SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RAD: 12-155801- -14 **FECHA:** 2016-05-12 16:16:13
DEP: 2020 DIRECCION DE NUEVAS CREACIONES **EVE:** 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II **FOLIOS:** 6
ACT: 519 TRASLADOINFORME

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
MAURICIO JARAMILLO CAMPUZANO

Asunto: Radicación: 12-155801- -14
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 5593
Folios: 6

Patente de Invención	**X**			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	**X**	Capítulo I	**X**	Capítulo II	
No. Solicitud Internacional	PCT/EP2011/052049				
Fecha de Presentación Internacional	11/02/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado N° 12-0155801-00000-0000	11/Septiembre/2012
Reivindicaciones:	Radicado N° 12-0155801-00000-0000	11/Septiembre/2012
	Radicado N° 12-0155801-00005-0000	03/Julio/2014
	Radicado N° 12-0155801-00009-0000	30/Diciembre/2014
Prioridad:	ES P201030199	12/Febrero/2010

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por la solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el

presente caso se estudiarán las reivindicaciones 1 a 10 del capítulo reivindicatorio (Radicado N°12-0155801-00009-0000).

Título de la solicitud: “Método de obtención de un extracto alergénico”.

El problema planteado en esta solicitud consiste en la necesidad de optimizar el proceso de purificación de alérgenos, útiles en la elaboración de medicamentos de inmunoterapias para tratar trastornos alérgicos.

Para resolver este problema técnico la solicitud en estudio proporciona un proceso para producir un extracto de alérgeno y un extracto alérgeno despigmentado polimerizado producido mediante dicho proceso.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 39/35, A61P 37/08.

4. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 carece de claridad y concisión por cuanto:

Todos los pasos están redactados en términos de un resultado a alcanzar, ignorando características técnicas del proceso, que ni siquiera están mencionadas en las reivindicaciones dependientes. Se recuerda a la solicitante que una reivindicación de procedimiento debe estar reclamada a partir de una serie de pasos ordenados donde se indiquen las condiciones necesarias para que cada uno de los pasos se lleve a cabo y todas las características técnicas que lo diferencian del estado de la técnica.

Según lo divulgado en la descripción, previo a la preparación del extracto (paso inicial) se lleva a cabo un proceso de desengrasado del material de origen por medio de acetona, sin embargo, esto no está incluido en las reivindicaciones (Pág. 22; Fig. 1; Pág. 26; Pág. 28; Pág. 30 – 31; Pág. 33; Pág. 35; Pág. 37).

El paso a) reclama de forma general poner en contacto un material de origen que comprende un alérgeno con un agente de extracción líquida, sin embargo, no se ha indicado la naturaleza de dicho agente de extracción.

El paso b) de la reivindicación 1, que consiste en someter la mezcla a una primera etapa de separación, no menciona la forma cómo se llevará a cabo dicha separación. El paso debe hacer mención a la técnica utilizada para lograr la separación. Lo mismo para el paso d).

El paso c), que hace referencia a la extracción, no define al agente de extracción.

El paso e) que consiste en eliminar las fracciones de bajo peso molecular, no menciona la técnica empleada para alcanzar ese resultado esperado. Además, el uso de membrana de 3.5 kDa no está soportado en la descripción, puesto que allí solo se menciona para esta etapa el empleo de membranas de 3kDa. El paso g), el cual incluye dos pasos en uno, también se refiere de forma general a "someter el extracto a una etapa de eliminación de fracciones de bajo peso molecular", sin indicar la técnica empleada de acuerdo a lo divulgado en la descripción. Se sugiere hacer las aclaraciones necesarias, también para el paso i).

El paso f) no revela ninguna característica técnica del proceso y solo describe un resultado a alcanzar. Se sugiere reemplazar por la etapa llevada a cabo para conseguir dicho resultado.

Por último, de acuerdo con los hallazgos descritos en el capítulo descriptivo, la concentración del aldehído y la velocidad de adición del aldehído son características técnicas esenciales del

proceso para lograr una polimerización óptima del extracto de alérgeno (Pág. 17), sin embargo, no están definidas en el paso h) del proceso. Por lo cual las reivindicaciones no definen la materia que se desea proteger (Art 30). Se sugiere incluir estas características esenciales en las reivindicaciones.

Se recuerda a la solicitante que en la reivindicación independiente deben estar reclamadas todas las características técnicas esenciales de la invención, que lo diferencian del estado de la técnica.

Descripción (Art 28 D 486)

En la solicitud presentada ante esta Oficina no se han incluido las figuras existentes en la solicitud internacional. Se sugiere hacer las correcciones necesarias.

5. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: febrero 12 de 2010, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO1994006821	"A process for the purification of aqueous extracts containing allergenically active proteins, extracts obtainable according to this process as well as their use"	31/Marzo/1994
D2	Casanovas *et al.* 2005	"Double-blind study of tolerability and antibody production of unmodified and chemically modified allergen vaccines of *Phleum pretense*"	20/Octubre/2005

El documento D1[1] divulga un proceso para la purificación de un extracto acuoso que contiene proteínas alérgicamente activas que comprende: proporcionar al extracto acuoso un material de partida, someter el material de partida a un tratamiento para remover compuestos no alergénicos y obtener extracto sustancialmente puro. (Pág. 2, líneas 33-37, Pág. 3, líneas 1-8).

