

RESEARCH ARTICLE

New toxicity testing of PQ grass allergy immunotherapy to support product development

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Abstract

PQ Grass represents an allergen-specific immunotherapy for pre-seasonal treatment of patients with seasonal allergic rhinitis (or rhinoconjunctivitis) with or without mild-to-moderate bronchial asthma. It consists of a native pollen extract for 13 grass species, chemically modified with glutaraldehyde, and adsorbed to L-tyrosine in a microcrystalline form with addition of the adjuvant Monophosphoryl Lipid A (MPL[®]). Previous non-clinical safety testing, including rat repeat dose toxicity in adult and juvenile animals, rat reproductive toxicity and rabbit local tolerance studies showed no safety findings of concern. A new Good Laboratory Practice compliant rat subcutaneous repeat dose toxicity study to evaluate a higher clinical dose and modified posology (once every 2 weeks for 13 weeks) showed no signs of toxicity. As seen in previous studies, relatively minor, immunostimulatory effects were seen such as reversible increased white cell count (notably neutrophils), increased globulin level (resulting in decreased A/G ratio) and increased fibrinogen as well as minor dose site reaction in the form of inflammatory cell infiltrate. These findings are likely due to the immunostimulatory nature of MPL and/or the presence of L-tyrosine within the adjuvanted vaccine. This new toxicity study with PQ Grass therefore supports longer posology with higher dose levels.

KEYWORDS

allergy vaccine, grass pollen, non-clinical testing, PQ grass, toxicity study

1 | INTRODUCTION

Allergic rhinitis is defined as symptoms of sneezing, nasal pruritus, air-flow obstruction and nasal discharge as well as ocular discomfort such as itching or watery eyes caused by IgE-mediated reactions against inhaled allergens and involving mucosal inflammation driven by type 2 helper T (Th2) cells (Bousquet et al., 2008; Klossek, Annesi-Maesano, Pribil, & Didier, 2012; Wheatley & Togias, 2015). Allergens of importance include seasonal grass pollens. Depending on climate and region, global sensitization rates to grass pollen can vary between 10% and 30% of the general population (Newson et al., 2014; Salo et al., 2014). PQ Grass is a grass-specific allergen immunotherapy

(AIT) for the pre-seasonal treatment of patients with proven type I hypersensitivity to cross-reacting grass pollens causing seasonal allergic rhinitis (or rhinoconjunctivitis) with or without mild-to-moderate bronchial asthma. AIT has been practised for many years in patients with pollen allergy and involves administration of increasing quantities of specific allergen extracts to induce a state of allergic non-responsiveness upon subsequent exposure (Akkoc, Akdis, & Akdis, 2011; Bousquet, Lockey, & Malling, 1998; Ebner, 1999; Larche, 2006). Thus, AIT downregulates the allergic response in an allergen-specific manner by causing decreases in mast cell and basophil activity and associated degranulation, with a reduced tendency for systemic anaphylaxis degranulation taking place (Akdis & Akdis, 2015).

This action is followed by generation of allergen-specific regulatory T and B (Treg and Breg) cells and suppression of allergen-specific Th2 cells, while immunoglobulin (IgE) is decreased. In addition, clinical studies using AIT have demonstrated correlation between increased allergen-specific IgG4 and clinical outcomes (Gehlhar, Schlaak, Becker, & Bufe, 1999; Shamji et al., 2017).

