Effects of sublingual immunotherapy in patients sensitised to *Ambrosia*. An open controlled study

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SUMMARY

Background: allergy to *Ambrosia* is a disease of growing importance in Europe. Injective and non-injective immunotherapy have been recognised as safe and effective but no evidence is currently available for sublingual immunotherapy (SLIT) in patients sensitised to *Ambrosia*. This study was planned to assess the effects and the safety of SLIT in patients clinically sensitised to *Ambrosia*.

Methods: 19 patients clinically sensitised to *Ambrosia* and treated with SLIT were compared to 14 patients treated only with drugs. Diary cards with symptoms and drug consumption were filled-in by patients during the pollen season whereas specific nasal challenge and skin prick test were run two months before and after the pollen season. Patients and doctors were also asked to express their subjective assessment about symptoms and drug consumption during the season.

Results: SLIT-treated patients had less symptoms and a significantly minor drug intake ($p = 0.04$) as compared to untreated patients. Nasal challenge test improved significantly in the SLIT group ($p = 0.0001$) but not in the control group ($p = 0.6875$) with a significant difference between groups at the end ($p = 0.0413$) but not at the beginning of the trial ($p = 0.213$). The decrease in skin reactivity was significant in the control group ($p = 0.0186$) and highly significant in the SLIT group ($p < 0.0001$), with no difference between groups ($p = 0.2987$). Subjective assessment from both patients and doctors was favorable to SLIT ($p = 0.0005$ for symptoms; $p = 0.0019$ for drug consumption). Only one minor local side effect was registered during SLIT.

Conclusions: according to our data, SLIT in patients allergic to *Ambrosia* is safe and able to improve both subjective and objective parameters.

Key words: *Ambrosia*. Skin prick test. Specific nasal challenge. Subjetive assessment. Sublingual immunotherapy.

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INTRODUCTION

Weeds of the genus *Ambrosia* belong to the *Compositae* family and include some species very important from the allergologic point of view: *Ambrosia artemisiifolia* (short ragweed), *Ambrosia bidentata* (southern ragweed), *Ambrosia psylostachya* (western ragweed), *Ambrosia aptera* (western giant ragweed), and *Ambrosia trifida* (giant ragweed). These species have different geographical distribution but they are largely cross-reactive and cause important respiratory symptoms from midsummer to early autumn in sensitised subjects, especially in Northern America. In the last two decades an increasing number of sensitisations and allergic symptoms have been reported also in the central part of Europe and a growing attention is now being paid to this genus.

Ambrosia has been firstly observed in Europe around 1915 (1) and later in Italy (2, 3) but in the eighties its diffusion followed an exponential growth, leading to a careful aerobiologic monitoring at least in Lombardy, Northern Italy (4-6).

The reasons behind this sudden and explosive diffusion are not clear, but the decreasing

importance of agriculture, the change from traditional cultures (wheat, maize) to sunflower (another species belonging to the *Compositae* family, whose seeds are often contaminated by *Ambrosia* seeds) and the increase of uncultivated areas can at least partly explain this evolution (7). Moreover, each individual *Ambrosia* is able to produce as much as 2.5 billion of pollen grains per day and to live from 5 to 20 years (7).

The first cases of patients with respiratory symptoms due to *Ambrosia* in Italy were reported in the eighties and since then the number of sensitised subjects has been progressively increasing (8).

Because allergens of *Ambrosia* are airborne not only by pollen grains but also by other particles of less than 10 μm in diameter, able to contribute to out-of-the pollination-season symptoms in sensitised subjects (9) and to penetrate in the lower respiratory tract, *Ambrosia* is highly asthmogenic (10).

Six different allergens from *Ambrosia artemisiifolia* (from Amb a 1 to Amb a 6, MW from 5 kDa to 38 kDa) have been purified and identified. Allergens from *Ambrosia* are known to cross-react with allergens from other *Compositae* like as *Artemisia* and with pollens from other taxonomically unrelated species (11-14). Because the pollination period of these genera is very close, a careful anamnesis is needed to establish a correct diagnosis (7).

Injective immunotherapy in patients sensitised to *Ambrosia* is largely used in the USA with the support of many positive studies (15-18). Some positive studies have been published on non-injective immunotherapies with this allergen (19-22) but none with sublingual immunotherapy (SLIT).

We designed therefore our study to assess safety and effects of SLIT in patients suffering from respiratory symptoms due to *Ambrosia*.

MATERIAL AND METHODS

Study plan

The trial was planned as a multicenter, parallel-group, open-controlled study. Thirty-three patients were selected according to the criteria outlined below.