El documento D2[2] da a conocer la importancia de la modificación química de alérgenos, por medio de la despigmentación y la polimerización, en tratamientos de inmunoterapia (Resumen).

6. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art 18 D486)

La reivindicación 1 consiste en: *"Un proceso para producir un extracto de alérgenos que comprende a) poner en contacto un material de origen…"* (Radicado Nº 12-0155801-00009-0000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

1 http://www.google.com/patents/WO1994006821A1

2 Casanovas, M. Sastre, J., Fernández-Nieto, M., Lluch, M., Carnés, J. and Fernández-Caldas, E. 2005. Double-blind study of tolerability and antibody production of unmodified and chemically modified allergen vaccines of Phleum pretense. Clinical & Experimental Allergy. 35(10): 1377–1383.

Características esenciales	D1	D2
Proceso para producir un extracto de alérgenos	Proceso para producir un extracto de alérgenos (Pág. 9-10)	Proceso para producir un extracto de alérgenos (Pág. 1378 subtítulo Allergen extractsforimmunotherapy)
a). Poner en contacto un material de origen que comprende un alérgeno con un agente de extracción líquida	Poner en contacto masa de polen desgrasada con un solvente acuoso (buffer de dilución, solución de bicarbonato amonio y agua destilada) (Pág. 9 ln. 33-37).	Extracción en PBS de polen desgrasado (Pág. 1378 subtítulo Allergen extractsforimmunotherapy)
b). Someter la mezcla a una primera etapa de separación	Centrifugar y colectar el sobrenadante (Pág. 10 ln. 1-2)	No menciona
c). Poner en contacto los residuos del material de origen con un agente de extracción de alérgenos	Poner en contacto los residuos insolubles con un agente de extracción (Pág. 10 ln. 2-3)	No menciona
d). Someter la mezcla a una segunda etapa de separación	Someter los extractos acuosos a filtración (Pág. 10 ln. 3-6)	No menciona
e). Someter al extracto de alérgenos bruto a una etapa de eliminación de fracciones de bajo peso molecular (eliminar las moléculas que tengan un tamaño molecular menor que 3.5kDa).	Someter al extracto de alérgenos bruto a una etapa de eliminación de fracciones de bajo peso molecular (diálisis o diafiltración usando membranas con un punto de corte de 5-10kDa) (Pág. 10 ln. 7-10)	Diafiltración en membranas de punto de corte de 3kDa. (Pág. 1378 subtítulo Allergen extractsforimmunotherapy)
f). Llevar a cabo la etapa e) hasta que el extracto tenga una conductividad menor que 1000uS/cm a 3-5°C	Llevar a cabo la etapa e) por 18h cambiando el buffer de diálisis muchas veces (Pág. 10 ln. 7-10)	
g). Acidificar el extracto de alérgenos nativos hasta pH 2-3.0 durante 5-30 min. Someter al extracto a una etapa de eliminación de fracciones de bajo peso molecular (eliminar las moléculas que tengan un tamaño molecular menor que 3.5kDa) y ajustar el pH a 7.0-8.0.	Acidificar el extracto de alérgenos nativos hasta pH 2. Re-dializar por 24 h. y ajustar el pH a 6.5 a 7.5. (Pág. 10 ln. 16-32)	Despigmentar el extracto nativo por tratamiento de ácido débil. Seguido de diálisis extensiva en mambranas de punto de corte de 3.5kDa (Pág. 1378 subtítulo Allergen extractsforimmunotherapy)
h). poner el extracto de alérgenos despigmentado obtenido en el paso anterior en contacto con glutaraldehído o formaldehído	No menciona	h). poner el extracto de alérgenos despigmentado obtenido en el paso anterior en contacto con glutaraldehído (Pág. 1378 subtítulo Allergen extractsforimmunotherapy)
i). Someter el extracto de alérgenos despigmentado a una etapa de eliminación de fracciones moleculares (eliminar las moléculas que tengan un tamaño molecular menor que 100 kDa)	No menciona	i). Someter el extracto de alérgenos despigmentado a una etapa de diálisis extensiva (eliminar las moléculas que tengan un tamaño molecular menor a 100 kDa) (Pág. 1378 subtítulo Allergen extractsforimmunotherapy)
j). Llevar a cabo la etapa i) hasta que el extracto tenga una conductividad menos que 210 uS/cm a 3-5°C y/o esté ausente de aldehído mediante barrido de UV o de luz visible.	No menciona	

El documento D1 se considera el estado de la técnica más cercano a la invención definida en la reivindicación 1 porque divulga un procedimiento para producir un extracto de alérgenos que consiste en poner en contacto masa de polen desgrasada con un solvente acuoso (buffer de dilución, solución de bicarbonato amonio agua destilada), centrifugar y colectar el sobrenadante, poner en contacto los residuos insolubles con un agente de extracción, someter los extractos acuosos a filtración, someter al extracto de alérgenos bruto a una etapa de eliminación de fracciones de bajo peso molecular (diálisis o diafiltración usando membranas

con un punto de corte de 5-10kDa) por 18h cambiando el buffer de diálisis varias veces, acidificar el extracto de alérgenos nativos hasta pH 2, re-dializar por 24 h y ajustar el pH a 6.5 a 7.5 (Págs. 9-10). Así mismo, el documento D1 da a conocer un extracto producido por el método descrito (Pág. 20).