The test article consists of pollen extract from 13 species of grass chemically modified with glutaraldehyde (forming an allergoid) adsorbed to L-tyrosine, a naturally occurring amino acid in a microcrystalline form with the addition of the adjuvant MPL[®], a detoxified derivative of lipid A of *Salmonella minnesota* R595. It has been shown that modification of allergen to allergoid reduces IgE reactivity with retention of IgG binding epitopes as shown from immunoreactivity and structural analysis testing with single or multiple mix grass allergoids (Moran, Wheeler, Overell, & Woroniecki, 1977; Starchenka, Bell, Mwange, Skinner, & Heath, 2017). Adjuvant use of microcrystalline tyrosine (MCT) allows controlled slow release of allergoid resulting in reduced incidence of local IgE-mediated allergic responses, while prolonging the antigenic response of the therapy (Leuthard et al., 2018). Thus, work with MCT showed that it protects from IgE-mediated reactivity in mouse models but acts independently of inflammasome (specific innate) and toll-like receptor signalling. Furthermore, testing in mice sensitized by injection of birch pollen extract with the same allergen adsorbed on MCT with up to five weekly doses (giving a total extract protein dose of 264 µg) showed IgG1 and IgG2a antibody responses were induced. The adjuvant activity of MPL arises through promotion of a Th1 response through stimulation of the innate immune response by binding to the toll-like receptor complex (Casella & Mitchell, 2008; Evans et al., 2003). Thus, switching away from an IgE-mediated allergic response, as evidenced by an IgG titre, occurs. A Th1-skewed effect with induced IgG2a and IgG2b responses but no IgE change was seen in mice treated with allergoid adsorbed to L-tyrosine and with MPL (Wheeler, Marshall, & Ulrich, 2001). Other work showed that allergoid (glutaraldehyde-modified ragweed pollen) MCT with MPL enhanced allergen-specific IgG1 and IgG2a antibody titre in mice with the combination of MCT and MPL generating a high and sustained IgG antibody response (Hutchings, Heath, & Skinner, 2017). Clinical studies with other PQ products have shown that this effect is mirrored in human populations demonstrating a significant increase in allergen-specific IgG and IgG4 immunoglobulin subtypes in response to treatment and dose level (Worm et al., 2018). Furthermore, this clinical work has indicated that a range of cumulative strengths and up-dosing schemes are clinically efficacious.

The proposed clinical regimen for PQ Grass is six subcutaneous injections given at intervals of up to 14 days as 900 standardized units (SU)/mL (first injection), 2700 SU/mL (second injection) and 6000 SU/mL (third to sixth injections) to give a cumulative dose of 27 600 SU. This proposed form has been preceded with other formulations used clinically including one of four injections with 300-2000 SU allergen extract, 20 mg L-tyrosine and 0.05 mg MPL at 0.5 mL or another of six injections with 900-8000 SU allergen extract, 20 mg L-tyrosine and 0.05 mg MPL at 1 mL. The new form of PQ Grass

enables higher doses of allergoid to be delivered per injection and higher cumulative doses to be achieved.

A number of non-clinical studies have been performed to support clinical development of the various forms of PQ Grass and these are summarized in Table 1. During this time, non-clinical evaluation followed the principles of available regulatory guidelines (EMA, 1998; ICH M3(R2), 2009; WHO, 2005; WHO, 2013) and advice from regulatory agency interactions. The toxicity study described herein with PQ Grass involved single subcutaneous doses in the rat given every other week for 13 weeks (seven doses) with an allergen extract strength of 8000 SU/mL using n (clinical number) + 1 subcutaneous doses to support longer dosing in the clinic at higher dose levels. The n + 1 dosing scheme follows current regulatory thinking (Matsumoto et al., 2016; WHO, 2013).

The toxicity study was performed at Covance Laboratories Ltd in 2017-2018 in compliance with Good Laboratory Practice (GLP).

2 | MATERIALS AND METHODS

2.1 | Test material and formulation

The PQ Grass 8000 SU/mL formulation is a 10% aqueous extract of equal quantities of pollen (harvested according to good agricultural practice and provided by audited and certified suppliers) from 13 grass species modified by glutaraldehyde, which is adsorbed by co-precipitation to L-tyrosine with addition of MPL. The identification of the grass species and composition of drug product are given in Tables 2 and 3, respectively.

The test material used for the repeat dose toxicity study was supplied as vials containing 8000 SU/mL of grass pollen allergoid, 50 µg/mL MPL adjuvant and 20 mg/mL tyrosine. The control material was supplied as vials of '2% Tyrosine suspension' and was identical to the test material except that grass pollen allergoid and MPL were not present.

Animal care and use was compliant with the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) recommendations and study designs underwent internal ethical review.

