

Safety and Efficacy of *Juniperus ashei* Sublingual-Swallow Ultra-Rush Pollen Immunotherapy in Cypress Rhinoconjunctivitis

A Double-Blind, Placebo-Controlled Study

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Key Words

Rush · Immunotherapy, sublingual · Rhinoconjunctivitis ·
Cypress · *Juniperus ashei*

Abstract

Background: The safety and efficacy of high-dose sublingual-swallow immunotherapy (SLIT) has been established in pollen rhinoconjunctivitis. This treatment has now been evaluated using an ultra-rush incremental dose regimen with a *Juniperus ashei* allergen extract in patients allergic to *Cupressus sempervirens* and *Cupressus arizonica*. **Methods:** Patients received either placebo or SLIT. Evaluation of safety was based on the frequency of adverse events during the incremental dose period (half a day) and during maintenance therapy (4 months). Evaluation of efficacy was based on symptom and medication scores at the pollen peak. **Results:** Seventy of the 76 patients included completed the study. There were no drop-outs during the rush procedure. One patient in the active group dropped out during the maintenance therapy due to adverse events: gastric pain and vomiting. There was also 1 drop-out in the placebo group due to pregnancy. Adverse events were infrequent,

local and mild. Symptom scores for rhinitis and conjunctivitis were not statistically different between groups, but there was a marked and significant ($p < 0.03$) decrease of the medication score (about 50%) and nasal steroid consumption (about 75%) in the active treatment group. An increase from baseline of serum IgE and IgG4 *J. ashei*-specific antibodies was only observed in actively treated patients ($p < 0.04$ and $p < 0.01$, respectively). **Conclusions:** The tolerability and safety of high-dose ultra-rush SLIT were comparable to those reported in previous SLIT studies. SLIT with *J. ashei* extract, due to its high Jun a 1 content, significantly reduced nasal steroid consumption in patients allergic to European cypress.

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Introduction

Injection immunotherapy, the reference immunological treatment for allergic rhinoconjunctivitis and moderate asthma, is generally administered in two successive sequences: a dose progression phase lasting several months followed by maintenance treatment lasting

several years. In the case of hymenoptera venom allergy, a potentially fatal situation, protective maintenance treatment can be achieved much more rapidly by using an accelerated protocol, under medical supervision in hospital, which limits the initial phase to a half-day [1]. This initial immunotherapy, called rush immunotherapy, has also been previously applied to other allergens, such as mites and pollens [2, 3], but the potential adverse effects that may occur in a less urgent situation than hymenoptera venom allergy has not allowed this method to proceed beyond the stage of clinical trials [4]. Some allergists use a cluster technique, midway between the 'classical' dose progression and 'rush' methods, which achieves maintenance treatment in 2 or 3 weeks, at a rate of several injections per day [5, 6]. However, the benefit-risk ratio of this method is still a subject of debate [7].

Sublingual-swallow immunotherapy (SLIT) is now recognized to be effective [8], with a similar efficacy to that of injection immunotherapy, but with a more favorable benefit-risk ratio [9]. This treatment also comprises two successive steps, a dose progression phase followed by maintenance treatment. A review of the literature reveals some studies claiming a 'rush' methodology via the sublingual route [10–13], but these studies are characterized by a 2-week dose progression phase, possibly with a morning dose and an evening dose, and with different concentrations of allergen extract. In 2002 we proposed another rush methodology via the sublingual route [14] using a 90-min induction, assuming a better safety than with an ultra-rush of 20 or 40 min duration [15–17].