La diferencia entre la invención, definida en la reivindicación 1 y el procedimiento divulgado en el documento D1 consiste en los pasos h) – j) de la presente solicitud.

El efecto que estos pasos le proporcionan al proceso reclamado, es que se consigue un extracto de alérgeno polimerizado.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar el procedimiento conocido en el documento D1 para conseguir el efecto técnico que la invención proporciona.

Sin embargo, los pasos de polimerización posteriores al paso de despigmentación de un extracto de alérgeno ya habían sido divulgados con anticipación en el documento D2, el cual enseña las etapas poner el extracto de alérgenos despigmentado en contacto con glutaraldehído y someter el extracto de alérgenos despigmentado a una etapa de diálisis extensiva, eliminando las moléculas que tengan un tamaño molecular menor a 100 kDa (Pág. 1378 subtítulo: Allergen extracts for immunotherapy).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría estos pasos finales del documento D2 en el procedimiento divulgado en el documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual, la solución propuesta se considera obvia y sin altura inventiva.

Vale la pena aclarar que el último paso del proceso, el cual consiste en asegurarse que el aldehído esté ausente, está revelado en el documento D2 de manera intrínseca, por el hecho de que tras usar membranas de 100kDa para eliminar las moléculas de menor peso molecular, el glutaraldehído haría sido eliminado del producto. De igual forma, el paso f) relacionado con obtener un extracto con una conductividad menor que 1000uS/cm, hace referencia a un resultado esperado que se consigue tras realizar la diálisis extensiva, con lo cual se reducen las sales de la solución (pasan libremente a través de las membranas) y con ello se reduce también la conductividad del extracto.

Puesto que las reivindicaciones dependientes 2 a 10 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Las reivindicaciones 1 a 10 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

7. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO1994006821	1 a 10	Nivel Inventivo
D2	Casanovas *et al.* 2005	1 a 10	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Si como respuesta a este requerimiento el solicitante llegara a modificar: i) el capítulo reivindicatorio, ii) la descripción de la invención a proteger, iii) o presente un nuevo capítulo reivindicatorio, y desee que se realice un nuevo examen de patentabilidad a esta modificación, no deberá pagar la tasa de modificación voluntaria pero sí la correspondiente a la tasa de examen de patentabilidad. En todo caso, el número de requerimientos por no patentabilidad estará supeditado a que el término del estudio de patentabilidad no sea superior a 18 meses, contados desde la publicación de la solicitud de patente de invención. Adicionalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones deberá pagar la tasa por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.

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Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2016-05-17

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Revisó: Lenin Roberto Guerrero

Clin Exp Allergy 2005; **35**:1377–1383 doi:10.1111/j.1365-2222.2005.02343.x

Double-blind study of tolerability and antibody production of unmodified and chemically modified allergen vaccines of *Phleum pratense*

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Summary

Background The physicochemical modification of allergen extracts provides a chance for administering higher doses of allergen vaccines.

Objective To evaluate the safety of a chemically modified (depigmented-glutaraldehyde polymerized) therapeutic vaccine of *Phleum pratense* administered at doses that are 10 times higher than those used in clinical practice, in comparison with conventional doses of the corresponding non-modified alum-adsorbed vaccine.

Materials and methods The design of the study was randomized, double-blind, parallel and included two groups of patients. Twenty-three patients were treated weekly during nine visits for the build-up phase, followed by two weekly maintenance doses (a total of 11 injections per patient). Twelve patients received a vaccine containing the standardized unmodified extract, at a maximum concentration of 308.5 mcg of freeze dried material/mL (Group A). Eleven patients received a standardized modified allergen extract (Group B). The maximum dose used was 2400 mcg/mL. Safety was evaluated recording all adverse events. Skin test results and specific antibody levels were evaluated at the beginning and at the end of the study.

Results Group A patients experienced three local immediate (two clinically irrelevant and one with a diameter $>$ 5 cm) and 18 delayed reactions (15 irrelevant and three with a diameter $>$ 10 cm), while Group B experienced six local immediate and 12 delayed reactions (all clinically irrelevant). Nine Group A patients experienced 12 systemic reactions (one immediate of grade 1, one of grade 2; and one delayed of grade 1; four of grade 2 and three of grade 3), while Group B patients experienced one immediate systemic reaction of grades 1, and 1 delayed reaction of grade 1.

Conclusions The modified extract of *P. pratense* is safe to treat sensitive patients, even at concentrations that are 10 times higher than those regularly administered in clinical practice. The majority of the local reactions were clinically irrelevant. No systemic reactions of grade 2, 3 or 4 were reported using the modified extract.

Keywords allergens, asthma rhinitis, depigmented–polymerized allergen extracts, immunotherapy, *Phleum pratense*, tolerability

Submitted 5 April 2004; revised 7 July 2005; accepted 19 August 2005

Introduction

Allergen immunotherapy is part of a broad-based category of therapy presently used to treat immunologic diseases, because it modifies, or downregulates, the immune response to foreign allergens [1]. Grass pollen immunotherapy improves the quality of life [2, 3], increases the threshold dose eliciting an immediate response using specific challenges [1] and reduces symptoms and/or the need for asthma and rhinoconjunctivitis medications [1]. A large series of injections is required, which consists of weekly injections, until the maintenance dose is reached after 3–4 months. The use of higher doses at the beginning can considerably shorten this process [4, 5]. However, this approach could be dangerous since a relationship between the dose administered and the appearance of IgE-mediated adverse events has been established [6, 7]. Regular injection of vaccines leads to an increase in specific IgG production. However, adverse events because of IgG overproduction with the use of allergen vaccines have not been reported.