2.2 | Repeat dose toxicity study

The study took the form of a 13-week intermittent dose subcutaneous toxicity study in the rat followed by a 4-week treatment-free period. Han-Wistar CrI:WI (Han) rats were obtained from Charles River Laboratories and were subjected to a 2-week acclimation period. A group of 15 male and 15 female rats were then given a single subcutaneous dose of PQ Grass at 1 mL/rat once every 2 weeks for 13 weeks (a total of seven doses). Each dose contained 8000 SU/mL of grass pollen allergoid, giving a cumulative quantity of 56 000 SU, which was well in excess of the proposed clinical cumulative dose of 27 600 SU. A further group of 15 males and 15 females received control material at 1 mL/rat. Four injection sites were used in rotation (first

TABLE 1 Non-clinical studies performed with PQ Grass

PQ Grass form (reference)	Design	Results
2000 SU/mL of 13 grass pollen allergoid, 50 µg/mL MPL adjuvant and 20 mg/mL tyrosine (Baldrick, Richardson, Wheeler, & Woroniecki, 2004)	Single subcutaneous or intraperitoneal dose to groups of 5 male and 5 female CD-1 mice (as well as controls) using 0.05 or 0.5 mL, respectively ^a	No study findings with subcutaneous dosing. Tremors, lethargy and unkempt appearance occurred with intraperitoneal dosing (including controls) and was attributed to the presence of phenol in the formulation
	Single subcutaneous or intraperitoneal dose to groups of 5 male and 5 female Han-Wistar rats (as well as controls) using 0.1 or 1 mL, respectively ^a	No study findings
	A total of 3 once-weekly subcutaneous doses to groups of 15 male and 15 female Han-Wistar rats (as well as a group lacking allergoid) with a 2-week recovery period using 0.1 or 1 mL	Key study findings of raised white blood cell count and neutrophils and/or lymphocytes, increased globulin and decreased A/G ratio and albumin and/or slightly increased spleen weight (all reversible) at 1 mL along with dose site findings of cellulitis/fibrosis/abscess, haemorrhage and/or granulomas (with evidence of recovery) at 0.1 and 1 mL (and in control). Such systemic and dose site findings have been reported in rat studies with MPL and L-tyrosine
12 000 SU/mL of 13 grass pollen allergoid, 50 µg/mL MPL adjuvant and 20 mg/mL tyrosine (Baldrick et al., 2004)	A total of 3 once-weekly subcutaneous doses to groups of 15 male and 15 female Han-Wistar rats (as well as a group lacking allergoid) with a 2-week recovery period using 0.1 or 1 mL	
4000 SU/mL of 13 grass pollen allergoid, 50 µg/mL MPL adjuvant and 20 mg/mL tyrosine (Baldrick, Hewings, & Skinner, 2011)	A total of 4 once-weekly ascending subcutaneous doses of 300, 800, 2000 and 2000 SU/0.5 mL to groups of 12 male and 12 female juvenile (commencing at 3 weeks of age) Sprague-Dawley rats (as well as a saline group and a group lacking allergoid) with a 4-week recovery period ^b	Key study findings of increased neutrophils and globulin, and decreased A/G ratio and albumin (all reversible) along with dose site findings of inflammatory cell infiltration/abscess (with evidence of recovery) (also in the group lacking allergoid)
	A subcutaneous dose 2 weeks before mating and on days 6 and 13 of gestation at 2000 SU/0.5 mL to groups of 24 female Sprague-Dawley rats (as well as a saline group)	No signs of reproduction toxicity was seen
2000 SU/mL of 13 grass pollen allergoid, 50 µg/mL MPL adjuvant and 20 mg/mL tyrosine (Baldrick et al., 2004)	A single subcutaneous dose of 2000 SU dosed 1 mL in a group of 4 male and 4 female New Zealand White rabbit with comparison with saline injection in the same animals	Dose site inflammatory response seen
4000 SU/mL of 13 grass pollen allergoid, 100 µg/mL MPL adjuvant and 20 mg/mL tyrosine (Reeve, Hewings, & Skinner, 2012)	Bacterial reverse mutation assay at up to 2000 SU/plate	Negative
	In vitro mouse lymphoma assay at up to 200 SU/mL	Negative (3- and 24-h treatment in the absence of exogenous metabolic activation -S9 fraction) but positive (3-h treatment in the presence of S9 fraction and possibly related to an artefact of the exogenous metabolizing system)
	Micronucleus test (bone marrow) in groups of 6 male Han-Wistar rats following subcutaneous doses of 10 000, 20 000 or 40 000 SU/kg on 2 consecutive days	Negative
	Comet assay (liver and kidney) in groups of 6 male Han-Wistar rats following subcutaneous doses of 20 000 or 40 000 SU/kg on 2 consecutive days	Negative

