Adenosine induces histamine release from human bronchoalveolar lavage mast cells

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ABSTRACT

Previous studies have shown that in vitro adenosine enhances histamine release from activated human lung mast cells obtained by enzymic dispersion of lung parenchyma. However, adenosine alone has no effect on histamine release from these cells. Given the evidence for direct activation of mast cells after endobronchial challenge with adenosine and previous studies indicating that mast cells obtained at bronchoalveolar lavage are a better model for asthma studies than those obtained by enzymic dispersion of lung tissue, the histamine-releasing effect of adenosine was examined on lavage mast cells. Bronchoalveolar lavage fluid was obtained from patients attending hospital for routine bronchoscopy (n = 54). Lavage cells were challenged with adenosine or adenosine receptor agonists (20 min, 37 °C) and histamine release determined using an automated fluorometric assay. Endogenous adenosine levels were also measured in lavage fluid (n = 9) via an HPLC method. Adenosine alone caused histamine release from lavage mast cells in 37 of 54 patients with a maximal histamine release of 20.56 ± 2.52% (range 5.2–61%). The adenosine receptor agonists (R)-N6-(2-phenylisopropyl)adenosine, 5'-N-ethylcarboxamido-adenosine and CGS21680 also induced histamine release from lavage mast cells. Preincubation of lavage mast cells with the adenosine receptor antagonist xanthine amine congener caused significant inhibition of the response to adenosine (P = 0.007). There was an inverse correlation between endogenous adenosine levels in the lavage fluid and the maximal response to in vitro adenosine challenge of the lavage cells. The findings of the present study indicate a means by which adenosine challenge of the airways can induce bronchoconstriction and support a role for adenosine in the pathophysiology of asthma. The results also suggest that cells obtained from bronchoalveolar lavage fluid may provide the ideal model for the testing of novel, adenosine receptor, targeted therapies for asthma.

INTRODUCTION

Asthma is a condition that can lead to a state of metabolic imbalance within the airways. Stimulation of bronchial mast cells and other inflammatory cells for mediator secretion and the subsequent contraction of bronchial smooth muscle increases oxygen and energy demand. Experiments in vitro have demonstrated that hypoxia or activation of human leucocytes or rat and dog lung tissue mast cells results in increased adenosine accumulation in the incubation supernatant [1–4]. Higher levels of adenosine have also been demonstrated in broncho-

Key words: adenosine, bronchoalveolar lavage, histamine release, mast cells.
Abbreviations: BAL, bronchoalveolar lavage; R-PIA, (R)-N6-(2-phenylisopropyl)adenosine; NECA, 5'-N-ethylcarboxamidoadenosine.
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alveolar lavage (BAL) fluid from subjects with asthma compared with normal individuals [5].

A potential role for adenosine in the modulation of allergic reactions was suggested by early work, which showed a potentiation of antigen-induced histamine release from sensitized guinea-pig uterine horns by adenosine [6]. Adenosine also enhances histamine release from rat peritoneal mast cells stimulated by a diverse variety of agents including the calcium ionophore A23187, concanavalin A, compound 48/80 and anti-IgE and modulates stimulated histamine release from dispersed lung mast cells and basophils [7,8]. Furthermore, stimulated mast cells release adenosine, suggesting a potential positive-feedback mechanism [1].

Adenosine provoked bronchoconstriction when inhaled by asthmatic individuals but not in non-atopic, non-asthmatic subjects, although high concentrations could not be used because of its poor solubility [9]. Later experiments involved inhalation of the more soluble AMP, which is rapidly converted to adenosine by the cell surface enzyme 5′-nucleotidase. Inhalation of AMP by atopic non-asthmatic subjects produced a degree of bronchoconstriction which was intermediate to that seen in asthmatic and normal subjects [10]. Thus, whereas the degree of bronchial hyperresponsiveness is the sole determinant of sensitivity to the direct-acting parasympathomimetic agent methacholine, the effect of AMP, and by inference adenosine, is related more to the allergic state. It was subsequently proposed that adenosine causes bronchoconstriction by an indirect mechanism mediated through an intermediate cell [10].