The objective of the present study was to evaluate the safety and efficacy of SLIT initiated at the beginning of tree pollen allergy responsible for rhinoconjunctivitis. Dose progression was done over 90 min. Another characteristic of this study is the choice of the allergen extract, *Juniperus ashei*, instead of *Cupressus sempervirens* and *Cupressus arizonica*, the pollens clinically responsible, due to the higher allergenicity of the *J. ashei* extract which has been standardized [18]. *C. sempervirens* and *C. arizonica* are widely used for ornamental purposes, for hedges and windbreaks in the south of France while *J. ashei* is only present in the Balkans and the USA. Indeed cypress pollen-allergenic extracts are difficult to produce and to standardize as shown by Arilla et al. [19], Mari et al. [20], and Hrabina et al. [18]. The physicochemical properties of cypress pollen grains could be responsible for the difficulty of extracting cypress pollen allergens. Mari et al. [20] and Di Felice et al. [21] on the basis of the high IgE cross-reactivity within cypress species already consid-

Table 1. Clinical data of randomized patients

	Treatment	
	SLIT	placebo
Number of patients	38	38
Gender		
Female	16	21
Male	22	17
Mean age (range), years	39 (22–60)	39 (19–60)
Mean duration of rhinitis, years	7.5	7.7
Conjunctivitis, n	37	32
Asthma, n	7	3
<i>Juniperus</i> -specific IgE (mean), kU/l	18.9	23.3

ered selecting a representative extract from one species that could be used for diagnostic and therapeutic purposes.

Material and Methods

Patients

Seventy-eight patients (mean age: 39 years; range: 19–60 years) were enrolled in this study and 76 were randomized. Patients reported typical symptoms of rhinoconjunctivitis, possibly associated with moderate asthma, during the cypress pollen season (January, February, March). Sensitization was confirmed by a positive skin prick test (wheal greater than or equal to 4 mm) to *C. sempervirens* or *C. arizonica* and to *J. ashei* extracts (Stallergènes, Antony, France) and serum specific antibody levels to *J. ashei* and *Cupressus* greater than or equal to class 2 as determined by CAP (Pharmacia, Uppsala, Sweden). Patients with perennial allergic rhinoconjunctivitis to mites or cockroaches or severe intermittent or persistent asthma were excluded. Patients sensitized to cat or dog dander and living with pets at home were also excluded. No patient had previously received immunotherapy or was treated with β -blockers. Other exclusion criteria were the known contraindications to immunotherapy according to the European Academy of Allergology and Clinical Immunology guidelines [22].

The study was approved by the Ethics Committee of Marseille, France, and the patients signed informed consent forms before starting the study. Seventy patients, 36 in the SLIT group and 34 in the placebo group, completed the study. The patients' baseline clinical data are shown in table 1.

Allergen Extract

A purified standardized *J. ashei* extract was manufactured by Stallergènes (Antony, France). The allergen extract was standardized with reference to an in-house reference standard by RAST inhibition, isoelectrofocusing and immunoblotting for determination of allergenic potency [18]. The biological activity of the in-house reference standard was determined by skin prick test

and the potency was expressed as the arbitrary index of reactivity (IR). A potency of 100 IR was designated as the strength of *J. ashei* extract that elicited a geometric mean wheal diameter of 7 mm on skin prick tests in 30 sensitive subjects. The Jun a 1 major allergen content was 76 µg/ml of the 100 IR allergen extract (determined by Terumi Midoro-Horiuti, Texas University, Galvestone, Tex., USA). Sublingual therapy was graded in a single concentration of 300 IR/ml (equivalent to 30 histamine equivalent potency, HEP) in 50% glycerol aqueous solution. Placebo consisted of 50% glycerol aqueous solution. No dosage of natural Cup s 1 as described by Arilla et al. [19] was performed but these authors showed a high homology with other cypress group 1 allergens, including Jun a 1 with 96% identical amino acids.

Study Design

This was a double-blind, placebo-controlled, parallel-group study. In order to analyze about 80 patients, patients were enrolled in the trial in both 2001 and 2002. They were assigned to either SLIT or placebo with a block size of four (two SLIT and two placebo preparations) starting at the beginning of the pollen season. Skin prick tests were performed before treatment. Blood samples were obtained for antibody analysis before and after treatment.

Immunotherapy Protocol

The dose progression phase was performed in hospital during a single morning in patients not receiving any other treatment. This was done a few days before the expected beginning of the pollen season and in some patients during the first days of the season. The treatment was started on day 1 with a titration within 90 min (30–90–150–300 IR). Maintenance treatment (300 IR) was given once a day before breakfast for 120 days in a one-off treatment.