MPL, Monophosphoryl Lipid A[®].^aThese acute studies were performed in 2000-2001 when such testing was routine.^bTo support use in a paediatric population based on recommendation from the European Medicines Agency.

dose into left shoulder, second into right shoulder, third into left hip, fourth into right hip and so on). Rats were approximately 6-7 weeks old and had bodyweights of 165-213 g (males) and 132-175 g (females) upon study initiation. Animals were maintained in an air-

conditioned room with 15-20 air changes/hour. The temperature and relative humidity ranges were maintained in the specified ranges of 20-24°C and 45%-65%, respectively and a 12-hour light/dark cycle was used. Standard animal diet and water were available ad libitum

TABLE 2 Grass species identification

Common name	Scientific name
Bent grass	<i>Agrostis capillaris/tenuis</i>
Foxtail (Meadow)	<i>Alopecurus pratensis</i>
Sweet vernal	<i>Anthoxanthum odoratum</i>
False oat	<i>Arrhenatherum elatius</i>
Brome	<i>Bromus</i> spp.
Dogtail (Crested)	<i>Cynosurus cristatus</i>
Cocksfoot	<i>Dactylis glomerata</i>
Fescue (Meadow)	<i>Festuca pratensis</i>
Yorkshire Fog	<i>Holcus lanatus</i>
Rye grass	<i>Lolium perenne/multiflorum</i>
Timothy grass	<i>Phleum pratense</i>
Meadow grass	<i>Poa pratensis/trivialis</i>
Cultivated rye	<i>Secale cereale</i>

throughout the study. Rats were housed two or three to a cage and were provided with wooden Aspen chew blocks, amber tunnels and dried pasta as forms of environmental and dietary enrichment.

Following the last dose, 10 male and 10 female animals from the control and test groups were killed after 13 weeks; the remaining rats were retained for a 4-week recovery period. The following study parameters were measured: mortality and clinical observations, bodyweights, food consumption, ophthalmoscopy, clinical pathology, organ weights plus macroscopic and microscopic examination. Cage-side observations were made daily, bodyweights were recorded weekly and food consumption was measured twice weekly. An ophthalmoscopic examination was performed predose, at week 12 and at the end of the recovery period; examination was carried out using an ophthalmoscope after induction of mydriasis. During week 13 and at the end of the recovery period, blood samples for haematology, coagulation and clinical chemistry assessment were collected from all animals after fasting overnight. Samples were collected in EDTA, trisodium citrate and lithium heparin tubes, respectively. Haematology parameters measured comprised: haemoglobin, red blood cell count, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, reticulocyte count, red cell

distribution width, total and differential white cell count and platelet count. Coagulation parameters measured comprised: activated prothrombin time, partial thromboplastin time and fibrinogen. Clinical chemistry parameters measured comprised: aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, alkaline phosphatase, total cholesterol, triglycerides, total bilirubin, total protein, albumin, globulin, albumin/globulin (A/G) ratio, sodium, potassium, chloride, calcium, inorganic phosphate, creatinine, urea, glucose and creatine kinase. The following urine parameters were measured: volume, colour, turbidity, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood and microscopy of sediment.

Rats were killed after 13 weeks of treatment or, after the 4-week recovery period, by isoflurane anaesthesia before exsanguination and then examined grossly for pathological changes. Selected organs were weighed and comprised: adrenal, brain, epididymis, heart, kidney, liver, ovary, pituitary, prostate, spleen, testis, thymus, thyroid with parathyroid, and uterus with cervix. Following processing to 5 µm sections and staining with haematoxylin and eosin for light microscopy, the following organs/tissues were examined histopathologically for all animals of the terminal control and test groups: adrenal, aorta, brain, caecum, colon, duodenum, epididymis, oesophagus, eyes, femur with bone marrow and femorotibial joint, gut-associated lymphoid tissue/Peyer's patch, gross lesions, heart, ileum, injection sites, jejunum, kidney, liver, lungs with mainstem bronchi and bronchioles, lymph node (axillary, mandibular and mesenteric), mammary gland, muscle (biceps femoris), nerve (optic and sciatic), ovary, pancreas, pituitary, prostate, rectum, salivary gland (mandibular and sublingual), seminal vesicle, skin and subcutis, spinal cord (at three levels), spleen, sternum with bone marrow, stomach, testis, thymus, thyroids with parathyroids, tongue, trachea, urinary bladder, uterus with cervix and vagina. Injection sites and any gross lesions were examined for all recovery group animals.