The efficacy of allergen immunotherapy is dose dependent; therefore, a careful balance between efficacy and safety is needed. Thus, the concept of optimal dose is used and defined as the dose of allergen vaccine inducing a clinically relevant effect in most patients without causing unacceptable side effects [8, 9]. To improve the safety of the original aqueous allergen vaccines, an initial physical modification step was introduced in the 1930s with the use of aluminium hydroxide [10]. However, these physically modified vaccines continued to express their allergenic properties. To overcome this disadvantage, allergen extracts modified with glutaraldehyde were developed in the 1960s, proving to be safer than

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unmodified vaccines, while retaining their clinical efficacy [11, 12]. The introduction of a depigmentation step before the polymerization process removes adsorbed pigments, enhances the solubility of the polymer and inactivates the enzymatic activity of certain allergens [13]. These modified vaccines maintain, or increase, specific IgG recognition, decrease specific IgE binding and induce smaller skin test reactions [14]. The clinical safety and efficacy of these modified allergen extracts has been proven for *Parietaria judaica* [15], *Olea europaea* [16], a mixture of *Dactylis glomerata* and *Olea europaea* [3] house dust mites (HDMs) [17, 18] and *Phleum pratense* [19]. The latter study was a dose-finding, efficacy and safety trial, which showed efficacy using a maximum concentration of 18.5 μg of freeze-dried material per millilitre.

The objective of this study was to evaluate the tolerability and safety of a chemically modified therapeutic vaccine of *P. pratense* during the build-up phase and at doses higher than those used regularly. As comparator, we used the respective non-modified vaccine.

Materials and methods

Study design

The study was randomized, double blind, parallel and included two groups of patients. The study was authorized by the Ethics Committee of the Fundación Jiménez Díaz, and the Spanish Health Authorities. All patients gave their witnessed written consent to participate in the study.

Patients

Twenty-three patients (11 men and 12 women; mean age 31 years), recruited from the outpatient clinic of the Allergy Department of the hospital, were included in the study. All met the following inclusion criteria: seasonal rhinoconjunctivitis and mild or moderate bronchial asthma, diagnosed according to Global Initiative for Asthma guidelines [20]; more than 2 years of evolution; detectable specific IgE to *P. pratense*; positive skin prick (weal diameter $\geqslant$3 mm) to a standardized *P. pratense* extract (30 histamine equivalent in prick-testing units (HEP)/mL; Laboratorios LETI, S.L, Tres Cantos, Spain) and negative to other common aeroallergens. The exclusion criteria were those outlined in the World Health Organization (WHO) position paper on allergen immunotherapy [1].

Patients were blindly randomized into two groups. Group A consisted of 12 patients (6 males and six females, mean age 28 years, range 19–39) who received treatment with the unmodified extract and group B of 11 patients (five males and six females, mean age 34 years, range 23–51) who received treatment with the modified extract.

Allergen extracts for immunotherapy

One hundred grams of pollen of *P. pratense*, previously deffated, were extracted 1 : 20 weight/volume in phosphate-buffered saline followed by diafiltration in 3 kDa Pellicon membranes (Millipore, Madrid, Spain). Afterwards, the extract was freeze dried (native extract). Part of this native extract was used to produce the treatments with the non-modified extract and the skin prick test preparations. The rest of the native extract was depigmented, following a patented procedure [21]. It consisted of a mild acid treatment followed by extensive dialysis in 3.5 kDa cut-off membranes against bidistilled water. This allergen preparation was treated with glutaraldehyde to produce the polymer (modified extract) using previously described methods [22, 23]. This step was followed by extensive dialysis in order to remove components with a molecular weight of less than 100 kDa. SDS-PAGE electrophoresis was performed with native, depigmented and polymerized allergen extracts in order to monitor each step of the production process.

Phl p 1 and Phl p 5 were measured by scanning densitometry [24]. The results were expressed as the percentage of major allergen per mg of freeze-dried extract. The protein contents were measured using the Bradford method [25]. The protein nitrogen units (PNUs) were determined using the micro-Kjeldhal method.

The IgG binding activity was measured by specific IgG inhibition assay [26]; allergenicity was evaluated by a capture-specific IgE-binding competition ELISA [27]. The results were expressed as micrograms of freeze-dried material needed to achieve 50% inhibition.

Allergen vaccines and immunotherapy schedule

Each treatment set consisted of three numbered vials (1, 2 and 3) of 10-fold increasing doses of standardized native or modified extract adsorbed onto aluminium hydroxide. The allergen concentration in each treatment vial administered to group A was that regularly used in clinical practice, having the maximum concentration (vial no. 3) of 308.50 mcg/mL, with a biologic potency of 100 HEP_L/mL. All patients were treated with the same batch. In Group B, the maximum concentration of the extract was 2400 mcg/mL, which was 10 times greater than the concentration regularly used in clinical practice. It was the result of depigmenting and polymerizing 30 850 mcg of the native extract. The contents of Phl p 1 and Phl p 5 were 3.14% and 4.37% of the native extract, respectively. All patients were treated with the same batch.