This daily dose in maintenance treatment corresponded to 228 µg Jun a 1. In the 2001 protocol, indwelling intravenous lines were used in all cases during the ultra-rush procedure.

Safety Parameters

Any event defined as an unpleasant or harmful occurrence, whether or not it was considered to be related to treatment, was considered to be an adverse event. A serious adverse event was defined as one of the following events: asthma attack, severe hypotension, laryngeal edema, giant urticaria, angioneurotic edema, or as any event requiring hospitalization, being life-threatening or death. Any event occurring during the rush treatment phase or during the maintenance phase was defined as: 0 = absent; 1 = local: itching and/or swelling of lips and mouth or abdominal pain; 2 = systemic reaction: rhinitis, conjunctivitis or asthma; 3 = severe reaction: giant urticaria, angioneurotic edema or anaphylactic shock. Blood pressure was measured during the rush phase (before and after each dose).

Symptom and Medication Scores

All patients recorded their daily symptom score and drug requirements from January to the end of March. The severity of individual symptoms was assessed on a scale from 0 to 3 for the nose (sneezing, blocking, running, itching) and eyes (redness, itching and watering): score 0 = absence of signs or symptoms; 1 = signs or symptoms present but not disturbing; 2 = signs or symptoms disturbing; 3 = signs or symptoms very disturbing and interfering with social life or work.

Rescue medication was scored each day as follows: one tablet (10 mg) of loratadine (2 points), one puff of triamcinolone in each nostril (2 points), one drop of antihistaminic or sodium cromoglycate per eye (1 point), and one tablet (20 mg) of prednisolone (18 points).

Determination of IgE and IgG4 Levels

Serum samples were analyzed for specific IgE using the EIA CAP system (Pharmacia Diagnostics, Uppsala, Sweden) and specific IgG4 were measured by a radioimmunoassay (Pharmacia Diagnostics) according to the manufacturer's instructions. Specific IgE levels are expressed as kilounits per liter and specific IgG4 levels are expressed as micrograms per liter.

Cypress Pollen Counts

Daily pollen counts between January 1 (week 1) and April 21 (week 16) in the Marseille area were obtained from the French National Network for Aerobiological Observation. The peak season was defined as a period of 4 continuous weeks including the maximum pollen count, i.e. week 7 to week 10 inclusive for 2001 and week 8 to week 11 inclusive for 2002. Pollens from the cypress family including *C. sempervirens* and *C. arizonica* cannot be differentiated by microscopy. There are no *J. ashei* trees in the studied area.

Statistical Analysis

The primary efficacy variables were the rhinitis and conjunctivitis symptom scores and the medication scores during the pollen peaks. The secondary efficacy variable was the number of days with asthma during the pollen peaks.

Comparability of the treatment groups was tested for relevant characteristics using χ^2 or Fisher's exact test for categorical variables and a Student t test for continuous variables. The normal distribution in each group was checked using a Shapiro-Wilk test. The Levene test was used to test homogeneity of variances. If the model assumptions were not satisfied, the treatment groups were then compared by the Wilcoxon two-sample test. All tests were two-tailed and the limit of significance was set at $p < 0.05$.

Results

Safety

Seventy-eight patients were preincluded in the study, but only 76 were randomized. There were no drop-outs during the rush procedure, but 6 patients dropped out during the maintenance treatment phase.

Table 2 shows the types of adverse events observed during the rush phase. Seven patients in the placebo group and 5 patients in the active treatment group reported adverse events, mostly local and of short duration. There were no variations in blood pressure.

There were 6 drop-outs during maintenance therapy, two in the active group (1 patient lost to follow-up, 1 adverse event: gastric pain and vomiting) and 4 in the pla-

Table 2. Safety during rush

Dose IR	Patient No.	Placebo	Patient No.	<i>Juniperus</i>	Total
30			7 20	Mouth itching Mouth itching	2 active
90			14 20	Mouth itching Itching of ear	2 active
150	2	Nose itching	14	Mouth itching	2 active
	8	Mouth itching	55	Tongue itching	5 placebo
	12	Mouth itching			
	43	Nasal obstruction			
	49	Nasal obstruction and eyes itching			
300	2	Nose itching	14	Mouth itching	4 active
	8	Mouth itching	20	Face hot flush	4 placebo
	21	Mouth itching	52	Tongue itching	
	24	Itching of eyes and nose	55	Tongue itching	

cebo group (1 patient lost to follow-up, 2 dropping out because of inefficacy, 1 because of pregnancy).