2.3 | Statistical analysis

Statistical analysis of bodyweight, food consumption, clinical pathology parameters and organ weights occurred with comparison of the control group with those receiving the PQ Grass. Probability values of $P < .05$, $P < .01$ and $P < .001$ were considered statistically significant.

TABLE 3 Composition of drug product

Ingredient	Function	Reference to quality standard	Quantity per mL of drug product proposed for clinical use		
			6 injections; cumulative dose: 27 600 SU		
Active pharmaceutical ingredient ^a					
Modified 13 Grass Extract	Active	In-house	900 SU/mL	2700 SU/mL	6000 SU/mL
Other components					
L-Tyrosine	Adsorbant base	USP/Ph. Eur.	20 mg	20 mg	20 mg
MPL	Adjuvant	In-house	0.05 mg	0.05 mg	0.05 mg

MPL, Monophosphoryl Lipid A[®]; SU, standardized units/mL.

^aFurther components include dipalmitoyl phosphatidylcholine, phenol, sodium chloride, disodium phosphate dodecahydrate, sodium dihydrogen phosphate dehydrate and water for injection.

3 | RESULTS

Single subcutaneous doses of 1 mL PQ Grass at 8000 SU/mL once every 2 weeks for 13 weeks were well tolerated. No obvious signs of systemic toxicity occurred and study findings were limited to a few clinical pathology parameters and dose site histopathological changes. Slightly higher bodyweight gain (4%) at termination for females (resulting in a 6% increase in bodyweight) compared with the control group was not seen after the recovery period and was considered coincidental. Haematology and coagulation parameter changes at termination are given in Table 4. Thus, there were minor increases in reticulocyte count and red cell distribution width (both sexes) and activated partial thromboplastin time (males only), which

may well have been coincidental as this parameter was decreased in females, along with increased white blood cell count (both sexes), notably for neutrophils and eosinophils (males only); lymphocytes were decreased (both sexes). Fibrinogen level was slightly increased (both sexes). Table 5 shows that clinical chemistry changes were associated with increased globulin level (both sexes) resulting in decreased A/G ratio and minor alterations in albumin (decreased in both sexes) and total protein (slightly increased in both sexes). Other findings comprised minor changes in aspartate aminotransferase (decreased mainly in males), calcium (increased mainly in males), phosphorus (increased in both sexes), urea (decreased in both sexes) and creatinine (increased in both sexes), all of which may well have been coincidental. Other than the calcium and urea alterations,

TABLE 4 Notable haematology and coagulation findings after 13 weeks of treatment with PQ Grass compared with control

Parameter (units)	Control males	Test males	Control females	Test females
Reticulocyte (10E9/L)	184.7 ± 21.81	247.9 ± 19.89 (+34%)*	167.5 ± 31.56	207.3 ± 32.53 (+24%)*
Reticulocyte (%)	2.0 ± 0.24	2.7 ± 0.23 (+35%)*	2.0 ± 0.37	2.5 ± 0.42 (+25%)*
Red cell distribution width (%)	12.2 ± 0.46	12.8 ± 0.49 (+5%)*	10.9 ± 0.43	11.6 ± 1.42 (+6%)
White blood cell (10E9/L)	6.3 ± 0.47	9.5 ± 1.35 (+51%)*	4.2 ± 0.55	5.7 ± 1.04 (+36%)*
Neutrophil (10E9/L)	1.56 ± 0.37	4.58 ± 0.74 (+194%)*	1.12 ± 0.3	2.78 ± 0.93 (+148%)*
Eosinophil (10E9/L)	0.07 ± 0.03	0.12 ± 0.04 (+71%)*	0.07 ± 0.02	0.07 ± 0.19 (0%)
Lymphocyte (%)	71 ± 4.8	48 ± 5.6 (-32%)*	68 ± 6.3	49 ± 11.2 (-28%)*
APTS (s)	18.9 ± 0.72	20.9 ± 1.02 (+11%)*	21.7 ± 8.28	18.3 ± 0.91 (-16%)
Fibrinogen (g/L)	1.68 ± 0.12	2.53 ± 0.14 (+51%)*	1.32 ± 0.16	2.44 ± 0.33 (+85%)*

