
Anti-allergic drug testing in an environmental challenge chamber is suitable both in and out of the relevant pollen season

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Background: An environmental challenge chamber (ECC) is a useful tool to expose allergic patients to relevant allergens in a controlled indoor setting and to test anti-allergic treatment. Hitherto, ECC studies with grass pollen are conducted primarily outside of the pollen season to avoid the influence of natural pollen exposure.

Objective: To investigate whether an established anti-allergic treatment, a combination of cetirizine (CET) and pseudoephedrine (PSE), shows an equivalent treatment effect within and outside of the grass pollen season when tested in an ECC.

Methods: In a randomized, placebo-controlled, double-blind, four-way crossover study, the effect of a combination of 10 mg CET and 120 mg PSE compared with placebo on nasal symptoms, nasal flow, and nasal secretion was investigated in 70 patients with seasonal allergic rhinitis. Subjects underwent four 6-hour pollen challenges in an ECC with administration of the drugs after 2 hours. Two challenges were conducted within the grass pollen season and two out of the grass pollen season.

Results: The active treatment significantly improved nasal symptoms and nasal flow and significantly reduced the amount of nasal secretion compared with placebo both within and outside of the pollen season ($P < .0001$ each). The treatment effect was not different between the seasons ($P > .05$).

Conclusion: Controlled allergen provocation in an ECC can be used to test anti-allergic treatment both within and outside of the grass pollen season.

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INTRODUCTION

Allergic rhinitis affects approximately 20% of the United States and European population and has the greatest prevalence of any respiratory disease,¹ which leads to an increasing need for new anti-allergic drugs. The gold standard to test novel anti-allergic treatments are double-blind, placebo-controlled field studies in which patients are exposed to natural pollen during the season. Field studies, however, face the problem that uncontrollable conditions such as weather and pollen concentration can lead to a great variability in symptoms. Therefore, multicenter studies with a high number of patients are needed to demonstrate treatment effects even in very effective treatment regimens, thus making these studies complex and expensive.

Alternatively, study designs have been developed using controlled allergen exposure in an environmental challenge chamber (ECC), in particular in proof-of-concept studies in the early clinical development of new anti-allergic drugs. The advantage lies in the ability to control environmental conditions such as temperature, humidity, and allergen concentration. It also allows simultaneous administration of medication under observation of medical personnel and ensures compliance in documenting symptoms. Thus, fewer participants are necessary to show significant treatment effects. Also, the onset of action of a drug can be determined and compared. Several ECCs in Europe, North America, and Japan have been used to test anti-allergic treatment in numerous trials in the past.^{2–7}

Hitherto, ECC studies with pollen are conducted primarily outside of the relevant pollen season to avoid the natural pollen exposure as a possible confounder, which may influence the variability of the individual's reactions. However, the model lacks the natural priming occurring during the season in which repeated contact with pollen over days and weeks enhances the inflammatory reaction in the nose and leads to a different and possibly stronger reaction.⁸ No study has directly investigated whether the natural pollen exposure during the season alters the treatment effect of anti-allergic drugs tested in an ECC.

The aim of this prospective study was to answer this open question by comparing the effect of a known and efficient

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anti-allergic treatment tested in an ECC once within and once outside of the pollen season, in the same patients. An equivalent treatment effect would allow the testing of anti-allergic treatment within the pollen season. Furthermore, it would answer the question of whether challenges outside of the pollen season provide methodically invalid results because of the lack of priming.

A combination of cetirizine (CET) plus pseudoephedrine (PSE) was chosen as an efficient and established anti-allergic treatment whose efficacy has been shown in previous ECC and field studies.^{9–11}

METHODS

Subjects

For this study, 70 adult subjects (aged 19–53 years) were randomized.

Inclusion criteria were history of seasonal allergic rhinitis due to grass pollen, positive skin prick test to *Dactylis glomerata* within 12 months of enrolment, a nasal congestion symptom score of at least 2, and a total nasal symptom score of at least 6 during a 2-hour screening challenge in the ECC, forced expiratory volume in 1 second (FEV₁) greater than 80% predicted, and smoking or non-smoking with a history of less than 10 pack years.