The adverse events reported during maintenance therapy are presented in table 3. No serious adverse events were observed. In 1 patient receiving active treatment, a transient reduction of the dosage was advised due to itching of the mouth.

Symptom and Medication Scores

The effects of SLIT and placebo on symptom and medication scores for all comparisons are shown in tables 4 and 5. The individual and total rhinitis and conjunctivitis scores were not significantly different between the SLIT group and the placebo group during the peak pollen season. However, the symptoms remained constantly very moderate, as they never exceeded a score of 1 on a scale from 0 to 3 for individual symptoms, or a score of 2 or 3 on a scale from 0 to 9 or 12 for total conjunctivitis or rhinitis scores.

However, the total medication score was significantly lower in the active treatment group than in the placebo group with a 60 or 40% reduction compared to median values, depending on the year considered. The variation of the median nasal dose of corticosteroids was equally marked and significant with a 70 or 88% reduction, depending on the year considered.

During the 2001 pollen peak, 2 days with asthma were recorded in the active treatment group versus 11 days with asthma in the placebo group. During the 2002 pol-

Table 3. Safety during maintenance therapy/types of adverse events

Adverse event	Placebo	<i>Juniperus</i>
Mouth itching		3
Gastric pain		2
Asthma		1
Diarrhea	1	2
Chest pain		1
Bone fracture		1
Urticaria	1	1
Rhinitis	3	1
Eczema	1	
Conjunctivitis	1	
Pregnancy	1	

len peak, the number of days with asthma was 15 in the active treatment group and 12 in the placebo group.

Serum IgE and IgG4 Levels

Pretreatment mean serum-specific IgE levels to *J. ashei* were not significantly different between the SLIT group and the placebo group. After treatment, the mean increase from baseline was 28.4 kU/l (SD: 27.8, median: 20.2) in the active treatment group and 8.2 kU/l (SD: 11.4, median: 4.9) in the placebo group. The between-group difference was significant ($p < 0.04$, Wilcoxon).

Table 4. Rhinitis and conjunctivitis symptom scores during pollen peaks

Symptom score	<i>Juniperus</i>	Placebo	p value
Sneezing (0–3)			
2001	0.83 ± 0.52 (0.92)	0.81 ± 0.44 (0.81)	0.61 NS ^a
2002	0.84 ± 0.50 (0.84)	0.70 ± 0.65 (0.61)	0.49 NS ^a
Rhinorrhea (0–3)			
2001	0.92 ± 0.51 (1.00)	0.72 ± 0.30 (0.69)	0.14 NS ^a
2002	0.82 ± 0.54 (0.86)	0.71 ± 0.65 (0.71)	0.62 NS ^a
Nasal itching (0–3)			
2001	0.45 ± 0.55 (0.15)	0.49 ± 0.33 (0.50)	0.57 NS ^a
2002	0.51 ± 0.39 (0.42)	0.56 ± 0.75 (0.30)	0.44 NS ^a
Nasal obstruction (0–3)			
2001	0.86 ± 0.70 (0.68)	0.58 ± 0.46 (0.67)	0.41 NS ^a
2002	0.50 ± 0.54 (0.37)	0.48 ± 0.66 (0.08)	0.43 NS ^a
Rhinitis total score (0–12)			
2001	3.06 ± 1.69 (2.88)	2.58 ± 0.91 (2.43)	0.43 NS ^b
2002	2.68 ± 1.64 (2.32)	2.44 ± 2.06 (2.25)	0.68 NS ^b
Redness of eyes (0–3)			
2001	0.56 ± 0.49 (0.56)	0.34 ± 0.42 (0.15)	0.27 NS ^a
2002	0.47 ± 0.53 (0.25)	0.50 ± 0.63 (0.07)	0.82 NS ^a
Itching of eyes (0–3)			
2001	0.45 ± 0.67 (0.08)	0.29 ± 0.31 (0.17)	0.97 NS ^a
2002	0.43 ± 0.47 (0.32)	0.37 ± 0.50 (0.04)	0.56 NS ^a
Watering of eyes (0–3)			
2001	0.56 ± 0.60 (0.47)	0.26 ± 0.33 (0.08)	0.18 NS ^a
2002	0.24 ± 0.36 (0.00)	0.36 ± 0.52 (0.07)	0.49 NS ^a
Conjunctivitis total score (0–9)			
2001	1.58 ± 1.58 (1.53)	0.89 ± 0.76 (1.11)	0.23 NS ^a
2002	1.14 ± 1.14 (0.84)	1.24 ± 1.40 (0.57)	0.95 NS ^a