APTS, activated partial thromboplastin time.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

Values in parenthesis are percentage change from control

TABLE 5 Notable clinical chemistry findings after 13 weeks of treatment with PQ Grass compared with control

Parameter (units)	Control males	Test males	Control females	Test females
AST (IU/L)	76 ± 6.1	64 ± 7.4 (-16%)*	72 ± 6.6	69 ± 4.8 (-4%)
Total protein (g/L)	68 ± 2.7	73 ± 1.8 (+7%)*	73 ± 3.3	76 ± 2.2 (+4%)
Albumin (g/L)	42 ± 1.2	40 ± 1.9 (-5%)	49 ± 2.5	47 ± 1.4 (-4%)*
Globulin (g/L)	27 ± 2.1	32 ± 2.0 (+19%)*	24 ± 1.8	29 ± 2.0 (+21%)*
A/G ratio	1.6 ± 0.12	1.2 ± 0.13 (-25%)*	2.1 ± 0.17	1.6 ± 0.13 (-24%)*
Calcium (mmol/L)	2.61 ± 0.07	2.73 ± 0.07 (+5%)*	2.66 ± 0.72	2.69 ± 0.54 (+1%)
Phosphorus (mmol/L)	1.4 ± 0.19	1.9 ± 0.20 (+36%)*	1.2 ± 0.19	1.4 ± 0.14 (+17%)*
Urea (mmol/L)	6.1 ± 0.69	4.8 ± 0.72 (21%)*	7.1 ± 0.85	6.7 ± 0.55 (-6%)
Creatinine (μmol/L)	29 ± 2.3	33 ± 2.8 (+14%)*	31 ± 5.0	35 ± 3.4 (+13%)

A/G, albumin/globulin; AST, aspartate aminotransferase.

* $P < .01$.

** $P < .01$.

*** $P < .001$.

Values in parenthesis are percentage change from control.

none of these clinical pathological changes was seen after the recovery period. Macroscopic examination showed occasional red areas/reddening or thickening/raised areas among animals of control and test groups at the left hip dose site, while a gelatinous appearance was noted at this site upon removal of overlying skin for a small number of animals administered PQ Grass. Histopathology findings are shown in Table 6. At the left hip dose site, inflammation was observed in both control and test groups and was characterized by a zone of inflammatory cells (predominantly neutrophils) surrounding a central amorphous area of injected material. In general, the severity of this finding was marginally higher for animals administered PQ Grass compared with the control group. At the other dose sites (right shoulder, left shoulder and right hip), focal areas of fibrosis were noted for a small number of animals in both control and test groups. After the recovery period, focal areas of fibrosis were observed at dose sites in single animals administered PQ Grass.

4 | DISCUSSION

PQ Grass AIT is an allergoid adsorbed to MCT with MPL for pre-seasonal treatment of patients with proven type I hypersensitivity to cross-reacting grass pollens causing seasonal allergic rhinitis (or rhinoconjunctivitis) with or without mild-to-moderate bronchial asthma. This paper reports the results of a toxicity study performed to characterize the safety of PQ Grass further to support clinical development. It was shown that subcutaneous doses of PQ Grass every second week on seven occasions over 3 months in the rat showed no obvious signs of systemic toxicity, with minor study findings reflecting use of an adjuvanted vaccine. Thus, increased white cell count (notably neutrophils), increased globulin level (resulting in decreased A/G ratio) and increased fibrinogen were seen as well as minor dose site reaction in the form of inflammatory cell infiltrate surrounding a central amorphous area of injected material at a marginally higher severity in animals administered PQ Grass and/or a few cases

TABLE 6 Incidence of injection site findings after 13 weeks of treatment and 4 weeks of recovery with PQ Grass compared with control

