Adenosine monophosphate and histamine induced bronchoconstriction: repeatability and protection by terbutaline

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Abstract

Background – Inhaled adenosine monophosphate (AMP) is thought to cause bronchoconstriction in asthmatic patients indirectly through mast cell mediator release. It may therefore be a more sensitive marker of airway inflammation in asthma and hence more specific for epidemiological surveys of asthma than challenges that act directly on airway smooth muscle such as histamine. There is some uncertainty as to how repeatable the measurement is and this is important if it is to be used for epidemiological studies.

Methods – The response to histamine and AMP challenges and the protection afforded by terbutaline (500 µg) against these two challenges was measured on two occasions two weeks apart in 20 subjects with asthma (19 completed the study). The response to histamine and AMP was measured as the provocative dose causing a 20% fall in forced expiratory volume in one second (PD₂₀) and the protection afforded by terbutaline in doubling doses (DD). Repeatability was assessed as the limits of agreement.

Results – Although terbutaline had a slightly greater protective effect against AMP than histamine on both the first (APD₂₀ = 2.66 versus 2.11 DD) and second occasion (2.56 and 2.15 DD), the differences were not statistically significant. The limits of agreement for the two histamine and two AMP challenges after placebo were from 3.06 to –3.5 and from 3.78 to –4.54 DD respectively, and these values did not differ significantly. The agreement limits between the first PD₂₀ histamine and PD₂₀ AMP values after placebo were similar, being from 3.73 to –3.72 DD after allowing for the 17.8-fold higher PD₂₀ values for AMP compared with histamine.

Conclusions – Terbutaline caused a slightly greater inhibition of the bronchoconstrictor response to AMP than histamine but the differences were small and non-significant. Any differences in repeatability between AMP and histamine challenges are small and in this study were not significant. The fact that the agreement between histamine and AMP PD₂₀ values was similar to the agreement between repeat histamine or repeat AMP PD₂₀ values suggests that, within an asthmatic population, PD₂₀ AMP may not be

providing different information from that provided by PD₂₀ histamine.

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Keywords: adenosine monophosphate, histamine, bronchial responsiveness.

Bronchial hyperresponsiveness, an exaggerated airway response to constrictor stimuli, is a key component of asthma.^{1,2} Constrictor stimuli can be divided into those that act predominantly through a direct effect on airway smooth muscle such as histamine and methacholine and those that act indirectly by stimulating neural pathways or release of inflammatory mediators. Adenosine monophosphate (AMP) is thought to cause bronchoconstriction by increasing the release of mediators from mast cells.³⁻⁶ It may therefore be a more sensitive marker of airway inflammation and hence more specific for asthma. In keeping with this, three studies have shown that β agonists cause greater bronchoprotection against AMP than histamine or methacholine challenge in patients with asthma.⁷⁻⁹ The specificity for asthma could be useful for epidemiological studies if the test was repeatable.

In a recent study the response to AMP appeared to be slightly less repeatable than that to histamine.¹⁰ We have therefore compared the repeatability of histamine and AMP challenge and their protection by terbutaline after a two week interval in patients with asthma. We measured the response to histamine and AMP as the provocative dose of AMP or histamine causing a 20% fall in forced expiratory volume in one second (PD₂₀).

Methods

SUBJECTS

Twenty subjects aged 22–56 years with stable asthma and no other significant illness were studied. All were current non-smokers and had mild asthma with a forced expiratory volume in one second (FEV₁) at least 60% predicted and requiring less than four puffs per day of a short acting inhaled β agonist and/or an inhaled corticosteroid. Twelve subjects were taking an inhaled corticosteroid and all were taking an inhaled β agonist. Nineteen subjects were atopic. Subjects gave written consent to the study which was approved by the Nottingham City Hospital ethics committee.

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MEASUREMENTS

FEV₁ was measured with a dry bellows spirometer (Vitalograph, Buckingham, UK) as the higher of two successive readings within 100 ml. The PD₂₀ AMP and histamine were determined using a breath activated dosimeter (MEFAR, Brescia, Italy). The nebuliser was set to nebulise for one second with a pause of six seconds at a pressure of 22 lb/in (152 kPa). Histamine and AMP (Sigma, Poole, Dorset, UK) were dissolved freshly in saline to produce a doubling concentration range of 0.115–118 µM for AMP and 0.021–21 µM for histamine. FEV₁ was measured one minute after each dose. Inhalation was stopped when the FEV₁ had fallen by 20% or more, when subjects had inhaled the highest dose of constrictor agent, or if side effects occurred. The PD₂₀ value was determined by interpolation between the last two points on the log dose–response plot. Extrapolation was allowed for one further dose.

