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Double-blind, placebo-controlled evaluation of grass pollen specific immunotherapy with oral drops administered sublingually or supralingually

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Summary
Forty-one patients suffering from grass pollen allergy underwent specific immunotherapy with standardized allergen extract consisting of six grass pollens (H-Al per os) administered either sublingually or supralingually for one year. In order to investigate clinical and immunological changes induced by the administration of allergens via the oral mucosa, the double-blind, placebo-controlled, randomized design of the trial with 30 other patients enrolled in placebo groups was applied.

Specific immunotherapy with oral drops administered sublingually or supralingually was performed in the same way, keeping the drops under or on the tongue, respectively, for 1–2 min before swallowing them; at the end of the trial the cumulative dose of the allergen was almost 20 times higher than that of the subcutaneous therapy with corresponding allergen preparation. Data about symptoms scores and drugs intake during grass pollen season, as well as skin reactivity, levels of specific IgG and IgE antibodies, before the study and after the study's completion, were obtained.

It was found that both routes of administration are effective according to subjective clinical parameters and drug consumption, with a highly significant reduction of symptoms and drug intake favoring sublingual administration where a reduction of more than 60% was achieved. Only sublingual active group showed a significant increase in *Dactylis glomerata*-specific IgG serum levels. Adverse effects were limited to a small number of generally mild local and/or systemic reactions.

The results suggest that the administration of allergens via the oral mucosa is safe and clinically effective, favoring the sublingual rather than supralingual route.

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Introduction

Over the last 20 years, interest in non-injection routes for allergen-specific immunotherapy has increased, especially in Europe.[13,14] These routes (oral, nasal, bronchial) have the overall aim of improving both safety and compliance.[1] During the past years particularly the oral route has become a promising way for administration of SIT, sublingual route being the most evidenced one. In spite of this, various manufacturers of vaccines still recommend slightly different ways of oral administration.

Administration of specific immunotherapy with allergen extract ''H-Al per os'' produced by Sevapharma a.s., Prague, Czech Republic, is recommended either sublingually or supralingually in the same way, keeping the drops under or on the tongue, respectively, before swallowing them.

This randomized double-blind controlled trial was aimed at assessing the clinical efficacy and safety of specific immunotherapy to grass pollen in adults and children with sublingual or supralingual administration. The primary efficacy criteria were symptom score and rescue medication consumption during the grass pollen season. *Dactylis glomerata* pollen specific IgG and IgE serum levels and skin reactions were considered to be secondary parameters.[4,5,9–11]

Grass pollen extract (H-Al per os) was chosen because grass pollen is the most important allergen in the Czech Republic and it represents the causative agent in about 70–90% of allergic patients.[16]

Material and methods

Patients

Seventy four patients were enrolled in the trial. Three patients were withdrawn during treatment (before the grass pollen season) for inadequate cooperation. The clinical trial was finished by 71 patients (41 male, 30 female, aged 7–50 years; mean 19.5 years).

All patients had seasonal rhinitis and/or conjunctivitis (without asthma) caused by grass pollen, had experienced symptoms in at least the previous two years and none of the patients had previous treatment with SIT within the last five years.

The diagnosis of allergy to grass pollen was made by clinical history, positive skin tests to standardized pollen extract – Grass mixture I and *Dactylis glomerata* (Sevapharma a.s., Prague, Czech Republic) – and the presence of specific IgE to the mixture of grass pollens shown by the CAP System (Pharmacia Diagnostics AB, Uppsala, Sweden) with class 3 as a minimum value (at minimum 3.5 kU/l).

The exclusion criteria included sensitization (evidenced by skin prick tests) to major inhalant allergens coinciding with the grass pollen season – ash (*Fraxinus excelsior*), lime (*Tilia cordata*), elderberry (*Sambucus nigra*), dandelion (*Taraxacum officinale*), plantain (*Plantago lanceolata*) and nettle (*Urtica dioica*), systemic immunologic or metabolic disease, malignancies, major anatomic alterations of the upper airways, severe atopic dermatitis, chronic corticosteroid or beta-blocker treatment, pregnancy, chronic or recurrent inflammation of oral mucosa and other contraindications of SIT.

All the patients, or parents of the minors, were informed in detail about the experimental procedure and provided written informed consent.

Allergen preparations

The standardized allergen extract ''H-Al per os'' (Sevapharma a.s., Prague, Czech Republic) used throughout the study was 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 concentrations of the solutions were as follows: 1, 10, 100, 1000 and 10 000 JSK/ml, respectively.