Patients

Patients were selected in three Centers in Lombardy in February-March 1998, according to the following inclusion criteria:

— Age between 15 and 60 years.

— History of respiratory allergy with rhino-conjunctivitis and/or asthma for at least 2 years.

— Skin prick test positive to a biologically standardized extract of *Ambrosia artemisiifolia* (100 BU/mL, ALK-Abellò) containing 18 μg/mL of the major allergen Amb a 1 (wheal area at least corresponding to the area obtained with the positive control, histamine 10 mg/mL).

— No other clinically relevant sensitization to inhalant allergens.

— No immunotherapy during the last 5 years.

— FEV1 at least 80% predicted in asthmatic patients.

Subjects with other absolute or relative contraindications (23) were excluded.

Treatment groups

Thirty-three patients were selected for the study. SLIT was suggested to all of them, but accepted by only 19 who were considered as the treated group. Fourteen patients not submitted to SLIT were considered as the control group.

SLIT

SLIT was performed with a commercially available extract (ALK-Abellò, Milan, Italy) prepared in 5 strengths (0.0144; 0.072; 0.36; 1.8 and 9 μg/mL of Amb a 1) in glycerosaline solution preserved with phenol (0.3% W/V).

The administration was started at the beginning of July 1998 (T1), before the pollen season. Drops were administered around 30 minutes before meals under the tongue and kept in the mouth for at least two minutes before swallowing.

During the build-up phase, drops had to be taken twice daily, starting with one drop of the lowest strength and increasing up to 5 drops and then repeating the procedure with the following vial until the top dose (5 drops of the top concentration) was reached on day 15. The top dose was afterwards repeated 5 times a week (Monday to Friday) without variation during the pollen season until the end of September 1998 (T2, end of the pollen season).

Drugs

All patients were instructed and allowed to take on need the following rescue drugs:

— Antihistamine (loratadine, 10 mg per tablet).

— Local corticosteroid (fluticasone, 50 μg/puff).
— Local β-2 agonist (salbutamol, 100 μg/puff).

Monitored parameters

Nasal Provocation Test (NPT)

All patients were submitted to specific NPT two months before or after the pollen season, i.e. in May (T0) and November 1998 (T3). The test was run using the same standardized extract of *Ambrosia artemisiifolia* used for skin prick test and for SLIT. After a check with saline diluent as negative control, an amount of 80 μL of allergen solution was sprayed into a nostril by a metered device beginning with the strength 0.36 μg/mL of Amb a 1. If negative, the test was repeated 15 minutes later with the strength 0.72 μg/mL of Amb a 1 and if negative again, with the strength 1.44 μg/mL of Amb a 1. The test was judged as positive according to clinical criteria, i.e. when at least two out of four objective symptoms (rhinorrea, sneezing, itching, nasal obstruction) appeared.

Skin reactivity (skin prick test)

All enrolled patients were submitted at T0 and T3 to skin prick test with a standardized extract (100 B.U./mL, 18 μg/mL of Amb a 1). Trained personnel did all tests at the same time of the day with the same device (ALK-Abellò plastic needle). Each wheal was outlined with a fine-tipped ball-pen and transferred by adhesive tape onto paper for the subsequent estimation of the area.

Diary cards

All patients have been instructed and asked to fill-in from T1 to T2 a diary for symptoms and drugs intake. Nasal symptoms (rhinorrea, sneezing, itching and obstruction), conjunctival symptoms (redness, edema and itching) were assigned a progressive score: 0 for no symptoms, 1 for mild symptoms, 2 for moderate symptoms and 3 for heavy symptoms. For respiratory symptoms the following scores were used: 0 for no symptoms; 1 for cough episodes without asthma; 2 for episodes of cough during day and night and slight breathlessness; 3 for persistent cough, breathlessness and nocturnal asthma; 4 for persistent cough and asthma. A score of 2 was assigned to each intake of oral drugs whereas a score of 1 was assigned to each administration of local drugs.

Table I

Demographic data

	SEX	AGE (years)	MEAN AGE (years)	PATHOLOGY
SLIT GROUP	12 F 4 M	21-46	34.75	5 R 4 RC 7 RCA
CONTROL GROUP	9 F 5 M	26-44	37.5	5 R 5 RC 4 RCA

R = rhinitis; RC = rhinoconjunctivitis; RCA = rhinoconjunctivitis and asthma.

Subjective evaluation

Patients and doctors were asked to give a subjective global evaluation of the therapy on a 0-10 scale at the end of the treatment.

