

Comparison of incremental and bolus dose inhaled allergen challenge in asthmatic patients

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Summary

Background Attenuation of airway responses to inhaled allergen is increasingly used to evaluate anti-asthma drugs. Many studies use different allergen challenge methods and the presence of the late asthmatic response can be identified by a screening challenge with inhalation of incremental doses of allergen. Once defined, subsequent challenges are often administered as a constant dose based on the dose from the screening challenge. Previously, constant dose challenges have been employed but never validated.

Objective A comparative study of two methods of delivering inhaled allergen by evaluating the responses of an incremental dose allergen challenge and the same cumulative dose administered as a bolus over a single inhalation.

Methods Thirty-five male patients with mild allergic asthma underwent incremental dose challenge followed 3–6 weeks later by a bolus dose challenge. Bronchoconstrictor responses were expressed as the maximum percentage fall in FEV₁ from baseline during the early (0–2 h) and late (4–10 h) asthmatic responses and area under the percentage change in FEV₁-time curve (AUC).

Results There were no significant differences between the challenges. The mean $\pm$ SEM fall in FEV₁ following incremental and bolus dose challenge was $33.1 \pm 1.8\%$ and $29.9 \pm 2.2\%$ during the early response, and $36.9 \pm 2.4\%$ and $34.0 \pm 3.1\%$ during the late response, respectively. The mean $\pm$ SEM AUC following incremental and bolus dose challenge was 35 ± 3 and 33 ± 3 $\Delta\%FEV_1/h$ for the AUC_{0–2h}, 147 ± 12 and 139 ± 16 $\Delta\%FEV_1/h$ for the AUC_{4–10h}, and 204 ± 14 and 190 ± 19 $\Delta\%FEV_1/h$ for the AUC_{0–10h}, respectively.

Conclusion Bolus dose allergen challenge is a safe method to administer inhaled allergen in clinical trials with a valid response when compared with incremental dose allergen challenge.

Keywords: asthma, bolus dose challenge, incremental dose challenge, inhaled allergen challenge, validity

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Introduction

Bronchial provocation with inhaled allergen is established in research studies, although its role in clinical practice is limited [1]. Despite numerous publications advocating the adoption of standard procedures for allergen challenge

[2–4], diverse methodological differences exist [4–12]. The implementation of uniform procedures is important as provocation with inhaled allergen may be potentially dangerous if a standardized and reproducible method is not used, especially if repeated allergen challenges are performed on the same patient over a protracted time course.

The response to inhaled allergen is variable, but in approximately 50% of sensitized adults it is characterized

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by a biphasic response [13,14]. The initial early asthmatic response (EAR), reflecting predominantly airway smooth muscle contraction is maximal between 10 and 30 min and resolves within 2 h after allergen inhalation. This is followed by the late asthmatic response (LAR) 3–8 h later, which reflects both airway smooth muscle contraction and inflammation, and may continue for 24 h or longer [1]. Development of the LAR is a useful clinical model of asthma to study inflammatory mechanisms [7,15–17] and is frequently employed to assess the efficacy, pharmacological and anti-inflammatory properties of therapeutic agents for the treatment of asthma [8,11,12,18–22].

Although allergen challenge methods are not uniform, protocols to assess the effect of novel agents on the LAR generally follow the same format: After an initial screening visit to define atopic status and an allergen challenge to demonstrate the presence of the LAR, a constant dose of allergen is administered on subsequent visits during active and placebo treatments. The initial screening challenge usually involves inhalation of incremental doses of allergen until an EAR is defined [4,6–9]. There is no universal agreement about the best method for constant dose allergen challenge and several methods have been employed; allergen may be administered as an incremental challenge in the same manner as the screening challenge, regardless of the change in FEV₁ [17,23,24] or as a single bolus dose representing either the cumulative allergen dose [25] or PD_{15–20} allergen [8,12] defined from the screening challenge. Alternatively, only the highest allergen concentrations producing a 20% or greater fall in FEV₁ during the screening challenge are administered [7,18].

Despite numerous studies using constant dose allergen challenges based on an initial incremental dose challenge there has been anecdotal concern about the use of constant dose challenges in asthmatic patients. Irrespective of the method used, no study has demonstrated that a constant dose allergen challenge produces the same response in terms of bronchoconstriction during the early and late asthmatic responses as incremental allergen challenge. This is important for two reasons: firstly, to ascertain whether constant dose allergen challenge reliably reproduces the bronchoconstrictor responses of incremental allergen challenge, and secondly to determine whether constant dose allergen challenge is safe and does not consistently produce greater bronchoconstriction than incremental dose challenge.

