

Safety and Immunological Changes During Sublingual Immunotherapy With Standardized Quality Grass Allergen Tablets

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Abstract. Immunotherapy is the only treatment for allergy that has the potential to alter the natural course of the disease. Sublingual immunotherapy for grass pollen-induced rhinoconjunctivitis has been developed to make immunotherapy available to a broader group of allergic patients. Here, a safe dose range and the safety during daily sublingual administration were investigated for a new tablet-based sublingual immunotherapy for grass pollen allergy. Simultaneously, immunological changes were monitored.

A randomized, double-blind, placebo-controlled phase I trial was undertaken, with stepwise dose-escalation during the dose-finding period, and afterwards with daily dosing 8 weeks prior to and 15 weeks during the grass pollen season (2500, 25 000, or 75 000 standardized quality tablet [SQ-T] units, or placebo). Fifty-two participants with grass pollen-induced rhinoconjunctivitis and a positive skin prick test and specific IgE to *Phleum pratense* entered the trial.

During the daily-dose treatment periods, 67% of the participants reported adverse events. The most frequent were itching in the mouth, eyes, or throat, and rhinitis, and most were mild and resolved within 1 day. Two participants withdrew due to adverse events (sting and blisters in the mouth and itching in the mouth). Time- and dose-dependent increases of *P pratense*-specific IgG, IgA, IgE, and IgE-competing components were found in serum during the first 8 weeks of daily dosing, indicating that the treatment had a significant allergen-specific effect on the immune system.

In conclusion, the grass allergen tablet, administered in a dose of 75 000 SQ-T once daily, was well tolerated and displayed systemic immunogenicity.

Key words: Grass pollen allergy. Immunological changes. Safety. Sublingual immunotherapy. Tablet

Resumen. La inmunoterapia es el único tratamiento en alergia con potencial para modificar el curso natural de la enfermedad. La inmunoterapia sublingual para el tratamiento de la rinoconjuntivitis inducida por polen de gramíneas se ha desarrollado para permitir el uso de este tipo de tratamiento a un grupo más amplio de pacientes alérgicos. En este estudio, se investiga el margen de seguridad durante la administración sublingual diaria de una nueva inmunoterapia sublingual en comprimidos para tratamiento de la alergia al polen de gramíneas. Al mismo tiempo se estudiaron los posibles cambios inmunológicos.

Se llevó a cabo un ensayo de fase I con diseño aleatorizado, doble ciego y controlado con placebo, con aumento escalonado de la dosis durante el período de determinación de la dosis tolerada y, posteriormente, con dosis diarias 8 semanas antes de la estación polínica y 15 semanas durante la misma (2.500, 25.000, 75.000 unidades SQ-T [comprimido de calidad estandarizada] o placebo). Se incluyeron 52 participantes con rinoconjuntivitis inducida por polen de gramíneas, con resultado positivo en la prueba cutánea e IgE específica frente a *Phleum pratense*.

Durante los períodos de tratamiento con dosis diarias, el 67% de los participantes notificaron acontecimientos adversos. Los más frecuentes fueron prurito en la boca, ojos y garganta y rinitis, la mayoría de los cuales fueron de

carácter leve y desaparecieron al cabo de un día. Dos participantes se retiraron del estudio debido a efectos adversos (llagas, ampollas y prurito en la boca). Se encontró un aumento dependiente del tiempo y de la dosis en la IgE, IgA e IgG específicas frente a *Phleum pratense* y componentes competitivos de IgE en el suero durante las 8 primeras semanas de la administración diaria, lo que indica que el tratamiento tuvo un efecto antigénico específico significativo sobre el sistema inmunitario.

En *conclusión*, el comprimido con alérgeno de gramíneas, administrado a una dosis de 75.000 unidades SQ-T una vez al día, se toleró bien y mostró inmunogenicidad sistémica.

Palabras clave: Alergia al polen de gramíneas. Cambios inmunológicos. Seguridad. Inmunoterapia sublingual. Comprimido

Introduction

Allergic rhinoconjunctivitis represents a global health problem, and in Western Europe the disease has prevalence of up to 34% [1]. Allergy to grass pollen is one of the most common inhalant allergies leading to impaired quality of life and increased expenditure in the health care system [2]. Specific immunotherapy (SIT) is the practice of administering allergens in order to increase systemic immunological tolerance to the allergen, and is currently the only treatment modality potentially able to change the mechanism of allergic disease and thereby prevent disease exacerbation [3].

