3.9)	3.5 (2.3-4.8)	2.6 (1.4 -3.9)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	0.1 (-1.2-1.3)	0.9 (-0.3-2.2)	-
	<i>P</i> -value ^{**}	-	0.913	0.147	-
	Difference (95% CI)	-		0.9 (-0.4-2.1)	-
	<i>P</i> -value ^{***}	-		0.179	-
Day 2	Mean $\pm$ SD	30.5 $\pm$ 15.9	24.7 $\pm$ 14.0	26.6 $\pm$ 13.7	26.0 $\pm$ 14.6
	Estimated	30.9	24.9	26.7	25.4
	Difference (95% CI)	-	6.1 (4.0-8.2)	4.3 (2.2-6.4)	5.5 (3.4-7.6)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	0.6 (-1.6-2.7)	-1.2 (-3.4-0.9)	-
	<i>P</i> -value ^{**}	-	0.602	0.250	-
	Difference (95% CI)	-		-1.8 (-3.9-0.3)	-
	<i>P</i> -value ^{***}	-		0.094	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(G) Nasal itching score

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	25.9 $\pm$ 10.3	21.4 $\pm$ 9.8	21.2 $\pm$ 10.0	22.1 $\pm$ 9.6
	Estimated	25.6	21.3	21.3	22.4
	Difference (95% CI)	-	4.3 (3.0-5.6)	4.4 (3.0-5.7)	3.3 (1.9 -4.6)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	1.0 (-0.3-2.4)	1.1 (-0.2-2.4)	-
	<i>P</i> -value ^{**}	-	0.122	0.106	-
	Difference (95% CI)	-		0.0 (-1.3-1.4)	-
	<i>P</i> -value ^{***}	-		0.944	-
Day 2	Mean $\pm$ SD	35.2 $\pm$ 15.3	26.3 $\pm$ 13.6	27.1 $\pm$ 12.7	27.1 $\pm$ 13.0
	Estimated	35.1	26.3	27.2	27.3
	Difference (95% CI)	-	8.8 (6.7-11.0)	7.9 (5.8-10.1)	7.8 (5.6-9.9)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	1.0 (-1.1-3.2)	0.2 (-2.0-2.3)	-
	<i>P</i> -value ^{**}	-	0.348	0.887	-
	Difference (95% CI)	-		-0.9 (-3.0-1.3)	-
	<i>P</i> -value ^{***}	-		0.426	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(H) Itchy eyes score

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	17.0 $\pm$ 11.9	13.9 $\pm$ 11.5	12.7 $\pm$ 9.9	13.8 $\pm$ 11.3
	Estimated	16.6	13.9	13.6	13.4
	Difference (95% CI)	-	2.7 (1.4-4.0)	3.0 (1.6-4.3)	3.2 (1.9 -4.5)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	-0.5 (-1.8-0.9)	-0.2 (-1.5-1.1)	-
	<i>P</i> -value ^{**}	-	0.497	0.741	-
	Difference (95% CI)	-		0.2 (-1.1-1.6)	-
	<i>P</i> -value ^{***}	-		0.729	-
Day 2	Mean $\pm$ SD	27.1 $\pm$ 17.0	18.8 $\pm$ 15.8	19.8 $\pm$ 14.9	21.4 $\pm$ 16.2
	Estimated	26.7	18.8	20.7	21.1
	Difference (95% CI)	-	7.9 (5.7-10.1)	6.0 (3.8-8.3)	5.6 (3.4-7.9)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	2.3 (0.1-4.5)	0.4 (-1.8-2.6)	-
	<i>P</i> -value ^{**}	-	0.044	0.719	-
	Difference (95% CI)	-		-1.9 (-4.1-0.3)	-
	<i>P</i> -value ^{***}	-		0.098	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

(I) Ocular tearing score

		Placebo (n = 126)	Bilastine 10 mg (n = 126)	Bilastine 20 mg (n = 126)	Fexofenadine (n = 126)
Day 1	Mean $\pm$ SD	11.5 $\pm$ 10.7	9.3 $\pm$ 10.2	8.0 $\pm$ 8.9	9.0 $\pm$ 10.03
	Estimated	11.0	9.1	9.0	9.0
	Difference (95% CI)	-	1.9 (0.7-3.0)	2.0 (0.8-3.1)	2.0 (0.9 -3.1)
	<i>P</i> -value [*]	-	0.001	0.001	0.001
	Difference (95% CI)	-	-0.1 (-1.3-1.0)	0.0 (-1.2-1.1)	-
	<i>P</i> -value ^{**}	-	0.811	0.962	-
	Difference (95% CI)	-		0.1 (-1.0-1.2)	-
	<i>P</i> -value ^{***}	-		0.849	-
Day 2	Mean $\pm$ SD	16.9 $\pm$ 16.2	11.6 $\pm$ 13.4	11.4 $\pm$ 11.6	12.5 $\pm$ 14.2
	Estimated	16.4	11.4	12.3	12.4
	Difference (95% CI)	-	5.0 (3.1-6.8)	4.1 (2.2-5.9)	3.9 (2.1-5.8)
	<i>P</i> -value [*]	-	< 0.001	< 0.001	< 0.001
	Difference (95% CI)	-	1.0 (-0.8-2.9)	0.1 (-1.7-2.0)	-
	<i>P</i> -value ^{**}	-	0.266	0.889	-
	Difference (95% CI)	-		-0.9 (-2.8-0.9)	-
	<i>P</i> -value ^{***}	-		0.333	-

Analysis of a linear mixed effect model with PPS population. Model had baseline, treatment, sequence and period as fixed effects and a random intercept for each subject. ^{*} vs. placebo, ^{**} vs. fexofenadine, and ^{***} vs. bilastine 10 mg.

