

Repeated high-dose inhalation allergen challenge in asthma

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Abstract

Introduction: Inhalation allergen challenge in humans is used to investigate lung pathophysiology and responses to novel therapies. However, the single high-dose allergen challenges that are commonly performed do not mimic repeated symptomatic environmental allergen exposure.

Objectives: To develop and evaluate the safety of a repeated high-dose symptomatic inhalation allergen challenge model.

Methods: Sixteen subjects with atopic asthma were recruited. Each underwent three inhalation allergen challenges using house dust mite (*Dermatophagoides pteronyssinus*) antigen at 48-h intervals with a target of symptom induction and an early asthmatic reaction fall in forced expiratory volume in 1 s (FEV₁) of 15% from baseline.

Results: All of the subjects completed the three-challenge protocol and the target immediate airway bronchoconstrictor response was achieved in all the subjects at all challenges. There were no adverse events recorded. The early asthmatic reaction was similar for the three challenges whether measured as mean maximal fall in FEV₁ or mean area under the curve. The late asthmatic reaction was also similar over the three challenges with no evidence of priming or desensitisation. Symptom scores and reliever medication use significantly increased over the time of the challenges. Baseline lung function and reversibility was unchanged 4 days after the last challenge.

Conclusion: We demonstrate that repeated high-dose inhaled house dust mite allergen challenge in human volunteers with mild asthma is safe, repeatable and acceptable. This allows the use of this model in further studies focused on understanding the pathophysiology of allergen induced asthma and the impact of therapeutic interventions.

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Key words

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Authorship

Both authors designed and performed the study, analysed the data and wrote the manuscript.

Ethics

The study was performed with the approval of the Southampton and South West Hampshire Research Ethics Committee (A) (reference 08/H0502/6) and written informed consent was obtained from all participants prior to enrolment.

Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

Introduction

Inhalation allergen challenge is used in asthma to investigate the impact of novel interventions on lung function and indices of airway inflammation (1, 2). Standardly, this is composed of a single allergen challenge targeted to achieve an FEV₁ drop of between 15 and 30% from baseline (2–6), and although these challenges are safe and repeatable (7) and correlate well

with single environmental exposures (8), there is debate as to whether they reflect and thus model repeated allergen exposure in the wider environment. Allergen challenge in the environment is likely to be repeated rather than single dose and may or may not produce repeated symptoms. To address the situation of repeated but essentially asymptomatic environmental allergen exposure, a repeated low-dose allergen challenge model has been developed in which there is

no target FEV₁ drop, rather a lower dose of allergen is repeatedly administered with the aim of inducing more sustained airway inflammation (9). This model has been used to investigate the effects of pretreatment (10) but does not consistently induce eosinophilic airway inflammatory changes. The situation of repeated symptomatic environmental allergen exposure has not been addressed experimentally in human subjects; by contrast, preclinical animal challenge protocols, designed to investigate novel asthma medications, use repeated high-dose allergen exposure. These models demonstrate sustained airway eosinophilic inflammation and structural airway remodelling changes (11–14). There is currently no direct human model that relates to these animal-based protocols, possibly on account of concerns over safety.

Current human allergen challenge protocols, both single high-dose and repeated low-dose models are useful in understanding the basis of disease, but have two particular insufficiencies that could be addressed by a repeated high-dose challenge model. Firstly, individuals may be exposed repeatedly to symptomatic doses of allergen from the environment, and secondly, the animal models, which have been so useful in developing our understanding of the disease, have often been developed with repeated allergen challenge (15); these models should be validated against human responses in order to support their previous findings and future use.

Thus, to evaluate the safety of repeated high-dose inhalation allergen challenge, prior to investigating the potential of such a model for the investigation of the mechanisms of allergen-induced airway remodelling in asthma, we undertook a repeated inhalation allergen challenge protocol. This was performed in volunteers with mild asthma and involved three allergen challenges each separated by 48 h, with assessment of lung function and symptom outcomes.

Here, we describe a safe, repeatable and clinically acceptable repeated allergen challenge protocol involving three allergen challenges each separated by 48 h. This challenge protocol will be useful in establishing human correlations with repeated allergen exposures in laboratory animals and also determining how repeated environmental allergen challenge differs from the established single allergen challenge model.

Materials and methods

Subjects

Sixteen subjects (four male) (mean age 24.1 years, range 21–40) with physician-diagnosed atopic asthma

were recruited by local advertisement and from a database of volunteers held by the department. The study was approved by the local research ethics committee, and all the subjects provided written informed consent. All of the participants were non-smokers, had an FEV₁ of >70% of predicted (16) and were only receiving treatment with short-acting inhaled β_2 agonists as needed. Additionally, all of the participants had a PC₂₀ to methacholine of <8 mg/mL [median value (interquartile range, IQR) 1.10 (1.76) mg/mL] and were skin prick test positive to house dust mite (HDM) (*Dermatophagoides pteronyssinus*) [median (IQR) wheal diameter 7.25 (2.9) mm]. If subjects were also pollen sensitive they were studied outside the relevant period of pollination in the winter months.