Patients were not pretreated before the administration of any dose. The subcutaneous administration was always done under medical supervision. After each administration, the patients remained for at least 30 min in the clinic [28] in order to detect any immediate adverse reaction. The patients were instructed to immediately inform the physician about all adverse events, related or not with the immunotherapy. A personal or telephone contact was established in the case of delayed adverse events.

Skin tests

Patients were skin-prick tested at baseline and at the end of the study using the same batch of native unmodified allergen extract. The SPTs were conducted in duplicate, on the volar surface of the forearm using four 10-fold dilutions of native extract of *P. pratense* (highest concentration 1 mg of freeze-dried extract per millilitre, with a potency of 30 HEP/mL). For each patient, the dose of allergen extract needed to produce the same weal size as the one induced by histamine HCl 10 mg/mL (10 HEP) was calculated [17].

Specific antibody response

Serum samples of each patient were collected at the first visit (baseline), after 2 months and at the end of the study (after 4 months), and stored in 0.5 mL aliquots at $-70\,^{\circ}C$ until use. Specific IgE was measured by capture ELISA [29], and specific IgG, IgG1 and IgG4 by ELISA [30, 31]; the results were expressed in optical densities (O.D.'s). The serum dilutions used to estimate specific IgE and IgG4 were 1 : 5, and for specific IgG and IgG1, 1 : 1000.

Safety monitoring

The reactions were categorized as immediate when the onset was during the first 30 min after the administration, and delayed when the onset was after this time. Local reactions were quantified measuring the diameter of the reaction. The immediate reactions with a diameter of less than 5 cm and the delayed reactions of less than 10 cm were considered as clinically irrelevant, since a reduction in dose is not recommended [32]. The systemic reactions were graded following the European Academy of Allergy and Clinical Immunology (EAACI) Position Paper [28]. The grades were as follows *grade 0*: no symptoms; *grade 1*: unspecic symptoms – reactions probably not IgE mediated, i.e. discomfort, headache or arthralgia; *grade 2*: mild systemic reactions – mild rhinitis or asthma responding adequately to antihistamines or β_2-agonist therapy; *grade 3*: non-life-threatening systemic reactions – urticaria, angioedema, or severe asthma, responding well to treatment; and *grade 4*: anaphylactic shock – rapidly evoked reaction of itching, ushing, erythema or bronchial obstruction, requiring intensive treatment. The study was designed to evaluate safety in a prospective manner. Therefore, the possibility of repeating or reducing the dose because of adverse reaction was not allowed.

Statistics

The sample size was established following the recommendations of André and Foulkes for tolerance studies in the development of vaccines [33]. The number of patients needed ($n = 22$) was calculated based on the assumption that 80% of the systemic reactions would occur in group A and 20% in group B, for an $\alpha = 0.05$ and a power of 80%. At the end, 12 patients were included in group A and 11 in group B. The SPSS software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. The value of the median and the interquartile (IQ) range was used for descriptive statistics. Contingency table was used to compare the number of adverse reactions and the non-parametric Mann–Whitney test was used to compare values between both groups. Wilcoxon and Friedman tests were used to evaluate the evolution of intragroup values.

Results

Characteristics of the therapeutic vaccines used

The 50% inhibition point of specific IgE binding was 154 ng for the native extract and 16.12 mcg (104.7 times more) for the resulting polymer. The amount of allergen extract needed to obtain a 50% inhibition of specific IgG binding was 289 ng for the native and 315 ng for the modified extract. Other characteristics of the vaccines used are shown in Table 1. Figure 1 shows the SDS-PAGE.

Table 1. Characteristics of the maximum concentration of native and modified vaccines of *Phleum pratense*

	Unmodified	Modified
Freeze-dried extract (mcg/mL)	308	2400
protein (mcg/mL)	100.1	1636.80
PNU/mL	2464.90	24696
Phl p1 (%)	3.14	ND*
Phl p5 (%)	4.37	ND*
Potency (HEP_L/mL)	100	33**

*Not detectable because of the modification process.
**Residual potency after the modification process. The initial potency of the native extract was 10 000 HEP_L.

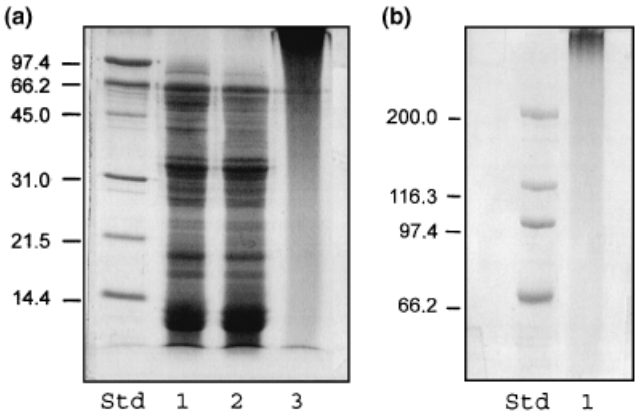

Fig. 1. SDS-PAGE electrophoresis patterns of the native, depigmented and depigmented–polymerized preparations of *Phleum. pratense*. (a) **Std**: low molecular weight markers; lane 1: native extract; lane 2: depigmented extract and lane 3: depigmented and polymerised extract. (b) lane 1: high Molecular weight markers: lane 1: depigmented and polymerized extract.