Tolerance

Patients were asked to report every kind of side effect or inconvenience possibly related to the administration of SLIT.

Statistics

Parametric data (skin prick test) have been analyzed with the Student's *t* test, whereas non-parametric data (NPT, scores and subjective judgement) have been analyzed with the Wilcoxon test (intragroup comparisons) or with the Mann-Whitney rank sum test (intergroup comparisons).

All statistical analysis has been run with a standard statistical software (BMDP Inc., Los Angeles, USA). A level of $p = 0.05$ was considered as statistically significant.

RESULTS

Patients

Thirty patients out of thirty-three concluded the study. One patient was excluded because of the intake of a non-allowed drug (chromone), one dropped-out for a new pathology not-related to the treatment and the last for local problems (itching of lips) after the administration of SLIT.

Complete demographic data of patients whose data were analyzed at the end of the study are shown in

table I. Groups turned out to be balanced for pathology, male/female ratio, age range and mean age.

Cumulative dose of allergen administered

Each patient was administered the cumulative dose of 107.6 µg of Amb a 1, i.e. 6.8 µg during the build-up and 100.8 µg during the maintenance phase.

Monitored parameters

NPT

Data obtained with NPT are shown in table II. No statistically significant difference could be shown between the two groups of patients at T0 (p = 0.2134) and in the control group at T0 *vs* T3 (p = 0.6875). Patients treated with SLIT showed at T3 a highly statistically significant increase in the NPT threshold dose as compared to T0 (p = 0.0001). This increase in the threshold dose was also statistically significant in comparison to the control group at T3 (p = 0.0413).

Skin reactivity

Skin reactivity was similar between groups at T0 (p = 0.7711). At T3 skin reactivity had a significant decrease in the control group (p = 0.0186) and a highly significant decrease in the SLIT group (p = 2.8 x 10^{-6}), but the difference between groups was not significant (p = 0.2987). Data are shown in table III.

Table II

Specific nasal provocation test

	RESULTS	
TIME	GROUP (number of patients)	Threshold [average value]
T0	Control (14)	5.000
	Treated (16)	3.875
T4	Control (14)	5.572
	Treated (16)	9.125
	STATISTICS	
TIME or GROUP	COMPARISON	p VALUE
T0	Treated *vs* Control	0.2134 ns
T4	Treated *vs* Control	0.0413*
Control	T0 *vs* T4	0.6875 ns
Treated	T0 *vs* T4	0.0001**

ns = not significant; * significant; ** highly significant; T0 = before the pollen season; T4 = after the pollen season.

Table III

Skin reactivity (wheal area after prick test)

	RESULTS	
TIME	GROUP (number of patients)	Average value ± standard deviation
T0	Control (14)	3.0801 ± 1.6118
	Treated (16)	2.7868 ± 1.3472
T4	Control (14)	1.8423 ± 0.8442
	Treated (16)	1.4366 ± 0.7626
	STATISTICS	
TIME or GROUP	COMPARISON	p VALUE
T0	Treated *vs* Control	0.7711
T4	Treated *vs* Control	0.2987
Control	T0 *vs* T4	0.0186*
Treated	T0 *vs* T4	2.8 x 10^{-6}**

ns = not significant; * significant; ** highly significant; T0 = before the pollen season; T4 = after the pollen season.

Scores and subjective evaluation

The SLIT group showed a lower symptom score (-11.03%) and a lower drug intake in comparison (-33.3%) to the control group. The difference for drugs was statistically significant (p = 0.0435).

Patients treated with SLIT gave also a very favorable subjective judgement as compared to the control group, for both symptoms (p = 0.0005) and drugs intake (p = 0.0019). The patients' evaluation was confirmed by the doctors' evaluation (p < 0.0001 for efficacy).

DISCUSSION

Efficacy of injective immunotherapy in patients sensitized to ragweed has been well-established (15-18). Creticos and coworkers set the optimal maintenance dose for the major allergen Amb a 1 between 0.6 and 12.4 µg per month following the change in mediators release (histamine and TAME-esterase: tosylarginine methyl ester-esterase) after specific nasal challenge in treated patients (24). Hedlin and coworkers were able to show that the significant decrease after specific nasal challenge of

the same mediators takes place when a cumulative dose of 0.22 μg of Amb a 1 is reached after 12 injections (25). For local nasal immunotherapy, Georgitis and coworkers set the effective treatment dose between 34 and 80 μg of Amb a 1 per month, whereas the dose of 2.7 μg of Amb a 1 per month was judged as clinically ineffective (26). Oral immunotherapy with microencapsulated (small intestine delivery) *Ambrosia* allergens has been studied in two trials with the administration of amounts of Amb a 1 between 144 and 576 μg per month (27-28). No evidence of clinical efficacy was reached, despite a minor seasonal increase of specific IgE and a sharp increase in specific IgG in the treated group as compared to the placebo group.

