A randomised placebo-controlled trial investigating efficacy and mechanisms of low-dose intradermal allergen immunotherapy in treatment of seasonal allergic rhinitis

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Scientific summary

Trial of immunotherapy for treatment of seasonal allergies

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Scientific summary

Background

In the UK an estimated 5 million people suffer moderate/severe persistent symptoms of allergic rhinitis that have an impact on quality of life, including disturbed sleep, disruption of leisure activities and impairment of performance at work/school. There is a substantial unmet need for both therapy and prophylaxis of seasonal allergic rhinitis.

In the UK, immunotherapy is indicated in patients with moderate or severe symptoms who fail to respond to conventional medications. Immunotherapy with grass pollen for treatment of season allergic rhinitis was first described in 1911, and the conventional approach involves the regular subcutaneous administration of allergen extracts at high doses (typically microgram quantities of group 5 grass pollen allergens). A significant body of evidence, including a Cochrane meta-analysis (Calderon MA, Alves B, Jacobson M, Hurwitz B, Sheikh A, Durham S. Allergen injection immunotherapy for seasonal allergic rhinitis. *Cochrane Database Syst Rev* 2007;**1**:CD001936), exists to support the clinical efficacy of high-dose subcutaneous immunotherapy. Grass pollen allergen may also be administered at high dose as sublingual tablets or drops, an approach that is further supported by a Cochrane meta-analysis (Radulovic S, Calderon MA, Wilson D, Durham S. Sublingual immunotherapy for allergic rhinitis. *Cochrane Database Syst Rev* 2010;**12**:CD002893). Both subcutaneous and sublingual high-dose immunotherapy have significant limitations: the vaccine products are expensive and the need for repeated administration in a specialist clinic (subcutaneous immunotherapy) or daily at home (sublingual immunotherapy) is associated with additional expense and/or inconvenience. Therefore, there is a continuing need to develop new and improved immunomodulatory therapies for allergic rhinitis.

We established 'proof of concept' for a novel low-dose intradermal immunotherapy regimen in subjects with grass pollen-induced allergic rhinitis. A feature of an intradermal allergen injection is the development of local swelling within 6 hours that persists for 24–36 hours. This 'late-phase response' is characterised by infiltration of inflammatory cells, notably activated T cells, eosinophils and basophils. We previously showed that six 2-weekly intradermal injections of grass pollen (containing only 7 ng of major allergen Phl p 5; 10 BU) resulted in a 93% suppression (mean of $n = 10$ subjects) in the cutaneous late-phase response, measured after 24 hours in response to these injections. This effect was systemic and antigen specific, and the magnitude of late-phase response suppression was comparable to that seen following treatment with a conventional high-dose subcutaneous grass pollen vaccine, and greater than that seen following sublingual immunotherapy. The concept of administering low-dose allergen immunotherapy by the intradermal route has been described in the medical literature dating back to 1926, and our own findings suggested the plausibility of this approach. A potential advantage of the novel intradermal regimen was that the effect on skin responses was seen with a low dose of allergen, which was not changed between visits. As adverse reactions to immunotherapy usually occur when doses are increased, this would offer significant clinical advantages over existing vaccines. Based on this, we initiated a randomised controlled trial (RCT) of low-dose intradermal allergen immunotherapy as a treatment for seasonal allergic rhinitis.

Objective

The objective of this study was to investigate the efficacy and mechanism of low-dose intradermal grass pollen immunotherapy in adults with seasonal allergic rhinitis ('hay fever').

Methods

We conducted a Phase II RCT comparing intradermal injection immunotherapy with grass pollen allergen extract or a histamine control.

Eligible participants were aged 18–65 years, with grass pollen-induced allergic rhinitis of at least 2 years' duration, with moderate or severe symptoms despite treatment with antihistamine drugs and/or nasal corticosteroid drugs. Participants were required to have a positive skin prick test response (> 3 mm to *Phleum pratense*, ALK Abelló, Reading, UK), a positive specific immunoglobulin E (IgE; > class 2) against *P. pratense*, and a pre-bronchodilator forced expiratory volume in 1 second (FEV_1) of > 70% of predicted value. Exclusion criteria included seasonal grass pollen-induced asthma requiring regular treatment and symptomatic seasonal allergic rhinitis and/or asthma caused by tree pollen, weed pollen or a perennial allergen to which the participant was regularly exposed, except for mild intermittent symptoms. Potential participants were also excluded if they had received treatment with grass pollen immunotherapy within the previous 5 years.

The intervention was a series of seven grass pollen or control injections, administered intradermally every 2 weeks into the forearm, before the 2013 grass pollen season. Each active intradermal allergen injection contained 10 BU [33.3 SQ-U (standard quality units)] of *P. pratense* soluble grass pollen extract (Aquagen SQ™ Timothy, ALK Abelló). The control drug was histamine only, administered at concentrations of 100 µg/ml (injections 1 and 2), 30 µg/ml (injections 3 and 4) and 10 µg/ml (injections 5–7). A reducing dose of histamine was used to help preserve blinding. Active and control study medications appeared identical.