Patients, immunotherapy schedule and safety There were no significant differences in age, gender, intital SPT size, specific IgE levels, symptom severity and asthma prevalence. The total number of injections administered was 114 in group A, and 121 in group B. Each patient received a maximum of 11 injections. Table 2 shows the schedule used and the total accumulated dose.

Three patients of group A and five of group B were free of local or systemic reactions. With the normal recommended doses used in clinical practice, nine patients of group B (82%) were free of reactions. The total number of local reactions was 21 in group A and 18 in group B ($P > 0.05$). Three immediate local reactions occurred in group A patients, and six in group B ($P > 0.05$); only one reaction in group A was evaluated as relevant (diameter > 5 cm). The total number of local delayed reactions was 18 in group A and 12 in group B ($P > 0.05$); three reactions were clinically relevant in group A (diameter > 10 cm), the rest were irrelevant.

Four immediate systemic reactions occurred in group A, two evaluated as grade 1, 1 of grade 2 and 1 of grade 3, whereas group B recorded 1 reaction of grade 1 ($P > 0.05$).

Table 2. Quantity of allergen extract and IgE potency (HEP_L) administered in each dose of the vaccine containing the native extract (Group A), and data corresponding to the modified extract, describing the amount of modified allergen extract administered per dose injected, the original IgE potency (HEP_L) before the process of chemical modification and the residual after this process (Group B)

	Unmodified					Modified				
						Potency			HEP_L/dose	
Vial	Units	Dose	ML	mcg/dose	HEP_L/dose	Before modification	Residual after modification	mcg/dose	Before modification	Residual after modification
1	1 HEP_L/mL	1	0.1	0.31	0.1	100 HEP_L/mL	0.3 HEP_L/mL	2.40	1	0.003
		2	0.3	0.92	0.3			7.20	3	0.010
		3	0.5	1.54	0.5			12.00	5	0.017
2	10 HEP_L/mL	4	0.1	3.08	1	1000 HEP_L/mL	3.3 HEP_L/mL	24.0	10	0.033
		5	0.3	9.24	3			72.0	30	0.100
		6	0.5	15.40	5			120.0	50	0.167
3	100 HEP_L/mL	7	0.1	30.80	10	10 000 HEP_L/mL	33.3 HEP_L/mL	240	100	0.333
		8	0.3	92.40	30			720	300	1.000
		9	0.5	154.00	50			1200	500	1.667
		10	0.5	154.00	50			1200	500	1.667
		11	0.5	154.00	50			1200	500	1.667
	Total μg accumulated			**615.69**				**4797.6**		

Table 3. Number and evaluation of clinically relevant adverse reactions distributed per vial of treatment

			Group A			Group B		
			Vial			Vial		
			1	2	3	1	2	3
Local	Immediate	∅ > 5 cm	0	0	1	0	0	0
	Delayed	∅ > 10 cm	0	2	1	0	0	0
Systemic	Immediate	Grade 2	0	0	1	0	0	0
		Grade 3	0	0	1	0	0	0
	Delayed	Grade 2	0	1	3	0	0	0
		Grade 3	0	0	4	0	0	0

The grey-shadowed area corresponds to the vial of maximum concentration used in regular clinical practices.

Delayed systemic reactions were 8 (1 of grade 1, 3 of grade 2 and 4 of grade 3) in group A and 1 (grade 1) in group B ($P = 0.03$). Nine of the 12 systemic reactions which took place in group A were produced when injecting the vial of maximum concentration (number 3). Four patients treated with the native extract were invited to stop their participation in the study because of a delayed systemic and one because of an immediate reaction. Table 3 shows the number and evaluation of clinically relevant adverse reactions per vial of treatment. Table 4 shows the description of systemic reactions. All the reactions were categorized as reactions because of the intrinsic characteristics of the vaccine preparation and the individual response. Systemic or local side-effects did not correlate with the severity of allergy.

Skin tests

The results are expressed as the amount of native, unmodified allergen extract necessary to induce a weal of the same size as the positive control. At baseline, the 10 HEP value for the group A was 10.31 mcg (IQ 3.4–34.58), and for group treated with the modified extract 1.48 (IQ 0.58–3.40). After the period of treatment, the results were 189.52 (IQ 101.05–417.79) and 237.96 mcg (IQ 94.22–386.82) respectively. Figure 2 shows the box plot of these results. The statistical analysis showed no statistical differences ($P > 0.05$) at baseline and at the end between both groups. The intragroup comparison showed that both groups needed a significantly ($P < 0.05$) higher quantity of allergen extract to achieve the 10 HEP value. At the end of the study, group A needed 18 times more quantity of native allergen extract to achieve the 10 HEP value, whereas group B needed 161 times more quantity.

Specific antibody response

Group A experienced a significant increase in specific IgG1, and group B in IgG and specific IgG1 levels after 4 months. Both groups did not experience any changes in specific IgE and IgG4 levels. When the results were analysed between groups, significantly higher levels of total specific IgG, IgG1 and IgG4 were detected in group B. Table 5 shows the descriptive and comparative statistics.

