

Both sublingual and supralingual routes of administration are effective in long-term allergen-specific immunotherapy

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ABSTRACT

The aim of this study was to confirm or refute the difference between efficacy of long-term specific immunotherapy (SIT) with standardized allergen vaccine consisting of six grass pollens (oat grass, orchard grass, fescue, rye grass, timothy grass, and rye) administered either by sublingual or by supralingual route. To investigate clinical and immunologic changes, 51 patients of a previous 1-year double-blind, placebo-controlled, randomized study were enrolled in an open randomized study that continued over the next 3 years. Sublingual or supralingual immunotherapy (SLIT) was performed in the same way, keeping the drops under or on the tongue, respectively, for 1–2 minutes before swallowing them. Data about symptoms scores and rescue medication intake during grass pollen seasons, as well as skin-prick test results, levels of specific IgG, and IgE antibodies were collected after each pollen season. It was clearly shown that both routes of administration were effective, leading to a significant decrease of clinical symptoms of grass pollen allergy after SIT lasting 3–4 years. No statistically significant difference between sublingually and supralingually treated patients was observed at the end of the study. Adverse effects were limited to a small number of generally mild local and/or systemic reactions with no significant difference between both administration ways of SIT. The significant therapeutic effect of both SLIT and supralingual immunotherapy lasting 3–4 years was clearly achieved. Despite no significant difference between efficacy of both administration ways of SIT, the onset of sublingual SIT effect seems to be slightly faster than that of supralingual SIT.

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Sublingual immunotherapy (SLIT) has been proven to be safe and effective for treatment of allergic rhinitis and asthma. Oral Langerhans cells probably play the significant role in the mechanism of SLIT.^{1,2} Their largest density in vestibulum, lingua, bucca, and palatum might favor the supralingual to the sublingual administration of immunotherapy.³

Allergen drops can be administered both sublingually and supralingually. Considering the results of a short-term 1-year clinical trial showing partial statistically significant differences between both routes, it was necessary to confirm or refute this finding by a long-term clinical trial corresponding to typical specific immunotherapy (SIT) duration.⁴

MATERIALS AND METHODS

Patients

Only patients participating in the previous 1-year trial⁴ were enrolled into this study. The clinical trial was completed by 51 of 61 enrolled patients who resigned a new informed consent; 10 patients withdrew because inadequate cooperation or pregnancy. The patients fulfilled the inclusion and exclusion criteria of the previous study⁴ that followed the generally accepted recommendations⁵ for SIT (Table 1).

Allergen Preparation

The standardized allergen extract “H-Al per os” (Sevapharma a.s., Prague, Czech Republic) contained a mixture of six species of grass pollens (grass mixture I): oat grass (*Arrhenatherum elatius*), orchard grass (*Dactylis glomerata*), fescue (*Festuca* sp.), rye grass (*Lolium* sp.), timothy grass (*Phleum pratense*), and rye (*Secale cereale*). The strength of active principle in aqueous solution was 1, 10, 100, 1000, and 10,000 JSK (jednotka standardní kvality [standard quality unit])/mL, expressed in biological units. The maximum dose (10 drops of 10,000 JSK/mL) contains ~11.2 µg of the grass pollen major allergen Phl p 5 according to the producer. The cumulative allergen dose in sublingual or supralingual administration was intermediate,⁶ almost 20 times higher compared with the cumulative allergen dose in sub-

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Table 1 Characteristics of subjects eligible for analysis

Parameter	4 yr Active Treatment		3 yr Active Treatment (1st yr placebo)		Total
	Sublingual	Supralingual	Sublingual	Supralingual	
No. of subjects	16	14	10	11	51
Male/female	8/8	8/6	6/4	7/4	29/22
Age distribution (yr), in the beginning of study <i>p</i> value (Kruskal-Wallis test)	17.1 ± 9.7	16.5 ± 10.3	14.3 ± 8.2	22.5 ± 11.1	17.6 ± 10.0
Sensitizations other than grass and major inhalant coinciding allergens	9	8	9	8	34

cutaneous immunotherapy with corresponding preparation.

Treatment

The study treatment was performed either by the way of sublingual or by the way supralingual administration of allergen vaccine (drops). The control groups of the previous study (formerly treated by placebo) started the treatment in this study with an initiation phase—increasing daily doses, from 1 to 10 drops, for each concentration until the maximum dose was reached. The maintenance phase of immunotherapy with a maximum therapeutic dose (10 drops of 10,000 JSK/mL three times a week) was then administered in a 3-year treatment. The active treatment groups of the previous study continued without interruption of the maintenance phase of the treatment for another 3 years (4 years together).

