

Adenosine bronchoprovocation: a promising marker of allergic inflammation in asthma?

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It is widely appreciated that asthma is an inflammatory disease of the airways associated with bronchial hyperresponsiveness and variable airflow obstruction.¹ A number of inflammatory cells and autacoid mediators have been implicated in the pathogenesis of this disease.

Adenosine is a purine nucleoside which may be produced in allergic inflammatory conditions upon appropriate stimulation. Indeed, increased amounts of adenosine in the bronchial lavage fluid have been demonstrated in subjects with asthma.² Once generated, adenosine promotes most of its effector functions by stimulating specific cell surface receptors, thus eliciting bronchoconstriction in asthmatic subjects³ and nasal symptoms mimicking rhinitis in subjects with stable allergic rhinitis.⁴

In recent years there has been a tendency for less importance to be attached to purine derivatives as mediators of allergic inflammation. However, the recent work by Nyce and Metzger⁵ in which adenosine induced bronchoconstriction is abolished by using antisense oligodeoxynucleotides to reduce the number of adenosine A1 receptors in the lung of an animal model of allergic asthma, and data from a number of sources which indicate that bronchial provocation with inhaled adenosine may be a more precise index of disease activity than conventional bronchoprovocation tests, have now led to a resurgence of interest in the role of this purine nucleoside in asthma.

In this article we will briefly review the putative role of adenosine as a mediator in airway inflammation, the current view of the mechanisms of adenosine induced bronchoconstriction, and the evidence for adenosine bronchoprovocation as a valuable diagnostic test for asthma.

Putative role of adenosine in airway inflammation

Adenosine is increased in inflammatory conditions of the airways. Adenosine levels have been reported to be significantly raised in lung lavage fluid from sensitised rabbit after allergen challenge.⁶ High concentrations of adenosine are also measured in the bronchoalveolar lavage (BAL) fluid of subjects with asthma and chronic bronchitis when compared with normal controls.² Taken together, these findings indicate that mechanisms for the production of adenosine are active during inflammatory disorders characteristic of asthma. However, the cellular source of adenosine in the airway fluids is not known. All cells contain adenosine and adenine nucleotides. Adenosine release has

been demonstrated to occur from rat peritoneal mast cells upon antigen challenge⁷ and mast cells may have been a source of adenosine released into airway fluid since concentrations tended to be higher in those asthmatic subjects who also had high histamine concentrations.² Besides mast cells, neutrophils and platelets are also found in increased numbers in the bronchial biopsy specimens of asthmatic subjects challenged with specific antigen.⁸ Platelets may be another potential source for adenosine. Platelet activation elicits release of ADP which could then be converted to adenosine. Similarly, neutrophils release AMP which is then converted to adenosine by a specific ecto-5'-nucleotidase.⁹ Epithelial injury is another prominent feature of asthma.^{10,11} It is possible that adenosine may be derived from adenine nucleotides which are released from damaged airway cells. Indeed, adenine nucleotides are present in large quantities in the cytoplasm of these cells and then converted to adenosine by the action of ectonucleotidase enzymes on the cell membranes.¹²

Once generated, adenosine has the capacity to promote a large variety of responses in the airways which are relevant to asthma. Aside from its potential role as a mediator of bronchoconstriction, adenosine may also function as a novel paracrine mediator that contributes to various aspects of the inflammatory response. Asthma is known to be a chronic inflammatory disease associated with the pathological findings of cellular infiltration and oedema of the bronchial mucosa. In this respect adenosine may have some important actions. Adenosine causes plasma exudation and increased bronchial blood flow.¹³ The ensuing presence of plasma proteins in the interstitium produces fluid efflux via osmosis and hence oedema. Adenosine may modulate inflammation by promoting neutrophil chemotaxis¹⁴ and by enhancing histamine release from immunologically activated human lung mast cells and circulating basophils via stimulation of specific cell surface receptors.¹⁵⁻¹⁷ Feoktistov *et al*¹⁸ have recently shown that adenosine elicits secretion of IL-8 (a potent leucocyte chemoattractant) from human mast cells through activation of A2b receptors. This finding suggests that another possible way by which adenosine could lead to the development of persistent airway inflammatory responses is through the release of mast cell derived cytokines.¹⁹