We have developed a protocol involving an incremental allergen challenge at screening designed to produce a 15% or more fall in FEV₁ during the EAR. We then administer the cumulative dose of allergen producing this fall as a single bolus dose in subsequent challenges during each treatment period [26]. We therefore undertook a comparison of these two allergen challenge methods by evaluating the early and late asthmatic responses following sequential

challenges with each challenge method in a group of mild asthmatic patients.

Materials and methods

Thirty-nine non-smoking male patients took part in the study. All patients had mild asthma with a FEV₁ greater than 70% of predicted and demonstrated a positive skin test in response to common airborne allergens (*Dermaphagoides pteronyssinus*, mixed grass pollen or cat fur). None had an exacerbation of asthma or a respiratory tract infection in the preceding 6 weeks and, apart from house dust mite, there was no current exposure to allergens to which the subjects were sensitized. Patients took no regular medications for their asthma apart from treating occasional symptoms with intermittent short acting β_2 -agonists. Written informed consent was obtained from each patient and the study was approved by the Ethics Committee of the Royal Brompton Hospital.

Study protocol

This was an observational study, undertaken in an open manner. Patients attended the laboratory at the same time in the morning on two occasions. At the first visit, after skin prick tests to define the appropriate allergen for challenge, an incremental dose allergen challenge was performed. At the second visit 3–6 weeks later a bolus dose allergen challenge was performed using the cumulative dose of allergen inhaled at the first visit. Following each allergen challenge FEV₁ was measured at intervals for 10 h. Incremental challenge always preceded bolus challenge as it defined the total dose required to produce a late allergic response.

Intradermal skin prick tests

Stock solutions of allergen extract (10 000 U/mL) for *Dermaphagoides pteronyssinus*, mixed grass pollen and cat fur (Aquagen SQ, ALK, Horsholm, Denmark) as well as a positive and negative control were applied intradermally by pricking the skin of the volar aspect of the forearm with a sterile lancet. The weal and flare response was measured 15 min later and the extract producing the largest weal used for allergen challenge.

Allergen challenge

Fresh dilutions of freeze-dried allergen extract (Aquagen SQ, ALK) were made up with 0.9% saline from a stock solution of 100 000 U/mL to give final concentrations of 200, 500, 1000, 2500, 5000, 12 500, 25 000 and 50 000 U/mL. Each solution was administered from a hand-held

nebulizer attached to a breath-activated dosimeter (Mefar, Brescia, Italy) with a delivery time of 1 s per breath. The nebulizer delivers particles with an aerodynamic mass median diameter of 3.5–4.0 μm at an output of 9 μL per breath. Pulmonary function was assessed by measurement of FEV₁ with a dry wedge spirometer (Vitalograph, Buckingham, UK).

A standard protocol was followed for each allergen challenge. Three recordings of FEV₁ were taken at 1 min intervals, the best of which was taken as baseline. Subjects then inhaled five breaths of saline-control nebulized from the dosimeter, by inspiring slowly from FRC to TLC over 3 s and then breath-holding for 6 s. A single measurement of FEV₁ was recorded 2, 3 and 4 min after saline inhalation and the highest reading taken as the reference (post-saline) value. Unless a fall in FEV₁ of >10% from baseline was seen, subjects then inhaled increments of the relevant allergen, in the same manner as saline-control, starting with a concentration of 200 U/mL. FEV₁ was measured 5 and 10 min after each concentration and the lower of these two values taken as the response. After each concentration of allergen, a fivefold increment was administered if the FEV₁ had not fallen by more than 10% from the post-saline value. If the fall in FEV₁ was between 10 and 15%, a 2.5-fold increment was administered. The challenge was terminated when a fall in FEV₁ of >15% from the post-saline value was achieved. After the final concentration of allergen, FEV₁ measurements were taken at 5, 10, 20, 30, 45 and 60 min and thereafter in duplicate at 30 min intervals up to 10 h. A late asthmatic response was defined as a fall in FEV₁ of greater than 15% from the post-saline value on at least three occasions, between 4 and 10 h after allergen exposure. Patients who did not demonstrate a late asthmatic response to allergen did not continue in the study.