The patients using sublingual or supralingual therapy were instructed to keep the drops under or on the tongue, respectively, for 1–2 minutes before swallowing them. All patients were allowed to use, when necessary, the following drugs as rescue medication: levocabastine, cromoglycate, azelastine, budesonide, beclomethasone, cetirizine, loratadine, prednisone, and/or salbutamol.

Study Design

The current study (Fig. 1), scheduled as open and randomized in relation to sublingual and supralingual administration, succeeded to the previous double-blind, randomized, placebo-controlled 1-year study of the sublingual and supralingual SIT parallel arrangement in patients randomized into four treatment groups (two active and two control groups).

During the study patients had to record the following data in patient diaries: investigational product intake, rescue medication intake, adverse events, concomitant treatment, and daily records of the presence and severity of symptoms during grass pollen season (from 19th to 33rd week). The primary end point of this study was defined by symptoms scores.⁷ In addition,

the rescue medication intake score, skin-prick tests (SPTs), and grass pollen-specific IgE and IgG antibodies were considered as secondary study outcomes similarly as in other studies.^{8–11} This clinical trial was conducted according to the rules of Good Clinical Practice and was approved by the Ethics Committee of the Czech Ministry of Health and State Institute for Drug Control (EudraCT 2004-001521-19).

Symptoms Scores

During grass pollen season of each study year, rhinitis symptoms (sneezing, nasal itching, watery runny nose, and nasal obstruction), conjunctivitis symptoms (ocular redness, ocular itching, tearing, and ocular swelling), and eventual bronchial symptoms (cough, presence of mucus, breathlessness, and wheezing) were recorded daily by all of the patients. Each symptom was rated by 4-point scale: 0, no symptom; 1, mild complaints (signs or symptoms present but not disturbing); 2, moderate complaints (signs or symptoms disturbing); and 3, severe complaints (very disturbing signs with impaired social or professional life, usual necessity of rescue medication). Patients who used a daily rescue medication had to have assigned 3 points to at least one daily symptom, but patients could also record a 3-point score without a symptomatic medication intake.

Medications Scores

Throughout the study, the patients were instructed to use symptomatic rescue medications if needed and to record their amounts in their diary. The daily use of this medication was assessed by a score of 1 or no use by a score of 0. The combined symptom medication score was calculated for each day during the pollen season and for each patient. The final combined score is the average of the daily combined scores.^{12,13}

Skin-Prick Tests

Quantitative SPTs were performed with grass mixture I allergen extract in a concentration of 1000

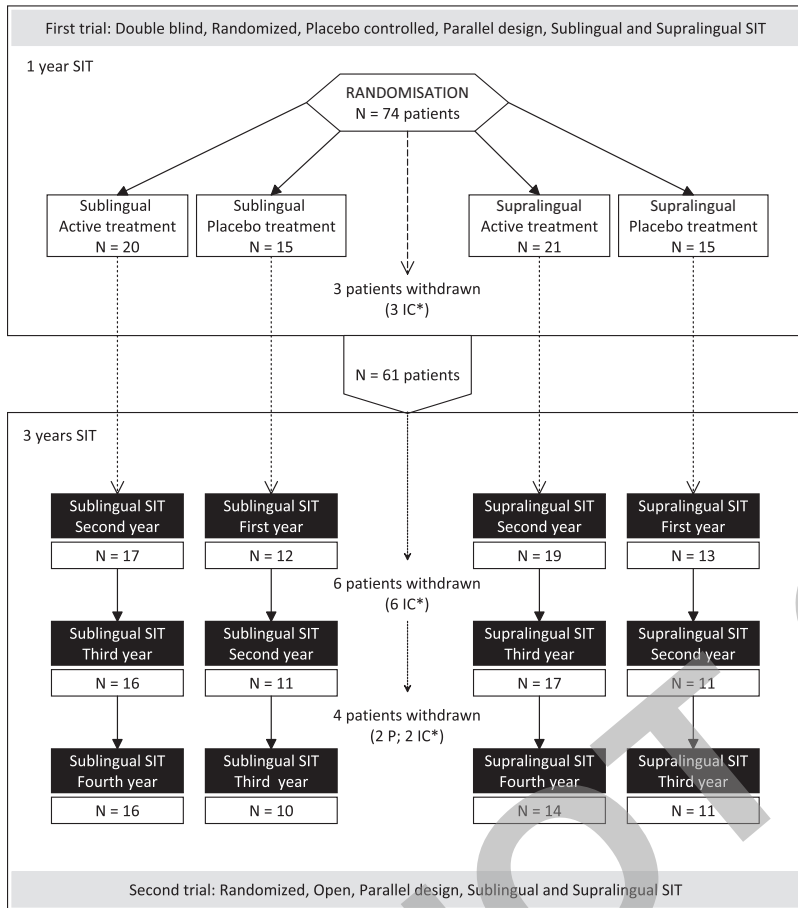


Figure 1. Scheme of both study designs. *IC, inadequate cooperation; P, pregnancy.