Traditionally SIT has been administered as subcutaneous injections every 4 to 8 weeks for 3 to 5 years and the efficacy has been confirmed in several trials [4-7]. Furthermore, a number of immunological changes have been observed in patients undergoing specific immunotherapy; among these are inhibition of allergen-induced late-phase reactions in skin, lungs, and nose, increase in serum allergen-specific IgG levels, which block the biological effect of IgE *in vitro*, alteration of the T helper (T_H)₂/T_H₁ balance in favor of T_H₁ responses, and induction of IL-10-producing T cells [10]. The clinical importance of these effects is still not fully elucidated.

In recent years, there has been a growing interest in the use of sublingual administration of allergens, which has shown promise in terms of efficacy [11, 12] and an excellent safety profile [12]. An orodispersible grass allergen tablet (GRAZAX[®], ALK-Abelló A/S, Hørsholm, Denmark) for sublingual administration has been developed to make immunotherapy available to a larger group of allergic patients by allowing self-administration at home. The active ingredient is a standardized allergen extract derived from grass pollen from timothy grass (*Phleum pratense*). As extensive cross-reactivity of allergenic components of grass pollens from different species has been shown [13], the clinical use of the grass allergen tablet is anticipated to be effective for the treatment of grass pollen allergy in general.

The objectives of this study were to evaluate the safety of a single sublingual administration of the grass allergen tablet in increasing doses, to evaluate the safety of 8 weeks of daily sublingual administration outside the grass pollen season, to evaluate the safety of 15 weeks of daily sublingual administration during the grass pollen season,

and to record changes in the participants' immunological status during daily sublingual administration of the grass allergen tablet based on laboratory analyses of serum.

Materials and Methods

Trial Design

This was a phase I, single-center, randomized, double-blind, placebo-controlled trial with sublingual administration of a grass allergen tablet (GRAZAX[®] *P pratense*, ALK-Abelló A/S, Hørsholm, Denmark). The trial design is illustrated in Figure 1. The trial included 3 successive treatment periods: a single-dose treatment period (period 1; stepwise dose-escalation of 2500, 25 000, 75 000, 125 000, 375 000 standardized quality tablet [SQ-T] units, or placebo); an 8-week multiple-dose treatment period (period 2) conducted prior to the grass pollen season in 2002 (daily dosing in parallel groups: 2500, 25 000, or 75 000 SQ-T, or placebo); and a 15-week multiple-dose treatment period (period 3) conducted approximately 4 weeks prior to and during the grass pollen season in 2002 (same dosing as during the 8-week period). In addition, participants attended a follow-up visit (period 4). The dose unit was defined on the basis of the amount of *P pratense* major allergen 5 (Phl p 5) in the allergen extract, with 100 000 SQ-T corresponding to 20 µg Phl p 5.

Period 1 included 4 groups (A, B, C, and D). In each group, participants were randomly allocated (2:1) to single doses of grass pollen tablets or placebo. Group A received 2500 SQ-T or placebo, and a second dose of 375 000 SQ-T or placebo, group B 25 000 SQ-T or placebo, group C 125 000 SQ-T or placebo, and group D 75 000 SQ-T or placebo (Group D included participants from the placebo groups A, B, and C). Before period 2, participants were stratified according to sex, symptoms during period 1, and level of specific IgE to *P pratense*, and re-randomized. Group E received 2500 SQ-T, group F 25 000 SQ-T, group G 75 000 SQ-T, and group H placebo, all daily for 8 weeks. After a 6-week pause in treatment, 32 participants continued to period 3; they received the same dose as in period 2 for 15 weeks during the grass pollen season. Immunological parameters were measured during period 2 (weeks 0, 4, and 8) and at follow-up (week 37 in period 4).