Figures represent mean ± SD with median values in parentheses.

^a Wilcoxon 2-sample test.

^b Student's t test.

Mean serum-specific IgG4 levels to *J. ashei* in the SLIT group increased from 171.8 µg/l (SD: 74.3, median: 150) to 481.6 µg/l (SD: 623.4, median: 218) after treatment. In the placebo group, mean specific IgG4 levels increased from 198.0 µg/l (SD: 165.3, median: 150) to 267.1 µg/l (SD: 370.4, median: 150). The between-group variation was significant ($p = 0.03$, Wilcoxon) (table 6). IgE and

Table 5. Medication scores during pollen peaks

Medication score	<i>Juniperus</i>	Placebo	p value
Loratadine 10 mg			
2001	0.55 ± 0.59 (0.42)	0.79 ± 0.64 (0.71)	0.33 NS ^a
2002	0.38 ± 0.34 (0.29)	0.46 ± 0.33 (0.50)	0.39 NS ^a
Prednisolone 20-mg tablets			
2001	0.12 ± 0.46 (0.00)	0.15 ± 0.15 (0.00)	1.00 NS ^a
2002	0.09 ± 0.16 (0.00)	0.11 ± 0.19 (0.00)	0.69 NS ^a
Triamcinolone (puffs/nostril)			
2001	0.62 ± 0.82 (0.20)	1.44 ± 0.78 (1.59)	0.04 ^a
2002	0.35 ± 0.51 (0.16)	0.85 ± 1.03 (0.50)	0.05 ^a
Eye drops (drops/eye)			
2001	0.18 ± 0.64 (0.00)	0.38 ± 0.77 (0.00)	0.53 NS ^a
2002	0.35 ± 0.68 (0.00)	0.17 ± 0.55 (0.00)	0.20 NS ^a
Total medication score			
2001	4.76 ± 10.15 (1.72)	5.09 ± 2.86 (4.35)	0.04 ^a
2002	3.39 ± 3.94 (2.00)	4.71 ± 5.00 (3.50)	0.03 ^a

Figures represent mean ± SD with median values in parentheses.

^a Wilcoxon 2-sample test.

Table 6. Serum *J. ashei*-specific IgE and IgG4

<i>J. ashei</i> -specific antibodies	<i>Juniperus</i>	Placebo	p value
IgE, kU/l			
Baseline	9.1 ± 11.1 (6.1)	11.3 ± 14.4 (6.4)	
After treatment	38.8 ± 35.1 (29.3)	20.4 ± 23.4 (15.2)	
Variation after treatment – baseline	28.4 ± 27.8 (20.2)	8.2 ± 11.4 (4.9)	0.04 ^a
IgG4, µg/l			
Baseline	171.8 ± 74.3 (150.0)	198.0 ± 165.3 (150.0)	
After treatment	481.6 ± 623.4 (218.0)	267.1 ± 370.4 (150.0)	
Variation after treatment – baseline	192.1 ± 328.2 (28.0)	61.0 ± 195.0 (0.0)	0.03 ^a

Figures represent mean ± SD with median values in parentheses.

^a Wilcoxon 2-sample test.

IgG4 levels to *Cupressus* did not change significantly after treatment (data not shown).