JSK/mL and with *D. glomerata* allergen extract in a concentration of 10, 100, 1000, and 10,000 JSK/mL together with positive and negative control after termination of grass pollen season of each study year. The diagnostic allergen extracts were identical to the investigational allergen.

The wheals were outlined with a fine-tip roller ball pen and transferred by means of transparent tape to the corresponding sheet of the Case Report Form. Wheal diameters were calculated from surfaces digitized by scanning.^{9,14} This regression line, allergen concentration by wheal diameter,⁴ was used to calculate the theoretic wheal diameter at a concentration of 1000 JSK/mL.

Specific IgE and IgG

The plasma samples from all patients were collected after termination of pollen season of each study pollen season. The samples were frozen and stored in the central laboratory at a temperature < -30°C. Serology was performed by the central laboratory at the end of the study. Levels of IgE specific to *D. glomerata* were determined by the CAP System (Phadia, Uppsala, Sweden) and specific IgG by ELISA (Dr. Focke Laboratorien GmbH, Neus, Germany).

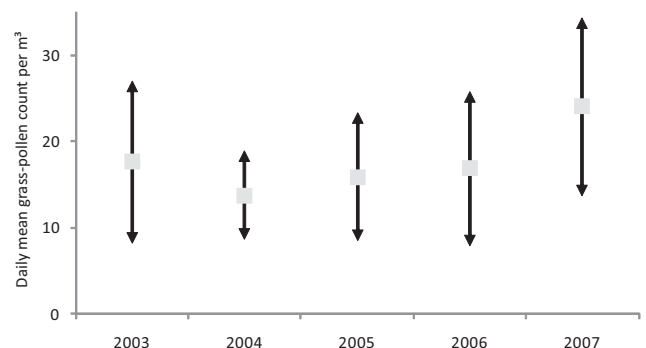


Figure 2. Daily mean grass pollen count per cubic meter (including 95% confidential limit) in the Czech Republic during four study seasons defined from the 19th to 33rd week of each year.

Pollen Counts

Despite heterogeneity of grass pollen seasons during the study years (2003–2007), no significant deviation in their durations and means of daily grain counts was found ($p = 0.6440$, Kruskal Wallis method; Fig. 2). The analyses were performed on primary and secondary outcomes recorded in diaries during the defined grass pollen seasons of each study year. Mean pollen grain counts from the area where the patients live (Czech

Republic) were recorded by the Czech Pollen Information Service.

Statistical Methods

The investigated parameters might not be normally distributed, and therefore the Mann-Whitney *U* test for intergroup comparison and the Wilcoxon test for intra-group comparisons were used when appropriate. All tests were two tailed, and the level of significance was set at 0.05. All of the statistical analyses and nonlinear regressions have been performed with the biostatistics software StatMate (Version 1.01i, 1998; GraphPad Software, San Diego, CA) and the curve-fitting and scientific graphing-in were performed with one comprehensive software Prism (Version 4.03, 2005; GraphPad Software).

RESULTS

Cumulative Dose of Allergen

The cumulative doses of administered allergen were calculated pursuant to daily records in patient diaries related to the content of returned drug bottles. At the end of the study, the mean cumulative dose reached 2,590,000 and 2,480,000 JSK in subjects with sublingual and supralingual SIT of 4 years, respectively (former active treatment groups of the previous study), and 1,820,000 and 1,970,000 JSK in subjects with sublingual and supralingual SIT of 3 years, respectively (former placebo groups of the previous study). The differences of mean cumulative doses among all four treated groups were not statistically significant with consideration of the SIT duration (method, Kruskal-Wallis; $p > 0.1$).

Study Outcomes

Data analysis of the primary and secondary end points such as combined or cumulative scores of total and particular symptoms and numbers of rescue medication intake days for the whole defined grass pollen seasons, SPTs as well as levels of specific IgG and IgE, were performed in the patients per protocol analysis randomized in four groups, using data of the previous study.⁴ The means of followed parameters decreased depending on SIT duration in each treated group except increasing IgG levels and invariant IgE levels. The statistical analysis confirmed significant changes mostly at the end of the study but because of relatively small size of each study group (see Fig. 1) statistical power (<50%) was weak.

Data obtained from patients of all four groups could be rearranged into only two groups related to the route of allergen administration, *i.e.*, sublingual or supralingual. The rearrangement was enabled by similarity of pollen seasons in the study years 2003–2007 and by similarity of the groups related to the SIT duration and

route of administration in the frequency of symptoms and rescue medication ($p > 0.1$).