Active principle is expressed in biologic units (JSK – jednotka standardní kvality – standard quality unit). The biologic activity is defined as follows: a prick test with an

Table 1 Characteristics of subjects eligible for analysis

	Active treatment		Placebo treatment	
	Sublingual	Supralingual	Sublingual	Supralingual
No. of subjects	20	21	15	15
Male/female	11/9	12/9	9/6	9/6
Age distribution (years)	17.4 ± 9.1	20.0 ± 10.4	24.4 ± 11.6	16.7 ± 8.9
Seasonal rhinitis in at least two previous grass seasons	20	21	15	15
Seasonal conjunctivitis in at least two previous grass seasons	15	14	9	10
Asthma during previous pollen seasons	0	0	0	0
Sensitizations other than grass and major inhalant coinciding allergens	10	14	12	11
Previous SIT interrupted for more than five years	0	0	0	0
Dactylis glomerata specific IgE before treatment >3.5 kU/l	20	21	15	15

extract containing 1000 JSK/ml produces a wheal with a mean diameter of 5.5 mm in a minimum of 20 patients sensitized to the allergen under consideration (definition of allergen producer). The maximal dose (10 drops of 10 000 JSK/ml) contains approximately 1.265 μg of the grass pollen major allergen Lol p I according to the producer.

Placebo preparations were identical to the active therapy in composition, appearance, presentation, taste, and color, but obviously contained no allergen.

Treatment

A scheme of increasing doses was used, with drops to be taken by the sublingual or supralingual route once daily, increasing from 1 to 10 drops for each vial until the maximal dose (10 drops of 10 000 JSK/ml) was reached.

The treatment was started in autumn and was followed by a maintenance therapy with 10 drops of 10 000 JSK/ml three times a week for one year.

The patients using sublingual therapy were instructed to keep the drops under the tongue for 1–2 min before swallowing them, while the patients using specific immunotherapy administered supralingually had to keep the drops on the tongue for 1–2 min before swallowing them.

Before the start of the grass pollen season (May 2004), the cumulative dose of allergen extract, received by each patient, was more than 350 000 JSK independently of the route of administration or on the preparation (allergen or placebo). The differences of the cumulative dose in all groups were not significant ($P = 0.69$).

According to the schedule, by the end of the trial (September 2004), an average total cumulative dose was more than 580 000 JSK. The difference among therapeutic groups was not significant ($P = 0.28$).

All patients were allowed to use, when necessary, the following drugs as rescue medication: levocabastine, cromoglycate, azelastine, budesonide, beclomethasone (local antiallergic medication), cetirizine or loratadine (oral antihistamines), prednisone and salbutamol (oral corticosteroid and inhaled beta 2-agonist).

Design of the study

This one-year clinical study was conducted according to the rules of Good Clinical Practice (GCP) and was approved by the Ethics Committee of the Czech Ministry of Health and State Institute for Drug Control. A multicenter, double-blind, randomized, placebo-controlled parallel design for both routes of administration (sublingually or supralingually) was used with no dummy arrangement. The trial was carried out in nine study centres.

Screening procedures prior to study inclusion/exclusion were conducted from May to August 2003. One-year specific allergen immunotherapy was performed from September 2003 to October 2004.[2,10]

Patients enrolled in the trial were randomized to receive either the active sublingual/supralingual therapy or placebo sublingual/supralingual therapy (see Table 1). Each suitable patient was assigned to the treated group due to the central randomization. The randomization key was generated by the GraphPad Software, Inc. 10855 Sorrento Valley Road 203 San Diego, CA 92121, USA.