The primary end point was a combined symptom and medication score (CSMS) during the grass pollen season period spanning 13 May to 31 August 2013. Daily symptoms (nose, eyes, mouth and lungs) and medication use (antihistamines, nasal steroid drugs, antihistamine eye drops and oral prednisolone) were recorded on diary cards. Symptom scores and medication scores for each participant were calculated as area under curve (AUC).

Secondary clinical end points were:

- overall symptoms during entire pollen season (AUC)
- overall medication scores over entire pollen season (AUC)
- Mini-Rhinitis Quality of Life Questionnaire (Mini-RQLQ) scores (measured three times during, and once after, the pollen season)
- health-related quality-of-life scores, evaluated using the European Quality of Life-5 Dimensions, 5-levels (EQ-5D-5L) questionnaire (measured three times during, and once after, the pollen season)
- visual analogue scale (VAS) scores for nasal and eye symptoms, recorded 2-weekly during the entire pollen season (AUC)
- global evaluation of symptoms, recorded once after the pollen season
- number of general practitioner visits for hay fever during summer 2013
- CSMSs during the peak of the 2013 grass pollen season (peak pollen season days defined in accordance with prespecified criteria)
- number of medication-free days during the grass pollen season
- number of symptom-free days during the grass pollen season
- individual symptoms scores (AUC) for each organ: nose, mouth, eyes and lungs
- total number of days during which prednisolone was taken during the grass pollen season
- frequency of adverse events (AEs).

Mechanistic studies

Sera were collected before and after intradermal grass pollen or control immunotherapy for measurement of grass pollen-specific immunoglobulins. Basophil activation tests were also performed following administration of the final intradermal allergen immunotherapy or control injection (May 2013). All

participants underwent intradermal skin challenge testing 4 months after the final intradermal allergen immunotherapy or control injection (September 2013). Participants were then randomised to undergo repeat follow-up testing 7, 10 or 13 months later to assess persistence of late-response suppression. The procedure for the intradermal skin challenge testing and the dose of allergen used were identical to that for an active intradermal allergen immunotherapy injection. Early- and late-phase responses were measured 15 minutes and 24 hours after challenge, respectively.

Forty participants (20 in each trial arm) were selected at random to undergo 3-mm skin punch biopsies immediately after measurement of late-phase responses (i.e. 24 hours after challenge) at the 4-month time point in September 2013. Biopsies were analysed for inflammatory cell infiltration by immunohistochemistry, and a proportion was also cultured as explants for analysis of cutaneous T cells by flow cytometry and microarray transcriptional profiling.

Statistical analysis

On the basis of data from a previous RCT of subcutaneous grass pollen immunotherapy, we estimated that with 35 participants in each group the study would have a power of 90% (alpha = 0.05) to detect a between-group difference in the primary outcome during the grass pollen season. For the purposes of sample size estimation, the treatment effect was conservatively estimated at only 80% of that observed with subcutaneous immunotherapy. To make allowance for the unknown distribution of the primary outcome, and based on the lower bound for the asymptotic relative efficiency of the Mann–Whitney *U*-test, the sample size was increased by a further 15% to 40 participants in each arm. To account for a post-randomisation dropout rate of up to 10%, a total sample size of 90 (45 each arm) was estimated as required.

Statistical analyses were performed on an intention-to-treat (ITT) basis, with data from all of the participants who could be assessed for the primary outcome. Summary measures for the baseline characteristics of each group were calculated as mean and standard deviation for continuous (approximate) normally distributed variables, medians and interquartile ranges (IQRs) for non-normally distributed variables, and frequencies and percentages for categorical variables. The AUC of the CSMSs was plotted against time as a summary measure of the primary outcome. The primary efficacy analysis, that is, the difference between the two arms in AUC of the CSMSs, was analysed on randomised patients using a stratified Mann–Whitney *U*-test (van Elteren test), adjusted for the baseline stratification factors of size of the skin test to grass pollen and presence or absence of rhinitis symptoms outside the grass pollen season. Median differences between the groups were calculated using the stratified Hodges–Lehmann method. Similar analyses were conducted for symptom scores, medication scores, symptoms in different organs and VASs. Linear mixed models were used to evaluate Mini-RQLQ and EQ-5D-5L scores in order to isolate the effect of the intervention on each arm after adjusting for stratification factors. Differences between the groups were reported with their 95% confidence intervals (CIs). All mechanistic between-group comparisons were performed by Mann–Whitney *U*-test, with the exception of serology and immunohistochemistry comparisons, which were analysed by analysis of covariance. Comparisons of serology between pre and post treatment, and skin biopsy immunohistochemistry between diluent control and allergen challenge were made by Wilcoxon signed-rank test.