Supplementary Table 2

Statistical analysis for TNSS, rhinorrhea, sneezing, nasal obstruction, and itching in per protocol set (PPS).

<Statistical analysis for TNSS: treatment vs placebo by Student's t-test>

group		Time after the first drug administration (hh:mm) on Day 1																
		00:00	00:15	00:30	00:45	1:00	1:15	1:30	1:45	2:00	2:15	2:30	2:45	3:00	5:00	12:00		
Bilastine	20mg	ns	ns	ns	*	**	**	**	**	**	**	**	**	**	**	**		
Bilastine	10mg	ns	ns	ns	ns	**	**	**	**	**	**	**	**	**	**	**		
Fexofenadine	60mg x 2	ns	ns	ns	ns	*	**	*	**	**	**	**	**	**	**	**		
group		Time after the first drug administration (hh:mm) on Day 2																
		22:00	22:15	22:30	22:45	23:00	23:15	23:30	23:45	24:00	24:15	24:30	24:45	25:00	25:15	25:30	25:45	26:00
Bilastine	20mg	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Bilastine	10mg	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Fexofenadine	60mg x 2	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*

<Statistical analysis for rhinorrhea: treatment vs placebo by Student's t-test>

group		Time after the first drug administration (hh:mm) on Day 1																
		00:00	00:15	00:30	00:45	1:00	1:15	1:30	1:45	2:00	2:15	2:30	2:45	3:00	5:00	12:00		
Bilastine	20mg	ns	ns	ns	ns	**	**	**	**	**	**	**	**	**	**	*		
Bilastine	10mg	ns	ns	ns	ns	*	**	**	**	**	**	**	**	**	**	*		
Fexofenadine	60mg x 2	ns	ns	ns	ns	ns	*	*	**	**	**	**	**	**	**	*		
group		Time after the first drug administration (hh:mm) on Day 2																
		22:00	22:15	22:30	22:45	23:00	23:15	23:30	23:45	24:00	24:15	24:30	24:45	25:00	25:15	25:30	25:45	26:00
Bilastine	20mg	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Bilastine	10mg	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Fexofenadine	60mg x 2	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	*

<Statistical analysis for sneezing: treatment vs placebo by Student's t-test>

group		Time after the first drug administration (hh:mm) on Day 1																
		00:00	00:15	00:30	00:45	1:00	1:15	1:30	1:45	2:00	2:15	2:30	2:45	3:00	5:00	12:00		
Bilastine	20mg	ns	ns	ns	*	*	**	**	**	**	**	**	**	**	**	*		
Bilastine	10mg	ns	ns	ns	ns	**	**	**	**	**	**	**	**	*	**	**		
Fexofenadine	60mg x 2	ns	ns	ns	ns	*	**	*	**	**	**	**	*	**	**	**		
group		Time after the first drug administration (hh:mm) on Day 2																
		22:00	22:15	22:30	22:45	23:00	23:15	23:30	23:45	24:00	24:15	24:30	24:45	25:00	25:15	25:30	25:45	26:00
Bilastine	20mg	ns	**	**	**	**	**	**	**	*	**	**	**	*	*	ns	**	*
Bilastine	10mg	*	*	ns	**	*	**	*	**	*	*	ns	**	ns	*	*	ns	ns
Fexofenadine	60mg x 2	ns	*	*	**	**	**	**	**	*	**	ns	**	ns	ns	*	*	ns

<Statistical analysis for nasal obstruction: treatment vs placebo by Student's t-test>

group		Time after the first drug administration (hh:mm) on Day 1																
		00:00	00:15	00:30	00:45	1:00	1:15	1:30	1:45	2:00	2:15	2:30	2:45	3:00	5:00	12:00		
Bilastine	20mg	ns	ns	ns	ns	*	ns	ns	*	*	*	**	**	**	**	ns		
Bilastine	10mg	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	**	*	**	**	*		
Fexofenadine	60mg x 2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**	*		
group		Time after the first drug administration (hh:mm) on Day 2																
		22:00	22:15	22:30	22:45	23:00	23:15	23:30	23:45	24:00	24:15	24:30	24:45	25:00	25:15	25:30	25:45	26:00
Bilastine	20mg	ns	*	ns	*	ns	ns	ns	*	*	*	*	ns	ns	ns	ns	ns	ns
Bilastine	10mg	*	**	**	**	*	*	**	**	**	**	**	**	**	*	*	*	*
Fexofenadine	60mg x 2	ns	*	*	*	*	*	*	**	*	*	ns	ns	ns	ns	ns	ns	ns

<Statistical analysis for nasal itching: treatment vs placebo by Student's t-test>

group		Time after the first drug administration (hh:mm) on Day 1																
		00:00	00:15	00:30	00:45	1:00	1:15	1:30	1:45	2:00	2:15	2:30	2:45	3:00	5:00	12:00		
Bilastine	20mg	ns	ns	ns	ns	*	**	**	**	**	**	**	**	**	**	*		
Bilastine	10mg	ns	ns	ns	ns	*	**	**	**	**	**	**	**	**	**	**		
Fexofenadine	60mg x 2	ns	ns	ns	ns	ns	*	*	**	**	**	**	**	**	**	*		
group		Time after the first drug administration (hh:mm) on Day 2																
		22:00	22:15	22:30	22:45	23:00	23:15	23:30	23:45	24:00	24:15	24:30	24:45	25:00	25:15	25:30	25:45	26:00
Bilastine	20mg	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Bilastine	10mg	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**
Fexofenadine	60mg x 2	*	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**

These statistical results correspond to Figure 2 and 3. $*P < 0.05$, $**p < 0.01$ for bilastine or fexofenadine vs. placebo (Student's t-test). ns, not significant