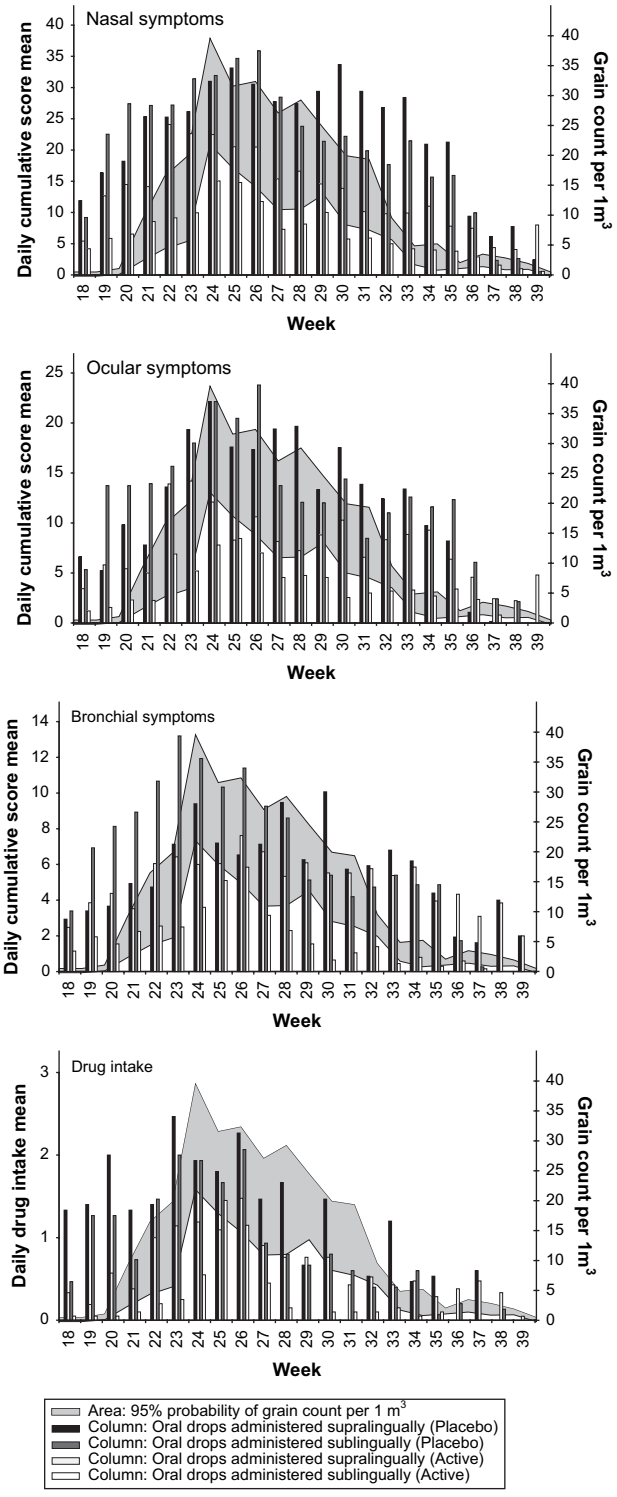

Figure 1 Daily cumulative score means of nasal, ocular and bronchial symptoms including daily drug intake mean in dependence of grass pollen season development (2004) in all treated groups.

Throughout the treatment, the patients had to record the following data in a diary: study treatment administration, rescue medication, adverse events and concomitant treatment. The patients also completed a daily record of

Table 2 Summary of cumulative scores of symptoms (nasal, ocular and bronchial) and mean frequency of rescue medication consumption in all treated groups for grass pollen season

	Oral drops administered sublingually		Oral drops administered supralingually	
	Active (20 subjects)	Placebo (15 subjects)	Active (21 subjects)	Placebo (15 subjects)
Total symptoms score				
Mean cumulative score of symptoms ± standard error (95%CI)	203.5 ± 49.34 (100.24—306.76)	611.07 ± 124.16 (344.74—877.39)	388.81 ± 67.59 (247.81—529.80)	624.33 ± 104.59 (399.99—848.67)
Improvement (%) — related to placebo group	66.7		37.7	
P (Mann—Whitney *U* test)	0.0029[a]	0.7130[b]	0.0891[a]	0.0285[c]
Power (%)	90[a]	3[b]	48[a]	57[c]
Nasal symptoms				
Mean cumulative score of symptoms ± standard error (95%CI)	111.35 ± 25.69 (57.57—165.13)	321.6 ± 54.54 (204.62—438.58)	204.71 ± 30.85 (140.37—269.06)	345.8 ± 49.31 (240.03—451.57)
Improvement (%) — related to placebo group	65.4		40.8	
P (Mann—Whitney *U* test)	0.0017[a]	0.7437[b]	0.0356[a]	0.0258[c]
Power (%)	95[a]	5[b]	70[a]	62[c]
Ocular symptoms				
Mean cumulative score of symptoms ± standard error (95%CI)	60.20 ± 18.17 (22.18—98.22)	185.67 ± 47.47 (83.84—287.49)	113.48 ± 23.01 (65.47—161.49)	194.00 ± 37.74 (113.05—274.95)
Improvement (%) — related to placebo group	67.6		41.5	
P (Mann—Whitney *U* test)	0.0130[a]	0.4937[b]	0.0832[a]	0.0951[c]
Power (%)	75[a]	3.5[b]	47[a]	42[c]
Bronchial symptoms				
Mean cumulative score of symptoms ± standard error (95%CI)	31.95 ± 10.66 (9.64—54.26)	103.80 ± 39.16 (19.80—187.80)	70.62 ± 21.75 (25.24—116.00)	84.53 ± 26.34 (28.04—141.02)
Improvement (%) — related to placebo group	69.2		16.5	
P (Mann—Whitney *U* test)	0.0299[a]	0.7400[b]	0.4800[a]	0.2184[c]
Power (%)	50[a]	6[b]	5[a]	33[c]
Rescue medication intake				
Mean frequency of drug intake ± standard error (95%CI)	4.60 ± 1.36 (1.75—7.45)	13.93 ± 4.60 (4.06—23.81)	10.33 ± 3.40 (3.24—17.42)	16.80 ± 4.74 (6.64—26.96)
Reduce (%) — related to placebo group	67.0		38.5	
P (Mann—Whitney *U* test)	0.0360[a]	0.6676[b]	0.2620[a]	0.1323[c]
Power (%)	56[a]	3[b]	20[a]	32[c]