Investigations have subsequently provided strong evidence that the mast cell is such an intermediate. The mast cell stabilizing agents disodium cromoglycate and nedocromil sodium provide, respectively, 7–10- and 20-fold protection against AMP-induced bronchoconstriction [11,12]. In subjects with mild asthma, two structurally unrelated H1-receptor antagonists, terfenadine and astemizole, both of which can completely inhibit the airway response to inhaled histamine, inhibited the bronchoconstrictor effects of AMP by 50% [13]. In the same study it was demonstrated that the inhibition of allergen-induced bronchoconstriction by H1-receptor blockade was much less than that after either AMP or histamine airway challenge. Instillation of AMP directly into an airway segment of asthmatic subjects leads to a rapid reduction in airway calibre with concomitant increases in levels of histamine, prostaglandin D2 and tryptase in BAL fluid [14]. Thus, observations in vitro and in vivo support a role for adenosine in the pathophysiology of asthma and have led to concerted efforts to determine both the extent of this role and the nucleoside’s mode of interaction with the mast cell.

Adenosine’s bronchoconstrictor effects are not mimicked by inosine, the deaminated metabolite of adenosine [10], or by the closely related purine guanosine [9].

indicating that adenosine-induced bronchoconstriction is mediated via an adenosine-specific receptor. In addition, theophylline, a methylxanthine known to inhibit receptor-mediated effects of adenosine, preferentially antagonizes adenosine-induced bronchoconstriction at concentrations that do not inhibit phosphodiesterases [15].

Since endobronchial challenge of asthmatic subjects with adenosine leads directly to mast cell activation and human lung tissue mast cells do not respond directly to this agent, the effect of adenosine on human mast cells obtained at BAL was examined.

**MATERIALS AND METHODS**

**Subjects**

The subjects were attending the day procedure unit at Belfast City Hospital for routine bronchoscopy. The majority of patients presented with haemoptysis or abnormal chest X-ray. Written informed consent was obtained from all subjects. The study was approved by the ethics committee of The Queen’s University of Belfast and carried out in accordance with the Declaration of Helsinki (1989).

**Lavage method**

All patients had a standardized lavage performed [16]. Topical lignocaine (4%) was applied to the oropharynx. The bronchoscope was passed through the nose and lignocaine (2%) applied via a cannula to the vocal cords. Further local anaesthesia was achieved with lignocaine (1%) as required. The bronchoscope was wedged in the right middle lobe. The lavage was performed with sterile isotonic saline (3 × 60 ml), inserted under minimum hand pressure. After a minimum dwell time the fluid was aspirated into a plastic container and the samples pooled into a polypropylene vessel. This was immediately placed on ice and transferred to the laboratory.

**Functional studies**

BAL fluid was centrifuged (200 g for 10 min at 4°C), washed twice in Tyrode’s buffer and resuspended in a volume appropriate for the experiment. The cells were pre-warmed (37°C for 5 min) and challenged (20 min) with adenosine or adenosine agonists and A23187 (1 µM; Calbiochem, Nottingham, U.K.). The reactions were stopped by the addition of ice-cold Tyrode’s buffer and the cells and supernatants separated by centrifugation. Histamine release was measured in the cell pellets and the supernatants using an automated fluorometric assay [17]. Histamine release was expressed as a percentage of the total cellular histamine content corrected for spontaneous release (i.e. that occurring in the absence of any stimulus). The mean spontaneous release was 12.9 ± 0.7% (n = 54). This value is comparable to that found previously by us.
Adenosine-induced histamine release

