Immunotherapy with sublingual birch pollen extract. A short-term double-blind placebo study

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SUMMARY

The aim of this double-blind placebo-controlled study was to evaluate the efficacy and tolerability of short-term birch pollen sublingual immunotherapy. Forty-one patients suffering from allergic rhinoconjunctivitis caused by Betula alba were included. Exclusion criteria were the following: undergoing immunotherapy within the last 2 years, contraindications to immunotherapy, pregnancy and nursing. The treatment schedule comprised a 28-day basic course, followed by a 3-month maintenance treatment. The evaluation of the parameters was performed before treatment and 4 months after the last maintenance dose. Skin prick test and conjunctival provocation test (CPT) in a dilution series were carried out to determine the threshold of the reaction. The objective parameters used were the diameter of the skin wheals and the lowest concentration of the allergen extract to induce the symptoms of itching and reddening of the eyes. The allergic reaction in general was evaluated with the help of a 2-h birch pollen challenge in the Vienna Challenge Chamber (VCC); nasal flow and resistance was measured by rhinomanometry, and nasal secretion was quantified by weighing used handkerchiefs. Bronchial reactions were objectified by spirometry; subjective symptoms of the eyes, the nose, and the bronchial tract were documented by the patients via a visual analog scale. Birch pollen specific IgE and IgG were evaluated by monoclonal antibody enzyme immunoassay before ($T_0$) and after ($T_1$) treatment. For statistics $p < 0.05$ was applied. At $T_1$ there was no decisive difference in the in vitro and in vivo results between the two groups. After the treatment period ($T_1$), actively treated patients showed a significantly higher tolerance to the birch pollen CPT ($p < 0.01$). The skin reaction was significantly lower than in the placebo group. Furthermore, actively treated patients produced less than half of the nasal secretion of placebo-treated patients during the challenge session. The rhinomanometry analysis during the challenge showed significant differences for verum and placebo in favor of the actively treated patients ($p = 0.039$). There was no significant difference in the specific IgE and IgG concentrations. The side effects and compliance during the treatment were comparable in both groups. In conclusion, sublingual immunotherapy is a well tolerated and clinically effective method of treatment.

Key words: Birch pollen - Challenge test - Placebo controlled study - Sublingual immunotherapy - Vienna challenge chamber (VCC)

INTRODUCTION

Birch polinosis is a seasonal allergy which occurs during the blossom period of birch and other cross-reacting trees, such as alder and hazel (1). Often there is an associated intolerance of apples, nuts, celery, carrots and kiwis (2) due to the similarity of their major allergens, thus aggravating the problem for the allergic patient.

Besides allergen avoidance, specific immunotherapy (IT) is the only causal treatment currently known for a hypersensitivity to airborne allergens (3). In terms of IT, allergenic extracts are usually applied subcutaneously, although this is not the only form of administration. Various other forms of application, e.g., oral or sublingual, have been tested in order to find a safer, more efficient and more comfortable way of treatment for the patient (4-7).

In recent years a number of randomized, controlled studies on sublingual immunotherapy (SLIT) have been published, the majority featuring useful results concerning effectiveness and safety (8-11). Despite its success, this form of desensitization is controversial: Piazza et al. (13) and Nelson et al. (14) found no improvement in their allergic patients, in their clinical picture, or in immunologic parameters. In any case, SLIT has gained an increasing interest throughout Europe, especially in Germany and France.
The aim of this study was to evaluate the tolerability of SLIT as well as the changes of in vivo and in vitro parameters during a short-term treatment with the *Betula alba* extract.

Clinical treatment efficacy was investigated by a physiologic-like birch pollen challenge in the Vienna Challenge Chamber (VCC) (15). The VCC model enabled us to generate a stable allergen climate, recreating the natural environmental allergen exposure. In this system up to 12 allergic patients can be challenged simultaneously with any airborne allergen under controlled and reproducible conditions.

**MATERIALS AND METHODS**

**Subjects**

Forty-one patients (aged 18-38) suffering from seasonal allergic rhinoconjunctivitis of at least 2 years duration were selected for this study. Our inclusion criteria were the following: a positive skin prick test with *Betula alba* extracts (100 BU/ml); a positive conjunctival test (itching and red eyes induced by one drop of a *Betula alba* test extract with 30 BU/ml); a positive nasal provocation test (at least moderate nasal symptoms induced by one sprayed dose of a test solution with 100 BU/ml); and increased specific IgE to *Betula alba* according to the RAST method.

Patients who had already received immunotherapy during the last 2 years, pregnant and nursing women and subjects with contraindications to immunotherapy were excluded from this study (3). All patients were informed about the study in advance and gave their written consent; an agreement from the local ethical committee was also obtained.