[a] Active—Placebo at the same administration route.
[b] Placebo—Placebo.
[c] Active—Active.

the presence and severity of symptoms during the grass pollen season (May 2004—September 2004).

Before treatment initiation (September—October 2003) and at study completion (after one year of treatment), quantitative prick tests to Grass mixture I and to *Dactylis glomerata* were performed, and blood samples were collected and stored frozen for antibody assays at study completion.[4,5,9—11]

The primary endpoint of this study was defined by symptoms scores. In addition, the rescue medication intake score, skin prick tests and grass pollen specific antibodies IgE and IgG were considered as secondary outcomes of this trial.

Symptoms scores

Throughout the grass pollen season, rhinitis, conjunctivitis and bronchial symptoms were recorded daily by the patients. Records were based on four symptoms: rhinitis symptoms — sneezing, nasal itching, watery runny nose, nasal obstruction; conjunctivitis symptoms — ocular redness, ocular itching, tearing and ocular swelling; bronchial symptoms — cough, presence of mucus, breathlessness, wheezing.

Each symptom was rated on the following four-point scale: 0: no symptom; 1: mild complaints (signs or symptoms present but not disturbing); 2: moderate complaints (signs or symptoms disturbing); 3: severe complaints (very disturbing signs with impaired social or professional life, usual necessity of rescue medication).

A very rigorous condition how to assess the symptom score while using permitted rescue medication was set in this trial. Patients who used the rescue medication had to have assigned 3 points to at least one symptom. On the other hand, the patients could record a 3 point score without using the symptomatic medication.