FEV₁ measurements and reversibility

Following screening, and at least 2 weeks prior to the repeated allergen challenge, the subjects underwent reversibility testing using 2.5 mg of salbutamol administered by an air-driven nebuliser (Respironics, Chichester, UK). This test was repeated 4 days after the last allergen challenge.

Allergen challenge

Three allergen challenges were performed at 48-h intervals. Fresh solutions of freeze-dried HDM allergen extract (Diagenics Ltd, Milton Keynes, UK) were made up with 0.9% saline to a concentration of 5000 U/mL. The solution was administered using a breath activated dosimeter connected to a controlling computer (APS pro, Jaeger, Höchberg, Germany) (7).

After measurement of baseline spirometry (FEV₁ and FVC) and providing these values were, respectively, $\geq 70\%$ and $\geq 80\%$ predicted, the subject underwent the challenge. Initially, 0.1 mg of 0.9% saline was administered with three recordings of FEV₁ each at 5 and 10 min post-challenge, with the best reading from each time point used for analysis. The lower of these readings at 5 or 10 min was then used as the baseline for further analysis. Initial dosing of allergen was then with 10 U. FEV₁ was recorded at 5, 10 and 15 min, as mentioned earlier. Further allergen was then incrementally administered at 15 min intervals, using 40, 160, 720, 2100 and 5000 U, with spirometric recording post-challenge, as mentioned earlier, until a fall in FEV₁ of $\geq 15\%$ compared with post-saline reference was obtained. If FEV₁ fell by between 10 and 15%, the previous dose was repeated rather than incrementing further. The cumulative dose required was recorded as

the PD₁₅. FEV₁ was then measured at 20, 30, 45 and 60 min, then at 30 min intervals up to 10 h.

On subsequent allergen challenges, the aforementioned protocol was followed, except that half the PD₁₅ from the previous challenge was used as the initial dose to account for possible sensitisation. If this did not cause a $\geq 15\%$ decrease in FEV₁, the same dose was administered again (giving the total PD₁₅ from the previous challenge). If a FEV₁ drop of $\geq 15\%$ was not achieved, the dose was increased according to the previously detailed protocol.

Symptom diary

The subjects completed a 14-day symptom diary morning and evening; the first challenge day was on day eight, allowing comparison for the week before challenge and the week during the repeated challenges. The minimum score, associated with no symptoms, was zero; the maximum score possible was 30. Total daily puffs of inhaled short acting β_2 agonist were also recorded.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Mac except for area under the curve (AUC) analysis, which was performed using Prism 3.0 for Windows (Graphpad Software Inc., La Jolla, CA, USA). All repeated challenge lung function and allergen dose measures were analysed using Friedman's test, while symptom scores and other paired data were analysed using Wilcoxon signed-rank test. A *P* value of <0.05 was taken as statistically significant.

Results

Safety

There were no serious adverse events such as significant worsening of asthma requiring hospital admission nor was there any requirement for the introduction of inhaled or oral steroids. All of the subjects completed the three-challenge protocol.

Dose of provocant

In order to detect priming or desensitisation, the dose of provocant required to induce the target early asthmatic reaction (EAR) was recorded and expressed as a percentage of the dose required in challenge 1. There was no significant change in the doses required at the three challenges; median (interquartile range) doses administered were 100% (0.0), 100 (42.5) and 100 (66.0) (*P* = 0.66).

Symptom score and reliever use

Mean daily symptom score and reliever use in the week of the challenges increased significantly compared with the preceding week. Mean (SD) daily symptom score increased from 0.9 (1.6) to 3.4 (2.7) (*P* = 0.002), while reliever use increased from a daily mean (SD) of 0.2 (0.4) to 1.7 (1.9) (*P* = 0.003) (Table 1).

Magnitude of responses

The required immediate airway bronchoconstrictor response was achieved in all subjects at all challenges. The EAR, defined as the fall in FEV₁ from 0 to 120 min following challenge, was similar for the three challenges whether measured as mean (SD) maximal fall in FEV₁ (20.7% (6.5), 22.5% (7.0), 21.8% (6.6), *P* = 0.83) or as mean (SD) AUC for that time period (1161 (379), 1302 (507), 1510 (685), *P* = 0.07). The late asthmatic reaction (LAR) was defined as the FEV₁ fall from post-saline baseline from 150 to 600 min post-challenge and was also similar, when measured as the mean maximal FEV₁ fall (20.7% (6.5), 24.2% (14.1), 26.5% (15.0), *P* = 0.18) or as the AUC for that time period (5455 (3980), 5881 (3712), 6645 (4205), *P* = 0.47) (Table 2 and Fig. 1).

