Evaluation of an Abbreviated Adenosine Monophosphate Bronchial Challenge*

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Rationale: Airway hyperresponsiveness to adenosine monophosphate (AMP) has been validated as a surrogate marker for airway inflammation. We wished to know whether an abbreviated challenge at the final threshold dose would produce the same fall in FEV\textsubscript{1} as a full, conventional dose-response challenge.

Methods: Seventeen patients with mild-to-moderate asthma (mean FEV\textsubscript{1}, 75.5\% predicted) attended for a full dose-response protocol, where the highest concentration of AMP to produce > 20\% fall in FEV\textsubscript{1} was noted, along with the maximum percentage fall and recovery time. Patients returned within 2 days for a further challenge, when they received only the highest concentration (as a single bolus) reached on the previous visit.

Results: The mean (± SEM) percentage fall in FEV\textsubscript{1} after the full challenge was 25.5 ± 1.3\%, and after the abbreviated challenge was 9.4 ± 2.4\%. The mean recovery after the full challenge was 28.13 ± 4.65 min, and after the abbreviated test was 10.81 ± 4.27 min.

Conclusion: An abbreviated challenge using a single bolus dose of AMP grossly underestimates bronchial hyperresponsiveness. Although the pharmacologic half-life of AMP is short (90 s), the lesser response and shortened recovery with the abbreviated challenge suggest a more prolonged physiologic half-life, which in turn may have implications for abbreviated challenge protocols

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Abbreviation: AMP = adenosine monophosphate

Increased bronchial hyperresponsiveness to non-specific pharmacologic agents such as histamine and methacholine is a hallmark of asthma, and guidelines for the use of direct bronchial provocation testing are well documented. Current recommendations for bronchial challenge are to employ a doubling regimen, that is, doubling the dose of inhaled agent at each step, with the best of three FEV\textsubscript{1} measurements obtained 90 s after each step. The challenge is continued until the FEV\textsubscript{1} falls by > 20\% of the initial baseline reading. In this manner, a dose-response curve can be plotted, and the provocative dose required to produce a fall in FEV\textsubscript{1} of 20\% can be calculated. This procedure has been shown to take from 35 to 65 min. There have been numerous efforts to reduce the length of bronchial challenge tests: (1) shortening the length of time between doses, (2) employing threefold or fourfold jumps rather than doubling steps, or (3) starting the challenge at a higher concentration.

A direct challenge using histamine at 1-min intervals relies on a cumulative dose to provoke bronchoconstriction; however, histamine administered at 5-min intervals has been shown to be entirely independent of cumulative dose. There are no data on the nature of adenosine monophosphate (AMP) indirect bronchial challenge. The pharmacologic half-life of AMP is approximately 90 s; therefore, each sequential concentration step should be unaffected by the previous concentration. If this were the case, only the highest concentration of AMP reached would have any effect on the fall in FEV\textsubscript{1} provoked. We proposed that, if this were to be true, a full protocol challenge would provoke the same degree of fall in FEV\textsubscript{1} as a single concentration of the maximum concentration reached in the full protocol. It is unlikely that this single concentration approach would be readily utilized in either clinical or research settings, as potential for a dramatic fall FEV\textsubscript{1}, with-
out warning is dangerous. However, if only the most recently administered concentration of AMP has any effect on FEV₁, it would be possible to construct a challenge protocol that can be shortened by the investigator. This could take the form of either beginning the challenge test at a moderate concentration, one that has fewer safety implications, or enabling the investigator to skip doubling concentrations if the lower concentrations are having little effect. Both would speed up the challenge process, without altering the interpatient and intrapatient correlation between challenges. However, if the fall in FEV₁ is due to the cumulative effect of the AMP, it is imperative that every patient who receives an AMP challenge receives the exact same challenge, and would question the validity of abbreviated challenges.

**Materials and Methods**

Seventeen atopic (a positive skin-prick test result to at least one common allergen) asthmatic patients were enrolled (mean [± SEM] FEV₁, 76.9 ± 3.16% predicted at screening; mean age, 47 ± 3 years). All patients were receiving inhaled corticosteroids (mean, 570 µg/d of chlorofluorocarbon-propelled beclomethasone dipropionate equivalent), six patients were receiving long-acting β₂-agonists, and two patients were receiving montelukast. All patients maintained their usual inhaled corticosteroid throughout the study; however, all long-acting β₂-agonists and montelukast were stopped 48 h prior to the first challenge, and were withheld until after the second challenge; short-acting β₂-agonists were withheld for 6 h prior to each challenge. All 17 patients attended the Asthma and Allergy Research Group, University of Dundee, for a full protocol AMP bronchial challenge from 0.09 to 500 mg/mL in almost doubling concentration steps, using the dosimetric technique described previously, to reach at least a 20% fall in FEV₁. All concentrations of AMP were administered at constant 2-min intervals, with FEV₁ measurements obtained 90 s after the final inhalation of each concentration of AMP. The highest concentration of AMP (Clinalfa; Merck Biosciences; Laufelfingen, Switzerland) administered to each patient was noted, along with the fall in FEV₁ at this concentration. Patients then recovered spontaneously, with FEV₁ measurements obtained at 2, 5, 10, 20, 30, 40, 50, and 60 min. Spirometry was carried out using a compact spirometer (Vitalograph; Vitalograph Ltd; Buckingham, UK) with a computer-assisted pneumotachograph head and pressure transducer. The spirometer was calibrated daily using a 1-L precision syringe (Vitalograph Ltd). The provocative concentration of AMP required to produce a 20% fall in FEV₁ was calculated by linear interpolation of the log-dose response. Patients returned to the department within 2 days, at which point they received only the highest concentration of AMP reached during the full protocol. The fall in FEV₁ was noted, and spontaneous recovery of FEV₁ was measured as before. Comparisons were made of the fall in FEV₁ and the time to spontaneous recovery, defined as time for FEV₁ to return to within 5% of baseline. Statistical comparisons were made by means of paired t-test. This study received ethical approval from the Tayside Committee on Medical Research Ethics on November 13, 2003 (reference 201/02).