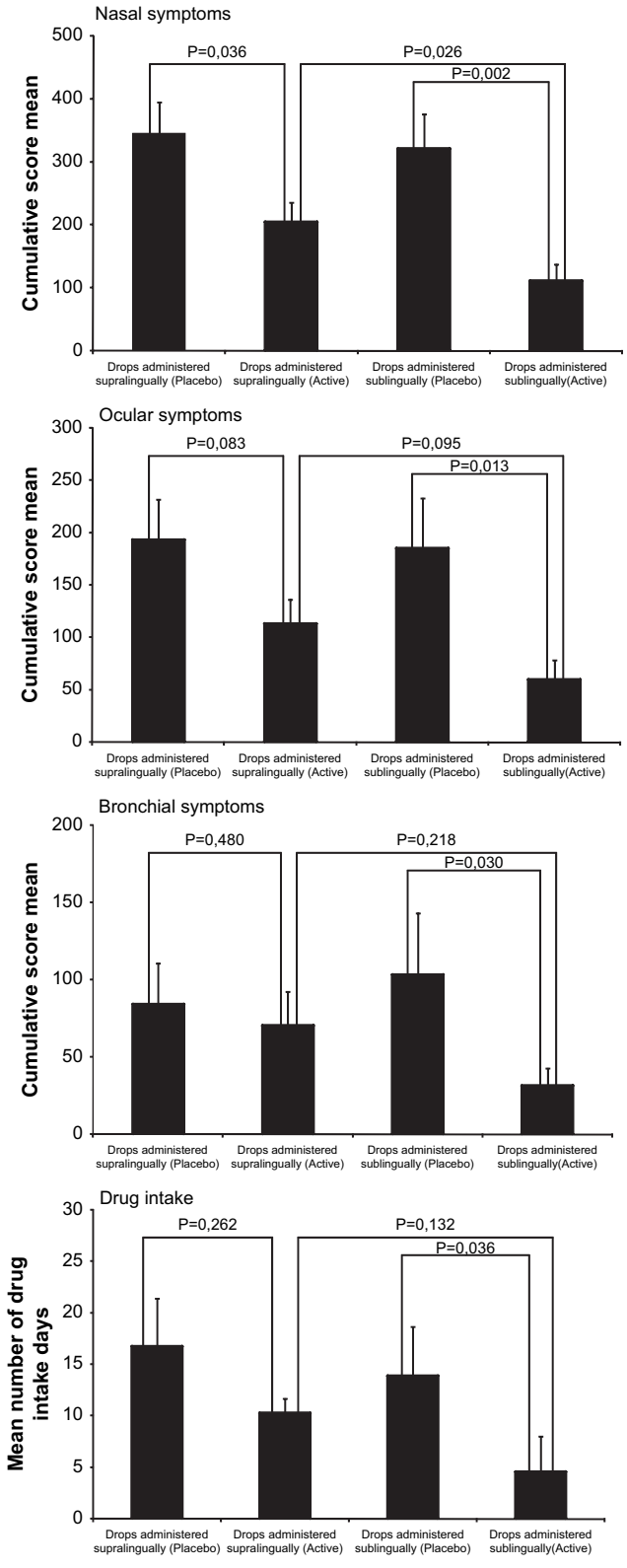

Figure 2 Cumulative scores of symptoms and mean number of drug intake days for grass pollen season in all treated groups.

Medication scores

Throughout the study, the patients were instructed to use symptomatic medications (rescue medication) if needed and to record the amounts used. The daily use of any permitted rescue medication was assessed by score 1. If subjects did not take any concomitant drug, the daily score was 0.

Skin prick tests

Quantitative prick tests were performed at inclusion and at treatment completion with Grass mixture I allergen extract at a concentration of 1000 JSK/ml and with *Dactylis glomerata* allergen extract at a concentration of 10, 100, 1000 and 10 000 JSK/ml. Prick tests were also performed with positive control and negative control.

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 (CRF). Wheal diameters were calculated from surfaces digitized by scanning.[5,8]

The least-squares method was used to calculate the following regression line equation: $y = a + b^{*}\log(c)$, where $y =$ diameter of the wheal, $a =$ intercept, $b =$ slope, $c =$ concentration. This regression line, which was the dose–response curve (concentration by wheal diameter), was used to calculate the theoretic wheal diameter obtained in response to a concentration of 1000 JSK/ml in each patient before and after treatment.

Specific IgE and IgG

Blood was taken at the inclusion of the patients and at the end of the study. Sera were frozen, collected and stored in the central site. The store temperature was less then $-30\,^{\circ}C$. Analyses were carried out by the central laboratory. *Dactylis glomerata*-specific IgE and IgG levels were determined by the CAP System (Pharmacia Diagnostics, Uppsala, Sweden) and the IgG ELISA Fooke test (Dr. FOOKE Laboratorien GmbH, Neus, Germany), respectively.

Pollen counts

The pollen season of the grasses in the Czech Republic starts at the beginning of May and lasts until the beginning of August (from the 21st week to the 32nd week). Pollen grain counts were recorded by the Czech Pollen Information Service (PIS).

Statistical methods

The investigated parameters might be non-normally distributed, and therefore the Mann–Whitney *U* test for intergroup comparison and the Wilcoxon test for intragroup comparisons were used when appropriate. All tests were two-tailed, and the level of significance was set at 0.05. All the statistical analyses have been performed with the biostatistics software StatMate (GraphPad, version 1.01i, 16/1/1998).

Results

Symptom scores and rescue medication

The daily cumulative score of nasal, ocular and bronchial symptoms including the daily frequency of symptomatic medication intake in the real grass pollen season (from the 21st week to the 32st week) are plotted in Fig. 1. The daily cumulative scores and daily drug intake mean in four treated groups (sublingual and supralingual active groups plus both placebo groups) coincide with the grass pollen peaks. Marked differences were evident in observed clinical symptoms and drug intake favoring both active treatments. These differences between both actives and both placebo groups are decreased with the grass grain count falling (roughly from the 32nd week).

The results, reported in Table 2 and Fig. 2, confirm the reduction of total symptoms occurrence and intensity of 38% and 67% in patients of the supralingual and sublingual active groups, respectively, related to patients of both placebo groups. The comparison between both active treated groups showed greater symptom reduction in the sublingual active group compared to the supralingual active group, with a significant difference ($P = 0.0258$) only in the clinical manifestation of nasal symptoms.

