Multiple daily administrations of low-dose sublingual immunotherapy in allergic rhinoconjunctivitis

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Background: Sublingual immunotherapy (SLIT) is an efficacious treatment for allergic rhinoconjunctivitis.

Objective: To investigate whether the number of daily administrations of SLIT can affect its efficacy.

Methods: In an open study, 64 patients with allergic seasonal rhinoconjunctivitis to grass or birch pollens were assigned to the following 2-year daily treatment schedules: "3–3" group, 1 drop 3 times daily for 2 years; "2–3" group, 1 drop twice daily in year 1 and 1 drop 3 times daily in year 2; "1–3" group, 1 drop once daily in year 1 and 1 drop 3 times daily in year 2; and control group, no treatment. One fifth of the allergen concentration recommended by the manufacturer as maintenance treatment was used throughout the study. Patients were monitored for skin reactivity to the allergen used for SLIT using an end point dilution technique and for drug use.

Results: No treatment-related adverse effects were observed. Skin reactivity to allergen decreased compared with controls in the first treatment year only in the "3–3" group and in all treated patients in year 2. Drug use decreased in the first treatment year in the "3–3" and "2–3" groups vs controls. This outcome extended to "1–3" patients in treatment year 2. Antihistamine use decreased significantly compared with baseline in year 1 in "3–3" and "2–3" patients and in all treated patients in year 2. No changes were observed in controls.

Conclusion: The number of daily administrations seems to correlate with the efficacy of SLIT.

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INTRODUCTION

Sublingual immunotherapy (SLIT) is a safe and efficacious treatment for allergic rhinoconjunctivitis.[1,2] The efficacy of SLIT has been reported with a wide range of treatment doses, 5 to 375 times those used for subcutaneous immunotherapy (SCIT).[3,4] It is unclear whether a dose-response relationship exists with efficacy, although at least 1 study[5] directly suggests that this is indeed the case. Furthermore, different weekly schedules are still used (3 times a week, every other day, and so on).[6] It was previously observed that patients treated according to a daily allergen administration schedule, despite a lower cumulative dose, showed a higher reduction in drug intake than patients treated with 3 weekly allergen administrations.[7] These data suggest that the frequency of SLIT administration could be crucial to its efficacy and possibly more so than the absolute amount of the administered dose. To evaluate the relevance of this assumption, we used markedly low-dose SLIT to evaluate whether this treatment is still efficacious when administered in protocols implying different numbers of daily dosages. We found a striking correlation between the number of daily dosages and both the clinical efficacy and the reduction in skin reactivity to allergen after only 1 year of SLIT. These results were consolidated in the second treatment year. These data suggest that caution should be maintained in attributing a critical role to SLIT doses to achieve clinical efficacy and that dedicated studies should instead be focused on the effect of the frequency of immunotherapy administration.

MATERIALS AND METHODS

Study Design

We conducted a 3-year open study of 64 patients (age range, 4–50 years) with allergic seasonal rhinoconjunctivitis to grass or birch pollens. Patients underwent an accurate anamnesis and clinical examination, including rhinologic examination with the evaluation of anatomical problems (such as nasal septum deviation), the nature of symptoms (rhinorrhea, nasal obstruction, associated ocular problems, cough, or asthma), and the seasonality, if any, of each symptom. A daily immunotherapy schedule was given, which was started before the pollen season in the first treatment year (in December 2000 and February 2001 for patients allergic to birch and grass, respectively) without a build-up phase. Immunotherapeutic treatment lasted 2 years.

Patient Enrollment

Patients were enrolled as volunteers after having been informed in full detail about the methods and aims of the trial. Patients or their parents signed an informed consent form. The procedures followed were in accordance with the ethical standards of the responsible institutional committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983.

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The study inclusion criteria were as follows: allergic seasonal rhinoconjunctivitis (without asthma) for at least 2 years; sensitization limited to either grass or birch pollens, as evaluated by means of skin prick testing and serum specific IgE measurement; living in northern Italy; and only partial control of allergic symptoms, never complete and satisfactory to the patient, with the use of antihistamines (either cetirizine or loratadine). The study exclusion criteria were as follows: asthma or nasal polyps or obstructive nasal septum deviation; a clinical history of significant symptomatic perennial allergic rhinitis or asthma caused by an allergen to which the patient was regularly exposed; a clinical history of significant recurrent acute sinusitis (defined as 2 episodes per year for the past 2 years, all of which required antibiotic drug treatment) or chronic sinusitis or chronic obstructive lung disease; and, at randomization, current symptoms of, or treatment for, upper respiratory tract infection, acute sinusitis, acute otitis media, or other relevant infectious processes.

