Bronchial provocation testing can be improved by using dry powder adenosine instead of nebulized adenosine monophosphate

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To the Editor,

Airway hyperresponsiveness (AHR) to adenosine has proven to be a good marker for eosinophilic airway inflammation in asthma and can be used to monitor disease activity and therapeutic effectiveness of inhaled corticosteroids (1–3). Adenosine is usually administered by nebulization of adenosine monophosphate (AMP), but the highest feasible concentration of AMP often fails to induce sufficient bronchoconstriction in subjects with asthma (4,5). We studied whether this limitation could be resolved by administering adenosine as dry powder formulation. We previously demonstrated the feasibility of this new bronchial provocation method in a small proof-of-concept study (6). The aim of the present study was to further validate the dry powder adenosine provocation test in a larger cohort of subjects with asthma.

Data were obtained from subjects recruited for the OLIVIA study (Effects of Extrafine Particle HFA-Beclomethasone versus Coarse Particle Treatment in Smokers and Ex-smokers with Asthma; clinical trial number: NCT01741285, www.clinicaltrials.gov). Sixty current or ex-smokers with asthma (34 females, 26 males) with FEV\(_1\) ≥50 predicted, who did not use inhaled corticosteroids for at least 4 weeks, underwent provocations with both AMP and dry powder adenosine as baseline measurements on subsequent visits (1–2 weeks apart), in addition to blood sampling, spirometry, body plethysmography, impulse oscillometry and multiple breath nitrogen washout measurements. The subjects’ mean (±SD) age was 45(±12) years, and their baseline FEV\(_1\) was 89(±16)% predicted.

AMP was administered by nebulization of doubling concentrations (0.04–320 mg/mL). Dry powder adenosine was administered with an investigational inhaler in doubling doses (0.04–80 mg) (6,7). We determined the provocative concentration (PC\(_{20}\)) of AMP and dose (PD\(_{20}\)) of adenosine that caused the FEV\(_1\) to drop by 20% by log-linear interpolation, and assessed which clinical characteristics were predictors of these parameters. Provocation tests were negative if no 20% drop in FEV\(_1\) was reached after administration of the highest concentration/dose, and values were censored to 640 mg/mL for PC\(_{20}\) AMP and 160 mg for PD\(_{20}\) adenosine for analysis. Calculations were performed with the base-2 logarithm (log\(_2\)) of PC\(_{20}\) AMP and PD\(_{20}\) adenosine to reflect the use of doubling dose steps and normalizing the distribution.

We calculated the agreement between the two tests with Cohen’s kappa and correlation analysis. We also performed a correlation analysis to assess associations between subject baseline characteristics and PC\(_{20}\) AMP/PD\(_{20}\) adenosine. Associations with a \(p\)-value <0.20 were considered for multiple linear regression analysis, although per baseline
measurement procedure, maximally one (the most significant) predictor was included to prevent multicollinearity. We performed a forced entry multiple linear regression analysis to determine which parameters independently predict the airway responses.

Forty subjects reached the predefined 20% drop in FEV$_1$ on both AMP and adenosine. Ten subjects had a positive adenosine test (PD$_{20}$ 5.4–39 mg) but negative AMP test (PC$_{20}$ >320 mg/mL), whereas two subjects had a negative adenosine test (PD$_{20}$ >80 mg) but positive AMP test (PC$_{20}$ 143 and 148 mg/mL). Seven subjects did not reach a 20% drop in FEV$_1$ on either stimulus. One subject, who had a negative AMP test, experienced severe cough during inhalation of dry powder adenosine, leading to early termination of the test. The total percentage of nonresponders was 30% (18 out of 60) for AMP and 15% (9 out of 59) for adenosine. Figure 1A shows PC$_{20}$ AMP and PD$_{20}$ adenosine values, clearly illustrating the higher rate of response to adenosine. PC$_{20}$ AMP and PD$_{20}$ adenosine were strongly correlated ($r_s = 0.799$; Figure 1B), yet had only a moderate agreement ($\kappa = 0.42$), mainly due to the larger number of non-responders to AMP.

![Figure 1](image)

**Figure 1.** (A) Comparison of the provocative concentration (PC$_{20}$) of adenosine monophosphate (AMP) and provocative dose (PD$_{20}$) of adenosine causing a 20% drop in FEV$_1$. The lines indicate the geometric means; *indicates negative test results, which were censored to PC$_{20}$ AMP = 640 mg/mL and PD$_{20}$ adenosine = 160 mg in the analyses. (B) Correlation analysis between PC$_{20}$ AMP and PD$_{20}$ adenosine, showing a strongly significant correlation between the two test results ($r_s = 0.799, p < 0.001$). $r_s$ = Spearman’s correlation coefficient.