The consumption of medication (the necessity of using rescue medication) was lower by 67% ($P = 0.036$) in sublingually treated patients compared to the placebo group, the same consumption reached a reduction of only 38.5% in supralingually treated patients compared to the placebo group. The difference in this case was not significant ($P = 0.1323$).

Skin prick tests

The mean wheal diameter size in response to *Dactylis glomerata* or Grass mixture I at a concentration of 1000 JSK/ml, predicted by the dose–response curve, was not significantly different among any of the active or placebo groups at baseline or after treatment completion. The summary of results is reported in Table 3.

Nevertheless, a decrease in wheal diameter was seen not only in both active treatment groups but also in the placebo groups. In the sublingual active group, there was no significant difference in variations from baseline to treatment completion for both allergens (*Dactylis glomerata* and Grass mixture I) but in the supralingual active group, there was a significant difference in mean wheal to *Dactylis glomerata* ($P = 0.01$) before and after treatment completion.

Table 3 Summary of *Dactylis glomerata* and grass mixture I skin prick test (SPT) results in all treated groups

	Oral drops administered sublingually		Oral drops administered supralingually	
	Active	Placebo	Active	Placebo
Dactylis glomerata SPT (mm)				
Mean wheal diameter ± standard error (95%CI)				
Before treatment	5.1 ± 0.38 (4.3–5.89)	5.34 ± 0.45 (4.38–6.3)	5.4 ± 0.33 (4.71–6.09)	4.91 ± 0.35 (4.17–5.66)
After one-year treatment	4.29 ± 0.5 (3.24–5.35)	4.28 ± 0.51 (3.2–5.37)	4.09 ± 0.36 (3.33–4.85)	4.48 ± 0.56 (3.27–5.69)
Change wheal diameter (mm) – related to diameter before treatment	−0.8 ± 0.43 (−1.7–0.1)	−1.06 ± 0.63 (−2.41–0.3)	−1.31 ± 0.42 (−2.19–0.43)	−0.44 ± 0.59 (−1.71–0.83)
Wheal diameter decrease: number of patients (%)	13 (65%)	10 (67%)	17 (81%)	10 (67%)
Wheal diameter increase: number of patients (%)	7 (35%)	5 (33%)	4 (19%)	5 (33%)
P (Wilcoxon matched-pairs test) – intragroup before–after	0.1054	0.0946	0.0101	0.5245
P (Mann–Whitney Test) – intergroup change wheal diameter	0.9867		0.3521	
Grass mixture I SPT (mm)				
Mean wheal diameter ± standard error (95%CI)				
Before treatment	7.33 ± 0.52 (6.24–8.42)	7.68 ± 0.69 (6.19–9.16)	7.8 ± 0.75 (6.25–9.36)	8.12 ± 0.92 (6.14–10.1)
After one-year treatment	6.37 ± 0.95 (4.39–8.36)	6.5 ± 0.87 (4.63–8.36)	6.36 ± 0.75 (4.79–7.93)	7.09 ± 1.08 (4.78–9.4)
Change wheal diameter (%) – related to diameter before treatment	−0.96 ± 0.83 (−2.69–0.77)	−1.18 ± 0.59 (−2.45–0.1)	−1.45 ± 0.71 (−2.93–0.04)	−1.03 ± 1.08 (−3.35–1.29)
Wheal diameter decrease: number of patients (%)	14 (70%)	11 (73%)	15 (71%)	11 (73%)
Wheal diameter increase: number of patients (%)	6 (30%)	4 (27%)	6 (29%)	4 (27%)
P (Wilcoxon matched-pairs test) – intragroup before–after	0.1769	0.0554	0.0595	0.2078
P (Mann–Whitney Test) – intergroup change wheal diameter	0.9336		>0.9999	

There was no correlation between skin prick tests and symptoms and/or drug intake scores within both active groups of patients and placebo groups similarly as elsewhere.[4,5,10]

IgE and IgG antibodies

Increase of IgE after one year of treatment was not significant in any treated group. No significant variation of specific *Dactylis glomerata* IgE was detected between the active and placebo groups before or after treatment completion.

Table 4 shows a significant increase ($P = 0.02$) in *Dactylis glomerata* specific IgG serum levels in the sublingual active group related to the corresponding placebo group after one year of treatment. The IgG level increase was achieved in 85% of the patients of the sublingual active group.

Safety

During the trial, no serious allergen immunotherapy-related adverse effect was observed.