Because of rearrangement, the number of data to analyze was nearly double in both treated groups (Table 2). The symptom scores and rescue medication intake before treatment were obtained from all subjects randomized in the control groups of the previous study who completed the current study, regardless of sublingual or supralingual placebo administration (*i.e.*, data of 21 subjects). The initial data of objective parameters (such as SPT, IgG, and IgE levels) were collected from measurements performed in all patients (51 subjects) in the beginning of the previous study and patients of placebo groups (21 subjects) at the end of the previous study.

Symptom Scores and Rescue Medication Intake

Statistical analysis of data depending on the SIT length exhibits a clear significant decrease of all symptoms occurrence and intensity, independently on route of administration (see Table 2, Fig. 3). The power of analysis reached 70–99%. Slight discrepancy of these results is observed in the 4th year of SIT because of the lower number of subjects in both treated groups (only subjects from the former active treatment groups of the previous study). This influence of small sample size was especially observed in patients with supralingual SIT, but the difference of means between both treated groups was not significant.

The finding of a significant decrease of the clinical symptoms depending on SIT duration led us to use a mathematical description of cumulative total symptom score on growing cumulative therapeutic dose (Fig. 4). The model data were obtained from means and SDs of cumulative doses and cumulative total symptoms scores for each monitored grass pollen season. The best-fit values of the parameters were obtained by using a one-phase exponential decay (nonlinear regression).

The plateaus of nonlinear regression reach the total symptoms score threshold of 45 (95% CI, 0–114) and 74 (95% CI, 39–109) after the long-term sublingual and supralingual therapy, respectively. The 95% confidence limits suggest no significant variance between both routes of SIT administration.

Skin-Prick Tests

After >1 year of therapy, the SPT wheals to *D. glomerata* and grass mixture I are reduced when compared with the same wheals before the SIT in both treated groups. The minimum 35% wheal reduction was recorded in >75% of patients after therapy of 3 or 4 years. The power of significant reduction was >88% ($p = <0.001$). Despite a greater wheal reduction in patients with sublingual SIT, a difference between sub-

Table 2 Summary of primary and secondary outcomes including cumulative therapeutic doses in sublingually and supralingually treated groups for all followed grass pollen seasons