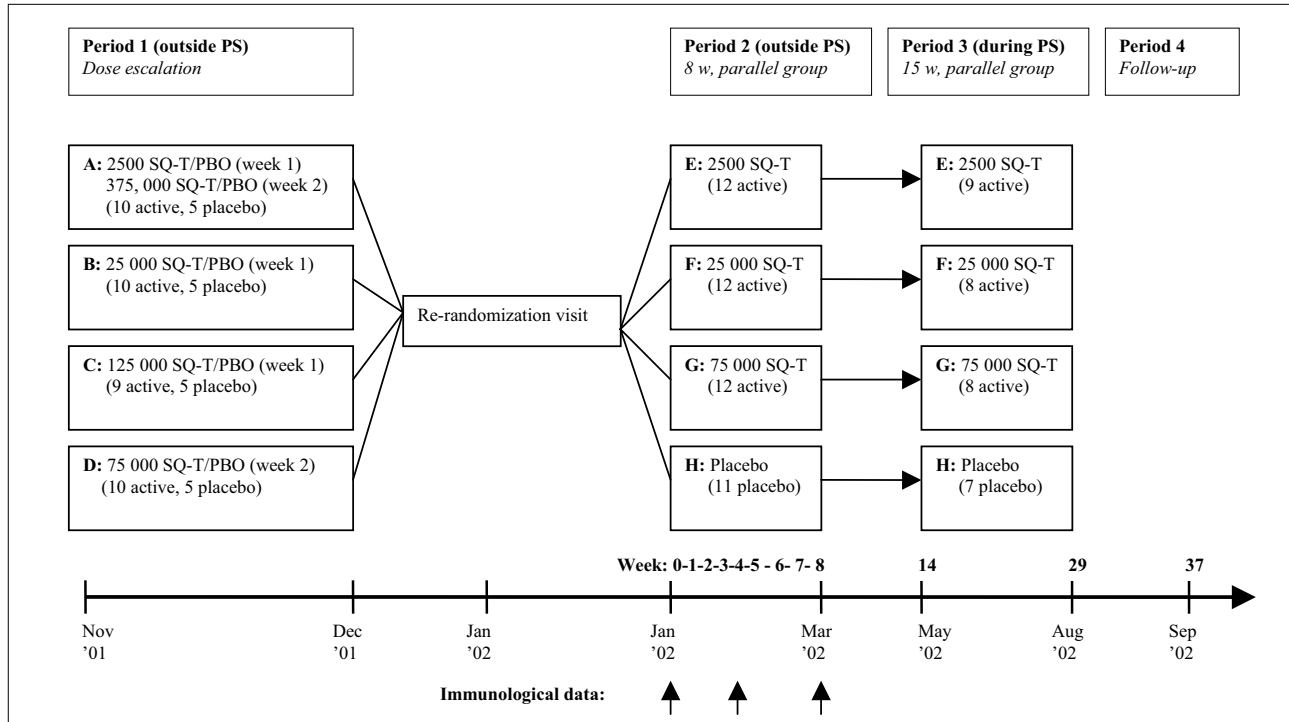


Figure 1. Trial diagram.

PS indicates grass pollen season; SQ-T, standardized quality tablet; PBO, placebo. Short arrows indicate the points at which immunological parameters were analyzed.

The primary endpoint was the number of adverse events (AEs), which were coded using the Medical Dictionary for Regulatory Activities [14]. Other endpoints included an assessment of the participant's tolerance of the trial medication by answering the question "How did you tolerate the treatment?" using a continuous visual analogue scale (VAS) score ranging from 0 (no symptoms) to 100 (severe symptoms), and a registration of the use of any concomitant medication since last visit. During the periods of daily dosing, participants also evaluated the severity of 12 prespecified symptoms on a daily basis and rated them as none, mild, moderate, or severe. During periods 2 and 3, severe prespecified symptoms and symptoms categorized as "other" were recorded as AEs.

Immunological parameters (*P pratense*-specific IgE, IgE-competing components [IgX], IgG, and IgA) were measured in serum samples collected during period 2 (weeks 0, 4, and 8) and at follow-up (week 37).