Results

Ninety-three participants were enrolled in the study and underwent randomisation. Study arms were well balanced for baseline characteristics. All of the 46 participants who were assigned to intradermal allergen immunotherapy completed the treatment course. Of the 47 participants who were assigned to control injections, one did not complete the treatment course for work-related reasons. Missing diary data for the primary end point were few, with 94% of participants supplying > 90% of daily diary card data. Five participants, all in the control arm, significantly deviated from the protocol in use of rescue medications. There was no evidence that participants were able to identify if they had received the active or control intervention.

Primary outcome

All 93 randomised participants were evaluated for the primary outcome and were included in the ITT analysis. There was no significant difference between the intradermal immunotherapy group (active intervention) and the control group for the primary end point, that is, the CSMS over the whole grass pollen season (difference in median AUC = 14; 95% CI –172.5 to 215.1; $p = 0.80$).

Secondary outcomes

There were no differences between the trial arms in the secondary end points of overall symptom scores (AUC; $p = 0.24$) or rescue medication use (AUC; $p = 0.44$) during the whole season, or the CSMSs during peak season (12 June to 26 July 2013) (AUC; $p = 0.99$). Among other secondary end points, allergic rhinitis symptoms measured by daily nasal symptom scores were 44% higher in the intradermal allergen immunotherapy group than in the control group, with a median difference in AUC values of 35 (95% CI 4.0 to 67.5; $p = 0.03$). There was also a trend for higher nasal symptoms measured by VAS in the intradermal allergen immunotherapy group, with a 28% median difference in AUC values (difference 53; 95% CI –11.6 to 125.2; $p = 0.05$). No significant differences were seen between groups in daily eye or lung symptoms, although there was a trend for mouth symptoms to be higher in the intradermal allergen group (difference in median AUC 10, 95% CI –3.8 to 24; $p = 0.05$). No significant group differences were observed in eye symptoms measured by VAS, or Mini-RQLQ scores, EQ-5D-5L scores, global evaluation of symptoms scores, numbers of symptom-free or medication-free days or number of days during which prednisolone was used as a rescue medication. There were few treatment-related AEs, with no difference between trial arms.

Outcomes of mechanistic studies

A seasonal fall in *P. pratense*-specific IgE occurred in the group that received control histamine injections (median change –5.4 kU/l, IQR –13.6 to –1.3; $p < 0.001$), but IgE levels were maintained in the active intradermal immunotherapy group [median change –1.0 kU/l IQR –7.3 to 2.4; $p = 0.23$ ($p = 0.001$ for between-group comparison)]. The same pattern was observed in levels of IgE that were specific for major allergens Phl p 1 and Phl p 5. A similar treatment effect was also seen on *P. pratense*-specific immunoglobulin G (IgG) titres, which fell in the control group ($p = 0.03$) but not the intradermal allergen group over the same period ($p = 0.26$ and $p = 0.007$ for between-group comparison), although this pattern was not seen with IgG4 (immunoglobulin G subclass 4) responses. Cluster of differentiation 4-positive ($CD4^+$) T cells that were expanded from grass pollen-challenged skin showed higher expression of T helper type 2 cell (Th2) surface marker CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells) in the intradermal allergen immunotherapy group [median 13.4% (IQR 6.3–25.4), $n = 10$] than those in the control group [6.3% (IQR 1.9–7.6%), $n = 9$] ($p = 0.04$), whereas expression of the T helper type 1 cell (Th1) marker CXCR3 [chemokine (C-X-C Motif) receptor 3] was lower in the intradermal allergen immunotherapy group (33.5%, IQR 24.7–47.3% vs. 56%, IQR 45.8–63.8%; $p = 0.01$). Microarray transcriptional profiling performed on skin T cells also identified higher expression of messenger ribonucleic acid for Th2 cytokine interleukin 5 in the intradermal immunotherapy group ($p = 0.03$).

Immunohistochemistry of skin biopsies showed grass pollen-induced recruitment of eosinophils, neutrophils, cluster of differentiation 3-positive T cells and $CD4^+$ T cells but no significant treatment effect. Furthermore, no significant treatment effect was seen on surface expression of peripheral blood basophil activation markers. Late-phase responses in the skin were still suppressed at 4 and 7 months after completing intradermal allergen treatment ($p = 0.03$ for both time points), but not at 10 or 13 months. In comparison with historical data, however, the degree of suppression at these times was less than that observed immediately after completing six injections, suggesting that the suppressive effect on late-phase responses was wearing off within 4 months.

Conclusions

In this study, we have demonstrated that preseasonal treatment with intradermal grass pollen injections was not clinically effective, as measured by the primary end point of a CSMS during the 2013 summer

grass pollen season. Although this trial was not specifically designed or powered to detect worsening of symptoms, analysis of secondary end points indicated that intradermal allergen immunotherapy was associated with worse allergic rhinitis nasal symptoms. Furthermore, we found evidence for immunological priming of IgE and Th2 cell responses. We conclude that novel immunotherapy strategies that promote dermal allergen exposure have the potential to be deleterious, even if local macroscopic responses appear to be suppressed by this approach.

Trial registration

This trial is registered as ISRCTN78413121.

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This report

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