PROTOCOL

The study had a placebo controlled crossover design. Subjects taking an inhaled steroid kept the dose constant throughout the study. All studies were carried out at the same time of day and after subjects had withheld inhaled β agonist treatment for at least eight hours.

Subjects attended the laboratory on four occasions within a week and after a two week interval on a further four occasions within a week. After resting for 15 minutes they inhaled placebo or terbutaline (500 µg) by dry powder inhaler (Turbohaler) followed 15 minutes later by an inhaled histamine or AMP challenge. All four combinations of placebo and terbutaline with the two challenges were given in random order on the four days before and on the four days after the two week interval. Treatment with placebo and terbutaline was double blind; the nature of the histamine and AMP challenge was known to the investigator but not to the subjects.

The number of subjects studied was based on the study by O'Connor *et al*⁶ in which a difference of 1.1 doubling doses between the

protection afforded by terbutaline on AMP and methacholine in 12 subjects was statistically significant.

ANALYSIS OF DATA

PD₂₀ values for histamine and AMP were log transformed for analysis and geometric mean values are given. Differences in PD₂₀ were measured in doubling doses (DD). The protective effect of terbutaline on histamine and AMP was measured in doubling doses for each subject by calculating

$$\frac{\log \text{PD}_{20} (\text{terbutaline}) - \log \text{PD}_{20} (\text{placebo})}{\log 2}$$

and compared by paired *t* test. The repeatability of histamine and AMP PD₂₀ measurements was assessed as the limits of agreement according to Chinn¹¹ as the mean (SD) difference in PD₂₀ difference × *t*_{0.05} and converted to doubling doses. The limits of agreement for histamine and AMP PD₂₀ were then compared by unpaired *t* test.

The limits of agreement for the first histamine and first AMP measurement were also computed as above after dividing PD₂₀ AMP values by 17.8 since the mean PD₂₀ AMP was 17.8 times higher on average than the mean PD₂₀ histamine value.

Results

Twenty subjects (nine men) aged 22–56 years were recruited. Nineteen completed all eight studies and one failed to return for the second set of four measurements. The mean initial FEV₁ was 2.62 litres (82% predicted). Details of the patients are presented in table 1.

On 20 of the 160 occasions a PD₂₀ could not be obtained because patients did not bronchoconstrict with the highest dose of constrictor agent after terbutaline (*n* = 16; one for histamine and 15 for AMP) or because of coughing after a lower dose of constrictor (*n* = 4). On these occasions the PD₂₀ value was censored to the highest dose of AMP or histamine given

Table 1 Details of age, sex, atopic status, spirometric measurements and treatment

Patient no.	Age	Sex	Atopic status	FEV ₁ (l)	FEV ₁ (% predicted)	Treatment
1	54	M	H,C,G	1.85	57	Sb,BDP 800 µg
2	37	M	H,C,G	2.85	84	Sb,Bud 400 µg
3	27	M	H,C	4.35	100	Sb,BDP 400 µg
4	35	M	H,C,G	3.40	90	Sb,BDP 400 µg
5	34	F	H,C,G	2.45	86	Sb
6	28	F	C,G	2.95	87	Tb
7	51	F	H	2.20	77	Sb,BDP 400 µg
8	38	M	H,C,G	2.70	63	Tb,BDP 400 µg
9	55	F	H,C,G	1.10	56	Sb,BDP 400 µg
10	34	M	H,C,G	4.15	95	Sb,BDP 400 µg
11	54	F	Negative	1.40	62	Sb,BDP 400 µg
12	55	F	H,C,G	2.40	95	Sb
13	56	F	Negative	2.00	87	Sb,BDP 800 µg
14	28	F	H,G	3.50	111	Sb
15	42	F	H	2.00	85	Sb,BDP 200 µg
16	40	M	H	3.75	85	Sb
17	54	M	H,C,G	2.05	63	Sb,BDP 400 µg
18	23	F	H,C,G	3.30	107	Sb,Bud 800 µg
19	24	F	H,C,G	2.70	79	Sb
20	22	M	H	4.50	105	Sb

FEV₁ = forced expiratory volume in one second; BDP = beclomethasone dipropionate; Bud = budesonide (dose is total daily dose); Sb = salbutamol as required; Tb = terbutaline as required.