We used in our trial a maintenance dose around 36 μg of Amb a 1 per month, i.e. around 6 times the optimal average dose indicated by Creticos for the injective immunotherapy (24). This is in good agreement with other studies with different allergens showing clinical efficacy of SLIT with monthly dosages of the major allergen between 1.44 to 24 μg, i.e. from 3 to 7 times higher than the corresponding standard injective therapy (29-31). Systemic immunological changes were also detected after SLIT in grass pollen allergic patients with the administration of extracts containing around 6.5 μg/month of the grass major allergen Group 5 (32). Our results showing a significant change in the NPT threshold in treated patients before *vs* after treatment and also in comparison to the control group are in good agreement with the findings of other authors in patients sensitized to *Ambrosia* (24, 25) or other allergens (29, 33). These results support and explain the lower drug consumption in the treated group as compared to the control group. The clinical relevance of the effects of SLIT in treated patients is not only merely statistically significant, but it is clearly reflected by the significantly better subjective assessment by patients and doctors in comparison to the control group.

Skin reactivity changed significantly in all patients during the trial, to an higher extent in the treated group but with no statistical difference between treated *vs* untreated patients. This result is not surprising, considering that the treatment was very short (3 months) and that the dimension of the sample at the end of the trial was reduced (30 patients on the whole). Significant changes in skin reactivity have been shown only in some SLIT studies with administrations lasting more than 12 months (34-36).

Tolerance was excellent and only one patient had local problems (itching of lips) after the administration of SLIT. It is interesting to underline that this patient had another sensitization to inhalant allergens judged as clinically irrelevant at enrollment.

We can conclude that SLIT with standardized extracts of *Ambrosia artemisiifolia* performed for three months according to a pre- and co-seasonal schedule with the administration of a monthly dose of 36 μg of Amb a 1 is safe and able to significantly modify subjective and objective parameters in comparison to a control group treated with drugs. Further studies with a larger sample of patients are needed to assess the clinical efficacy of this treatment and to confirm the potential benefits shown by our experience.

RESUMEN

Antecedentes: la alergia a *Ambrosia,* es una enfermedad de importancia creciente en Europa. Tanto la inmunoterapia subcutánea como la no inyectada, han demostrado ser seguras y eficaces, pero actualmente no se dispone de evidencia sobre la inmunoterapia sublingual (ITSL) en pacientes sensibles a *Ambrosia.* Este estudio se desarrolló para valorar los efectos y la seguridad del ITSL en pacientes clínicamente sensibles a *Ambrosia.*

Métodos: 19 pacientes sensibles a *Ambrosia* y tratados con ITSL, se compararon con 14 pacientes tratados solamente con medicación antialérgica.

Durante la época de polinización los pacientes rellenaron diariamente cartillas indicando los síntomas y la medicación consumida. Se realizaron pruebas del pinchazo cutáneo y pruebas de provocación nasal específica dos meses antes y después de la polinización. Tanto los pacientes como los médicos evaluaron subjetivamente los síntomas y medicación durante este período.

Resultados: los pacientes tratados con ITSL mostraron menor sintomatología y una reducción significativa del consumo de medicamentos ($p = 0,04$) comparado con los pacientes no tratados.

La provocación nasal mejoró significativamente en el grupo tratado con ITSL ($p = 0,0001$) pero no en el grupo control ($p = 0,6875$) con una diferencia significativa al final ($p = 0,0413$) pero no al principio del ensayo ($p = 0,213$). La disminución de la reactividad de la piel fue significativa en el grupo control ($p = 0,0186$) y altamente significativa para el grupo ITSL ($p < 0,0001$), con poca diferencia entre ambos grupos ($p = 0,2987$).

La valoración subjetiva tanto de médicos como de pacientes fue favorable para la ITSL ($p = 0,0005$ en los síntomas, y $p = 0,0019$ en el consumo de medicamentos).

Solo se registró un caso de reacción local leve con la ITSL.

Conclusiones: según nuestros datos, la ITSL en pacientes alérgicos a *Ambrosia* es seguro, y capaz de mejorar tanto los parámetros subjetivos como los objetivos.

Palabras clave: Ambrosia. Prueba del pinchazo cutáneo. Prueba de provocación nasal específica. Valoración subjetiva. Inmunoterapia sublingual.

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