Local adverse effects were documented in three patients of the sublingual active group (11 events) and in five patients of the supralingual active group (22 events), whereas only together in three patients from both placebo groups 5 events were found. The local adverse effects consisted of undesirable taste, difficulty in swallowing, tongue or lips swelling, burning of the lips or mouth, itching of the tongue, throat or mouth.

Systemic adverse effects were observed only in patients of both active groups except for one case reported in one patient of the placebo group. Nine patients from the supralingual and six patients from the sublingual active group reported 33 events and 34 events of systemic adverse effects, respectively. These were represented by rhinitis, painful or difficult breathing, breathlessness, cough, conjunctivitis, sneezing, ear itching, abdominal pain, headache, heartburn, nausea and fatigue.

The number of patients with at least one local adverse effect was not significantly different between the both active and placebo groups (Fisher's exact test). On the other hand, the number of patients with at least one systemic adverse effect was significantly higher in both active groups than in the placebo groups ($P = 0.027$ and $P = 0.0245$ for the group with sublingual and supralingual SIT, respectively). Occurrence of these systemic effects was in both active group with no significant difference.

The documented local and systemic adverse effects correspond to the side reactions reported in the leaflet

Table 4 Summary of *Dactylis glomerata* specific IgG and IgE results in all treated groups

	Oral drops administered sublingually		Oral drops administered supralingually	
	Active	Placebo	Active	Placebo
Dactylis glomerata specific IgG (μg/ml) *GMT ± standard error (95%CI)*				
Before treatment	27.13 ± 17.46 (15.38—88.47)	43.62 ± 16.77 (43.26—115.22)	56.97 ± 22.79 (44.06—139.16)	53.96 ± 12.01 (44.05—95.59)
After one-year treatment	47.82 ± 13.68 (38.64—95.88)	51.72 ± 25.39 (38.5—147.43)	67.67 ± 21.49 (55.25—144.93)	46.64 ± 8.77 (37.93—75.56)
Mean change of IgG (%) — related to IgG before treatment	138.8 ± 46.85 (40.74—236.85)	63.09 ± 40.84 (−24.52—150.7)	74.12 ± 40.8 (−11—159.24)	7.88 ± 22.73 (−40.89—56.65)
IgG decrease: number of patients (%)	3 (15%)	6 (40%)	9 (43%)	9 (60%)
IgG increase: number of patients (%)	17 (85%)	9 (60%)	12 (57%)	6 (40%)
P (Wilcoxon matched-pairs test) — intragroup before—after	0.0240	0.5614	0.3038	0.2078
P (Mann—Whitney Test) — intergroup change IgG	0.0620		0.1581	
Dactylis glomerata specific IgE (U/ml) *GMT ± standard error (95%CI)*				
Before treatment	57.04 ± 19.8 (59.29—142.18)	26.71 ± 13.65 (23.77—82.33)	47.21 ± 14.79 (46.55—108.24)	36.63 ± 13.27 (31.98—88.9)
After one-year treatment	57.69 ± 17.63 (62.44—136.24)	39.06 ± 17.94 (43.83—120.79)	53.48 ± 20.81 (48—134.82)	38.14 ± 14.32 (31,44—92.89)
Mean change of IgE (%) — related to IgE before treatment	39.15 ± 19.58 (−1.83—80.13)	555.41 ± 425.05 (−356.31—1467.14)	40.31 ± 18.2 (2.35—78.28)	27.19 ± 23.08 (−22.33—76.7)
IgE decrease: number of patients (%)	6 (30%)	3 (20%)	8 (38%)	8 (53%)
IgE increase: number of patients (%)	14 (70%)	12 (80%)	13 (62%)	7 (47%)
P (Wilcoxon matched-pairs test) — intragroup before—after	0.3683	0.0637	0.1373	0.8469
P (Mann—Whitney Test) — intergroup change IgE	0.1994		0.4042	