		Control male	Test male	Control female	Test female
Tissue and finding	Level (SU/mL)	0	8000	0	8000
Terminal kill					
Subcutaneous, left shoulder	No. examined:	10	10	10	10
Fibrosis	Grade –	10	8	9	8
	1	0	2	1	2
Subcutaneous, right shoulder	No. examined	10	10	10	10
Fibrosis	Grade –	10	8	10	5
	1	0	2	0	4
	2	0	0	0	1
Subcutaneous, left hip	No. examined	10	10	10	10
Inflammation	Grade –	3	2	2	0
	1	3	2	6	0
	2	4	2	2	4
	3	0	4	0	6
Subcutaneous, right hip	No. examined	10	10	10	10
Fibrosis	Grade –	9	10	10	10
	1	1	0	0	0
	2	0	0	0	0
Recovery kill					
Subcutaneous, left shoulder	No. examined	5	5	5	5
Fibrosis	Grade –	5	5	5	4
	1	0	0	0	1
Subcutaneous, right shoulder	No. examined	5	5	5	5
Fibrosis	Grade –	5	4	5	5
	1	0	1	0	0
Subcutaneous, left hip	No. examined:	5	5	5	5
Fibrosis	Grade –	5	5	5	4
	1	0	0	0	1
Subcutaneous, right hip	No. examined	5	5	5	5
Fibrosis	Grade –	5	5	5	5
	1	0	0	0	0

–, finding not present; 1, minimal; 2, slight; 3, moderate; F, female; M, male.

of focal areas of fibrosis, which was also seen in controls (same vaccine formulation except grass pollen allergoid and MPL were not present). Recovery after the 4-week non-dose period was generally seen for study findings. As shown in Table 1, an array of non-clinical studies performed with PQ Grass confirms its safety profile. Thus, there was no evidence of general toxicity, reproduction toxicity or genotoxicity. As in the present study, a few limited clinical pathology and dose site findings were seen in toxicity testing in the rat, which were related to dosing with an adjuvanted vaccine. Furthermore, toxicity work with other allergoid MCT with MPL products (with household mite and ragweed pollen extracts) and their component parts (L-tyrosine and MPL) have shown consistent findings associated with immunostimulatory changes (including increased white blood cell values and altered globulin levels) in the rat (Baldrick, Richardson, & Wheeler, 2002a, 2002b; Baldrick, Richardson, Elliott, & Wheeler, 2002; 2007). Thus, both systemic and dose site effects are likely due to the action of the immunostimulatory nature of MPL and/or the presence of L-tyrosine within the adjuvanted vaccine. More recently, a review of toxicity studies with 30 different adjuvanted vaccines showed common study findings of altered clinical pathology parameters (commonly raised blood neutrophil count and increased globulin levels, but also raised fibrinogen) and evidence of inflammation at the dose site (Baldrick, 2016). It was concluded that clinical pathological and injection site changes can be interpreted as related to an expected reaction after vaccine dosing, with generation of an immune response largely because of the presence of adjuvant, although direct vaccine antigen involvement was also occasionally demonstrated by the presence of a slightly increased inflammatory response seen over adjuvant treatment only.

Overall, repeated subcutaneous doses of PQ Grass in the rat revealed no safety concerns at a cumulative quantity of 56 000 SU grass pollen extract, which was well in excess of the proposed clinical cumulative dose of 27 300 SU. This lack of toxicity has been supported by clinical use as widespread use of PQ Grass and a grass allergoid vaccine (an earlier form without the presence of MPL) has shown that these products are well tolerated with the majority of adverse drug reactions being resolvable local reactions (in-house data).

In conclusion, an additional repeat dose toxicity study in the rat to supplement other available toxicity studies with PQ Grass at a higher cumulative dose has shown that there were no safety findings of concern. Expected, and relatively minor, immunostimulatory effects were seen following repeated dosing (once every 2 weeks for 13 weeks) in the form of reversible increased white cell count (notably neutrophils), increased globulin level (resulting in decreased A/G ratio) and increased fibrinogen, as well as minor dose site reaction in the form of inflammatory cell infiltrate. These findings are likely due to the immunostimulatory nature of MPL and/or the presence of MCT.

CONFLICT OF INTEREST

Paul Baldrick assisted in preparation of the manuscript under contract with Allergy Therapeutics Ltd. Tom Martin was Study Director and

Chris Gray was Study Pathologist for the repeat dose toxicity study. All other authors: none to declare.

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