**Study design**

For this randomized double-blind parallel group trial patients were divided into two groups, 20 receiving placebo and 21 receiving an active treatment (verum). Figure 1 shows our study schedule. At the beginning of the trial (T₀) the evaluation of all objective and subjective parameters was performed to get an initial report. During the 28-day basic course the patients received a daily increasing dose of medication until the maintenance dose was reached. Maintenance treatment then lasted 3 months. After the last maintenance dose (T₁) evaluation of all parameters was performed a second time.

**Material**

During the whole study a biologically standardized extract of *Betula alba* provided by Alergia e Inmunología Abelló S.A. was used for diagnostic and treatment purposes. The standardization method is described in reference 16. The active allergen extract (*Betula alba*) and the placebo, a saline solution, contained 50% glycerin, 0.4% phenol and 0.01% menthol. The bottles for the treatment and their contents were identical in taste and layout in order to guarantee the double-blind character of the trial. The contents was unknown to the research team and the patients. The basic course started with

![Study timetable indicating treatment course and evaluation time at the beginning of the trial (T₀) and after the last maintenance dose (T₁).](image-url)
Table 1
Study timetable, indicating treatment course and evaluation time $T_o$ and $T_1$.

<table>
<thead>
<tr>
<th>Initiation of treatment</th>
<th>Vial</th>
<th>Days</th>
<th>Drops</th>
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<tbody>
<tr>
<td>I (4 STU/ml)</td>
<td>1</td>
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<td></td>
<td>10</td>
<td>2</td>
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<tr>
<td>II (20 STU/ml)</td>
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<td></td>
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<td>III (100 STU/ml)</td>
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<td>24</td>
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<tr>
<td>IV (500 STU/ml)</td>
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<td>26</td>
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<td>27</td>
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<td>28</td>
<td>6</td>
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</table>

Maintenance treatment

IV (500 STU/ml) Ten drops administered every other day: Monday, Wednesday, Friday

One drop of a 4 STU/ml (standardized treatment units according to Abelló S.A. manufacturer-specific units; 16) solution, and one drop of placebo respectively. The dose was increased daily according to the treatment schedule (Table 1) so that the patients received 10 drops at the end of the week. This schedule was repeated with the next higher concentrations of 20, 100 and finally 500 STU/ml of the solution. After reaching the maintenance dose of 10 drops of the 500 STU/ml solution (respectively placebo) the extract was administered every other day, i.e., three times a week, for 3 months.

Methods

In vivo tests

Three fivefold dilutions of glycerinated extract containing 150, 30 and 6 BU/ml (17) birch pollen were applied via a skin prick test on the ventral side of the forearm. For interpretation the wheals were traced after 15 min and copied onto a paper to measure their sizes. Any medication that could have influenced the skin reaction was withdrawn in time (18). The development of the skin sensitization was determined by means of a parallel line assay (PLA) (19) by checking the regression of the skin reaction and parallel reactions. At the beginning of the study all skin reactions were labelled $T_1$ and $T_2$ after 4 months, according to the study design.

At time $T_1$, the first conjunctival provocation test (CPT) in the form of a dilution series was performed. The lyophilized extract for the CPT was stored at 4°C and reconstructed shortly before use. The dilution series started at 0.24 BU/ml and were quintupled until a positive reaction was reached. The test was positive if itching or redness of the conjunctiva occurred after 10 min.

Vienna Challenge Chamber (VCC)

In order to obtain objective data on the patients’ allergic reactions, two sessions (at $T_o$ and $T_1$) in the VCC were performed. The VCC (15) is an enclosed room where up to 12 patients can be challenged simultaneously with a determinable amount of pollen allergens under stable and reproducible conditions. The chamber is filled with fresh air that is supplied through an air conditioning system. The air is enriched continuously by birch pollen in a concentration of 1,000 grains per m³ of air. This concentration has been experimentally evaluated by former investigations as being definitely over the threshold of reaction and not leading to severe side effects. It can be kept constant for hours and the dispersion remains homogeneous throughout the challenge. The concentration is checked in 5-min intervals.

The patients undergo two exposure tests ($T_o$ and $T_1$) of 2 h each. Symptom scores, visual analog scale and nasal flow are measured every 15 min while forced expiratory volume (FEV₁) and nasal secretion are quantified every 30 min. Nasal flow and nasal resistance are measured by active anterior rhinomanometry at 150 Pascal pressure difference between both ends of the nose (20). By weighing used handkerchiefs the nasal secretion is quantified. As a precaution, objective respiration parameters are taken by spirometry in order to avoid asthma attacks in patients with an unknown bronchial hyperreactivity, though these are extremely rare with such a low pollen concentration. Ocular, bronchial and nasal complaints are recorded on a visual analog scale (VAS) by the patients themselves.