At the second visit baseline FEV₁ had to be within 15% of that prior to the first allergen challenge. If this was not achieved the visit was rescheduled for a week ahead. For this visit the cumulative concentration of allergen producing the initial >15% fall in FEV₁ was made up as a single concentration from stock (100 000 U/mL) allergen solution with 0.9% saline. This was then administered over five breaths after saline-control. FEV₁ was then measured at the same intervals as visit 1.

Reconstitution and dilution of allergen solutions and all allergen challenges were undertaken by the same investigator (DAT). A medically qualified doctor experienced in the treatment of anaphylaxis and acute asthma was in attendance for the whole of the 10 h following challenge. Patients were encouraged not to use rescue medication during this time, however, this was not withheld if requested by the patient and administered if deemed necessary by the attending physician. Any patient requiring rescue medication during this time was withdrawn from the study. All

patients remained in the unit overnight for observation and the following morning received nebulized salbutamol 2.5 mg and budesonide 1 mg prior to discharge.

Analysis of data

All results were expressed as means $\pm$ standard error of the mean (SEM) unless otherwise stated. At each time-point after challenge airway responses to allergen were expressed as percentage change from the post-saline value and the values after both challenges compared using a paired *t*-test with the Bonferroni correction for multiple comparisons. For each patient the bronchoconstrictor response to allergen was expressed as the maximum percentage fall in FEV₁ from baseline and the area under the percentage fall in FEV₁-time curve (AUC). The AUC was calculated using the trapezoidal method and determined for the early (AUC_{0–2 h}), late (AUC_{4–10 h}), and entire measured response to allergen (AUC_{0–10 h}). Differences between these variables after each challenge were evaluated by paired *t*-tests. Agreement between the two challenge methods was determined by calculating the intraclass correlation coefficient (*r*_i) [27]. Statistical significance was taken as *P* < 0.05.

Results

Sixty-four patients underwent incremental allergen challenge at visit 1 of which 39 demonstrated an LAR. Four of these patients were withdrawn from the analysis as they demonstrated an EAR after the first dose of allergen and did not therefore receive an incremental challenge. Characteristics of the remaining 35 subjects are given in Table 1. The mean cumulative concentration of allergen required to produce an EAR following the incremental allergen challenge was 10 142 $\pm$ 1705 U/mL; the most frequent cumulative concentration administered was 6200 U/mL and no patient required more than 31 200 U/mL. No patient required rescue medication prior to the last FEV₁ measurement at 10 h. On three occasions, involving two patients, FEV₁ fell by approximately 60% during the EAR. This effect was transient and on each occasion the FEV₁ recovered to 80% of baseline within 10 min. Apart from wheeze both patients felt no discomfort, did not request rescue medication, and this was felt acceptable by the attending physician. One patient developed a fall in FEV₁ of greater than 70% at the final 10 h measurement of the LAR during both visits. Likewise he did not describe any discomfort but was rescued at this point with nebulized salbutamol.

Both incremental and bolus dose allergen challenges produced similar reductions in FEV₁ from baseline during the early and late asthmatic responses, there being no statistical difference in the fall in FEV₁ after both challenges at each time-point (Fig. 1). The mean maximum fall during

Table 1. Patient characteristics

Patient	Age (years)	Asthma duration (years)	FEV ₁ (% pred.)	Allergen (type)	Allergen dose* (units/mL)
1	25	16	92	Cat	31 200
2	24	14	93	HDM	31 200
3	23	20	81	Cat	16 200
4	27	2	87	Cat	6200
5	28	20	91	GP	1200
6	23	13	85	HDM	6200
7	26	24	83	Cat	3700
8	22	13	89	HDM	6200
9	21	11	98	HDM	3700
10	20	4	93	HDM	31 200
11	25	11	107	HDM	6200
12	28	17	84	HDM	6200
13	28	7	101	GP	3700
14	31	27	103	GP	1200
15	32	20	92	HDM	8200
16	24	17	112	GP	1200
17	27	14	80	Cat	1200
18	31	28	80	Cat	18 700
19	25	21	98	Cat	1200
20	24	17	86	HDM	700
21	38	33	87	GP	18 700
22	26	14	85	GP	6200
23	25	21	72	Cat	1700
24	20	12	93	HDM	6200
25	29	19	93	HDM	18 700
26	22	20	74	GP	21 200
27	30	23	89	Cat	8700
28	23	10	93	HDM	700
29	27	19	105	Cat	3700
30	23	21	73	Cat	700
31	22	18	91	GP	18 700
32	21	19	91	HDM	31 200
33	23	21	88	HDM	1700
34	25	23	93	HDM	31 200
35	20	18	94	HDM	31 200
Mean	25	17.5	90	10 142	
SEM	1	1	2	1705	