Atopic status describes positive skin prick tests to H = house dust mite, C = cat, G = grass.

Figure 1 Difference between the first and second measurements of (A) $\log PD_{20}$ AMP and (B) $\log PD_{20}$ histamine plotted against the mean PD_{20} measurements in 19 subjects with asthma. The relation between the first PD_{20} AMP and PD_{20} histamine values after placebo are also shown in (C) after the AMP values* have been divided by 17.8 (see text).

after extrapolation by one dose.¹¹ The protective effect of terbutaline was then assessed in two ways – in the 10 subjects who had no censored values and by including all subjects and including the censored values. The mean PD_{20} values on each occasion are given in table 2.

REPEATABILITY

The agreement between the first and second PD_{20} histamine values and between the first and second AMP values is shown in fig 1 according to the method of Bland and Altman.¹² The agreement limits for the two PD_{20} histamine and two AMP measurements after placebo were from 3.06 to –3.5 and from 3.78 to –4.54 doubling doses, respectively, and the agreement limits for the two challenges did not differ significantly ($p < 0.05$).

RELATION OF PD_{20} HISTAMINE TO PD_{20} AMP

Following placebo the mean PD_{20} AMP value was 17.8 times higher than the mean PD_{20} histamine value ($p < 0.001$). The relation between the first PD_{20} histamine and the first PD_{20} AMP values following placebo is shown in fig 1, the AMP values having been divided by 17.8. The agreement limits for comparison of the first histamine and first AMP PD_{20} measurements after allowing for the 17.8-fold difference in mean PD_{20} values were 3.73 to –3.72 doubling doses.

PROTECTIVE EFFECT OF TERBUTALINE

Terbutaline had a slightly greater protective effect against AMP than histamine on both the first and second occasions (terbutaline induced changes in PD_{20} were 2.70 and 2.56 DD for

AMP compared with 2.11 and 2.15 DD for histamine). The mean difference between the protective effect of terbutaline on histamine and AMP PD_{20} was 0.58 DD (95% CI –0.28 to 1.44; $p = 0.17$) on the first occasion and 0.41 DD (95% CI –0.53 to 1.56; $p = 0.37$) on the second. When the results from the 10 subjects with no censored values were analysed in the same way the difference between the protective effect of terbutaline against AMP and histamine induced bronchoconstriction remained non-significant on both the first (AMP 2.82 DD, histamine 2.64 DD; mean difference 0.18 DD; 95% CI –0.58 to 0.94; $p = 0.6$) and second occasion (AMP 3.14, histamine 2.46; mean difference 0.68 DD; 95% CI –0.94 to 2.29 DD; $p = 0.37$).

There was no significant difference in the change in PD_{20} with terbutaline for either AMP (2.27 versus 2.22 DD) or histamine (1.9 versus 2.44 DD) between the 13 patients who were taking an inhaled corticosteroid and the seven who were not.

Discussion

The mechanism underlying the bronchoconstriction caused by inhalation of AMP in patients with asthma is not certain but increased release of mast cell mediators is probably involved. Adenosine and its synthetic analogues such as AMP potentiate IgE dependent release of the preformed mediators histamine and hexosaminidase from rodent¹³ and human⁶ mast cells in vitro, and drugs that inhibit mast cell activation (sodium cromoglycate and nedocromil sodium)^{14,15} or inhibit the effect of mast cell products (the selective H_1 receptor antagonists, terfenadine and astemizole^{16–18}) have been shown to protect against AMP induced bronchoconstriction in both atopic¹⁶ and non-atopic¹⁷ subjects with asthma. Bronchoconstrictor prostanoids such as prostaglandins D_2 , $F_{2\alpha}$ and thromboxane A_2 may also be involved since indomethacin and lysine acetylsalicylate inhibit adenosine and AMP induced bronchoconstriction in asthmatic subjects.^{19–21} The action of adenosine may also involve neural reflexes since adenosine potentiates the constrictor response to nerve stimulation via an A_2 -receptor mechanism²²

Table 2 Geometric mean PD_{20} histamine and AMP values after placebo and terbutaline

Measurement		$PD_{20}(\mu\text{mol})$	
		Histamine	AMP
Placebo	First	0.4	6.97
	Second	0.42	7.65
After terbutaline	First	1.71	45.2
	Second	1.86	40.0