Discussion

One of the first publications devoted to SLIT, although using a very low dose, raised doubt about the safety of this type of treatment in view of the high incidence of asthma attacks, sometimes severe ones, observed in children [23]. Nevertheless, the high level of safety of this method has now been established in both adults and children [8, 24] by more than 20 complementary studies. However, these previous studies used a very wide range of dosages and it was therefore possible that SLIT started immediately at the highest concentration with a half-day dose progression phase could be associated with adverse effects. The results of the present study show that very few patients treated with *J. ashei* developed adverse reactions during the dose progression phase. All reported adverse effects were subjective symptoms and not objective signs, if itching of the ear is considered to be due to the extension of an oral reaction via the Eustachian tube. Only one episode of facial hot flushes could correspond to a benign systemic effect. The tolerability and safety of high-dose ultra-rush SLIT with *J. ashei* allergen extract in patients allergic to *C. sempervirens* and *C. arizonica* were not statistically different from those reported in previous studies using various extracts from the same producer [24].

It is impossible, at the present time, to explain the rather unexpected excellent clinical safety of the ultra-rush dose progression phase up to a dose of 20 drops, i.e. 1 ml of an allergen extract with a concentration equivalent to 30 HEP. However, a preliminary study already demonstrated that it was possible to start grass pollen immunotherapy with an initial dose of 30 IR, i.e. one tenth of the maximum maintenance dose generally used [25]. As in conventional desensitization, the rush procedure comprises administration of increasing doses of allergen, but this method is more closely related to drug tolerance protocols, in which the therapeutic dose of a drug to which the patient is sensitive, without necessarily being allergic, is reached in several hours or several days. Routes of administration that can be used in drug tolerance protocols include the oral, subcutaneous, and intravenous routes and even nasal or bronchial nebulization [26]. Good examples of the efficacy of these protocols have been reported in AIDS patients [27–29]. Although the mechanisms responsible for this tolerance

to rush administration are unknown, it can be hypothesized that the mechanisms involved in conventional immunotherapy are subsequently activated, as illustrated by the marked increase of IgG4 production observed after 4 months in the present study. In the context of drug intolerance, reintroduction of treatment following its discontinuation requires a new dose progression phase. It remains to be determined whether a similar modality is required in the case of allergen immunotherapy.

The adverse effects described during maintenance treatment were infrequent and mainly consisted of local reactions, i.e. affecting the mucous membranes directly in contact with the allergen, i.e. the mouth, stomach and intestine, in line with the known safety data concerning SLIT [8, 24].

Few studies, not always conclusive ones, have been devoted to *Juniperus* pollen injection immunotherapy [30–35] and, to our knowledge, the present study is the first to analyze SLIT for cypress allergy. The effect of treatment on the clinical features was not significant, but, despite pollen seasons of normal intensity, the patients' symptoms generally remained present but were not too irritating. As in a previous study on grass pollen rhinitis [36], the efficacy of SLIT is demonstrated by the medication score, which was considerably and significantly decreased in terms of both the global score and nasal steroid consumption. This therapeutic efficacy obtained for coseasonal immunotherapy can probably be explained by the high daily dose administered, which makes up for the absence of a cumulative dose prior to the pollen season. SLIT has only been validated for high doses [37] and a dose-effect relationship has already been demonstrated for ragweed [38].

In practice, it therefore appears that SLIT can be started in patients who present shortly before or even at the beginning of a pollen season, while standard practice in this situation is to defer immunotherapy until the following year.

Finally, the original feature of this study concerns the treatment of patients with a pollen extract with which they have never previously been in contact, due to its superior allergenicity. A comparison of 1/20 w/v extracts of *J. ashei*, *C. sempervirens* and *C. arizonica* by in vitro RAST inhibition shows that the *J. ashei* extract is 20 times more allergenic than *C. sempervirens* and 11 times more allergenic than *C. arizonica*. The wheal diameter on in vivo skin tests also reveals highly significant differences with diameters of 8.34, 6.33 and 6.58 mm, respectively [39]. This can be explained by the high level of cross-an-

tigenicity between these varieties of cypress and by the abundance of Jun a 1 in *J. ashei*. As this difference of biological potency is due to the Jun a 1 major allergen, treatments based on a single or a limited number of natural or recombinant allergenic molecules could therefore also be effective.

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