*Cumulative allergen dose producing > 15% fall in FEV₁ after final allergen concentration determined at visit 1. HDM = house dust mite, GP = grass pollen.

the early (0–2 h) and late (4–10 h) asthmatic responses did not significantly differ (Table 2 and Fig. 2). After incremental and bolus allergen challenges this was $33.1 \pm 1.8\%$ and $29.9 \pm 2.2\%$ (mean difference 3.2% [95% CI: 0.9–7.3]), respectively, for the early response, and $36.9 \pm 2.4\%$ and $34.0 \pm 3.1\%$ (mean difference 2.9 [95% CI: 1.2–7.1]), respectively, for the late response. Mean area under the percentage change in FEV₁-time curves did not

significantly differ (Table 2). The mean difference was 1.88% (95% CI: 2.1–5.9) change in FEV₁/h for the AUC_{0–2h}, 8.6% (95% CI: 15.0–32.1) change in FEV₁/h for the AUC_{4–10h}, and 13.6% (95% CI: 15.7–43.0) change in FEV₁/h for the AUC_{0–10h}.

Agreement between the two challenge methods was satisfactory for the mean maximum fall in FEV₁ during the LAR ($r_1 = 0.75$), AUC_{0–2} ($r_1 = 0.78$), AUC_{4–10}

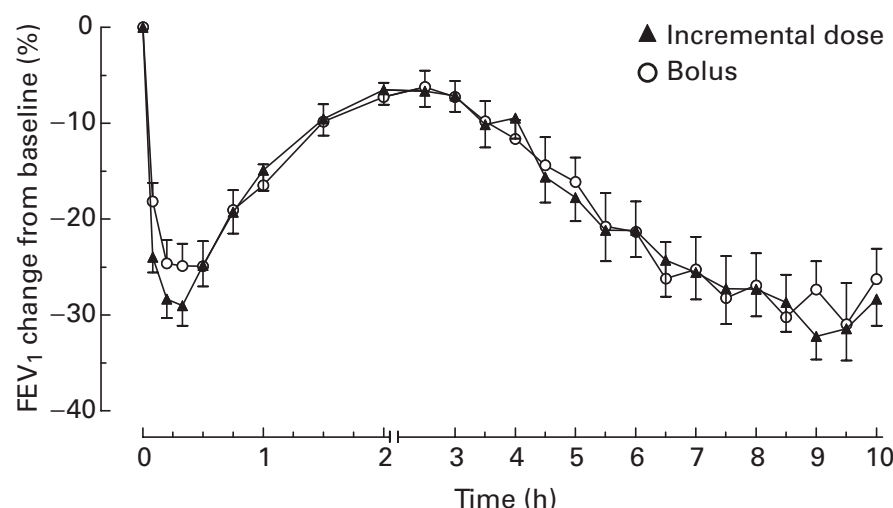


Fig. 1. Effect of inhaled allergen on lung function following incremental and bolus dose allergen challenge; expressed as percentage change in FEV₁ from the post-saline value over time. Data are mean $\pm$ SEM.

($r_i = 0.70$) and AUC_{0-10} ($r_i = 0.67$) (Table 2). Agreement was poorer for the mean maximum fall in FEV₁ during the EAR ($r_i = 0.55$) (Table 2).

Discussion

An important feature of all investigation methods is within-subject reproducibility, especially if different methods for the same test are used in one study, when it is termed validity. This is particularly the case for inhaled allergen as not only will reproducible or valid responses indicate whether drug effects are real, but a significantly different response may be dangerous. We have demonstrated that the bronchoconstrictor response to inhaled allergen administered as a bolus dose closely matches the response of the same dose administered more slowly over gradual

increments. In addition, bolus administration of allergen, which may be perceived as potentially more dangerous than incremental dose allergen, is well tolerated and if anything tends to produce a smaller degree of bronchoconstriction during the early asthmatic response, the time at which allergen-induced complications are most likely to occur [3].