Exclusion criteria were structural nasal defect, nasal polyps, infection of the upper airways within 4 weeks of study entry, sinusitis, asthma (except mild intermittent asthma using β_2 -agonists only), pregnancy, breastfeeding, immunotherapy within the last 2 years, known hypersensitivity, allergic reactions or intolerance to CET, PSE, or any of the other ingredients, and participation in another clinical trial within 30 days of study enrollment.

The washout periods for drugs before screening were 4 weeks for inhaled and 8 weeks for oral, intravenous, or dermal steroids, 1 week for cromolyn, antihistamines, anti-rhinitis, or hay fever medication, and 2 weeks for monoamine oxidase inhibitors.

The study was conducted at the Fraunhofer ECC in Hannover, Germany.¹² Each subject gave written informed consent before study participation. The study was approved by the ethics committee of the Medical School of Hannover. It was conducted in accordance with the Declaration of Helsinki (Somerset West Amendment, 1996) and the ICH Guideline on Good Clinical Practice (Note for Guidance on Good Clinical Practice [CPMP/ICH/135/95], Jan 17, 1997). The ClinicalTrials.gov identifier is NCT00474890.

Study Design

This was a single-center, randomized, placebo-controlled, double-blind, four-way crossover study in which adult subjects with intermittent allergic rhinitis were repeatedly challenged with grass pollen in the Fraunhofer ECC (4,000 grains/m³ air, *Dactylis glomerata*, Biopol Laboratory Inc, Spokane, Washington). A concentration of 4,000 grains/m³ is equivalent to the concentration measured over a grass pollen meadow on a summer day¹³ (and own unpublished data). This

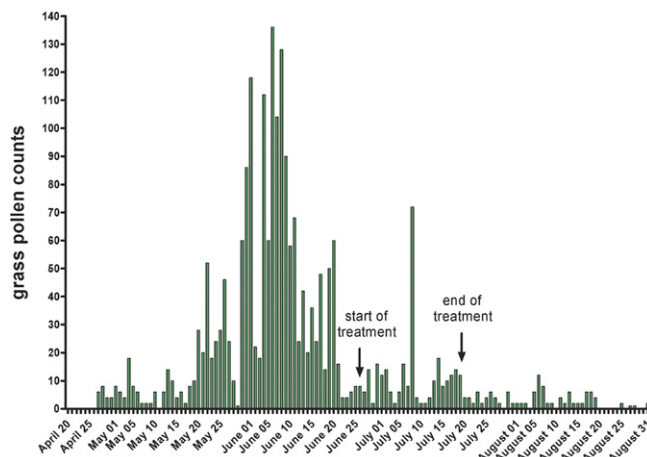


Figure 1. Official local grass pollen counts in 2007. Please note that these counts reflect the pollen burden per cubic meter in the city center as a mean over 24 hours. The results are considerably lower than those over a meadow on a summer's day, which is simulated in the environmental challenge chamber.

pollen concentration cannot be directly compared with the official grass pollen counts, which reflect the pollen burden per cubic meter in the city as a mean over 24 hours and are therefore considerably lower. The concentration of pollen in the ECC is closely monitored and stable over the complete duration of the challenges.¹²

After a screening visit including a 2-hour screening pollen challenge, the subjects were challenged at four different visits for 6 hours in the ECC. Two challenges were conducted within the grass pollen season (June/July 2007), and 2 were conducted out of the season (November/December 2007). Figure 1 shows local official grass pollen counts from April to September 2007.

Two hours after challenge initiation, subjects received either CET+PSE (CET: 10 mg ratiopharm GmbH, Ulm, Germany; PSE: 120 mg Thornton & Ross Ltd, Huddersfield, West Yorkshire, United Kingdom) or placebo under the supervision of medical personnel. The subjects were randomized to four different treatment sequences (A-B-A-B, A-B-B-A, B-A-A-B, or B-A-B-A) at a 1:1:1:1-ratio according to a computer-generated code to prevent a confounding influence of the treatment sequence. The washout period between visits was at least 7 days.