Time Point	Oral Drops Administered Sublingually				Oral Drops Administered Supralingually				Sublingually vs Supralingually (power; <i>p</i> value)
	No. Subjects	Mean Value ± SE (±95% CI)	Improvement/Change* (power; <i>p</i> value)	No. Subjects	Mean Value ± SE (±95% CI)	Improvement/Change* (Power; <i>p</i> value)			
Combined score#									
Before SIT	21	47.3 ± 6.8 (33.1–61.4)	×	21	47.3 ± 6.8 (33.1–61.4)	×			
After 1 yr SIT	26	20.3 ± 4.9 (10.3–30.3)	57% (89%; 0.0003)	25	32.4 ± 7.7 (16.5–48.3)	31.4% (28%; 0.0189)			(26%; >0.1)
After 2 yr SIT	26	13.2 ± 5.8 (1.2–25.2)	72.1% (96%; <0.0001)	25	20.7 ± 5.6 (9.2–32.1)	56.3% (85%; 0.0002)			(<20%; >0.05)
After 3 yr SIT	26	15 ± 7.6 (–0.6–30.5)	68.3% (85%; <0.0001)	25	15.4 ± 5.5 (3.9–26.8)	67.5% (95%; <0.0001)			(<10%; >0.1)
After 4 yr SIT	16	3.9 ± 1.2 (1.3–6.4)	91.8% (>99%; <0.0001)	14	21.7 ± 10.2 (–0.3–43.6)	54.2% (69%; 0.0013)			(43%; >0.1)
Total symptoms score									
Before SIT	21	224.7 ± 34.9 (151.8–297.6)	×	21	224.7 ± 34.9 (151.8–297.6)	×			×
After 1 yr SIT	26	96.3 ± 23.1 (48.7–143.8)	57.2% (87%; 0.0006)	25	135.9 ± 25.4 (83.4–188.4)	39.5% (53%; 0.0158)			(<20%; >0.05)
After 2 yr SIT	26	59.4 ± 26 (5.9–112.9)	73.6% (97%; <0.0001)	25	90.3 ± 22.7 (43.6–137.1)	59.8% (90%; 0.0001)			(<20%; >0.05)
After 3 yr SIT	26	66.5 ± 33 (–1.4–134.4)	70.4% (89%; <0.0001)	25	62.6 ± 19.9 (21.5–103.8)	72.1% (>99%; <0.0001)			(<10%; >0.05)
After 4 yr SIT	16	18.6 ± 5.7 (6.5–30.8)	91.7% (>99%; <0.0001)	14	82 ± 33.5 (9.5–154.5)	63.5% (77%; 0.0008)			(48%; >0.05)
No. of days of rescue medication intake									
Before SIT	21	18.3 ± 4.7 (8.4–28.2)	×	21	18.3 ± 4.7 (8.4–28.2)	×			
After 1 yr SIT	26	8.2 ± 2.6 (2.8–13.6)	55.2% (48%; 0.0394)	25	20.1 ± 9.2 (1.1–39.1)	–9.8% (<10%; 0.4397)			(24%; >0.05)
After 2 yr SIT	26	5.9 ± 2.1 (1.5–10.3)	67.8% (70%; 0.0097)	25	14.5 ± 5.6 (2.9–26.1)	20.6% (<10%; 0.1093)			(30%; >0.05)
After 3 yr SIT	26	7.1 ± 3 (0.9–13.3)	61.1% (52%; 0.0077)	25	12.4 ± 6.1 (–0.2–24.9)	32.4% (10%; 0.0121)			(<20%; >0.05)
After 4 yr SIT	16	1.6 ± 0.7 (0–3.1)	91.5% (84%; 0.0005)	14	19.3 ± 11.6 (–5.8–44.4)	–5.5% (<10%; 0.2185)			(35%; >0.05)
GMT: <i>Dactylis glomerata</i> -specific IgG levels (μg/mL)									
Before SIT	72	44.5 ± 10.3 (24.3–64.6)	×	72	44.5 ± 10.3 (24.3–64.6)	×			
After 1 yr SIT	26	63.2 ± 23.7 (16.9–109.6)	42.3% (<20%; 0.2466)	25	80.6 ± 23.6 (34.4–126.7)	81.2% (36%; 0.0304)			(<10%; >0.05)
After 2 yr SIT	26	129.9 ± 47.2 (37.4–222.4)	192.2% (72%; <0.0001)	25	114.8 ± 50.9 (15–214.6)	158.4% (52%; 0.0007)			(<10%; >0.05)
After 3 yr SIT	26	167.3 ± 67.1 (35.8–298.8)	276.3% (79%; <0.0001)	25	140.4 ± 33.7 (74.4–206.5)	215.9% (94%; <0.0001)			(<10%; >0.05)
After 4 yr SIT	16	110.9 ± 24.3 (63.3–158.6)	149.6% (75%; 0.0027)	14	112.7 ± 77.9 (–40–265.4)	153.5% (37%; 0.0152)			(<10%; >0.05)
GMT: <i>Dactylis glomerata</i> -specific IgE levels (U/mL)									
Before SIT	72	47.7 ± 8.4 (31.3–64.1)	×	72	47.7 ± 8.4 (31.3–64.1)	×			×
After 1 yr SIT	26	50.1 ± 14.2 (22.3–77.9)	5% (<10%; 0.4141)	25	46.5 ± 18 (11.3–81.7)	–2.6% (<10%; >0.9999)			(<10%; >0.05)
After 2 yr SIT	26	42.6 ± 10.3 (22.4–62.7)	–10.8% (<10%; 0.7173)	25	24.7 ± 9.9 (5.3–44.2)	–48.1% (31%; 0.0512)			(23%; >0.05)
After 3 yr SIT	26	44.3 ± 11.7 (21.3–67.2)	–7.3% (<10%; 0.7023)	25	28.7 ± 12.1 (5–52.5)	–39.8% (22%; 0.1106)			(<10%; >0.05)
After 4 yr SIT	16	47.8 ± 15.8 (16.9–78.8)	0.2% (<10%; 0.7827)	14	21.9 ± 17 (–11.5–55.3)	–54% (23%; 0.0804)			(<20%; >0.05)
<i>Dactylis glomerata</i> SPT (mm)									
Before SIT	72	6.6 ± 0.3 (6.1–7.1)	×	72	6.6 ± 0.3 (6.1–7.1)	×			×
After 1 yr SIT	26	5.2 ± 0.4 (4.4–6)	21.6% (81%; 0.0013)	25	5.7 ± 0.4 (4.8–6.6)	13.6% (42%; 0.0474)			(<20%; >0.05)
After 2 yr SIT	26	4.7 ± 0.4 (3.9–5.6)	28.6% (96%; 0.0004)	25	4.9 ± 0.4 (4.1–5.6)	26.6% (94%; 0.0006)			(<10%; >0.05)
After 3 yr SIT	26	4 ± 0.3 (3.4–4.7)	38.8% (>99%; <0.0001)	25	4.2 ± 0.3 (3.6–4.8)	36.1% (>99%; <0.0001)			(<10%; >0.05)
After 4 yr SIT	16	3.9 ± 0.4 (2.9–4.8)	41.5% (>99%; <0.0001)	14	4.3 ± 0.5 (3.1–5.5)	34.7% (95%; 0.0005)			(<10%; >0.05)