Discussion

Herein we present the safety profile, the action on skin sensitivity and specific antibody production of a depigmented-glutaraldehyde-polymerized vaccine of *P. pratense* used at high doses. The highest concentration of the modified allergen injected (2.4 mg/mL) was calculated based on the yield of the modification process. It was the result of chemically modifying 30.8 mg, which is 100 times greater than the maximum concentration regularly used with the corresponding non-modified vaccine. In this case, the yield of the process from native to modified extract was 7.8%. The maximum concentration of Phl p 5 in the unmodified allergen extract administered in this study was similar to the one used by

Table 4. Systemic reactions: description of the reactions, time of appearance (immediate or late), grade, dose that provoked the reaction, vial and quantity (mL) injected

Patient ID	Reaction	Group	Visit	Vial	mL	Grade	Description
20298	Delayed	A	8	3	0.1	1	Unspecific symptoms
20298	Immediate	A	9	3	0.3	2	Nasal-ocular symptoms, dyspnoea, dizziness, and cough *
21895	Immediate	A	4	2	0.5	1	Perioral itching
22687	Immediate	A	9	3	0.3	2	Wheals and rhinoconjunctivitis *
25039	Delayed	B	7	3	0.5	1	Headache and nasal obstruction
33274	Delayed	A	8&9	3	0.3 and 0.5	3	Naso-ocular symptoms, abdominal pain, diarrhoea and headache
53278	Delayed	A	9	3	0.5	2	Rhinoconjunctivitis
61156	Immediate	A	7	3	0.1	3	Wheals, rhinoconjunctivitis and bronchospasm *
65456	Immediate	B	10	3	0.5	1	Palatal itching
79508	Delayed	A	8	3	0.3	3	Pharyngeal discomfort, headache, and generalized wheals *
81767	Delayed	A	10	3	0.5	3	Asthma, wheals, fatigue, and dry cough *

*The patients were invited to stop the participation in the study after the adverse reaction.

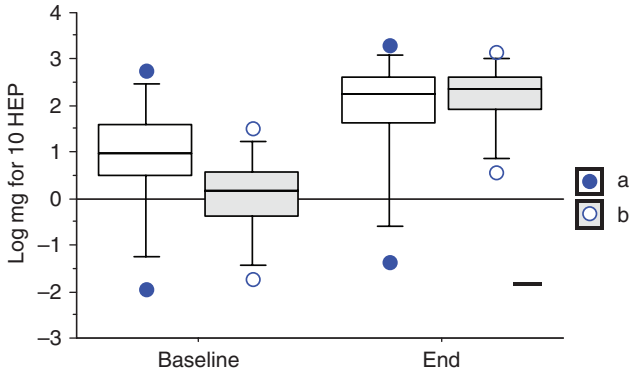

Fig. 2. Box plot view of the skin-prick tests (SPTs) results. The *X*-axis shows SPT results of both groups before and after the study. The *Y*-axis shows the micrograms (log transformed) needed to produce a wheal of the same size as the one produced by histamine HCl at 10 mg/mL (10 HEP). A and B denote Groups A and B.

Walker et al. [2] (20 mcg/mL of Phl p 5) and to the doses described in the WHO Position Paper on Allergen Immunotherapy [1], supported by the study of Østerballe et al. [34]. However, the maximum dose of the modified preparation was 12 348 PNU, greater than the 3300 PNU given by Grammer et al. [35] in an accelerated dosage schedule using glutaraldehyde polymerized extracts of grasses.

Placebo was not used following the Note of Clarification (paragraph 29) added by the World Medical Association Declaration of Helsinki [36]. It is stated that care must be taken in using placebo-controlled trials and that this methodology should only be used in the absence of an existing proven therapy. Thus, as comparator we used the respective non-modified vaccine. The study was conducted following the recommendations of the Committee for Proprietary Medicinal Products in the clinical evaluation of new vaccines [37], which indicates that, whenever feasible, besides an overall safety comparison, an additional comparison with the appropriate control vaccines should be made. The study was designed to evaluate the safety profile of a modified allergen therapeutic vaccine under experimental conditions. Thus, all reactions were recorded, including those that would not have been recorded under normal clinical conditions.

In the study, both groups of patients experienced a significant reduction in skin sensitivity in a short period of time. Both extracts showed no effect on specific IgE and IgG4 production, which could be because of the short duration of the study. The effect on specific IgG was significant for both

Table 5. Median, first and third quartiles of specific IgE, IgG, IgG1 and IgG4 of both groups, and results of the comparative statistics intragroup (Friedman's test) and intergroups (Mann–Whitney's test)

Group		Baseline	2 months	4 months	*P* (Friedman)
		IgE			
	1st quartile	0.34	0.49	0.20	
A	Median	1.43	1.40	1.46	>0.05
	3rd quartile	1.73	1.85	1.95	
	1st quartile	0.48	0.62	0.52	
B	Median	1.18	1.27	1.25	>0.05
	3rd quartile	2.49	2.75	1.82	
	P (Mann–Whitney)	>0.05	>0.05	>0.05	
		IgG			
	1st quartile	0.59	0.91	0.83	
A	Median	1.06	1.20	0.98	>0.05
	3rd quartile	1.57	1.66	1.76	
	1st quartile	0.83	0.93	2.05	
B	Median	1.06	1.30	2.56	0.002
	3rd quartile	1.34	2.04	3.12	
	P (Mann—Whitney)	>0.05	>0.05	0.01	
		IgG1			
	1st quartile	0.17	0.20	0.24	
A	Median	0.20	0.26	0.31	0.007
	3rd quartile	0.29	0.41	0.60	
	1st quartile	0.14	0.22	0.75	
B	Median	0.20	0.37	1.72	<0.001
	3rd quartile	0.30	0.86	2.00	
	P (Mann–Whitney)	>0.05	>0.05	0.002	
		IgG4			
	1st quartile	0.16	0.20	0.47	
A	Median	0.69	0.76	1.04	>0.05
	3rd quartile	2.44	1.67	1.88	
	1st quartile	0.96	0.77	1.30	
B	Median	1.67	1.49	2.30	>0.05
	3rd quartile	2.21	2.37	2.96	
	P (Mann–Whitney)	>0.05	>0.05	0.041	