The concentration of *P pratense*-specific IgE antibodies in participant serum samples (kU/L, arbitrary units) was measured using an ADVIA Centaur Immunoassay System (Bayer Healthcare, NY, USA) as described by Petersen et al [15]. The inhibitory capacity of IgE-competing components for the reaction between IgE and *P pratense* allergens, termed IgX, was estimated as a ratio between IgE measured using a modification of the Petersen et al protocol (excluding the first washing step, thus allowing non-IgE antibodies to compete with IgE for the allergen) and IgE measured using the conventional protocol.

The level of *P pratense*-specific IgG and IgA

antibodies was determined by a direct enzyme-linked immunosorbent assay (ELISA) method. ELISA plates (Maxisorp™, Nunc, Roskilde, Denmark) were coated with *P pratense*, serial dilutions were made of each serum sample, and detection was performed using either horseradish peroxidase (HRP)-labeled mouse antihuman IgG (Zymed, San Francisco, USA) or HRP-labeled rabbit antihuman IgA (DAKO, Copenhagen, Denmark). Serial dilutions of a standard serum were added to each ELISA plate and optical density (OD) values used in a 4-parameter logistic regression using GraphPad Prism version 4.02 (GraphPad software, San Diego, USA). The standard curves were used to standardize the OD titer of the serum samples and to adjust for assay-to-assay and plate-to-plate variations.

Participants

Forty-seven participants (all Caucasian) were enrolled in the trial. The trial was performed with the approval of local ethics committees and in accordance with guidelines for good clinical practice and the Declaration of Helsinki. Written informed consent was obtained from all participants.

The main inclusion criteria were as follows: 18 to 65 years of age, clinical history of moderate to severe grass pollen-induced allergic rhinoconjunctivitis, positive skin prick test, and specific IgE against *P pratense*, no clinical history of significant rhinitis, sinusitis, and/or asthma outside the grass pollen season, no allergic symptoms to

birch in the period January to March, no daily contact with animals to which the participant was sensitized, no use of medication that was known to interfere with adrenaline, no history of diseases that could influence the result of the trial or the health of the participant, no severe atopic dermatitis, and no SIT with *P pratense* extract during the previous 5 years.

set (the full analysis set) was used. Baseline comparability, immunological responses and safety assessments were evaluated by summary statistics and frequency tables. A nonparametric analysis (Wilcoxon) was used to test the differences in specific antibodies between the pretreatment visit (week 0) and the follow-up visit (week 37). *P* values of less than .05 were considered statistically significant.

Statistical Analysis

Results were analyzed using SAS® version 8 (SAS Institute Inc, Cary, North Carolina, USA). One analysis

Results

The trial was conducted between 15 November 2001 and 27 September 2002. There were no significant differences in