Second measurement was made two weeks after the first.

and antimuscarinic drugs have a minor protective effect against adenosine induced bronchoconstriction in asthma.²³

The bronchoconstrictor response to adenosine therefore involves one, or possibly two, pathways which may be abnormally activated in asthma, suggesting that it may be a more specific marker of asthma than bronchoconstrictor challenges such as histamine and methacholine. This could be of value for epidemiological studies where the lack of specificity of histamine and methacholine in distinguishing asthma from other lung diseases limits their value. Using AMP to measure bronchial responsiveness would only be of value if the test was repeatable and this was questioned in a previous study.¹⁰ The present study shows no significant difference in the repeatability of the response to AMP when compared with that of histamine challenge.

Previous studies have shown that a single dose of terbutaline (500 µg)⁷⁻⁹ or chronic treatment with budesonide²⁴ provide greater protection against bronchoconstriction induced by AMP than that induced by methacholine. This difference has aroused interest since it may reflect drug effects other than airway smooth muscle relaxation such as inhibition of mast cell mediator release by β agonists and an anti-inflammatory effect of inhaled corticosteroids. The protection against histamine induced bronchoconstriction in our study was similar to that seen with the same dose of terbutaline against methacholine challenge by O'Connor *et al.*⁸ whereas the effect of terbutaline against AMP was less and the difference in protection against the two challenges was not statistically significant. The effect of terbutaline against AMP induced bronchoconstriction may have been underestimated in our study since several subjects had censored PD₂₀ AMP values following terbutaline, although the fact that the difference in the protective effect of terbutaline was not significant in the 10 subjects without a censored value argues against this.

The differences between our findings and those in previous studies could reflect differences in asthma severity, or in the contribution of mast cell mediator release to bronchoconstriction, or in previous treatment. The asthma severity appears to be similar to that in two of the previous studies^{7,9} since patients had identical mean FEV₁ percentage predicted values (92%) and similar consumption of β agonists (average two puffs per day) before the study. In the third study⁸ subjects had a higher FEV₁ and β agonists were replaced with ipratropium for one week before the study. Corticosteroid treatment differed between the studies with 13 patients taking an inhaled corticosteroid in a constant dose in our study compared with none in the studies by O'Connor *et al.*^{8,9} and five of nine subjects in the study by Phillips *et al.*⁷ We found no difference, however, in the relative protection of terbutaline against AMP and histamine between those taking and those not taking an inhaled corticosteroid. Finally, we have considered whether tachyphylaxis to histamine or AMP may be relevant since this has been seen over

several hours following AMP²⁵ and histamine,²⁶ though usually in normal subjects or patients with mild asthma. To our knowledge, it has not been seen after 24 hours in patients with asthma. Our studies were carried out at least a day apart as were the studies by O'Connor *et al.*⁸ and as their patients had, if anything, milder asthma, tachyphylaxis would have been more likely. Since we have also been unable to confirm that regular inhaled budesonide results in greater protection against AMP than histamine induced bronchoconstriction,¹⁰ we conclude that any difference in the ability of terbutaline or regular budesonide to provide greater protection against AMP is small in subjects with relatively mild asthma.

The agreement between a single PD₂₀ AMP and PD₂₀ histamine measurement after placebo was similar to the repeatability of AMP and histamine after allowing for the 17.8 times higher mean values for AMP. This suggests that PD₂₀ AMP might not be adding anything further to the information given by PD₂₀ histamine in patients with asthma and that both are reflecting the same pathophysiological process in the airways. A study in children suggested that PD₂₀ AMP was better able to distinguish asthma from COPD than methacholine.²⁷ Further studies that include non-asthmatic patients are needed to determine whether PD₂₀ AMP provides greater specificity for asthma in adults in an epidemiological setting.

In conclusion, we have shown reasonable agreement between PD₂₀ AMP and PD₂₀ histamine in 20 patients with asthma and similar repeatability between AMP and histamine PD₂₀ values in these patients. We have been unable to confirm the previous finding of a greater protective effect of terbutaline against AMP compared with a challenge that is not mast cell dependent. These findings suggest that AMP PD₂₀ gives similar information to histamine PD₂₀ in an asthmatic population; further field studies are needed to determine whether it is more specific for asthma in a population of adults containing non-asthmatic patients.

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