FEV₁ prior to repeated challenges and airway reversibility

At screening, mean (SD) percentage predicted FEV₁ was 94.3% (14.2). Mean (SD) percentage predicted

Table 1. Symptom score and reliever use in week prior to and week of repeated inhaled house dust mite allergen challenges

	Week before repeated challenges	Week of repeated challenges	<i>P</i> value
daily symptom score	0.9 (1.6)	3.4 (2.7)	0.002
daily reliever use	0.2 (0.4)	1.7 (1.9)	0.003

P values calculated by Wilcoxon signed-rank test. Symptom scores in arbitrary units. Values are means with SD in parentheses.

Table 2. Dose of provocant required, changes in FEV₁ and area under the curve for three repeated inhaled house dust mite allergen challenges at 48-h intervals

	Allergen challenge number			<i>P</i> value
	1	2	3	
Dose of provocant	100 (0.0)	100 (42.5)	100 (66.0)	0.66
Baseline FEV ₁	91.1 (12.3)	88.7 (12.2)	87.7 (12.7)	0.09
EAR FEV ₁	20.7 (6.5)	22.5 (7.0)	21.8 (6.6)	0.83
EAR AUC	1161 (379)	1302 (507)	1510 (685)	0.07
LAR FEV ₁	22.8 (13.6)	24.2 (14.1)	26.5 (15.0)	0.18
LAR AUC	5455 (3980)	5881 (3712)	6645 (4205)	0.47

P values are calculated using Friedman's test. Values are means with SD in parentheses, except dose, which is median, with interquartile range in parentheses. EAR, early asthmatic reaction (0 to 120 min); LAR, late asthmatic reaction (150 to 600 min); Baseline FEV₁, percentage predicted FEV₁ prior to saline inhalation; EAR/LAR FEV₁, maximal change in FEV₁ from post saline baseline; AUC, area under the curve.

FEV₁ 4 days after final challenge was 95.5% (14.1), which was not different from screening (*P* = 0.53). There was no significant change in the baseline pre-challenge FEV₁ with repeated allergen challenge. The mean (SD) percentage predicted baseline FEV₁ prior to each repeated challenge was 91.1% (12.3), 88.7% (12.2) and 87.7% (12.7), respectively (*P* = 0.09) (Table 3).

Mean (SD) FEV₁ reversibility to 2.5 mg nebulised salbutamol was similar prior to and following repeated challenge, at 7.5% (5.9) prior to and 9.9% (6.4) after challenges (*P* = 0.12) (Table 3).

Discussion

This study has demonstrated that repeated high-dose inhaled HDM allergen challenge in human volunteers with mild asthma is safe, repeatable and acceptable.

Previously, only limited information has been available regarding repeated allergen challenge in human asthmatic volunteers and none of it sufficiently strong to give confidence in the safety of a repeated high-dose allergen protocol. One small study reported the effects of two challenges in eight volunteers (17). In this study, inhalation allergen challenge, targeted to produce a fall in FEV₁ of 15% from baseline, was repeated after an

interval of 48 h. No changes in the intensity of the EAR and LAR were identified. This study, additional to its sample size, was, however, limited in that the study population was mixed, in that different allergens (HDM and grass pollen) were used in different individuals. It has recently been demonstrated that these allergens have a different propensity to induce a LAR response (18), and this has implications for severity of response. Similarly, one other study that evaluated the impact of four successive allergen challenge days on priming or desensitisation (19) recruited those with varying allergic sensitivity, with the predominant incorporation of ragweed pollen sensitive subjects. Although this study found no deleterious effect, it only included two subjects who had late reactions as defined solely by symptom reporting with no spirometric measures.

In our study, all of the subjects were studied with the same HDM challenge, and despite only having approximately a mean 20% fall in FEV₁ during the immediate reaction, had a mean late reaction fall in FEV₁ of 20%–30%, with 14 of the 16 subjects having a late reaction as defined by a fall in FEV₁ of $\geq 15\%$ from baseline at some time between 150 and 600 min post-challenge. The LAR has been linked to eosinophilic airway inflammation and enhanced bronchial hyperresponsiveness (3). The late reaction is thus considered better repre-

Table 3. Percentage predicted FEV₁ and reversibility prior to and following repeated allergen challenge

	Prior to repeated challenges	Four days after repeated challenges	<i>P</i> value
Percentage predicted FEV ₁	94.3 (14.2)	95.5 (14.1)	0.53
Percentage FEV ₁ reversibility	7.5 (5.9)	9.9 (6.4)	0.12

P values calculated by Wilcoxon signed-rank test. Reversibility measured using 2.5 mg nebulised salbutamol. Values are means with SD in parentheses.