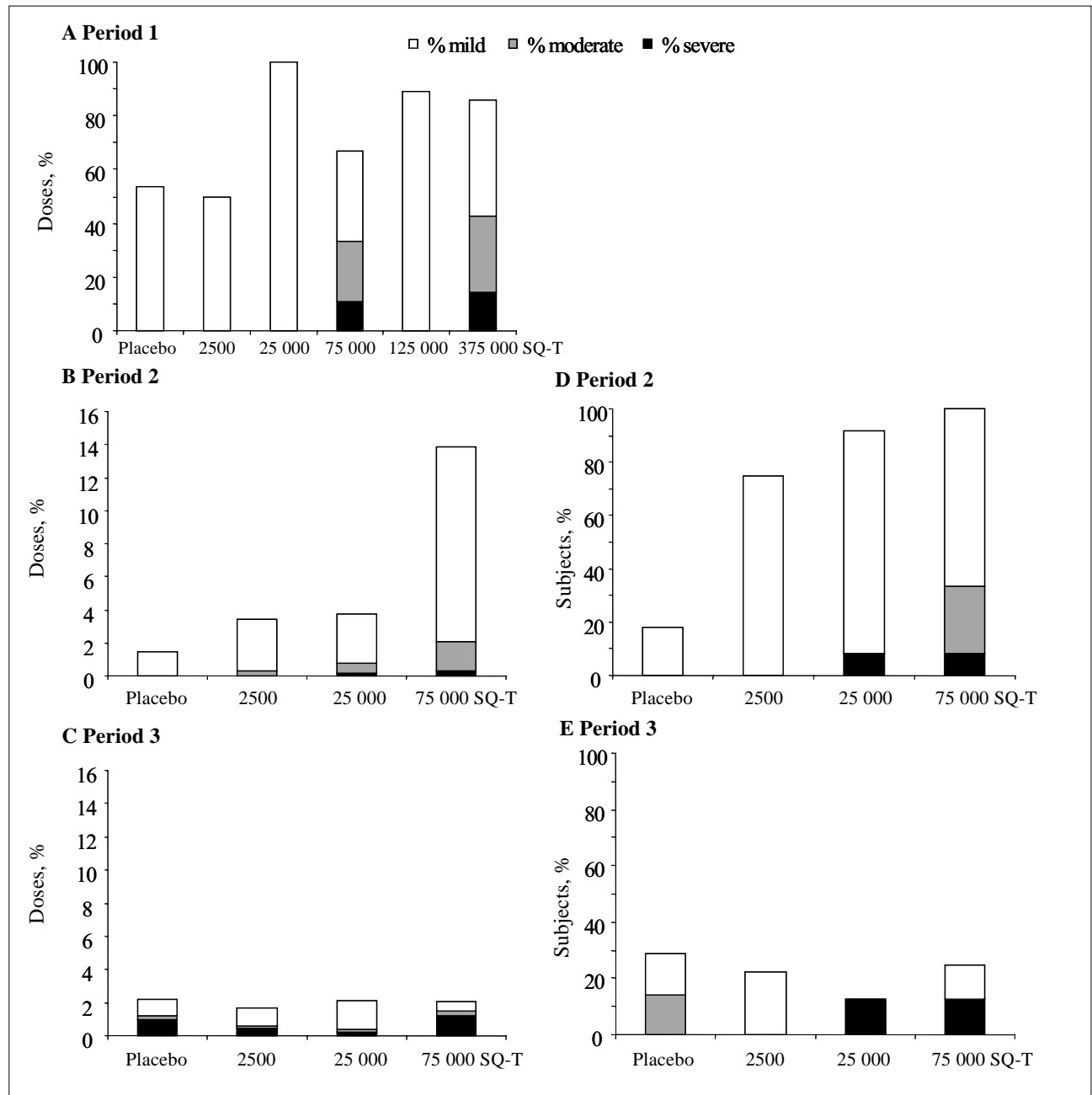


Figure 2. Adverse events summarized by severity relative to number of doses administered (A-C) or number of participants (D, E).

patient characteristics (sex, age, body mass index, pulse, blood pressure) or in allergic history between the treatment groups in period 1 or in periods 2 to 4.

In total, 52 participants entered the trial. Forty-three participants completed period 1, 44 completed period 2 (5 additional participants entered period 2 prior to initiation of the period), 28 completed period 3, and 28 attended the follow-up visit.

The AEs are summarized in Figure 2A-D. In Figure 2A-C, the number of doses administered is calculated from the number of participants included in the period and the weeks of treatment; all AEs are included regardless of severity and relationship to trial medication. In Figure 2D-E, only the most severe AE in the period is listed for each participant. Only definite or possible related AEs are included.

A total of 73 AEs (64.7% of the participants) occurred during period 1, 150 (76.6% of the participants) during period 2, and 67 (53.1% of the participants) during periods 3 to 4. The most frequently reported AEs were "itching mouth" (41.2% of the participants) and "itchy throat" (16.2% of the participants) in period 1, and "itching mouth" (46.8% of the participants), "itching eyes" (17% of the participants), and "itchy throat" (10.6% of the participants) in period 2. During period 3 (the grass pollen season), most of the AEs were hay-fever related, the most frequent being "itching eyes" and "rhinitis" (both reported by 18.8% of the participants). The majority of AEs (82.2% in period 1 and 86% in period 2) were mild and resolved within 1 day. In period 3, 53.7% of all AEs were reported as mild, 11.9% as moderate, and 34.3% as severe. Out of all AEs reported, 93.2% were considered possibly or definitely related to trial medication in period 1, 88.0% in period 2, and 50.7% in period 3.