Figure 1 Chromatograms of BAL fluid

A is a representative chromatogram of BAL fluid obtained from a study subject. The arrows indicate the retention time of adenosine standards. Chromatogram B shows the result when the same BAL fluid was incubated with 5 units/ml adenosine deaminase for 20 min at 37 °C. The disappearance of the peak with a retention time of 5 min confirms its identity as adenosine. Inosine has a retention time of around 2.5 min and an increase of the inosine peak can also be observed. Chromatogram C shows BAL fluid from the same subject after addition of 2 μM adenosine standard.

in a similar non-preselected group of patients attending for a clinically indicated bronchoscopy [18]. CGS21680 was dissolved in 1% DMSO/Tyrode’s buffer and A23187 in 0.5% DMSO/Tyrode’s buffer. Other compounds were dissolved in Tyrode’s buffer.

Adenosine determination

Immediately after aspiration an aliquot (9 ml) of the lavage fluid was placed in a polystyrene tube containing 1 ml of stopping solution designed to inhibit the metabolism and the cellular uptake of adenosine. The stopping solution was composed of 2.3 μM erythro-9(2-hydroxy non-3-yl)-adenine and 20 μM dipyridamole. After separation of the cell fraction, the lavage fluid was frozen at −70 °C before analysis.

The method for measurement of adenosine in cell-free lavage fluid was adapted from that of Driver et al. [5]. The prepared supernatant (1 ml) was filtered through a 0.45 μM clarification filter to remove particulate impurities. Adenosine was measured in 100 μl of the filtered fluid using a Waters U6K HPLC system (Waters Assoc., Milford, MA, U.S.A.) with a Waters 990 photodiode array detector (Waters Assoc.) set at a wavelength of 254 nm. The sample was injected onto an adsorbosil C18 column (4.6 mm × 15 cm) (Alltech, U.K.) and eluted with a mobile phase consisting of 20% (v/v) methanol/KH2PO4 (4 mM) at pH 5 and a flow rate of 1 ml/min. The presence of adenosine was confirmed by the addition of a known amount of adenosine to augment the adenosine peaks and enzymic shift through the use of adenosine deaminase which converts adenosine to inosine (Figure 1). In this assay system, adenosine appeared as a single distinct peak with a retention time of 5–5.6 min. Adenosine concentrations were calculated by measuring peak heights and comparing these with peaks produced by adenosine standards of known concentration. The detection limit for the assay was 0.5 μM.

Statistical methods

All values are given as means ± S.E.M. unless otherwise stated. Correlation coefficients were calculated using Spearman’s rank method. A P value of less than 0.05 was considered statistically significant.

RESULTS

Subjects

A total of 54 subjects were investigated (33 male). The mean age of subjects was 58.5 ± 1.8 years. The majority of subjects were smokers (25 of 38 where data were available). The mean volume of lavage return was 62.4 ± 2.5 ml.

Response to adenosine and adenosine analogues

Addition of adenosine alone caused histamine release from human BAL mast cells in 37 of 54 samples. There was a high degree of inter-individual variation in both the extent of histamine release and the concentration of
Figure 2 Representative responses of individual human BAL cell preparations to NECA (○) and the corresponding response to adenosine (■)
Histamine release is expressed as a percentage of the total cellular histamine content and corrected for spontaneous release.

Figure 3 Representative responses of individual human BAL cell preparations to R-PIA (■) and the corresponding response to adenosine (○)
Histamine release is expressed as a percentage of the total cellular histamine content and corrected for spontaneous release.