In vitro tests

Before starting the IT (at $T_o$) and after 4 months (at $T_1$) blood samples were taken from all the patients and were stored at -20°C to be analyzed at the end of the study. The specific IgE and IgG to Betula alba were determined by monoclonal antibody enzyme immunoassay (21-23).
Statistics

The CPT as well as the in vitro definitions (specific IgE and IgG against *Betula alba*) were calculated according to the nonparametric method; the comparison between the groups according to the Mann-Whitney method; the comparison between T₀ and T₁, according to the Wilcoxon method (BMDP, 3D program). A software program (AIASA, CRS-PLA) was developed for the comparison of the results of the skin tests according to the PLA (19). Cutaneous tolerance index (CTI) (17) is the factor by which the concentration of the skin prick test extract has to be multiplied after treatment in order to get the same skin response as before the treatment. If the CTI is higher than one, a decrease in the skin sensitivity is given.

The testing of the hypothesis of differences of the primary efficacy parameter (the nasal sensitivity during the physiologic challenge in the VCC at time T₀ and T₁) between the verum test preparations and placebo was planned to be done by means of dependent *t*-tests. For the nasal flow at 150 Pa the AUC of the individual values during each challenge period was calculated. If normal distribution and homogeneity of variance could not be accepted, a nonparametric test method (Wilcoxon matched pairs signed rank test) was used. The Bonferroni-Holm procedure was used to maintain a global alpha of 0.05.

RESULTS

Homogeneity of the groups

Forty-one patients, 15 males and 26 females, suffering from allergic rhinoconjunctivitis due to birch pollen were included in this trial; 34 patients finished the study as outlined in the protocol. The two groups were similar in age (33 ± 15 years in the active group, 32 ± 16 in the placebo group) and clinical data (a duration of illness of 9 years on average) and comparable concerning sensitivity in CPT and skin prick test to birch pollen. Even the skin test sensitivity to early spring pollens was similar in both groups. At the beginning of the trial (at T₀) there was no decisive difference in the in vitro and in vivo results between the active and the placebo group. During the first challenge in the VCC at time T₀ the report was similar according to objective and subjective parameters. There was no significant difference between the groups in T₀.

Immunotherapy

The administered number of doses was similar in both groups (verum and placebo), on average between 68.1 and 69.7 applications. The accumulated dose was also similar, with a total of 225 STU/ml in the active group and corresponding 234 STU/ml in the placebo group; all but one reached the maintenance dose. In the active group three patients abandoned the study because of personal reasons; in the placebo group four patients abandoned the study.

Side effects

Seven patients from the active and five from the placebo group reported side effects. Two patients from the placebo group and two from the active group reported itching of the tongue and/or in the mouth. Ocular itching was reported by two patients in the active group and by three patients from the placebo group. Three actively treated patients complained about runny nose and/or sneezing after medication. One patient in the active group suffered from gastrointestinal discomfort and somnolence, which was probably not caused by the immunotherapy. No significant difference was noted between the treatment groups.

Development of the CPT

Conjunctival sensitivity decreased significantly at T₁ (p < 0.01) in patients that received the treatment (Table 2) in contrast to placebo.

<table>
<thead>
<tr>
<th></th>
<th>T₀</th>
<th>T₁</th>
<th>Evolution*</th>
</tr>
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<tbody>
<tr>
<td>Active</td>
<td>1.7 ± 1.67</td>
<td>6.5 ± 9.16</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>Placebo</td>
<td>1.7 ± 1.59</td>
<td>2.7 ± 3.36</td>
<td>p = 0.125</td>
</tr>
</tbody>
</table>

*Wilcoxon test.

Changes in the skin prick test

In the active group the skin reactions remained unchanged during the IT (CTI = 0.92), whereas a significant rise of the cutaneous response (p < 0.05) was observed at T₁ in the placebo group (CTI = 0.54) (Fig. 2).

In vitro results

The specific IgE and IgG concentration to *Betula alba* increased slightly in the active group, though a slight decrease was observed in the placebo group. However, these changes were not statistically significant (Table 3).

Results of the efficacy parameters in the VCC

At T₀ the nasal flow, evaluated by active anterior rhinomanometry, decreased during the first hour of the
allergen challenge in both groups and stabilized for the second hour (Fig. 3). At $T_1$, a similar pattern was observed in the placebo-treated patients, but the active group did not experience a marked flow reduction. The baseline levels of nasal flow were notably lower than in $T_0$ ($p < 0.01$) in both groups with great differences between verum and placebo. A cross-over analysis of variances shows different developments for verum and placebo ($p = 0.033$). The area under the flow curve proves the significant difference ($p = 0.028$) between the active and the placebo group in favor of the active one.

After the treatment period, placebo-treated patients produced more than twice the nasal secretion than the actively treated during the challenge session. However, the difference was not significant.

The subjective parameters, the symptom score as well as the VAS were consistent; the symptoms worsened during the first hour of the challenge and remained stable or even decreased slightly in the second hour. At $T_1$, the placebo group reported worse symptoms on the VAS than the active group (Fig. 4); however, they were statistically insignificant.