Patients who met the study criteria and who were willing to participate in the study were consecutively assigned to 1 of 3 treatment schedules: "3–3" group (n = 18), 1 drop 3 times daily for 2 years; "2–3" group (n = 18), 1 drop twice daily in year 1 and 1 drop 3 times daily in year 2; and "1–3" group (n = 18), 1 drop once daily in year 1 and 1 drop 3 times daily in year 2. Patients who met the inclusion criteria but who did not want to perform immunotherapy served as untreated controls (n = 10). Patients were not informed that different numbers of drops were prescribed to different patients to prevent the possibility that "the more the better" hypothesis could affect the individual perception of the need for antihistamines. Patient characteristics are summarized in Table 1.

Skin Testing and Allergen Extracts Used for Diagnosis

The skin response to the sensitizing allergen was quantified using skin prick tests with mixed grasses (*Poa pratensis, Phleum pratense, Dactylis glomerata, Festuca pratensis,* and *Lolium perenne*) and mixed Betulaceae (*Betula alba, Alnus glutinosa,* and *Corylus avellana*) commercial extracts (ALK Abellò, Milan, Italy) by means of an end point dilution technique. Each allergen extract was prepared in a saline solution containing glycerol, 50% vol/vol, and phenol, 0.4% wt/vol, and was preliminarily used undiluted to evaluate the monosensitization of the patient. Then, the previously described end point technique was used.[7] Briefly, this method implied 12 consecutive half dilutions in a saline solution of the diagnostic extract prepared in sterile solution immediately before the assay. Saline solutions and histamine hydrochloride (ALK-Abellò) were used as negative and positive controls, respectively. All the skin tests were performed by the same individual (V.B.).

To avoid any interference due to exposure to the relevant environmental allergen, the skin end point test was performed outside the pollination period of the sensitizing species (ie, in February and December for patients allergic to grass and trees, respectively). The test was performed at the beginning of the first (observational) year to evaluate baseline reactivity and was repeated in the same months after the symptomatic seasons of each treatment year. Positivity was expressed as the highest dilution yielding a wheal diameter greater than 3 mm. Using this end point dilution skin test, all patients included in the study scored positive at the 1:64 dilution (or above) at baseline.

Immunotherapy Extract

In each treatment group, mixed birch or grass allergen extracts (ALK Abellò) were used at one fifth of the concentration recommended by the manufacturer as maintenance therapy (ie, 200 standard treatment units/mL). For each administration, a single drop of the extract at this concentration was used 1, 2, or 3 times a day, according to the experimental group. In the conventional immunotherapy protocol, this dose would have to be scaled up to 5 drops and followed by a further step to a dosage 5 times higher to reach maintenance. The timing of immunotherapy administration was as follows: (1) 8 AM for the once daily schedule (the first year of treatment in the "1–3" group), (2) 8 AM and 8 PM for the twice-daily schedule (the first year of treatment in the "2–3" group), and (3) 8 AM, 2 PM, and 10 PM for the 3 times daily schedule (the first year of treatment in the "3–3" group and the second year treatment in all the treated groups).

Drug Use Score

As stated, at enrollment, all patients had a history of at least 2 years of allergic symptoms, which required exclusively the administration of oral antihistamines. Drug use was quantified as the number of days during which the antihistamine (cetirizine or loratadine) was taken in each symptomatic season. In the geographic area where patients were living, the average duration of the allergic season is approximately 90 days for birch and grass pollens.

Table 1. Characteristics of 64 Patients With Allergic Seasonal Rhinoconjunctivitis to Grass or Birch Pollens*

Group	Patients, No.	Sex, M/F, No.	Age, mean (range), y	Allergic patients, No.	
				Grasses	Trees
"1–3"	18	10/8	18.5 (7–32)	10	8
"2–3"	18	10/8	19.1 (4–50)	12	6
"3–3"	18	10/8	18.8 (7–33)	13	5
Controls	10	5/5	18.8 (6–33)	7	3
Total	**64**	**35/29**	18.8 (5–40)	**42**	**22**

* See the "Patient Enrollment" subsection for definitions of the treatment groups.

Statistical Analysis

Statistical analysis was performed by means of nonparametric tests (the Wilcoxon test for intragroup comparison and the Mann-Whitney test for intergroup comparison) because none of the examined data could be considered for normal distribution either directly or following common mathematical transformations. The statistical analysis was performed using a software program (GraphPad; GraphPad Software, San Diego, CA). $P \leq .05$ was considered statistically significant.