adenosine required to elicit a maximal response (Figures 2–4). Maximal histamine release in response to adenosine challenge was 20.56 ± 2.52% (range 5.2–61%, n = 37). The adenosine receptor agonists (R)-N’-(2-phenylisopropyl)adenosine (R-PIA), 5’-N-ethylcarboxamido-adenosine (NECA) and CGS21680 also caused histamine release from BAL mast cells. As with adenosine, the response to these agonists varied greatly between individual BAL preparations. The maximal histamine release induced by NECA was 12.7 ± 1.1% (range 11.3–18.3%, n = 6 of 8) (Figure 2). The maximal response to adenosine in the same BAL samples was 16.9 ± 4.4% (range 6.8–37.7%). The maximal response to R-PIA was 32.0 ± 11.4% (range 5.9–75.7%, n = 6 of 14) (Figure 3), with a corresponding response to adenosine of 24.5 ± 6.9% (range 12.2–61.0%). CGS21680 caused a maximal histamine release of 22.3 ± 5.7% (range 8.5–36.2%, n = 4 of 5) (Figure 4) with a corresponding response to adenosine of 13.1 ± 5.14% (range 31.8%). The response to the vehicle (DMSO) did not differ from spontaneous release. There was no significant difference between the maximal response to adenosine and the corresponding maximal response to any of the agonists tested. When used to challenge the same BAL preparations there was no consistent relationship between the potency of adenosine and any of the receptor agonists tested.

Effect of xanthine amine congener on the response of BAL mast cells to adenosine
Preincubation of the BAL cell preparations with xanthine amine congener (1 μM) did not alter basal release (11.06 ± 1.3% and 10.28 ± 1.8% respectively), but caused a significant inhibition of the response to adenosine. Maximal histamine release was reduced from 18.4 ± 2.1% to 6.2 ± 2.7% (P = 0.007, n = 5) (Figure 5).

Effect of endogenous adenosine levels on the response of BAL mast cells to adenosine challenge
The median BAL fluid adenosine concentration was 1.4 μM (range 0.8–17.5 μM) and that of the maximum adenosine response was 24.3% (range 10.2–42.6%) (Table 1). Comparing individual subjects, there was a negative correlation between the maximal response to adenosine and endogenous BAL fluid adenosine levels.
Adenosine-induced histamine release

Figure 4 Representative responses of individual human BAL cell preparations to CGS21680 (■) and the corresponding response to adenosine (○)
Histamine release is expressed as a percentage of the total cellular histamine content and corrected for spontaneous release.

Figure 5 Effect of xanthine amine congener (XAC, 1 μM) on the maximum histamine release induced by adenosine (Ado) in BAL cell preparations of five individuals
Histamine release is expressed as a percentage of the total cellular histamine content and corrected for spontaneous release.

(r = -0.75, P = 0.025, n = 9). There was no significant correlation between BAL fluid adenosine concentration and release induced by A23187 (1 μM) (r = -0.59, P = 0.097, n = 9) or spontaneous histamine release (r = 0.54, P = 0.13, n = 9).

DISCUSSION

In contrast to observations in vitro with other mast cell types, including those enzymically isolated from human lung parenchymal tissue, adenosine alone induced histamine release from BAL mast cells [7,19–21]. These data are similar to those previously reported for substance P, where the human lung tissue mast cells are also refractory to the stimulus but mast cells retrieved by BAL respond to the peptide [18,22].

The BAL fluid employed does not provide a pure mast cell population and the cells form part of a complex milieu of cells and their mediators. Therefore, the present data cannot establish whether the observed histamine release is a result of the direct action of adenosine on mast cells or a secondary effect due to stimulation of other cell types within the preparation. However, given the strong evidence that mast cells are involved in the bronchoconstrictor effects of adenosine, the simplest hypothesis is that mast cells alone are sufficient to respond to adenosine. If this is the case, the present data suggest either that this is a manifestation of mast cell heterogeneity (compare with the substance P data), or that there is an additional signal present in the BAL preparation, not present in human lung tissue mast cell preparations, which promotes mast cell stimulation by adenosine. The latter hypothesis is supported by data indicating histamine release from mast cells after administration of adenosine in vivo to rats [23,24]. This suggests that the BAL cell preparation more accurately reflects the in vivo state of the mast cell.