groups. In group B, in spite of the high doses injected and the significant increase in specific IgG levels, these levels did not reach the threshold value, which could have induced an inflammatory response because of a type III hypersensitivity reaction, as observed in massive local reactions occurring 2–8 h after a tetanus toxoid injection [38, 39]. The absence of these reactions suggests the possible tolerogenic properties of allergen extracts treated with glutaraldehyde. This fact has been demonstrated in rats that were exposed parenterally or pergastrically to glutaraldehyde-polymerized collagen from bovine nasal septal cartilage and became resistant to the subsequent induction of disease with arthritogenic collagen administered intradermally in Freund's incomplete adjuvant [40]. We acknowledge that more studies with allergens are needed to confirm this possibility.

In the study, 12 systemic reactions in nine patients were reported in group A (8.8% of the injections). Seven occurred during the up-dosing process with the vial of maximum concentration. Because of the characteristics of the study, which was designed to evaluate safety with the imposibility of stepping back the dose administered in case of moderate-severe adverse reactions, five patients were invited to stop their participation, two because of immediate systemic reactions, and three due to delayed reactions. Two systemic reactions occurred in group B, 1 delayed (0.75% of the injections) graded as 1, with the injection of 0.5 mL of vial no. 2, and 1 immediate of grade 1 with vial 3. Although group A patients experienced more local reactions than group B patients (21 vs. 18), this difference was not significant. The majority of the local reactions were clinically irrelevant; in Group B there were no clinically relevant local reactions.

Overall, the rate of systemic reactions using aqueous or alum-adsorbed pollen extracts is in the range of 0.01%–70% of the patients [12]. In one prospective study [41] using native alum-adsorbed grass-pollen extracts, 8.3% of the systemic reactions were immediate of grade 2, and 20.8% of grade 3, and 12.5%, 54.2% and 4.2% delayed of grades 2, 3 and 4, respectively. In our study, in group A, 10% of the systemic reactions were immediate of grade 1% and 10% of grade 2% and 10%, 40% and 30% were delayed of grade 1, 2 and 3, respectively. All these reactions were mild and resolved spontaneously without the need for rescue medication. No reactions of grade 4 were recorded. The incidence systemic delayed reactions of grades 2 and 3 detected in our study were similar to the ones found in this study [41].

It has been shown that formaldehyde-modified extracts elicit almost as many systemic reactions as native extracts when not fractionated by molecular weight size. However, these reactions were usually less severe [12]. The safety records of two types of glutaraldehyde–polymerized extracts have been reviewed [12]. High molecular weight preparations did not induce systemic reactions, but systemic reactions using unfractionated allergoids were not uncommon. In our study, the doses of the modified extract used in commercially available treatments only produced one systemic reaction of grade 1 (non-IgE mediated).

An equally modified preparation of *D. pteronyssinus* showed a similar safety profile as the one described in this study [42]. In a prospective multicentre observational cohort study [43], a total of 2261 injections were administered to 175 patients with rhinoconjunctivitis, with or without asthma, sensitized to grass pollen (55%), tree pollen (18%), tree and grass pollen mixture (14%), HDMs (11%) and others (2%). Local reactions with a diameter > 10 cm occurred in 0.1% of the injections. Thirteen patients experienced systemic reactions, all judged to be mild.

This study demonstrates that depigmented-and glutaraldehyde-polymerized allergen extracts of *P. pratense* used at concentrations which are 10 times higher than those regularly administered in clinical practice are safe to treat *P. pratense*-sensitive patients, confirming the wide therapeutic margin for this chemically modified allergen vaccine and the safer profile than regular vaccines based on native unmodified extracts.

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INFORME DE BÙSQUEDA DE PATENTES PARA EL No de Radicación: 12-155801	
CRITERIOS PARA LA BÙSQUEDA (anexar pantallazo o reporte en PDF, junto con los boleanos utilizados)	
Palabras clave utilizadas	producing allergen extract diafiltration allergen vaccines AND glutaraldehyde polymer
CLASIFICACIONES INTERNACIONALES UTILIZADAS PARA LA BÚSQUEDA	

BASES DE DATOS CONSULTADAS.			
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INFORMES DE EXÁMENES REALIZADOS EN OTRAS OFICINAS (documentos patentes, literatura no patente etc…)

Oficina	Fecha del examen o de reporte de búsqueda de documentos	Anterioridades encontradas para el examen o reportados en el examen de búsqueda(relacionarlos todos)	Reiv. afectadas	RELEVANCIA (requisito que afecta)	FUERON TENIDOS EN CUENTA PARA EL EXAMEN EN LA OFICINA COLOMBIANA	
					SI	NO
EUROPEA		EP1834648	1-17	NI		X
		WO94/06821	1-17	NI	X	
USPTO		EP1834648	1-17	NI		X
		WO94/06821	1-17	NI	X	

DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÙSQUEDA.

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