**DISCUSSION**

Allergic rhinoconjunctivitis is not a fatal condition, but restricts the patients enormously. Their quality of life is markedly diminished (24-26), influencing not only their well-being and increasing their risk of consequential illnesses such as headache or sinusitis, but also affecting their whole social life. Often a chronic tiredness due to disturbances in their sleep (27) can be observed and a loss or impairment of smell (28) is a logi-

| Table 3 |
|------------------|------------------|------------------|------------------|
| Mean dose (U/ml) and standard deviation of birch pollen specific IgE and IgG. | | | |
| **IgE** | **IgG** | **Evolution** |
| **(HRU/ml)** | **Active** | **Placebo** | **Active** | **Placebo** | **p** |
| T0 | 4.1 ± 4.42 | 6.2 ± 7.18 | 4.9 ± 4.22 | 4.5 ± 4.21 | 0.53 |
| T1 | 610 ± 410 | 727 ± 328 | 637 ± 426 | 695 ± 284 | 0.06 |
| Evolution | $p = 0.62$ | $p = 0.51$ |
atmosphere at the time of the second challenge. These allergens are cross-reactive to birch pollen and induce the priming effect in increasing not only nasal but also skin sensitivity.

This could also explain the generally higher nasal secretion and lower nasal flow during the second challenge session in the VCC at T₁. Unlike authors of previous studies in which changes of the in vitro parameters were found after 1 year of treatment (10), we have not detected such modifications after our short-term treatment of only 4 months.

The specific IgG concentration to *Betula alba* increased after the active treatment but was statistically insignificant.

The various parameters of the VCC showed a distinct behavior: before the start of the therapy, the subjective parameters and objective measurement of the nasal function (nasal flow at 150 Pa as well as nasal secretion) were similar in both groups. After 4 months of sublingual immunotherapy, the subjective parameters as well as the amount of nasal secretion showed advantages of the active therapy over placebo. The primary efficacy parameter of the nasal flow proved that SLIT is significantly more effective than placebo (*p* = 0.028) after 4 months of treatment.

Apart from a few cases with minimal side effects, the tolerance of the treatment was positive throughout. In comparison with the SLIT with *Dermatophagoides pteronyssinus* (10), *Betula alba* was tolerated much better. Itching of the tongue or lips has also been observed by other authors (9, 10) and is attributed to ingredients such as glycerin, phenol or menthol. These side effects, however, subside shortly and are of no significance.

Interpreting the results of the study one arrives at the conclusion that a sublingual treatment with *Betula alba* extract is tolerated very well despite the limited observation time. Skin, conjunctival and nasal reactions decreased significantly. The positive results of this form of administration have also been observed by various other authors (8-10).

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**RESUMEN**

El objetivo de este estudio a doble ciego y controlado con placebo es valorar la eficacia y tolerancia de una pauta a corto plazo de inmunoterapia sublingual frente a polen de abedul. Incluimos 41 pacientes diagnosticados de rinoconjuntivitis alérgica por sensibilización frente a *Betula alba*. Entre los criterios de exclusión está: el tratamiento inmunoterápico en los últimos 2 años, las contraindicaciones propias de la inmunoterapia, embarazo y la lactancia. El esquema de tratamiento comprendía 28 días en la fase de inicio seguido por una pauta de mantenimiento de 3 meses de duración. La evaluación de los diferentes parámetros se realizó al inicio del tratamiento (*T₀*) y tras finalizarlo (*T₁*), es decir transcurridos 4 me-
ses. En la fase previa al tratamiento (T₀) no encontramos diferencias significativas tanto en los resultados in vivo como in vitro entre ambos grupos. Finalizado el tratamiento (T₄), los pacientes tratados activamente mostraban una mayor tolerancia a la prueba de provocación conjuntival realizada con polen de abedul. La reactividad cutánea era significativamente menor que en el grupo placebo. Además, los pacientes que habían recibido inmunoterapia sublingual desarrollaban menos de la mitad de la secreción nasal con respecto al grupo placebo durante la prueba de provocación en la Cámara de Provacación Viennense (VCC). El análisis de la rinometría durante dicha provocación en la VCC mostró diferencias significativas entre ambos grupos a favor de los pacientes tratados activamente (p = 0.033). No encontramos diferencias significativas en las concentraciones de IgE e IgG específicas. Los efectos secundarios y la aceptación durante el tratamiento resultaron ser similares en ambos grupos. En conclusión, la inmunoterapia por vía sublingual es un método de tratamiento clínicamente eficaz y bien tolerado.

Palabras clave: Polen de abedul - Test de provocación - Estudio control-placebo - Inmunoterapia sublingual - Cámara de Provacación Viennense

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