The appreciation of the potential role of adenosine receptors in the pathogenesis of asthma raises the possibility that blockade of adenosine receptors may be exploited as a novel approach to the treatment of asthma. Indeed,

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blockade of A2b receptors may be one of the mechanisms that explains the beneficial effect of theophylline in asthma. Theophylline, which also acts as an adenosine receptor antagonist, has been shown to produce a greater protection against adenosine-induced bronchoconstriction than against histamine-induced bronchoconstriction.²⁰ One difficulty with this view has been the anti-asthmatic activity of the methylxanthine phosphodiesterase inhibitor, enprofylline, which has previously been thought to lack adenosine receptor antagonist activity.²¹ However, it has recently been shown that this drug does, in fact, possess A2b receptor antagonist properties.¹⁸

At present it is premature to implicate purines in the pathogenesis of asthma. Their contribution will only be established through the use of potent and specific adenosine antagonists which are now in clinical development.

Mechanisms of adenosine induced bronchoconstriction

It is now more than a decade since Cushley *et al*³ first reported that adenosine provoked concentration related bronchoconstriction when administered by inhalation to asthmatic subjects but not to normal volunteers. In addition, inhalation of its related nucleotide, adenosine 5'-monophosphate (AMP), produces an almost identical effect on the airways²² as this is dephosphorylated to yield adenosine. Since these observations were made, considerable effort has been directed at elucidating the mechanism by which adenosine mediates bronchoconstriction and its importance as a mediator in clinical asthma. Although there are no adenosine antagonists that have acceptance for use in humans, alternative pharmacological approaches have suggested that it is unlikely that adenosine acts directly on smooth muscle cells *in vivo*, but indirectly through activation of specific receptors on intermediary inflammatory cells such as mast cells or on afferent nerve endings.

That mast cell derived mediators are involved in the bronchoconstrictor response to inhaled adenosine is indicated by *in vitro* studies which have shown that adenosine markedly enhances histamine release¹⁶ and prostanoid generation¹⁷ elicited from immunologically primed human lung mast cells. Moreover, a recent study has now produced evidence that adenosine can directly stimulate histamine release from human mast cells obtained by BAL.²³ But what *in vivo* evidence is there to support this? The principal drugs with mast cell inhibitory properties available for *in vivo* use in humans are sodium cromoglycate (SCG), nedocromil sodium, and the β_2 agonist, salbutamol.²⁴ In a series of studies conducted in asthmatic subjects SCG, nedocromil sodium, and salbutamol have been found to inhibit substantially AMP induced bronchoconstriction by 9.6-, 22.2-, and 26.6-fold, respectively.^{25,26} In addition, premedication with H₁-histamine receptor antagonists^{27,28} and potent inhibitors of cyclooxygenase and 5-lipoxygenase²⁹⁻³² have been shown to inhibit the acute bronchoconstrictor

response to inhaled AMP in asthmatic subjects. To speculate further, the recent demonstration of a protective effect of inhaled heparin in AMP induced bronchoconstriction may be ascribed to blockade of the intracellular 1,4,5-inositol triphosphate (IP3) dependent pathway of mast cell degranulation.³³

More direct evidence that mediators released from airway mast cells are critical for adenosine induced responses comes from a study in which venous plasma histamine levels were measured after allergen and AMP challenge in a group of atopic subjects. A small but significant increase in histamine levels was detected following adenosine challenge.³⁴ We have since carried out a study in which AMP was directly instilled into a segmental airway of a group of asthmatic subjects and the segment was then lavaged to sample for mediator release. A significant increase in lavage concentrations of mast cell derived histamine and tryptase were observed.³⁵ In addition, a recent study using a model of nasal provocation with AMP indicates that this purine nucleotide is also able to induce an immediate rise in histamine levels in the nasal lavage fluid.³⁶ These studies provide strong support for the notion that a major component of AMP induced bronchoconstriction results from mast cell degranulation, probably via stimulation of cell surface purinoceptors.