Baseline variables included in multiple linear regression analysis for PC$_{20}$ AMP were age, smoking status, blood eosinophils, FEV$_1$, residual volume (RV), and the ventilation heterogeneity of the conductive lung zone ($S_{cond}$). For PD$_{20}$ adenosine, the variables were age, blood eosinophils, FEV$_1$, and RV. The models obtained by multiple regression
analysis were largely similar for $PC_{20}$ AMP and $PD_{20}$ adenosine with predictive powers of 34% and 30% respectively (Table 1). Only age (AMP and adenosine) and FEV$_1$ (adenosine) were found to be independent predictors ($p < 0.05$). Age and FEV$_1$ were positively associated with both $PC_{20}$ AMP and $PD_{20}$ adenosine, whereas blood eosinophils and RV exhibited a trend toward an inverse association.

Table 1. Baseline predictors for $PC_{20}$ AMP and $PD_{20}$ adenosine obtained by multiple linear regression analysis

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Baseline predictor</th>
<th>B</th>
<th>95% CI</th>
<th>p-value</th>
<th>R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\log_2 PC_{20}$ AMP</td>
<td>Age, yr</td>
<td>0.111</td>
<td>0.035; 0.187</td>
<td>0.005</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Current smoking status</td>
<td>-0.028</td>
<td>-1.82; 1.77</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eos blood, % total</td>
<td>-0.306</td>
<td>-0.686; 0.074</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEV$_1$, % predicted</td>
<td>0.047</td>
<td>-0.011; 0.104</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RV, % predicted</td>
<td>-0.018</td>
<td>-0.054; 0.017</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$S_{vel}$ L$^{-1}$</td>
<td>-1.94</td>
<td>-42.1; 38.2</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>$\log_2 PD_{20}$ adenosine</td>
<td>Age, yr</td>
<td>0.059</td>
<td>0.007; 0.112</td>
<td>0.027</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Eos blood, % total</td>
<td>-0.244</td>
<td>-0.542; 0.055</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEV$_1$, % predicted</td>
<td>0.052</td>
<td>0.009; 0.096</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RV, % predicted</td>
<td>-0.024</td>
<td>-0.050; 0.002</td>
<td>0.073</td>
<td></td>
</tr>
</tbody>
</table>

AMP = adenosine monophosphate; CI = confidence intervals; Eos blood = blood eosinophils as percentage of total leukocytes; $PC_{20}$ = provocative concentration causing a 20% drop in FEV$_1$; $PD_{20}$ = provocative dose causing a 20% drop in FEV$_1$; FEV$_1$ = forced expiratory volume in 1 s; RV = residual volume; $S_{vel}$ = ventilation heterogeneity of the conductive lung zone.

The present work shows that bronchial provocation with dry powder adenosine is a suitable method for measuring AHR in asthmatic subjects. Moreover, the new testing method allowed us to administer higher doses, resulting in fewer false-negative test results, and the degree of AHR to dry powder adenosine correlated well with the degree of AHR to nebulized AMP. Despite the greater sensitivity of the dry powder adenosine provocation test, there were still nine subjects with a negative test result. Although the order of the provocation tests was non-randomized, with AMP administered first and dry powder adenosine administered second 1-2 weeks later, and refractoriness has been shown to occur after AMP provocation(8), we consider any remaining effect 1-2 weeks later to be unlikely given the findings of Singh et al(9). Some patients may have developed a component of chronic obstructive pulmonary disease or asthma-chronic obstructive pulmonary disease overlap, since this study examined current or former smokers. There was, however, no relationship apparent between measures of airway obstruction at baseline and $PD_{20}$ adenosine (e.g. only two out of nine had an FEV$_1$/FVC ratio <70%) or with their smoking status (four were current and five were former smokers). Therefore, we expect that increasing the top dose, which was now arbitrarily
chosen at 80 mg, could further reduce the number of false-negatives and thus increase the test’s sensitivity even more. However, we cannot rule out the possibility that some subjects with asthma will remain unresponsive to even higher doses inhaled adenosine. This issue requires further investigation.

The subjects did not appear to react more severely to dry powder adenosine than anticipated from their responsiveness to AMP, indicating that the test is safe to use. Severe cough, a side effect that has been shown to hinder applicability of the mannitol provocation test(10), another indirect measure of AHR, was only reported in one subject. No other side effects were observed.

We previously reported that AHR to AMP is associated with eosinophilic inflammation(1). In the present study, we included blood eosinophils in the prediction models, although their individual contributions were not significant for either PC_{20} AMP or PD_{20} adenosine (p = 0.066 and p = 0.11 respectively). This can be explained by the fact that in the present study we investigated eosinophilic inflammation in blood rather than sputum, by the smaller study population compared with the previous work (60 versus 120 patients(1)), and the nonparametric distribution due to the high number of nonresponders, especially to AMP. Alternatively, differences in the smoking behavior of the subjects may have played a role. Smoking has been shown to blunt eosinophilic inflammation, as demonstrated by lower numbers of eosinophils in the sputum and blood of smokers and ex-smokers compared to never-smokers(11). Further studies in never-smokers are therefore warranted.

In conclusion, we have shown that bronchial provocation with dry powder adenosine is a suitable alternative to provocation with nebulized AMP, considering the good agreement between the tests and comparable baseline predictors. Moreover, dry powder adenosine appears to offer an improvement over nebulized AMP, because of its higher sensitivity for less hyperresponsive subjects with asthma.

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REFERENCES


PART II

Molecular Phenotyping