Based on 2 events of "throat constriction" in the 375 000 SQ-T group (1 severe and 1 moderate) the dose escalation was stopped and the highest dose chosen for the following periods was 75 000 SQ-T.

One serious AE (rectal hemorrhage) was reported in period 3 in a participant receiving 25 000 SQ-T. The serious AE was judged as unlikely to be related to trial medication.

Two participants (3.8%) withdrew due to AEs: 1 participant receiving 75 000 SQ-T withdrew due to sting and blisters in the mouth, and 1 receiving placebo withdrew due to itching in the mouth. Both incidences were judged as having a possible relation to trial medication.

The mean VAS scores in period 1 (single doses) increased with dose from 5.2 to 40.9 (placebo to 375 000 SQ-T; Figure 3A). During periods 2 and 3 the mean VAS score decreased during treatment in all active groups (Figure 3B).

Time- and dose-dependent changes of grass pollen allergen-specific IgE, IgX, IgG, and IgA were found in blood, indicating that the treatment had an effect on the immune system.

The mean concentration of *P pratense*-specific IgE increased rapidly during period 2, especially in the 75 000 SQ-T groups (Figure 4). At the follow-up visit, a

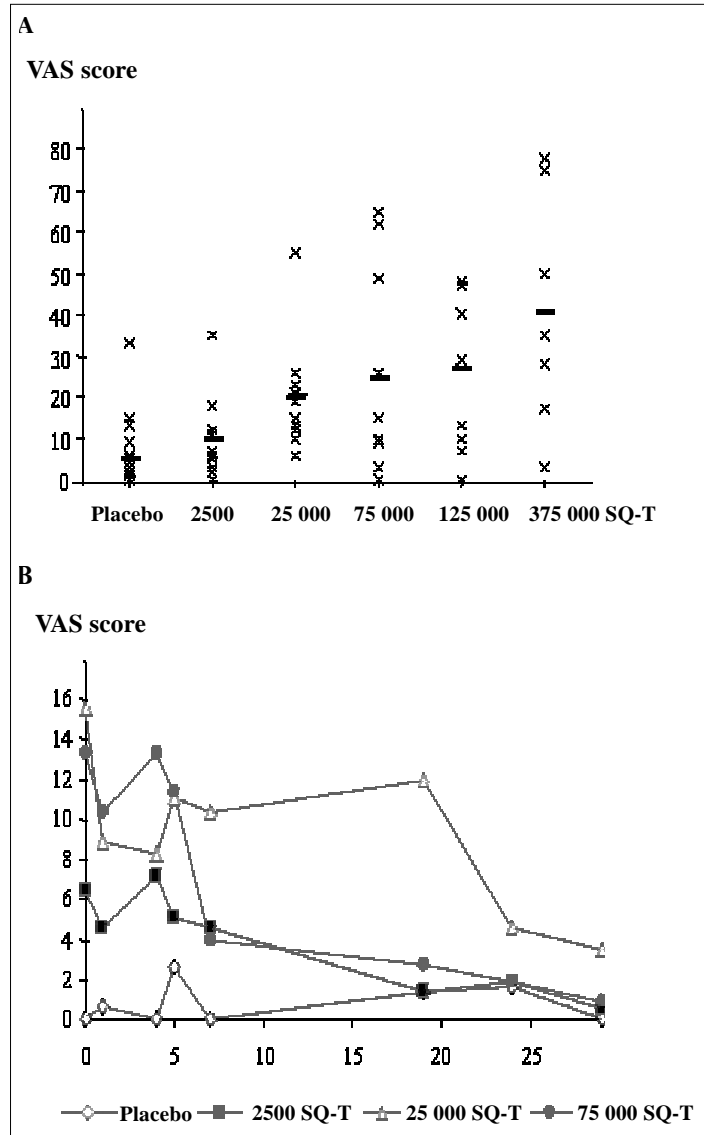


Figure 3. Visual analogue scale (VAS) scores. A) Individual (X) and mean (-) VAS scores in period 1. B) Mean VAS scores during periods 2 (weeks 0-8) and 3 (weeks 14-29).

decrease was observed in mean IgE concentration in the 75 000 SQ-T group, although the level was still higher than before period 2 ($P = .016$). No changes were observed in the placebo group, whereas there were increases in the 2500 and 25 000 SQ-T groups.