Activation of neural pathways may also contribute to the contractile airway response to these autacoids in asthma. The possibility of a reflex cholinergic component was originally indicated by data from studies in inbred rats³⁷ and later confirmed in a clinical investigation in which the anticholinergic drug ipratropium bromide administered by inhalation to subjects with stable asthma significantly attenuated the airway effects of adenosine.³⁸ Further support for a neural contribution to AMP induced bronchoconstriction comes from observations made with the loop diuretics frusemide and bumetanide. Inhalation of these drugs inhibits the bronchoconstrictor response of AMP without affecting the response to histamine.³⁹⁻⁴¹ Inhalation of loop diuretics has a similar effect on bronchoconstriction provoked by inhalation of sodium metabisulphite⁴⁰ and bradykinin⁴² and it is believed to act by modulating sensory nerves responses in the airways. In guinea pigs *in vivo* the synthetic adenosine analogue, 2-chloroadenosine, provoked bronchoconstriction which was attenuated by capsaicin.⁴³ Moreover, in BDE rats adenosine elicits increased pulmonary resistance by stimulating neuropeptide producing nerves via prior mast cell activation.⁴⁴ These observations indicate that release of contractile neuropeptides from sensory nerve endings might be of some importance in mediating the airway effects of purine derivatives. In support of this view, repeated challenge with bradykinin sufficient to cause a 15-fold reduction in response to the kinin results in a small attenuation of a subsequent AMP response without affecting airway responsiveness to histamine.⁴⁵ However, inhibition of NEP by inhaled phosphoramidon failed to elicit any significant enhancement of

the bronchospastic response provoked by AMP, suggesting that release of endogenous neuropeptides has little importance in the airway response to adenosine in asthma.⁴⁶

The bronchoconstrictor response to adenosine therefore involves one, or possibly two, pathways which may be abnormally active in asthma, suggesting that it may be a more specific marker of asthma than bronchoconstrictor challenges such as histamine and methacholine.

Airway responsiveness to adenosine as a marker of disease activity

Bronchial hyperresponsiveness (BHR), the exaggerated bronchoconstrictor response to various stimuli, is frequently associated with bronchial asthma.⁴⁷ The extent of this susceptibility has been related to the degree of airway inflammation, as reflected by the number and state of activation of various inflammatory cells.^{11,48-50} Although inflammation of asthmatic airways is often linked with BHR, the clinical and diagnostic relevance of airway responsiveness as currently defined is unclear. Indeed, standard bronchoprovocation tests with inhalation of histamine or methacholine are not specific for confirming a diagnosis of asthma as they do not exclude chronic obstructive pulmonary disease (COPD) or other respiratory disorders. The relatively low specificity of histamine or methacholine bronchoprovocation for asthma indicates that other tests might offer greater specificity. Assessment of airway hyperresponsiveness with indirect bronchoconstrictor stimuli such as adenosine may fill this gap.

Confirmation of the *in vivo* bronchoconstrictor response in asthma has been obtained *in vitro* where bronchi from asthmatic subjects were more sensitive to adenosine than those obtained from non-asthmatic controls.⁵¹ In this regard, adenosine may be unique since *in vitro* hyperresponsiveness was not demonstrated with other mediators such as histamine and leukotriene C₄.⁵¹ In humans the airway response to inhaled adenosine is not accompanied by a late phase of bronchoconstriction or an increase in non-specific bronchial responsiveness.⁵² Responsiveness of the airways to inhaled adenine derivatives correlates only weakly with more direct indices of airway responsiveness measured with such agonists as histamine²² and methacholine.⁵³