Between-subject variability was relatively wide in our study, particularly for the EAR, and it is likely this occurred because we did not attempt to predict the concentration of allergen required to produce a 15% fall in FEV₁ during the EAR. Other allergen challenge protocols predict the amount of bronchoconstriction during the EAR, and hence the starting concentration of inhaled allergen, from the degree of skin prick test response and airway responsiveness to methacholine or histamine prior to allergen challenge [6]. As such studies using this protocol tend to have slightly less variability between subjects in the maximum FEV₁ fall during the EAR [23] than the method we describe. With our protocol we do not assess airway responsiveness, and the skin prick test only defines which allergen the patient is most sensitive to and does not quantify the degree of sensitivity with a range of concentrations. Because we do not make such predictions, and to avoid potential severe bronchoconstriction, all patients start by inhaling the same low concentration of allergen (200 U/mL). It is clear however, that the EAR is slightly more variable between subjects by this method, but in all cases was well tolerated. On three occasions we observed maximum falls in FEV₁ of approximately 60% during the EAR. It is important to emphasize that all patients were accompanied by a respiratory physician during each allergen challenge and for the 10 h after. In these three instances the effects were transient and no patient requested rescue medication or felt any discomfort apart from mild wheeze.

Inman *et al.* [23], assessed the reproducibility of

Table 2. Maximum fall in FEV₁ and area under the percentage change in FEV₁-time curve following allergen challenge ($n = 35$)

	Allergen challenge method		
	Incremental	Bolus	r _i
<i>Maximum Fall in FEV₁</i> (% change from baseline)			
EAR	33.1 ± 1.8	29.9 ± 2.2	0.55
LAR	36.9 ± 2.4	34.0 ± 3.1	0.75
<i>AUC (% change FEV₁/h)</i>			
AUC ₀₋₂	35 ± 3	33 ± 3	0.78
AUC ₄₋₁₀	147 ± 12	139 ± 16	0.70
AUC ₀₋₁₀	204 ± 14	190 ± 19	0.67

Data are mean $\pm$ SEM, r_i = Intraclass correlation coefficient.

incremental dose allergen challenge demonstrating intraclass correlation coefficients of -0.03 and 0.67 for the maximum FEV_1 fall during the early and late asthmatic responses, respectively. Similarly, we have demonstrated what appears to be poor validity of the maximum FEV_1 fall during the early ($r_i=0.54$) but not the late ($r_i=0.73$) asthmatic response between two different allergen challenge methods. The fact that the intraclass correlation coefficient is influenced by the variability of the subjects over which it is defined may be important in this respect. If the subjects are highly variable the coefficient will tend to be high, whereas for a more homogeneous group it will be low. The between-subject variability of the maximum fall in FEV_1 during the early response after incremental challenge was restricted as, by definition, the method is designed to produce a decrease in $FEV_1 \geq 15\%$ (range 18–60%). In contrast, bolus dose allergen challenge produces a wider range of measurements, as there is no restriction on the minimum FEV_1 fall (range -2 to 63%). In agreement with Inman *et al.* [23], we believe that the narrower range of measurements of maximum fall in FEV_1 after incremental dose challenge prevents a high intraclass correlation coefficient during the early asthmatic response. We believe that the more appropriate parameter to measure is the area under the response-time curve (AUC) as this takes into account all data points within a given time period. Intraclass correlation coefficients for the early and late response AUCs demonstrated good agreement between the two methods.

To obtain a valid response following the constant dose allergen challenge it is essential the same dose of allergen is inhaled as that defined from the screening challenge, since the early and late phase responses are directly related to the cumulative dose of inhaled allergen [28]. The administration of a bolus dose representing the PD_{20} , interpolated from the log-dose response curve, or an incremental challenge using the highest two to three allergen concentrations derived from the initial challenge may give a lesser bronchoconstrictor response than the screening allergen challenge, since the latter usually produces a fall of greater than 20% during the EAR. Furthermore, as we have demonstrated in our study, since there is more between-subject variability in the maximum FEV_1 fall during the early response after bolus dose challenge, there is also a significant possibility that a greater proportion of subjects will not obtain an EAR at all. We have demonstrated that if the cumulative allergen dose defined from the screening challenge is administered in total as a bolus dose over five 1 s inhalations a valid early