RESULTS

Adverse Effects

No SLIT-related adverse effects were observed in any of the treated groups. Although no build-up phase was performed at the beginning of the study, neither local (oral) adverse effects nor systemic symptoms (rhinoconjunctivitis, asthma, urticaria, or pruritus) were observed. No dropouts were observed.

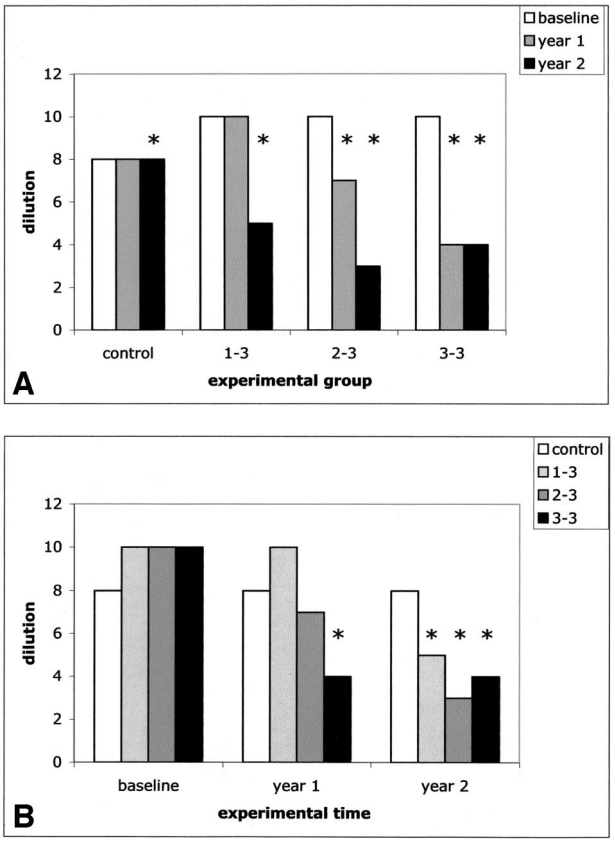

Figure 1. Median values of skin reactivity at the 3 considered experimental times. The median values of the reverse of dilution yielding wheals with a diameter greater than 3 mm are indicated for representative purposes. A, The intragroup analysis shows the modification across time of skin reactivity in each experimental group. B, The intergroup analysis shows the comparison at each experimental time of skin reactivity in the 3 experimental groups. Asterisks indicate the significant differences of the considered values compared with baseline values of the same experimental group (A) or compared with time-matched values of controls (B).

Skin Test Reactivity

In the intragroup analysis, skin reactivity significantly decreased compared with baseline in only "3–3" and "2–3" patients after the first treatment year ($P < .001$ and $P = .002$, respectively) and in all treated patients after the second treatment year ($P < .001$) (Fig 1A and Table 2). In contrast, in the control group, skin reactivity did not change in the first year and increased compared with baseline in the second year of follow-up ($P = .03$). In the intergroup analysis, values of skin reactivity to each sensitizing allergen were homogeneous in the 4 experimental groups at baseline (Fig 1B). After 1 year of treatment, skin reactivity decreased compared with controls in the "3–3" group ($P < .001$) but not in the "2–3" and "1–3" groups. After 2 years of treatment, skin reactivity decreased compared with controls in all the treated groups ($P < .001$) (Fig 1B and Table 2).

Comparing skin reactivity in the "1–3" group vs the "2–3" group, the "1–3" group vs the "3–3" group, and the "2–3" group vs the "3–3" group at baseline, no differences were obtained, indicating a homogeneous level of allergen-specific sensitization at the recruitment of patients. At year 1, "2–3" and "3–3" patients reached a lower level of sensitization compared with "1–3" patients ($P < .001$). Furthermore, at year 1, "3–3" patients lowered their sensitization compared with "2–3" patients ($P < .001$). At year 2, "2–3" and "3–3" patients reached a lower level of sensitization compared with "1–3" patients ($P < .001$), whereas no difference occurred between "2–3" and "3–3" patients.

Drug Use

In the intragroup analysis, antihistamine use decreased significantly compared with baseline in the first treatment year only in the "3–3" and "2–3" groups but not in "1–3" patients ($P < .001$) and in all treated patients in the second treatment year ($P < .001$). No changes were observed in controls (Fig 2A and Table 2). In the intergroup analysis, at baseline, treated patients were similar to controls regarding antihistamine use (Fig 2B and Table 2). In contrast, in the first

Table 2. Skin Reactivity and Drug Use in the Experimental Groups*

Group	Value, median (IQR)		
	Baseline	Year 1	Year 2
Skin reactivity (reverse of dilution)			
Controls	8 (6.5–8)	8 (8–8)	8 (8–10)
"1–3"	10 (8–10)	10 (8–10)	5 (4–6)
"2–3"	10 (8–11.5)	7 (6–8)	3 (2–4)
"3–3"	10 (10–10)	4 (4–6)	4 (2–4)
Drug use (days with antihistamine therapy)			
Controls	60 (45–60)	60 (45–60)	60 (60–60)
"1–3"	60 (60–90)	60 (40–87.5)	20 (10–20)
"2–3"	60 (40–82.5)	30 (20–55)	10 (2.5–10)
"3–3"	50 (40–60)	20 (10–30)	0 (0–7.5)

Abbreviation: IQR, interquartile range.