Table 2 Continued

Time Point	No. Subjects	Oral Drops Administered Sublingually		No. Subjects	Oral Drops Administered Supralingually		Sublingually vs Supralingually (power; <i>p</i> value)
		Mean Value $\pm$ SE ($\pm$ 95% CI)	Improvement/Change* (power; <i>p</i> value)		Mean Value $\pm$ SE ($\pm$ 95% CI)	Improvement/Change* (Power; <i>p</i> value)	
Grass mixture I SPT (mm)							
Before SIT	72	7.2 $\pm$ 0.4 (6.4–8)	×	72	7.2 $\pm$ 0.4 (6.4–8)	×	×
After 1 yr SIT	26	5.4 $\pm$ 0.8 (3.9–7)	24.1% (56%; 0.0047)	25	6.5 $\pm$ 0.7 (5–8)	9.4% (<10%; 0.1736)	(<20%; >0.05)
After 2 yr SIT	26	4.6 $\pm$ 0.5 (3.6–5.6)	36.2% (94%; 0.0003)	25	5.2 $\pm$ 0.5 (4–6.3)	28% (75%; 0.0062)	(<10%; >0.05)
After 3 yr SIT	26	4.7 $\pm$ 0.3 (4.1–5.4)	33.9% (93%; 0.0002)	25	4.8 $\pm$ 0.5 (3.8–5.9)	32.9% (88%; 0.0013)	(<10%; >0.05)
After 4 yr SIT	16	4.5 $\pm$ 0.6 (3.2–5.8)	37.3% (95%; 0.0021)	14	4.5 $\pm$ 0.5 (3.4–5.6)	37.2% (79%; 0.0029)	(<10%; >0.05)

*Improvement/change of parameter related to the value before SIT.

#Combined symptom–medication score according to the World Allergy Organization recommendation.

GMT = geometric mean titre; SIT = specific immunotherapy; SPT = skin-prick test.

lingually and supralingually treated patients was not found ($p > 0.05$; power <10%; see Table 2 and Fig. 3). The nonsignificant differences between groups in both SPT wheals to *D. glomerata* and grass mixture I were evidenced by the plateau values generated by best-fit nonlinear regression (see Fig. 4).

IgE and IgG Antibodies

IgG levels specific to *D. glomerata* were increased in >90 and 70% of subjects in sublingual and supralingual SIT therapy of 3–4 years, respectively. The increase of IgG levels compared with the levels before treatment was very significant ($p < 0.001$) with the power of 79 and 94% in sublingually and supralingually treated patients, respectively (see Table 2 and Fig. 3). The IgG levels are probably dependent on the cumulative therapeutic dose and SIT duration and they are not affected by administration route (see Fig. 4).

In contrast with IgG levels, the IgE antibodies specific to *D. glomerata* did not show any changes related to SIT duration or cumulative dose. The statistical analysis shows the independence of this parameter in the way of the treatment.

Safety

During both trials, no serious allergen immunotherapy-related adverse effect was recorded. Local adverse reactions were documented in seven patients of both sublingual and supralingual groups during the SIT of 3–4 years duration. The local adverse effects consisted of undesirable taste; difficult swallowing; tongue, lips, or plicae sublinguales swelling; and mouth, tongue, or throat burning, and/or itching.

Systemic adverse effects were observed in 8 and 13 patients of sublingual and supralingual group, respectively. Recorded were rhinitis, painful breathing, cough, conjunctivitis, eyes or ear itching, sneezing, rash, abdominal pain, headache, heartburn, fatigue, and dyspepsia. The difference in number of patients with systemic reactions between both groups is not statistically significant ($p = 0.1599$; Fisher's exact test).

DISCUSSION

The significant decrease of clinical symptoms of grass pollen allergy characterized by nasal, ocular, and bronchial symptoms and reduction in rescue medication intake in patients treated by sublingual SIT was shown in many studies.^{15–18} This study shows that also long-term supralingual SIT is similarly effective. Treatment with placebo for 4 years may be controversial from the ethical point of view. That is why the previously used design of the study was applied—only the 1st year of treatment was placebo-controlled and during the following 3 years