Phillips *et al.*⁵⁴ have shown that atopic subjects, when compared with non-atopic controls, are relatively more responsive to inhaled adenosine than they are to methacholine, indicating that the airway response to these purines may be an index of mast cell priming. In this context, it is of interest that adenosine potentiates the release of inflammatory mediators when human mast cells are immunologically primed *in vitro*.^{16,17} Increased adenosine responsiveness in the form of heightened histamine release has been shown in sensitised mice compared with non-sensitised controls.⁵⁴ Indeed, nasal challenge with AMP elicits rhinitic symptoms and an immediate rise in histamine levels in the lavage fluid with the

greatest increase occurring in atopic compared with non-atopic volunteers.³⁶ This indicates that atopic status is the single most important determinant of enhanced adenosine induced histamine release and that this response may be used as an index of mast cell priming *in vivo*. The capacity of adenosine to augment mediator release from mast cells in the presence of a "low level" second stimulus, which may be immunological or non-immunological, raises the possibility that this nucleoside elicits mediator release in asthmatic human airways by interacting with cytokine "primed" mast cells on the surface of inflamed airways. Thus, purine induced bronchoconstriction in asthmatic and normal atopic subjects may well depend on the state of airway mast cell priming and might be useful as an *in vivo* test for this.

On the basis of these observations, we believe that adenosine bronchoprovocation may have an application in differentiating asthma from other diseases of the airways, and might provide an index that could be used to survey disease progression, monitor treatment, and assess prognosis.

Non-smoking adults with COPD are significantly less responsive to inhaled adenosine than non-smoking asthmatic subjects, whereas the sensitivity to methacholine is similar in both groups.⁵⁵ In children bronchoprovocation tests with inhaled adenosine discriminates asthma from paediatric COPD with a sensitivity and specificity of 85-90%, in contrast to methacholine challenge which fails to distinguish between the two.⁵⁶ Taken together these findings indicate that adenosine challenge may be a useful tool in the differential diagnosis of asthma and COPD in patients of all ages in whom the diagnosis is clinically uncertain. In addition, the specificity of adenosine bronchoprovocation for asthma, together with the high repeatability of this test, could be useful for epidemiological studies.

The view that adenosine responsiveness may be a more robust marker of disease activity in relation to allergic airway inflammation than other non-specific stimuli such as histamine or methacholine is supported by a number of clinical studies. In subjects with active allergic rhinitis we have recently shown that airways responsiveness to AMP, but not methacholine, is strongly correlated to sputum eosinophilia.⁵⁷ A series of clinical studies have confirmed the potential usefulness of AMP in detecting inflammatory changes in adult and paediatric asthma. These authors have demonstrated that regular treatment with inhaled corticosteroids results in a significantly greater reduction in AMP responsiveness than direct (methacholine and histamine) and neurally acting stimuli (sodium metabisulphite and bradykinin).⁵⁸⁻⁶⁰ 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.^{26,61,62} Van Velzen *et al.*⁶³ have recently shown that improvements in clinical asthma occurred in a group of 16 allergic asthmatic children admitted to a high altitude clinic. This was believed to be due to the lower allergen levels

encountered and was accompanied by a significant reduction in airway responsiveness to AMP but, interestingly, not to methacholine. Taken together these studies support the view that AMP bronchoprovocation is a potentially useful marker of disease activity with a closer relationship to the underlying inflammatory processes in asthma than is the case for histamine or methacholine, and as such deserves further assessment in this application.

Concluding remarks

Although adenosine is an important pro-inflammatory mediator in asthma, the definitive appreciation of its potential role in the pathogenesis of asthma has to await the availability of potent and specific adenosine antagonists for clinical use. Investigations of the mechanisms of the adenosine induced bronchoconstriction has provided abundant evidence that responses produced by this purine derivative are not a mere reflection of non-specific airways hyperresponsiveness but involve a selective interaction with activated inflammatory cells in diseased airways and may be strictly related to atopy. In this respect, the airway response to adenosine may provide a useful tool to explore further the inflammatory and immunological processes in asthma.

Although adenosine bronchoprovocation may provide greater diagnostic specificity for asthma, further work is needed to define the value of this unique stimulus in an epidemiological setting and in following disease activity and response to treatment.

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