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Revisión

A Th1 Inducing Adjuvant, MPL®, Safely Assists in Reducing the Length of a Pollen Allergy Vaccination Course

Background and aims: The mechanism of successful allergy vaccination is thought to be associated with a promotion of Th1 cell activity. A Th1-inducing adjuvant was incorporated in a grass pollen allergy vaccine with the aim of improving the efficiency of allergy vaccination such that only four injections would be required. *Materials and methods:* The adjuvant, 3-deacylated monophosphoryl lipid A (MPL®, Corixa, USA) was formulated in a standardised vaccine employing a tyrosine-adsorbed glutaraldehyde-modified grass pollen extract. This new therapy was evaluated in a phase III double-blind, placebo-controlled study of grass-pollen sensitive patients (81 actively-treated, 60 on placebo). *Results:* Significant improvements were found in nasal ($p = 0.016$) and ocular ($p = 0.003$) symptoms and combined symptom and medication scores ($p = 0.013$). Grass pollen-specific IgG antibody was elevated by active treatment ($p < 0.01$). No seasonal rise in specific IgE was observed in the actively-treated group in contrast to the placebo group ($p = 0.002$). Local adverse events were higher in the actively-treated group, but there were no group differences with generalised adverse events. *Conclusion:* A new grass pollen allergy vaccine incorporating a Th1-inducing adjuvant was shown to be well-tolerated and efficacious after only four injections. The vaccine is available in a number of countries as Pollinex® Quattro or Quattro MPL®. This new approach supports the use of immunotherapy more widely for the effective treatment of specifically diagnosed type 1 allergy. This treatment is now being extended to treat allergy to other pollens and to house-dust mites.

Key words: Allergy vaccine. MPL®. Monophosphoryl lipid A. Grass pollen. Th1.

Un adyuvante inductor de Th-1, MPR®, ayuda de forma segura a reducir la duración de un ciclo de inmunoterapia con polen de gramíneas

Antecedentes y Objetivos: Se considera que el mecanismo de una vacuna antialérgica consiste y está asociado a una promoción de la actividad de las células Th1. Se incorporó un adyuvante inductor Th1 a una vacuna antialérgica frente a los pólenes de las gramíneas con el propósito de mejorar la eficacia de la vacunación de manera que únicamente se requieran 4 dosis. *Materiales y métodos:* El adyuvante, 3-deacy-

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lated monophosphoryl lipid A (MPL®, Corixa, USA) se formuló en una vacuna estandarizada empleando un extracto de pólenes de gramíneas modificado con glutaraldehido adsorbido con tirosina. Esta nueva terapia se evaluó en un ensayo Fase III, doble-ciego, frente a placebo, en pacientes sensibles al polen de gramíneas (activo: 81, placebo: 60). *Resultados:* Se detectaron mejoras significativas en los síntomas nasales (p = 0,016) y oculares (p = 0,003) y en la combinación de síntomas y necesidades de medicación (p = 0,013). Los niveles de IgG específicas al polen de gramíneas se incrementaron por el tratamiento activo (p > 0,01). No se observaron incrementos de las IgE específicas durante la estación en el grupo activo, a diferencia del grupo placebo (p = 0,002). Las reacciones locales fueron más alta en el grupo activo pero se no observaron diferencias entre los grupos en relación a reacciones adversas generalizadas. *Conclusión:* La nueva vacuna antialérgica por incorporación de un nuevo adyuvante Th1 inductor, se mostró bien tolerada y eficaz con sólo 4 dosis. La nueva vacuna está disponible en diversos países como Pollinex®, Quattro o MPL®, Quattro. Esta nueva aproximación respalda el empleo de la inmunoterapia como tratamiento eficaz de la alergias tipo I específicamente diagnosticadas. Este tipo de tratamiento se está desarrollando para tratar alergias inducidas por otros pólenes y por ácaros domésticos.

Palabras clave: Inmunoterapia alergológica. PML®. Monofosforil lípido A. Polen de gramíneas. Th-1.

Therapeutic allergy vaccination (AV) for treatment of respiratory allergy is sometimes used sparingly because of perceived lack of efficacy and potential side effects, particularly in unskilled hands. Well-managed AV is considered by many allergists as superior to symptomatic treatment in appropriately diagnosed cases by modifying the underlying disease process[1]. AV has continued success in many countries, fewer serious reactions being reported following improvements in product standardisation and training in its use. There are pharmacoeconomic advantages of AV, even with long courses of treatment[2]. However, the often long duration of treatment is costly in time for both the clinician and patient. Ideal therapeutic goals would comprise vaccine formulations having shorter injection regimes with enhanced safety and efficacy profiles.