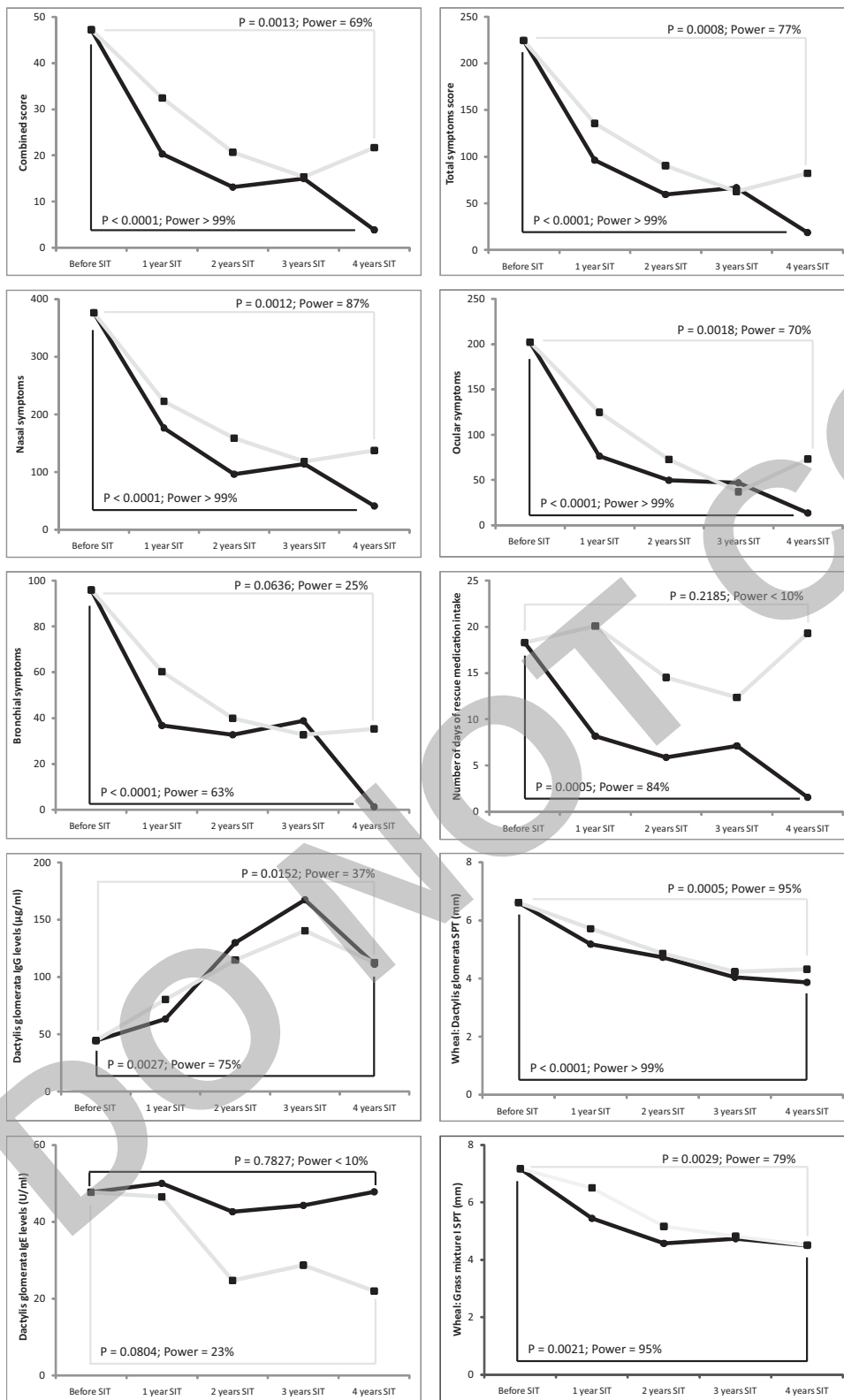


Figure 3. Primary and secondary end points and cumulative dose in dependence on the sublingual and supralingual specific immunotherapy (SIT) duration ("before SIT" = data from initial placebo groups; ●, oral drops administered sublingually [SIT]; ■, oral drops administered supralingually [SIT]).

both groups (former active treatment and former placebo) were treated with active substance. Nevertheless, the trends of symptom and medication scores in both groups are quite convincing. Moreover, changes in SPT reactivity support the efficacy of the treatment. After

long-term SIT, the significant reduction of SPT wheals related to reactions before treatment was recorded in most of the patients. The changes of SPT wheals conform to the decline of clinical symptoms depending on the cumulative therapeutic dose.