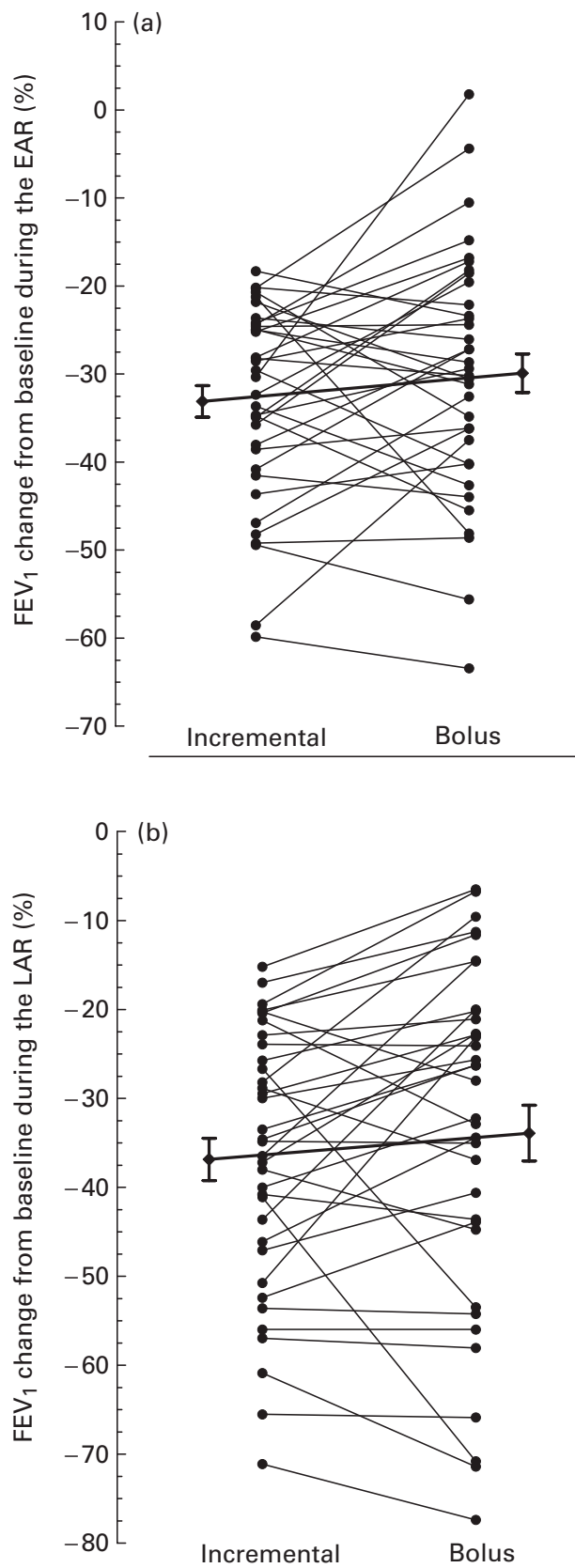


Fig. 2. Individual maximum change in FEV_1 after incremental and bolus dose allergen challenge during (a) early asthmatic response (EAR) and (b) late asthmatic response (LAR). Group mean $\pm$ SEM given.

and late phase response is observed when compared with the incremental challenge. In clinical studies investigating the effect of drug treatment on allergen responses one advantage of a bolus dose challenge is the ability to allow exact and equal timing of allergen administration between subjects. This may be particularly relevant in studies where precise timing of the constant dose allergen challenge relative to drug administration is required, as following incremental dose allergen challenge the final dose of allergen may be inhaled at different times by each patient.

In conclusion, we provide data demonstrating the safety and validity of a constant dose allergen challenge based on an incremental dose challenge in asthmatic patients. We do not advocate this method as any way superior to other well-established and reproducible methods [6,23]. However, it is clear that allergen challenge protocols of the type we describe are becoming increasingly used, particularly in the assessment of anti-inflammatory properties of novel therapeutic agents for the treatment of asthma. With greater numbers of patients being exposed to allergen challenge it is essential we critically analyse these methods to avoid potential dangers and pitfalls which may arise if safety is based on anecdotal experience rather than formal evaluation.

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