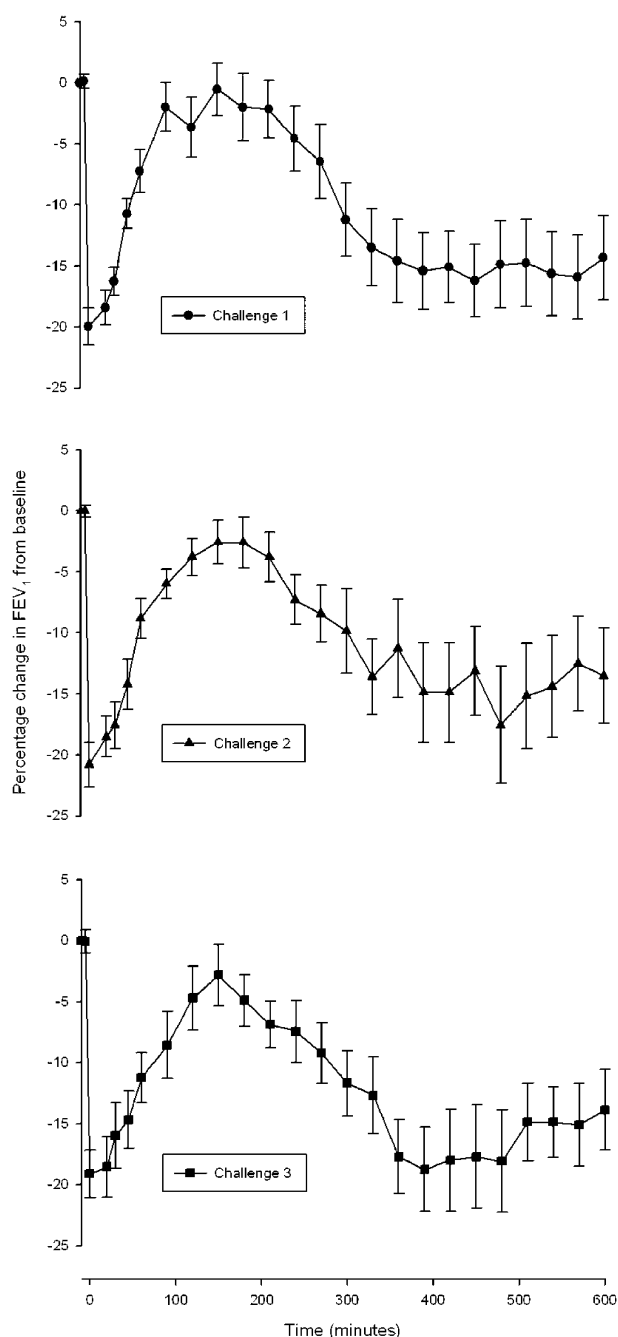


Figure 1. Mean percentage change in FEV₁ from baseline over time following three inhaled house dust mite allergen challenges at 48-h intervals. Bars = standard error of the mean.

sentative of clinical asthma, and by selecting HDM-sensitive subjects, we have biased our study to selecting those who may potentially deteriorate with repeated allergen challenge. Our study did not specifically investigate the impact of repeated allergen challenge on bronchial reactivity in case reactivity measures them-

selves had some confounding impact. Similarly, we did not induce sputum to assess airway inflammatory cell changes and other markers of ongoing airway inflammation. As the safety of this model has been established, it will now be possible to assess the impact of this repeated inhalation allergen challenge protocol on these outcome measures and investigate their relationship to the change in symptoms.

The repeated allergen challenge induced an increase in symptoms and an increased need for short-acting beta-agonist use. There was, however, no indication of priming or indeed of desensitisation in that there was no significant difference in allergen dose administered in any of the three separate challenges. Our safety evaluation was additionally based on lung function measures. No significant difference was evident on the third challenge day in either baseline lung function pre-challenge, early or late lung function changes in response to the challenge or in baseline lung function and reversibility 4 days post-challenge. There was a trend towards a greater AUC for the early reaction ($P = 0.07$) with repeated challenge so if a study protocol was planned that involved more than three repeated challenges, this trend towards a reduction in FEV₁ could become both statistically and clinically significant and would need evaluating. However, this study has shown that three repeated allergen challenges in human asthma volunteers designed to produce a mean early fall in FEV₁ of 15% is safe and repeatable. This thus allows the use of this model in further understanding the pathophysiology of allergen-induced asthma and the impact of therapeutic intervention such as in the development of new asthma treatments or the assessment of efficacy of immunotherapy.

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