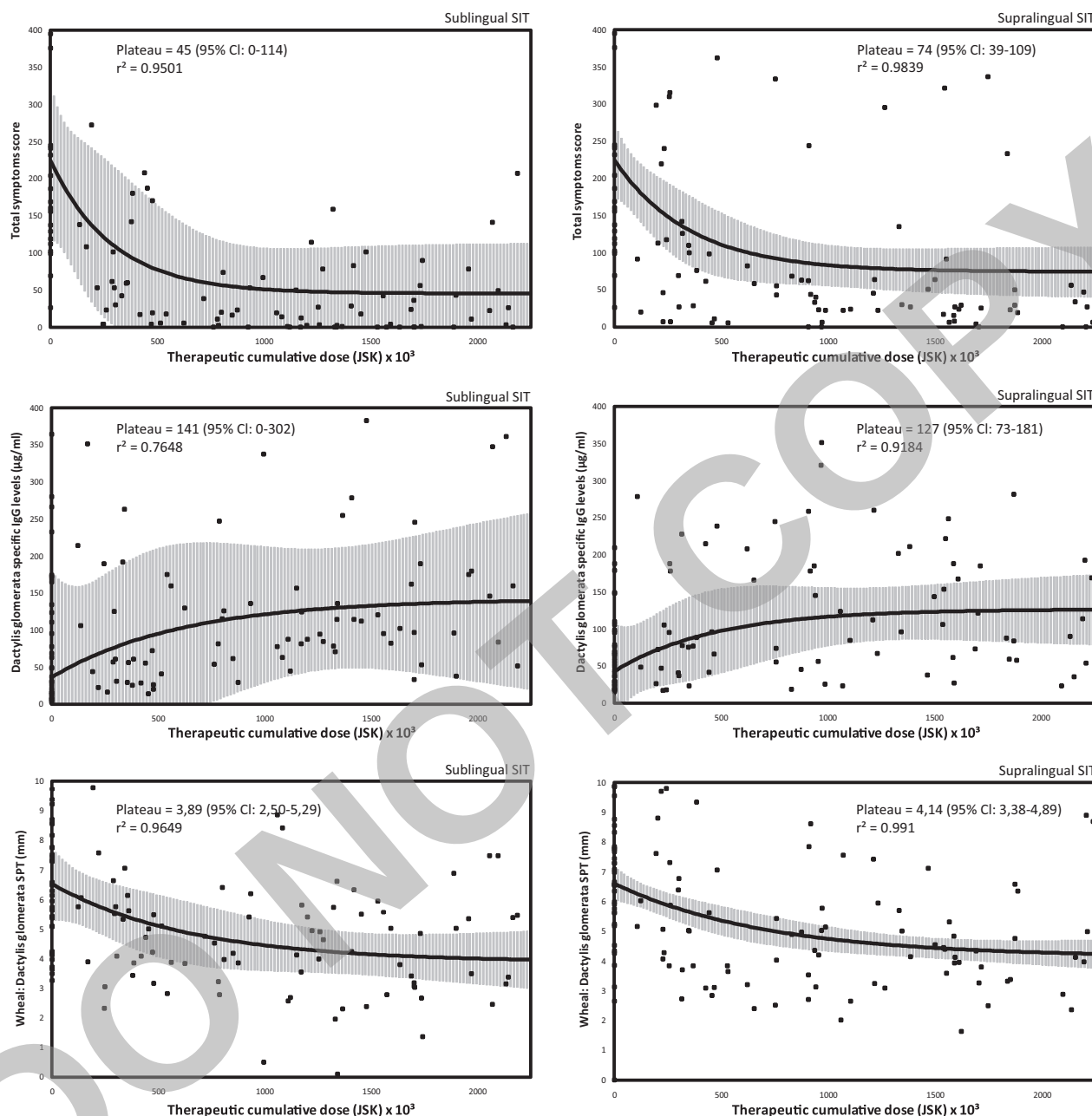


Figure 4. Nonlinear best-fit curves of parameters depending on therapeutic cumulative dose (including area of 95% confidential limits and measured data). ■, Measured data; —, nonlinear fit curve; □, $\pm 95\%$ confidential limit.

The SIT length and the cumulative therapeutic dose affect the increase of IgG specific to *D. glomerata*.^{19,20} On the contrary, the levels of IgE specific to *D. glomerata* remain invariant during all of the SIT periods with no significant changes.²¹

Statistical and nonlinear regression analyses did not show any significant difference between both allergen routes of administration (sublingual and supralingual) in primary and secondary study end points. A partial diversity of both therapies was recorded in the 1st years of treatment.⁴ Probably, faster onset of the clinical effect of sublingual treatment than that of the su-

pralingual one determined by earlier regression of especially nasal and ocular symptoms was found. Despite the lower means of followed parameters in sublingually treated group at the study end, the differences of means between the both groups are not significant. The sublingual administration of SIT could be likely considered to bring faster effects and to be individually a more suitable treatment than the supralingual one.⁴

The tolerance and safety of both routes of allergen administration are similar and very high. The documented local and systemic adverse effects correspond to the side reac-

tions reported in the leaflet of the product and they were expected. The incidence of adverse effects was lower in this study compared with the antecedent 1-year study. The reason for this finding was probably higher induced drug tolerance during the maintenance phase of the immunotherapy of the previous study in some of the patients.

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