The additional signal required for direct activation by adenosine may be constitutively present in the BAL cell preparation and indeed in vivo, or may be generated as a result of adenosine application. Many substances, such as cytokines or neuropeptides, which activate mast cells may be present at subthreshold concentrations; in the presence of adenosine this threshold may be lowered and mast cell degranulation may occur. The adenosine receptor antagonist xanthine amine congener inhibited the response of BAL mast cells to adenosine, indicating the participation of an extracellular adenosine receptor in the activation process. These data do not indicate the receptor subtype involved as, unlike the rodent A$_2$ adenosine receptor, the human equivalent is sensitive to this antagonist [25].

A characteristic of adenosine-induced histamine release from BAL mast cells is the high level of interindividual variation in both the maximal histamine release and the concentration of adenosine at which this occurs. Many BAL preparations exhibited ‘bell shaped’ or even
The present study examined the possibility that the cells were obtained from non-preselected patients attending for a clinically indicated bronchoscopy. Thus the underlying pathologies differed between subjects. Previously we have found a wide variation in response to substance P in such patients, although the variation in response was not found in further studies with a clearly defined asthmatic population [18,22]. The inter-individual variability in response to adenosine and adenosine agonists is therefore most probably due to variation in the clinical histories of this non-preselected population. However, some of our patients may have been prescribed theophylline, which could modulate the response to adenosine.

It is unlikely that the variation in response is due to differing rates of adenosine metabolism in these preparations. Firstly, the variation was also seen in response to adenosine analogues which do not undergo metabolism. Secondly, levels of adenosine deaminase previously detected in BAL fluid have been negligible [5]. The present study also examined the possibility that different endogenous BAL fluid adenosine levels may be responsible for the observed variation in response to challenge with adenosine. Within the nine individuals included in the study there was a negative correlation between BAL fluid adenosine concentrations and response to exogenous adenosine. Given that adenosine levels are increased in the BAL fluid of asthmatic subjects, the present results may appear contradictory to the mast cell mediator-induced bronchoconstriction after AMP challenge of asthmatic subjects [14]. However, the hyperresponsive airways of asthmatic individuals will require less mediator secretion to induce bronchoconstriction. Furthermore, airways become tachyphylactic to the effects of AMP after repeated inhalation [26]. It is unlikely that this refractoriness is due to desensitization of the airways to the effects of histamine as there is no cross-tachyphylaxis between AMP and histamine. In addition, repeated exposure to AMP potentiates rather than reduces the effect of allergen provocation, which suggests that depletion of preformed mediators from mast cells is an unlikely explanation [27]. The refractoriness to adenosine therefore appears to be specific to the nucleoside. The present data suggest a down-regulation of mast cell responsiveness to adenosine after exposure to high levels of the nucleotide. This is supported by the demonstration in vitro that mast cell responses to adenosine are inhibited after chronic exposure to adenosine receptor agonists [28].

Overall, the data presented here indicate a means by which adenosine challenge of the Airways can induce bronchoconstriction and support a role for adenosine in the pathophysiology of asthma. Given that adenosine is released after allergen challenge [4], it is likely that adenosine acts as an autocoid agent in the disease.

Recently much attention has been directed towards adenosine receptors as therapeutic targets in asthma [29,30]. The present study provides no clear evidence as to the adenosine receptor type involved in the activation of BAL mast cells, but these cells may prove a valuable model for testing the therapeutic potential of potent and selective adenosine receptor antagonists in the future. The promising preliminary finding that adenosine can directly activate lavage mast cells has encouraged us to investigate further the action of adenosine on BAL cells using carefully defined subject groups of atopic asthmatic subjects, atopic non-asthmatic subjects and non-atopic, non-asthmatic controls.

**Table 1** Data for subjects in whom BAL adenosine levels were measured

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<th>Patient</th>
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<th>Sex</th>
<th>Volume return (ml)</th>
<th>Adenosine concn. (μM)</th>
<th>Maximum HR in response to adenosine (% of total)</th>
<th>Concn. of adenosine causing maximal HR (M) (μM)</th>
<th>HR in response to A23187 (% of total)</th>
<th>Spontaneous HR (% of total)</th>
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REFERENCES


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