The mean ratios of *P pratense*-specific IgX showed decreases in the mean values during the treatment period in a dose-dependent manner (ie, most pronounced in the 75 000 SQ-T group; Figure 4). Thus, the treatment led to higher levels of IgE-competing antibodies. No changes were noticed in the lower treatment groups.

Regarding *P pratense*-specific IgG, participants receiving 75 000 SQ-T showed an increase in mean IgG concentrations during period 2, whereas only slight changes were seen in the lower dose groups (Figure 4). Also, for *P pratense*-specific IgA, increases were mainly seen in the 75 000 SQ-T treatment group (Figure 4).

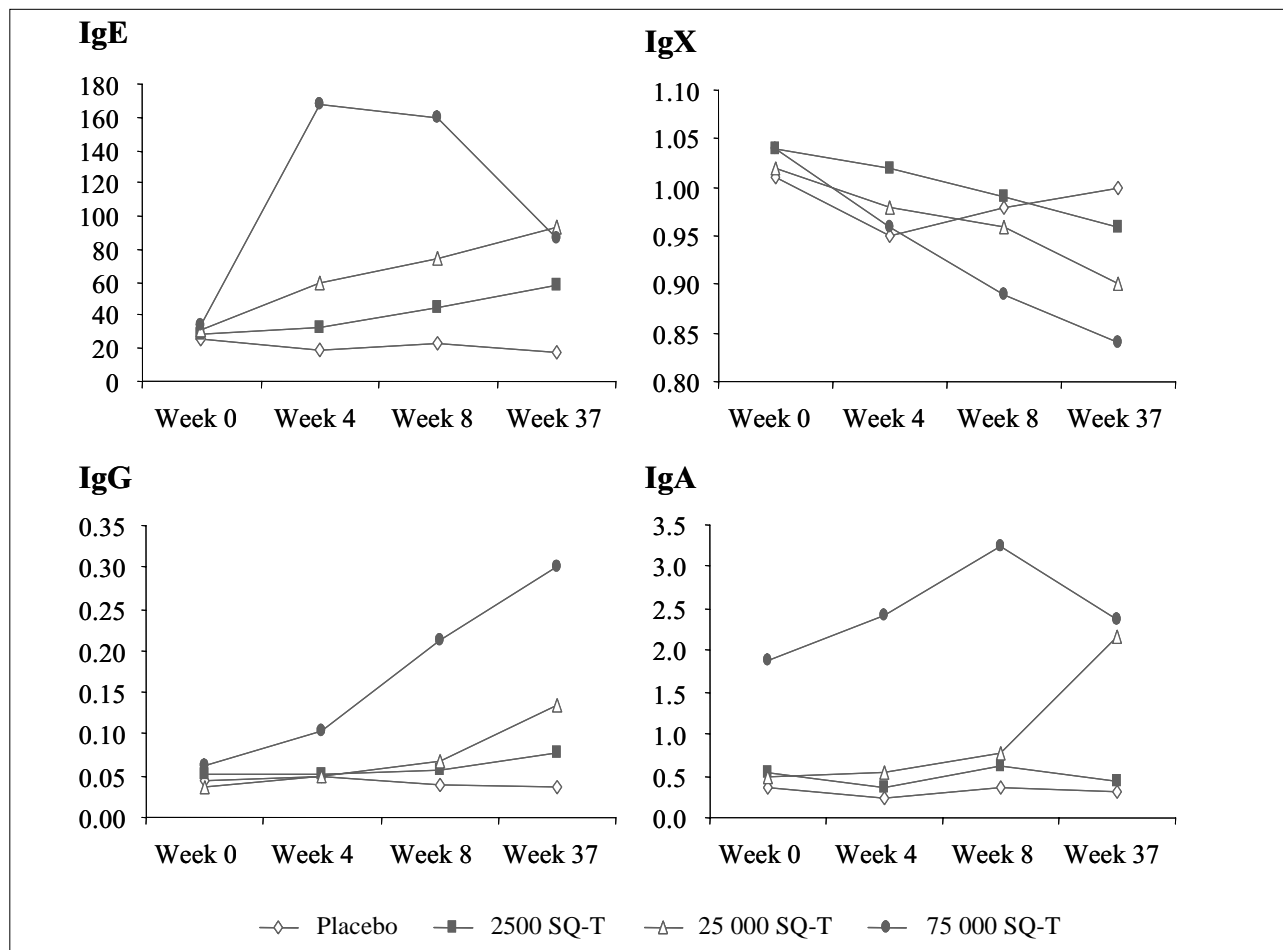


Figure 4. Time and dose dependency of *Phleum pratense*-specific antibodies (IgE, IgX [IgE-competing components], IgG, and IgA).

Discussion

The first part of the trial was a dose-finding study with single administration of increasing doses of the grass allergen tablet, starting with 2500 SQ-T and proceeding to a predefined higher dose if no important AEs occurred. Due to 2 events of "throat constriction" in the 375 000 SQ-T group the dose escalation was stopped and for safety reasons the highest dose chosen for the following daily-dose periods was 75 000 SQ-T.

The cumulative dose over 6 weeks for 75 000 SQ-T grass allergen tablets corresponds to approximately 600 μ g Phl p 5. This dose is 30 times higher than the maintenance dose for Alutard SQ[®] *P pratense* (ALK-Abelló A/S), which has the same active substance as the grass allergen tablet.

The AEs and symptoms seen outside the grass pollen-season were mainly related to local reactions in or around the mouth. The majority of AEs and symptoms were mild and disappeared spontaneously within hours of tablet intake. During the grass pollen season, hay fever symptoms were most predominant, but this was most likely due to the natural exposure to grass pollens (ie, events which were part of the underlying disease and not caused by the treatment). However, reactions related to

the areas in or around the mouth were also reported. Again, the majority of AEs and symptoms were mild and disappeared spontaneously.

As seen from the number of treatment-related AEs and the participants' assessment of how they tolerated the treatment, side effects of treatment with the grass allergen tablet were significantly reduced with increased length of treatment, possibly due to local desensitization.