The primary study objective was to compare the effect of CET+PSE within and out of the season on the total nasal symptom score (TNSS). The secondary objective was to compare the effect of CET+PSE within and out of the season on the symptom nasal congestion assessed by a visual analog scale (VAS), on nasal flow, and on nasal secretion.

Physical and nasal examinations, clinical laboratory tests, vital signs, and pregnancy tests for females were conducted at the screening visit. Vital signs measurements and pregnancy tests were also conducted before each allergen challenge. At each visit, subjects were asked by the study physician

whether they had experienced any adverse events or deterioration in their well-being or medical condition since the start of treatment. Any use of concomitant medication was recorded.

Assessment of Subjective Parameters

Nasal symptoms (congestion, rhinorrhea, sneezing, and itching) were self-assessed before and every 20 minutes during the allergen challenge in the ECC. For each symptom, the subjects recorded scores on an electronic diary sheet according to the following 4-point severity scale: 0 = none; 1 = mild; 2 = moderate; 3 = severe. The TNSS was calculated by adding the scores of the 4 individual nasal symptoms.

Subjects ranked their nasal congestion before and every 20 minutes during the allergen challenge by using a 100-mm VAS (0: no congestion, 100: worst imaginable congestion).

Assessment of Objective Parameters

The nasal flow rate was measured by rhinomanometry (Via-sys Healthcare, Höchberg, Germany) before and every 60 minutes during challenge. Rhinomanometry is an active anterior measuring technique using a face mask. Inspiratory flow rates (mL/s) at 150 Pascal were obtained separately for the right and left nostril. The sum of the flow rates of both nostrils was calculated.

Nasal secretion was collected using preweighed paper tissues. Used and unused tissues were saved in a plastic bag by each subject and collected every 60 minutes. The amount of nasal secretion was determined by weighing these tissues. The subjects were provided with a new package of tissues for each hour in challenge.

Safety Parameters

Lung function (FEV₁) was measured using the portable electronic asthma monitor "AM-1" (VIASYS Healthcare, Höchberg, Germany) at the screening visit and before and every 60 minutes during allergen challenge. The subjects continued to measure their lung function at home every 2 hours until retiring to bed and on the following morning. They received a salbutamol aerosol as rescue medication in case of a decrease of FEV₁ > 20%.

Statistics

Sample size calculation. The sample size considerations were based on the assumption that no period and no carryover effects were present. According to the results of previous studies, the verum effect was expected as 10.2 (area under the curve [AUC]) and the within-subjects standard deviation as 5.27. The study was designed to detect a difference of the verum effects between the seasons of 20% (corresponding to 2.04) at a 2-sided alpha level of 0.05 with a power of greater than 80%. With 60 patients, the power was calculated as 85% (nQuery Advisor 6.0). A total of 70 patients were randomized to allow for dropouts.

Statistical analysis. The in-season and out-season TNSS, VAS, nasal flow, and nasal secretion were compared in the per-protocol population for timepoint 0 (before challenge)

and timepoint 2 hours (before dosing). The means of the 2 in-season and the 2 out-season challenges were computed for each of the parameters, and a paired *t* test was performed for descriptive comparison of group means. *P* < .05 was regarded as showing a significant difference.

The treatment effect was analyzed in the intent-to-treat population. The AUC was computed for the TNSS, VAS, nasal flow, and nasal secretion from time of medication intake (+2h) until the end of observation (+6h). For each parameter, the value at medication intake was entered as a fixed covariate into the linear mixed model with the AUC as dependent variable, season, period, and treatment within each season as fixed, and the intercept as random effect. Treatment effects were compared between the two seasons by linear contrasts. The analysis was performed using the PROC-MIXED procedure of SAS 9.1 (SAS Institute, Cary, North Carolina).