* See the "Patient Enrollment" subsection for definitions of the treatment groups.

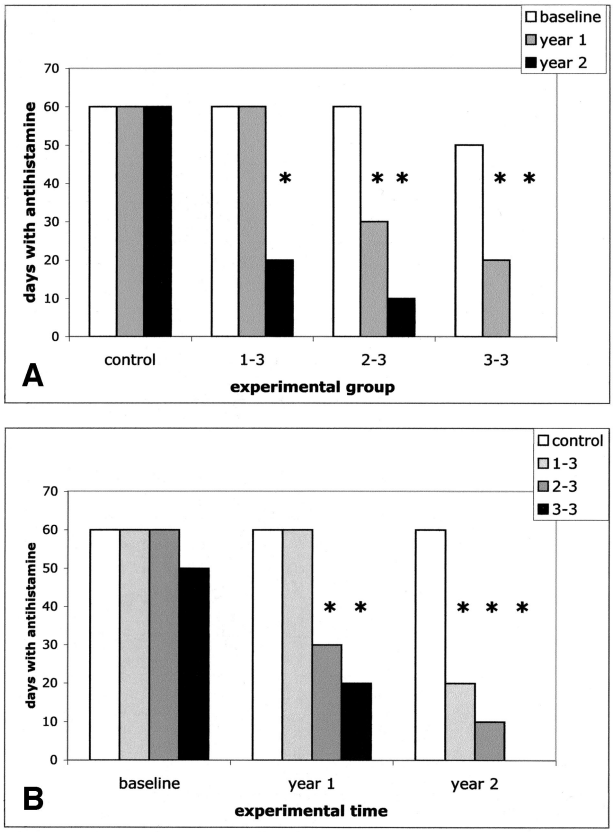

Figure 2. Median values of antihistamine use at the 3 considered experimental times. The number of days with antihistamine use is indicated. A, The intragroup analysis shows the modification across time of drug use in each experimental group. B, The intergroup analysis shows the comparison at each experimental time of drug use in the 3 experimental groups. Asterisks indicate the significant differences of the considered values compared with baseline values of the same experimental group (A) or compared with time-matched values of controls (B).

treatment year, "3–3" and "2–3" patients reached lower drug use compared with controls ($P < .001$ and $P < .02$, respectively). The reduction in drug use compared with controls extended to "1–3" patients in the second treatment year ($P < .001$) (Fig 2B and Table 2).

Comparing the use of drugs in the "1–3" group vs the "2–3" group, the "1–3" group vs the "3–3" group, and the "2–3" group vs the "3–3" group at baseline, no differences were observed, indicating a homogeneous level of clinical severity at the recruitment of patients. At year 1, "2–3" and "3–3" patients decreased drug use compared with "1–3" patients ($P < .001$). Furthermore, at year 1, "3–3" patients had lower drug use compared with "2–3" patients ($P < .002$). At year 2, "2–3" and "3–3" patients had lower drug use compared with "1–3" patients ($P < .003$ and $P < .001$, respectively). Furthermore, at year 2, "3–3" patients used fewer drugs than "3–3" patients ($P < .04$).

DISCUSSION

Sublingual immunotherapy is a safe and effective alternative to injection immunotherapy for respiratory allergic diseases.[8–16] A rigorous meta-analysis[1] of evidence-based studies has recently established this conclusion. Herein, we confirm the efficacy of SLIT, which induced the reduction of 2 end points (skin reactivity to allergen and antihistamine use) in monosensitized patients with seasonal allergic rhinoconjunctivitis.