A mechanism possibly responsible for successful allergy vaccination is the induction of a switch in cytokine production of allergen-specific T lymphocyte helper cells from a more Th2-like to a more Th1-like profile, leading to down regulation of the Late Phase Reaction, associated inflammation and an eventual reduction in IgE antibody. Recently, Th1 cell induction has been shown to be favoured by a new adjuvant, 3-deacylated monophosphoryl lipid A (MPL®), a purified, detoxified glycolipid extracted from the cell walls of *Salmonella minnesota*[3]. This effect has been shown in both pre-clinical[4] and clinical[5] studies with different microbial antigens. A recent study demonstrated reduction of IL-4 and IL-5 levels together with an increase in interferon-gamma following therapy with tree pollen allergoid augmented with MPL®[6]. MPL® adjuvant has proven to be well tolerated and safe in a considerable number of patients to date in several infectious disease and cancer vaccines[7].

In this study, the efficacy of allergy vaccines incorporating MPL® was evaluated in a course containing a reduced number of injections. The active ingredient was glutaraldehyde-modified grass pollen extract. Chemically modified allergens (termed allergoids) are safer to use because they react less with IgE antibody, whilst other properties such as specific IgG induction and T-cell reactivity are not similarly reduced. The poorly soluble amino acid L-tyrosine was used as a depot base to adsorb the allergoid and otherwise soluble MPL®. The product was manufactured in an audited GMP facility, controlled and standardised as required by the European regulatory authorities[8,9].

Extensive toxicology studies were performed as agreed with the Paul Erlich Institute, the regulatory agency for Germany. The studies showed no abnormalities preventing its use in clinical trials as judged by the 'Toxicology Expert'. Phase 1 and 2 clinical studies were completed successfully.

MATERIALS AND METHODS

A phase 3, multicentre, double blind, placebo controlled study was performed in Germany and Austria with subjects allergic to grass pollen. Studies were in accordance with GCP principles and The Declaration of Helsinki and appropriate ethical approvals were obtained. Ethical committees in Germany required a 2:1 ratio of active *(verum)* to placebo treated subjects.

Subjects were randomised using randomisation computer software into active (allergoid adsorbed to a 2% L-tyrosine suspension with MPL®) and placebo (2% L-tyrosine suspension) groups. The allergoid preparation was standardised and composed of semipurified glutaraldehyde-modified extracts from a mixture of pollens from 12 temperate zone grasses and *Secale cereale* (cultivated rye). An independent statistician selected 74 (from 81) subjects in the active group and 50 (from 60) in the placebo group to provide suitably analysable data.

Subjects in the active therapy group received 3 preseasonal subcutaneous injections at weekly intervals of increasing strengths of product followed by one further injection at top strength. The doses of allergoid injected were: 300, 800 and 2000 Standardised Units (SU). The top dose contains an approximate equivalent of 24μg of Group 1 allergens. All active doses contained 50μg of MPL®. The placebo group received four doses of L-tyrosine suspensions at the same time as the active group. Subjects completed daily diary cards during the main grass pollen season scoring symptoms by a standardised system. This consisted of recording whether the symptoms for that day were: none (0), mild (1), moderate (2) or severe (3). Clear written guidance was given to symptom scoring. Medications were provided to control pollen allergy, excluding corticosteroids and long acting anti-histamines. Scoring for medications was done according to a predetermined scheme.

An unstratified Wilcoxon Test (two-sided) was used to analyse the data. This form of statistical analysis indicates a very high degree of confidence associated with differences. It was shown statistically that there were no pre-treatment differences between groups in: age, sex, age at symptom onset, symptom type, other sensitivities, RAST class, skin prick test sensitivity to grass pollen, medications taken during the previous year and time off work due to allergy.

Serum samples were taken for antibody assays at the following times: before therapy (baseline), after therapy, at the middle of the assessment period and after the pollen season. Specific IgG and IgE antibodies against a grass pollen mixture and *S. cereale* were quantified using a liquid-phase immunoassay (AlaSTAT, Diagnostic Products Corporation).

RESULTS

The analysis of symptom and medication scores is summarised in table I. Symptoms during the pollen season were significantly higher in the placebo group in the nose and eyes and a combination of these and the lung. Lung symptoms alone showed no significant difference; few subjects suffered from pollen-induced asthma.

Subjects in the placebo group did not take statistically significantly more medication to control the higher level of symptoms they experienced. However, the combination of medication and symptom scores indicated that the active group significantly improved during the pollen

Table I. Symptom and Medication Statistics

	Eyes		**Nose**		**Medication**		**Eyes, nose and lungs**		**Eyes, nose, lungs and medication**	
Significance level	***p* = 0.003**		***p* = 0.016**		***p* = 0.29**		***p* = 0.003**		***p* = 0.013**	
	P	A	P	A	P	A	P	A	P	A
Mean	1.12	0.82	1.46	1.21	0.71	0.54	0.95	0.75	0.83	0.65
± SD	0.52	0.58	0.51	0.65	0.77	0.71	0.41	0.44	0.47	0.48
Median	1.13	0.71	1.43	1.09	0.34	0.23	0.9	0.65	0.71	0.54
Difference of the medians							-28%		-24%	
Effect Size							-0.48		-0.40	
95% Confidence limits of Effect Size							0.14-0.87		0.04-0.77	