Table 5 Summary of adverse effects

	Supralingual active group		Sublingual active group		Supralingual placebo groups		Sublingual placebo groups	
	Cases	Patients	Cases	Patients	Cases	Patients	Cases	Patients
Local adverse effects								
Undesirable taste	0	0	0	0	2	1	0	0
Difficult swallowing	2	1	0	0	0	0	0	0
Tongue swelling	1	1	3	1	0	0	0	0
Plicae sublinguales swelling	0	0	3	1	0	0	0	0
Lips swelling	2	1	0	0	0	0	0	0
Lips burning	3	1	0	0	0	0	0	0
Mouth burning	0	0	0	0	1	1	0	0
Tongue burning	0	0	1	1	0	0	0	0
Throat burning	1	1	0	0	0	0	0	0
tongue itching	5	2	0	0	0	0	0	0
Throat itching	2	2	0	0	0	0	0	0
Mouth itching	6	3	4	2	1	1	1	1
Total local AE	22	5	11	3	4	3	1	1
Systemic adverse effects								
Rhinitis	7	3	19	4	0	0	0	0
Painful breathing	1	1	0	0	0	0	0	0
Difficult breathing	0	0	4	2	0	0	0	0
Breathlessness	0	0	1	1	0	0	0	0
Cough	1	1	1	1	0	0	0	0
Conjunctivitis	10	4	7	3	0	0	0	0
Sneezing	1	1	0	0	0	0	0	0
Ear itching	5	1	0	0	0	0	0	0
Abdominal pain	0	0	0	0	1	1	0	0
Headache	4	2	1	1	0	0	0	0
Heartburn	1	1	0	0	0	0	0	0
Nausea	3	1	0	0	0	0	0	0
Fatigue	0	0	1	1	0	0	0	0
Total systemic AE	33	9	34	6	1	1	0	0

of the product and they were expected. Only one special local effect, swelling of sublingual ridges (plicae sublinguales) was observed in one patient of the sublingually treated group. In three of the patients of the sublingual group, respiratory difficulties were represented by morning breathlessness and difficult or painful breathing. Because of the short and temporary duration of these difficulties, allergen immunotherapy was not discontinued. The adverse events that occurred in the trial are presented in Table 5.

In active groups, 0.32% of local and 1.00% of systemic adverse effects were registered from a number of 3392 sublingually administered doses and 0.57% of local and 0.86% systemic adverse effects of 3856 supralingually administered doses.

Discussion

This multicentric study compared a six grass pollen allergen extract administered sublingually or supralingually as oral drops with placebo. The number of patients evaluated was sufficiently large and the study population was well defined, with the baseline characteristics of both treatment groups and placebo groups being similar both at the onset and upon completion of the study.

The results of this trial support the evidence that a slow absorption and processing of the locally retained allergen occurs through the local oral immune system.[2] In addition, the allergen absorption seems to be considerably higher for allergens placed under the tongue (sublingual administration) rather than on the tongue (supralingual administration).

Clinical improvement assessed by means of symptom scores and rescue medication consumption supports the statement mentioned above because the results were highly significant in the sublingually treated patients vs. the placebo group, whereas in supralingually treated patients, there was a significant difference only in the nasal symptom score compared to placebo-treated patients. Moreover, the symptom scores and drug consumption were almost twice as high in patients with supralingually administered oral drops as in patients of the sublingual active group.

Also the percentage of increase of *Dactylis glomerata* specific IgG levels achieved in sublingually treated patients

(138.8%) in comparison to the supralingual active (74.1%) and placebo groups (63.1% and 7.9%) corresponds to this reasoning.

Moreover, symptom scores and drug intake, especially during the peak of the grass pollen season, showed a significant difference with reduction of over 60% in patients treated with the sublingual active therapy. It can be supposed that the unambiguous significance was achieved due to the high cumulative dose[5,8,10] and rule of scoring in diary: Patients received allergen doses (cumulative dose of about 600 000 JSK in one year of immunotherapy) almost 20 times higher than those generally administered by the parenteral route using the corresponding allergenic preparation. Rule of symptom scoring requires at least one symptom with 3 points score to allow use of rescue medication. These results are not inconsistent with the results reported elsewhere in the literature.[3–7,11,12,15]

The significant *Dactylis glomerata* specific IgG level increase observed in this study and in the literature[7,12] provides evidence that sublingual immunotherapy has an effect on the immune system. Because of the influence of two factors, the effect of specific immunotherapy and the exposure to grass pollen,[5] the increase in specific IgE was greater in both placebo groups than in both active groups whereas the increase of specific IgG was greater in the sublingual active group than in the supralingual and placebo groups. These data should be interpreted with respect to the mild grass pollen season (daily mean of pollen count was 16.9 grain/m^3).

Most of the adverse events were mild local reactions concerning the oral cavity that did not require special treatment. All the active treated patients showed a very good tolerance of the immunotherapy, and the safety of the sublingual and supralingual routes was also associated with good clinical efficacy in favor of sublingual treatment.

In conclusion, this trial demonstrated that both of these routes of administration are safe and provide clinical benefits including significant improvements in rhinitis, conjunctivitis and eventually bronchial symptoms on reduction of drug intake in favor of sublingual immunotherapy.

Conflicts of interest

The authors have no conflict of interest.

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