While an up-dosing period is essential in subcutaneous immunotherapy in order to minimize potential serious AEs, the safety data of the sublingual grass allergen tablet indicate that the up-dosing regimen might be eliminated to achieve a simpler and more convenient administration schedule. No negative safety issues were revealed for this modality and the relevance of up-dosing in sublingual immunotherapy (SLIT) in general should be reevaluated in relation to the dose needed for optimal efficacy.

From trials investigating the immunological changes induced by SLIT, increases in IgG₁ and IgG₄ have been reported, although quantitatively smaller than with subcutaneous immunotherapy [16-18]. Consistent changes in IgE have not been observed. SLIT has also been shown to reduce the proliferative response of T lymphocytes [17].

In the present trial, sublingual grass allergen tablet

treatment induced a dose- and time-dependent increase in allergen-specific IgE, IgG, and IgA antibody responses during the first 8 weeks of daily dosing. The IgE-competing components (IgX) likewise exhibited a dose- and time-dependent increase in competitive capacity towards IgE for the interaction with the allergens. This suggested that the treatment had a significant allergen-specific effect on the immune system, and the antibody responses indicate that allergen-specific T lymphocytes were affected.

Some participants responded strongly to the treatment, whereas others had a slight increase in IgE. The reason for this difference is not clear. The single allergen dose given in the first period did not seem to influence the level of IgE in the second period, such that the high IgE responders were not those who received a high dose during the first period (data not shown).

There was not enough data to link the antibody responses to the clinical data in terms of the number and severity of AEs or the VAS score. However, it has been shown that inhibitory components to allergen-IgE binding are associated with clinical improvement [19]. It will be important in future trials of SLIT to keep looking for parameters to predict tolerability and also the clinical outcome in individual participants.

In conclusion, the doses administered on a daily basis before and during the grass pollen season (2500 SQ-T, 25 000 SQ-T, and 75 000 SQ-T) were considered well tolerated and safe for further studies. The grass allergen tablet had a dose-dependent effect on the immune system in terms of changes in specific IgE, IgG, IgA, and IgX.

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