RESULTS

Study Subjects

Seventy subjects with seasonal allergic rhinitis were randomized (see Table 1 for subject characteristics). During the course of the study, 73 adverse events were observed in all subjects. The most frequent was headache (22/73), with an almost equal distribution between active treatment and placebo. The events resolved by the end of the study. Three serious adverse events occurred (hospital admissions) that were unrelated to the study medication or any study procedure. Seventeen participants dropped out during the course of the study, 11 because of a respiratory tract infection, 1 because of a deterioration of atopic dermatitis, 1 because of a drug eruption, and 4 withdrew their consent. Three more patients missed a single challenge day during the course of the study. The remaining 50 subjects completed the study per protocol. For the efficacy analysis, the data of the intent-to-treat group were analyzed.

Safety Results

Eight subjects experienced a decline in FEV₁ between 20% and 40% compared with their personal best FEV₁ value

Table 1. Participants' Characteristics

Variable	
Sex n [%]	
Male	40 (57.1)
Female	30 (42.9)
Age [years]	
Mean ± SD	35 ± 9
Range	19–53
Body weight [kg]	
Mean ± SD	79 ± 16
Range	48–144
Mild intermittent asthma n (%)	11 (15.7)
FEV ₁ [L]	
Mean ± SD	3.92 ± 0.77
Range	2.09–5.97

within 12 hours after the allergen challenge and had to use their rescue medication (salbutamol aerosol). Seven of those 8 subjects had a decline in FEV₁ on a single occasion, and 1 subject on 2 separate occasions. Five of the subjects experiencing a decline in FEV₁ had not been diagnosed with asthma before the study.

None of the subjects experienced a persistent decline in FEV₁. One subject experienced a decline of more than 40% (40.3%), which was successfully treated with rescue medication. This patient was known to have asthma. Seven declines in FEV₁ occurred during the in-season part of the study, and 2 in the out-season part of the study.

Period Effect and Carryover Effect

A period or a carryover effect was not observed. The order of dosing did not influence the study results. The washout period of at least 7 days between challenge days was sufficient.

Efficacy Results

In the per-protocol-population, nasal symptoms (TNSS; nasal congestion assessed by VAS) were slightly higher ($P < .05$) within the season (0.6 ± 0.8 and 6.7 ± 7.0 , respectively) compared with out-of-season (0.3 ± 0.5 and 5.0 ± 5.3 , respectively) at timepoint 0, just before the start of the challenge. Likewise, the symptoms reached a slightly higher level ($P < .05$) within the season (6.8 ± 1.9 and 48.5 ± 23.0 , respectively) compared with outside the season (6.2 ± 1.9 and 39.3 ± 18.5 , respectively) before dosing at timepoint 2 hours. Nasal secretion was higher ($P < .0001$) within the season (9.0 ± 5.3 g) compared with outside the season (6.9 ± 4.7 g) at timepoint 2 hours. Also, nasal flow was lower ($P < .05$) within the season (214.5 ± 166.0 mL/second) compared with outside the season (265.2 ± 188.0 mL/second) at timepoint 2h, but not at timepoint 0, before challenge ($P > .5$).

Treatment with CET+PSE significantly improved the TNSS, VAS, and nasal flow and significantly reduced

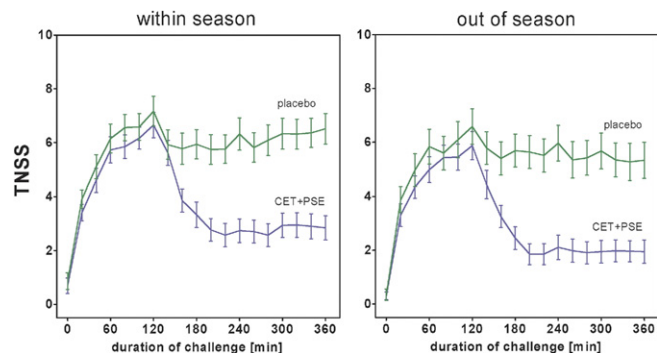


Figure 2. Total nasal symptom score (TNSS) during 6 hours of challenge within the grass pollen season (left graph) and outside of the grass pollen season (right graph). Data are displayed as mean and 95% confidence interval. Statistical analysis showed a significant treatment effect compared with placebo ($P < .0001$; AUC_{2-6h}). No difference is seen in treatment effects within and outside of the pollen season ($P > .05$).