P= Placebo group, A= Actively treated group

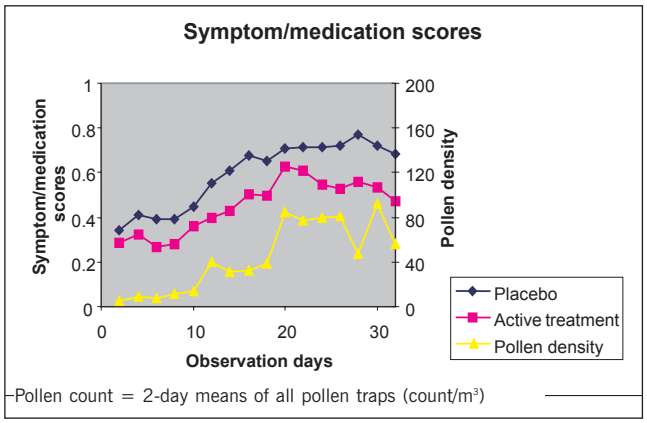

Fig. 1. Combined median symptom and medication scores with pollen count.

season. Effect sizes are shown for two significantly different measurements. It was chosen to present these calculations for the combined symptom scores and the combination of symptom and medication scores as the most pertinent indicators of efficacy. Effect sizes of 0.40 and 0.48 were seen, regarded statistically as moderate to large, which is also reflected by the 95% confidence limits. The combined scores are displayed in figure 1, plotting medians for two-day datapoints together with pollen counts (as a mean of the levels observed in several centres). This visual impression of how the two groups fared during the pollen season shows a marked widening of differences, commencing after day 20. Co-incidentally, the pollen levels were considerably elevated in days 20-32.

The clinicians assessment of the subjects at the final visit after the pollen season concluded that those on active treatment had fared better.

Antibody assay results are shown in table II. The active treatment induced a highly significant rise of grass pollen specific IgG over the placebo group for all timepoints except baseline ($p < 0.01$). In contrast, there was no increase in specific IgE levels comparing to placebo; furthermore, the placebo level rose in comparison to the active group at the middle of the assessment period ($p = 0.002$).

Local reactions (redness/swelling or pain/itching) were found more frequently in the actively-treated group ($p < 0.01$). No treatment was required beyond measures such as cooling. Systemic reactions, generally mild rhinoconjunctivitis, were equally distributed between the two groups; no serious systemic reaction occurred. Other adverse events (that were unlikely to be treatment-related) were evenly distributed between the groups.

DISCUSSION

This study has shown that a satisfactory clinical outcome was achieved as judged by a significant reduction of symptoms from the eyes and nose respectively, and from combined data for the eyes, nose and lungs. Additionally, when these three organ scores were combined with medication scores there was a significant reduction. Baseline symptom scores indicated that the subjects were presenting symptoms before the onset of the pollen season; consequently, it was unlikely that the specific therapy would totally eliminate their condition.

In vitro (antibody assay) data supported the clinical findings. Specific IgG induction, although not necessarily directly related to efficacy, is regarded as an indicator of a potent allergy vaccine. The elevation of specific IgG antibody was particularly rapid and pronounced, with a

Table II. Specific antibody measurements

Patient group/ specific antibody	Timepoint 0 (baseline)	Timepoint 1 (after therapy)	Timepoint 2 (middle of assessment)	Timepoint 3 (after pollen season)
Active IgG (mg/L)	4.6 ± 4.7 ($p = 0.55$)	11.1 ± 13.0 ($p < 0.01$)	13.8 ± 11.3 ($p < 0.01$)	7.5 ± 4.9 ($p < 0.01$)
Placebo IgG (mg/L)	4.1 ± 3.3	2.3 ± 2.3	5.9 ± 3.5	5.3 ± 3.1
Active IgE (IU/mL)	18.5 ± 26.3	16.4 ± 21.2	25.2 ± 29.3	21.8 ± 24.7
Placebo IgE (IU/mL)	23.2 ± 28.9	15.1 ± 18.7	37.3 ± 35.7	31.0 ± 31.0

P= Placebo group, A= Actively treated group

threefold rise in the actively-treated group. The active treatment did not induce specific IgE antibody, which was in contrast to the seasonally induced strong rise of IgE antibody seen only in the placebo group. The absence of an increase of specific IgE levels in the active group that was found with the placebo group endorses a view of change in the ratio of Th1/Th2 activity caused by the treatment.

The clinical and *in vitro* results are clearly encouraging, considering the therapy consisted of only four injections. The clinical efficacy that was obtained in spite of a small number of injections must be attributed at least in part to the Th1-cell inducing activities of MPL® adjuvant which was present in the vaccine formulation. The safety and tolerance outcomes were generally good, with local reactions higher in the active group but certainly comparative to the reported responses from treatments with other allergy vaccines. Other events were equally divided between the two groups.

It is hoped that the success of this therapy will encourage more widespread use of this new formulation, and that the studies will extend to the treatment of other type 1 hypersensitivities, such as house-dust mite allergy.

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