**Results**

The geometric mean concentration of AMP required to produce a 20% fall in FEV₁ was 18.1 ± 13.3 mg/mL, representing nine doubling concentrations. The mean duration of challenge was 38 min. Mean baseline FEV₁ was 2.38 ± 0.12 L (79 ± 3% predicted) prior to the full challenge, and 2.35 ± 0.13 L (78 ± 3% predicted) prior to the abbreviated challenge. There was no significant difference between the two prechallenge baseline FEV₁ readings.

The mean percentage fall in FEV₁ after the full challenge was 25.5 ± 1.3%, and after the abbreviated challenge was 9.4 ± 2.4% (p < 0.001) [Fig 1, top, a]. The mean recovery after the full challenge was 28.13 ± 4.65 min, and for the abbreviated test was 10.81 ± 4.27 min (p = 0.01). The median recovery times were 30 min and 2 min for the full and abbreviated challenges, respectively (Fig 1, bottom, b).

**Figure 1.** Top, a: Individual data for the full and abbreviated challenge protocols, showing maximum fall in FEV₁. Bottom, b: Percentage of patients who recovered to within 5% of prechallenge FEV₁ for the full and abbreviated challenge protocols.
DISCUSSION

The use of an abbreviated challenge using the approach described grossly underestimates bronchial hyperresponsiveness. AMP is an indirect bronchial provocation agent. It exerts its action by degranulation of bronchial mast cells, leading to the release of inflammatory mediators, such as histamine and the leukotrienes. In this way, it reflects better the degree of inflammation within the bronchial tree and also the response of inflammatory changes to inhaled corticosteroids. In this respect, it may have more potential as a clinical tool for use outside the research environment, to monitor response to treatment, to assess disease severity, and to determine the degree of disease control. Sont et al used airway provocative agent, predicts failure of step-down of inhaled corticosteroids. An indirect challenge may sensitivty reduces exacerbations. Furthermore, a positive bronchial challenge using mannitol, an indirect provocative agent, predicts failure of step-down of inhaled corticosteroids. An indirect challenge may be a useful way to determine accurately the inflammatory status in the airways of asthmatics.

If the AMP challenge is truly cumulative, the total dose of AMP administered in a full, doubling-dilution protocol will be almost double that of the final dose administered. If the dose-response curve were linear, then one would expect the response to the full protocol to be almost double the response to the single concentration protocol outlined. The results show that the mean response was almost three times greater in the full protocol than in the single-concentration protocol. Although it is known that AMP acts directly on mast cells, causing degranulation, it in not clear if the lower doses of AMP administered in the full protocol may act to prime these mast cells, promoting increased release of inflammatory mediators. This may explain the disparity in results between the full and abbreviated protocols.

We carried out the second challenge within 2 days of the initial challenge. It is recognized that after allergen challenge, there is a period of time during which responses to subsequent allergen challenges are damped down. This is thought to be due to repriming of mast cells, and “immune recovery.” It is possible that there is a similar phenomenon with AMP challenges. However, it is known that the action of AMP is specific, acting on A2 receptors on mast cells, unlike the generalized allergic response that occurs during and after allergen challenge. Recovery from allergen challenge can be prolonged; however, recovery from the challenges using both protocols was short (28 min and 10 min for the full and abbreviated challenges, respectively), suggesting that there is little prolonged effect on bronchial responsiveness after challenge. However, it remains possible that the first AMP challenge does indeed damp down the response to the second challenge, and as such further work is warranted to compare the results of two identical challenges carried out at a 48-h interval.

Although the pharmacologic half-life of AMP is short (90 s), our data suggest that the physiologic half-life may be significantly longer, resulting in a cumulative effect over a full protocol challenge. This has implications for both abbreviated and full protocol challenges. Attempts to shorten the challenge must be made in the knowledge that it is important that all bronchial provocation tests be carried out to strict timings, as any alteration from the time between concentrations as per protocol can result in differing cumulative effects from the provocative agent used. Likewise, the concentration steps should remain consistent, particularly throughout successive challenges, if the challenge is to be utilized as a monitoring tool. It may be reasonable to start an AMP challenge at a higher concentration, to shorten the protocol, but care must be taken to choose a sensible starting concentration to avoid a precipitous fall in FEV1 in particularly susceptible patients. In summary, our data do not support the use of a single-dose, abbreviated AMP challenge protocol, and throws in to doubt the validity of other abbreviated challenges.

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