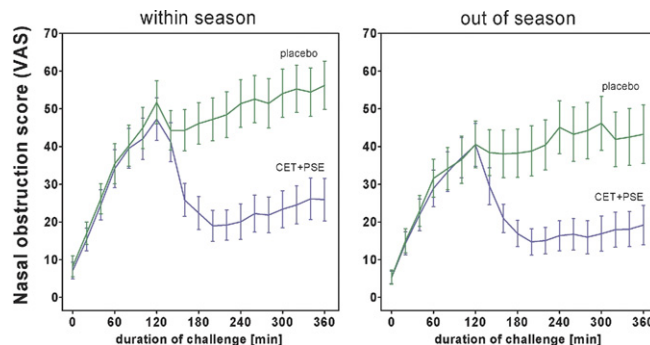


Figure 3. Nasal congestion score assessed on a visual analog scale (VAS) during 6 hours of challenge within the grass pollen season (left graph) and outside of the grass pollen season (right graph). Data are displayed as mean and 95% confidence interval. Statistical analysis showed a significant treatment effect compared with placebo ($P < .0001$; AUC_{2-6h}). No difference is seen in treatment effects within and outside of the pollen season ($P > .05$).

nasal secretion compared with placebo both within and outside of the pollen season in the intent-to-treat population (Figs 2–5; $P < .0001$ each). In the in-season (out-season) part of the study, TNSS was reduced from timepoint 2 hours to 6 hours by 57.1% (66.8%) in the treatment group compared with 9.2% (19.0%) in the placebo group. Likewise, nasal congestion assessed by VAS was reduced by 45.1% (52.5%) in the treatment group compared with an increase of 8.7% (6.6%) in the placebo group. The CET + PSE also improved nasal flow by 34.8% (29.5%), whereas nasal flow was reduced by 34.8% (22.7%) in the placebo group. Nasal secretion was reduced by 63.4% (76.6%) in the treatment group compared with 23.8% (30.3%) in the placebo group.

The treatment effects on TNSS, VAS, nasal flow, and nasal secretion did not show a statistically significant difference; in other words, the treatment effect was the same within and outside of the pollen season ($P > .05$ each).

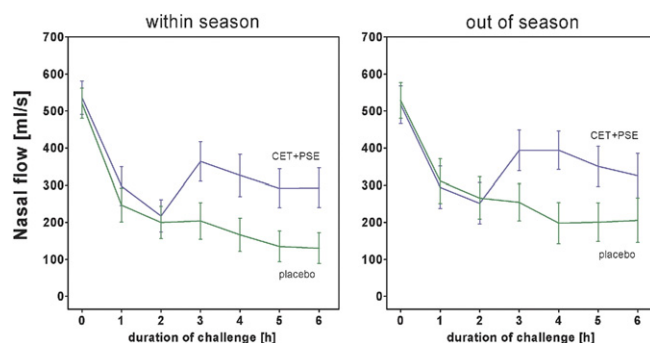


Figure 4. Nasal flow measured by rhinomanometry during 6 hours of challenge within the grass pollen season (left graph) and outside of the grass pollen season (right graph). Data are displayed as mean and 95% confidence interval. Statistical analysis showed a significant treatment effect compared with placebo ($P < .0001$; AUC_{2-6h}). No difference is seen in treatment effects within and outside of the pollen season ($P > .05$).

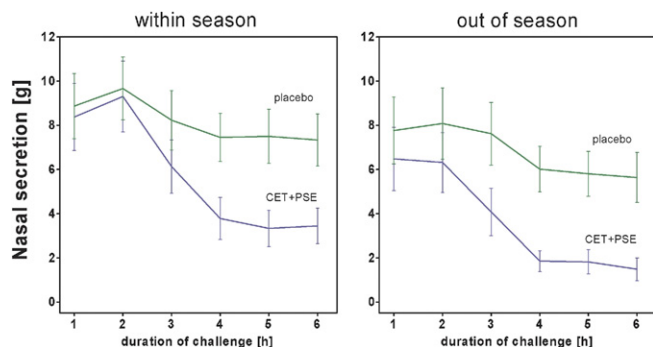


Figure 5. Nasal secretions during 6 hours of challenge within the grass pollen season (*left graph*) and outside of the grass pollen season (*right graph*). Data are displayed as mean and 95% confidence interval. Statistical analysis showed a significant treatment effect compared with placebo ($P < .0001$; AUC_{2-6h}). No difference is seen in treatment effects within and outside of the pollen season ($P > .05$).