We found a direct correlation between the improvement of end points and the number of daily administrations. To make it unlikely that the slightly different allergen doses related to the different number of daily dispensations could explain the results, we administered an amount of allergen approximately one fiftieth of that recommended by the manufacturer (namely, 2%, 4%, and 6% of the daily recommended dose in the first treatment year of "1–3," "2–3," and "3–3" patients, respectively). As a whole, the administered dose corresponded to 0.56, 0.37, and 0.18 times the cumulative monthly SCIT dose in the "3–3," "2–3," and "1–3" groups, respectively. The SLIT/SCIT ratio of dosages is an imperfect way to express the allergen dose, which should be accepted with some caution until the content in recombinant allergen is provided for all available extracts. However, these figures are far below the lower value of the wide dose range (5–375 times), which seemed efficacious in a meta-analysis[3] of evidenced-based SLIT studies with schedules implying single daily administrations. Indeed, in a consensus document from the Allergic Rhinitis and Its Impact on Asthma group, efficacy was attributed to even higher SLIT doses, in the 50 to 100 SLIT/SCIT ratio, or above.[17] In contrast to these arguments, with the extremely low doses we used, the impact of SLIT on skin reactivity to the relevant allergen and on the drug use scores was critically dependent on the number of daily assumptions. Indeed, despite these remarkably low doses, a formally more appropriated experimental design would have been to compare the usual once-daily dose with the same amount divided into 2 or 3 doses rather than simultaneously changing frequency and dose. However, the results suggest that in determining SLIT efficacy, allergen persistence represents a far more relevant factor than its concentration.

Although this is an open study, the fact that such an objective end point as skin reactivity was modulated in parallel to drug use suggests that indeed the immune response mutated in response to the number of daily drops taken by patients, bringing about the observed clinical consequences. Furthermore, although symptom scores were not collected with daily diaries, a clear-cut improvement in "3–3" patients was observed after a single year of treatment compared with the previous season in terms of overall self-evaluation of symptoms and with respect to answers to questions about quality of life. Furthermore, this improvement extended to the other groups when the "3–3" dosage was applied to them.

Recently, the dose-response dependence of the efficacy and severity of adverse effects has been considered in 2 dedicated studies,[5,18] although within a narrow dose range, and in 1 meta-analysis.[19] Furthermore, the possibility that precoseasonal treatment may be as efficacious as a perennial treatment has been reported.[6,20] However, the relevance of the number of daily administrations in obtaining efficacious clinical results has never been addressed directly. It was previously observed that patients treated according to a daily allergen administration schedule, despite a lower cumulative dose, showed a significantly greater decrease in skin reactivity, a higher rate of no use of any drug, and a more than 50% reduction in yearly drug intake compared with patients treated with 3 weekly allergen administrations.[7] These data were obtained as side results in a study focused on the modulation of skin reactivity to the allergen. Nevertheless, it was indicating that the number of daily administrations of a sublingual vaccine could be more critical in obtaining a favorable modulation of immunity to allergens than the amount of the administered allergen itself. The biological mechanisms that underlie SLIT efficacy are still largely unknown and remain a matter of speculation.[21] Thus, it should not be surprising that dose-response rules cannot be applied tout-court to the allergic response involving such an immune-privileged site as the oral mucosa. Indeed, our results can easily be integrated with the established knowledge on oral allergen absorption in SLIT. In fact, a study from Bagnasco et al[22] demonstrated that when performing SLIT, only a minimal amount of allergen is absorbed orally, but this has to be considered critical to the efficacy of SLIT. In contrast, the gastrointestinal absorption, which is prevalent in SLIT itself and represents the exclusive modality of absorption in oral immunotherapy, is not associated with any clinical improvement.[23–28] Along this line, the allergen persistence in the oral mucosa may be a far more relevant factor for gaining efficacy than allergen concentration. It would be interesting to experimentally compare with radiolabeled allergens the amount that is actually absorbed through the oral mucosa when using a dose as low as one fiftieth of the recommended one.

We suggest that multiple daily doses may provide a much greater impact on the overall allergen absorption through the oral mucosa than the concentration of the allergen or its cumulative amount. In principle, it cannot be excluded that extremely high concentrations of allergen used in some SLIT trials may also imply longer persistence of the extract in the mouth. This increased persistence could be responsible for relatively greater efficacy[5] rather than the dose itself, but at a given point this would be achieved at the cost of many and annoying oral and gastrointestinal adverse effects.[3,29]

In conclusion, these data indicate that SLIT is safe and efficacious in patients with allergic rhinoconjunctivitis also at a low-dose regimen. The number of doses seems to be far more crucial for SLIT efficacy than the absolute amount of administered allergen. Two daily doses are necessary to achieve clinical improvement, which is more consistent with 3 doses. Adoption of the concentration used in this study would also lower the cost of treatment and avoid the induction phase. This issue deserves to be investigated more extensively in double-blind, placebo-controlled studies of homogenous cohorts (ie, either adults or children with sensitization to a single allergen). This approach should parallel current efforts with high-dose regimens.[5,18]

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