DISCUSSION

The aim of this study was to assess whether the treatment effect of cetirizine plus pseudoephedrine is equivalent within and outside of the grass pollen season when tested using controlled allergen exposure in an ECC. An equivalent treatment effect would validate the use of Challenge Chambers within the relevant pollen seasons.

As expected and shown in numerous previous studies,^{9–11} the combination of cetirizine and pseudoephedrine showed a marked treatment effect compared with placebo. The treatment effect is not different within and outside of the season, in both subjective and objective parameters. This means that the natural pollen exposure within the season did not influence the magnitude of the treatment effect assessed under controlled allergen exposure in the ECC.

The subjects reached their peak of symptoms after 2 hours, before dosing. One can observe slightly higher nasal symptoms, a stronger decline in nasal flow, and more nasal secretion within the season compared with out of the season. This was expected, because the priming with natural pollen within the season is well able to induce a higher response to the challenge atmosphere in the ECC. This priming effect is even mimicked in some ECC studies by repetitive challenges with the aim to induce higher symptoms.^{14,15}

Again, this difference did not influence the magnitude of the treatment effect.

The subjects' in-season symptoms as well as nasal flow before the start of challenge were clinically almost the same as outside the season. One would expect to see a higher level of baseline symptoms within the season. That this is not the case is most likely because the in-season part of study was conducted after the peak of the grass pollen season, that is, after May/early June in Germany, when pollen counts were moderate. Furthermore, the challenges started early in the morning, leaving the participants only very little time for natural pollen exposure in the open before they reached the

site. A study start after the peak of the season is favorable, because it allows the participating subjects to refrain from anti-allergic medication more easily in a placebo-controlled study design, whereas a study during the season's peak would be largely influenced by the use of uncontrolled anti-allergic rescue medication. More importantly for this study, a start after the season's peak ensured that there had been enough time for immunological priming.

Nine incidences of a decline in FEV_1 occurred in 8 subjects, which was initially expected in a population including patients with mild asthma. However, that 5 of those 8 subjects had not been diagnosed with asthma before the study was surprising. In all subjects, the bronchial reaction occurred after the end of challenge within the following 12 hours, thus presenting a late asthmatic response. Because all incidences could be self-treated by the subjects with a beta-2-agonist aerosol, they did not present a safety hazard. However, the fact that some of the so-believed non-asthmatic subjects experienced a decline in FEV_1 points out that lung function monitoring after challenges is necessary even in a study population of subjects without asthma.

Seven of the 9 incidences occurred within the season, suggesting that priming with natural pollen increases the risk of adverse pulmonary events. This has to be considered when designing studies within the season.

The rate of dropouts in this study was more than expected, 20 subjects instead of 10. However, the treatment effect was analyzed in the intent-to-treat population, and data from the dropouts could still be analyzed in the linear mixed model used. Therefore, the relatively high dropout rate did not leave the study underpowered.

The study clearly showed that the combination of antihistamines and decongestants can be tested by using controlled allergen exposure in an ECC both within and outside of the grass pollen season, with the limitation that this was not tested for the peak of the season. That this holds true for single treatment with either antihistamines or decongestants is likely, however, not proven by this study, because the single treatment was not tested.

The study proved that studies outside of the season are not compromised by the lack of priming and show the same treatment effect as if performed during the season. However, these findings cannot be generalized to every treatment. Whether these conclusions hold true for steroids and other immune-modulating agents that alter the inflammatory reaction in the nose is unclear. The actions of these compounds might be affected by the priming status of the patients when the inflammation of the nasal mucosa is enhanced. Further studies are needed to investigate this.

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