OCCASIONAL REVIEW

Evolving concepts on the value of adenosine hyperresponsiveness in asthma and chronic obstructive pulmonary disease

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Adenosine is a purine nucleoside which mediates a variety of cellular responses relevant to asthma and COPD through interaction with specific receptors. Administration of adenosine by inhalation to patients with asthma and COPD is known to cause concentration related bronchoconstriction. Responses elicited by this purine derivative in asthma and COPD should not be considered as a mere reflection of non-specific airways hyperresponsiveness. Evaluation of airways responsiveness by adenosine induced bronchoconstriction may be valuable in differentiating asthma from COPD, monitoring of anti-inflammatory treatment in asthma, surveying disease progression, and assessing disease activity in relation to allergic airways inflammation.

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Adenosine is a purine nucleoside which has the capacity to elicit a variety of cellular responses relevant to asthma and chronic obstructive pulmonary disease (COPD) through interaction with specific cell surface purinoreceptors as indicated by the ability of the adenosine uptake inhibitor, dipyridamole, to enhance adenosine induced effects.[1–3] On the basis of molecular cloning and ligand affinity data, adenosine receptors are currently classified into four subtypes—A_1, A_{2A}, A_{2B}, and A_3—each with their unique patterns of tissue distribution and signal transduction.[4 5]

In 1983 Cushley *et al*[6] were the first to administer aerosolised adenosine to a group of asthmatic subjects. Whereas the nucleoside had no discernible effect on airway calibre in normal individuals, the asthmatics experienced concentration related bronchoconstriction with a maximum effect at 5 minutes and subsequent slow recovery that was complete by 45–60 minutes.[6] Ten years later Oosterhoff *et al*[7] reported hyperresponsiveness to adenosine administered by inhalation in 28 out of 30 patients with COPD. The severity of their response was significantly higher in the patients with COPD who smoked than in the non-smoking COPD patients, whereas no discernible difference in methacholine hyperresponsiveness was observed between the two groups.

Since these initial observations, a role for adenosine in asthma and COPD has been postulated and there have been several reviews which have set out in detail the key evidence supporting this view.[8–10] Elucidation of the fine mechanisms of adenosine induced bronchoconstriction has provided convincing evidence that responses elicited by this purine derivative in asthma and COPD are not a mere reflection of non-specific airways hyperresponsiveness but involve a selective interaction with activated inflammatory and structural cells.

This paper reviews the mechanism(s) by which adenosine mediates bronchoconstriction in asthma and COPD, the evidence in favour of the hypothesis that airway response to adenosine may better discriminate the inflammatory and immunological processes in asthma and COPD, and the possibility that adenosine responsiveness may represent a distinctive marker of disease severity and progression.

MECHANISM OF ADENOSINE INDUCED BRONCHOCONSTRICTION IN ASTHMA AND COPD

Despite the evidence that inhaled purine derivatives elicit dose related bronchoconstriction in patients with asthma and COPD,[6 7] the action of adenosine on airway smooth muscle in vitro is conflicting, varying between species and, in the same species, varying with the type of preparation, the initial level of smooth muscle tone, and the concentration of the nucleoside used. In isolated guinea pig airway with high resting tone induced by carbachol, adenosine causes relaxation via an A_2 receptor mechanism,[11 12] whereas constriction occurs when the preparation is maintained at intrinsic tone.[13] In isolated human airway preparations the predominant effect of the nucleoside is contractile, although the effect is weak.[14] However, bronchial preparations obtained from asthmatic subjects were more sensitive to the contractile effects of adenosine than those obtained from non-asthmatic controls,[15] and when inhaled by asthmatics adenosine provoked bronchoconstriction that was not elicited in normal individuals.[6]

The adenosine nucleotides AMP and ADP are equipotent with the parent nucleoside.[16] As neither has any effect on adenosine receptors,[17] but both can be rapidly converted to adenosine by 5'-nucleotidase, it is likely that these nucleotides act in vivo after prior conversion to adenosine. Since AMP in particular is more soluble in aqueous solution, allowing higher concentrations of agonist to be delivered by aerosolisation, it has replaced adenosine as the most frequently used purine nucleoside bronchoprovoking agent.

Since these observations were made, considerable effort has been directed at elucidating the mechanism by which adenosine mediates bronchoconstriction in asthma and COPD. Although no adenosine antagonists 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 purinoreceptors expressed on intermediary inflammatory cells such as mast cells or on afferent nerve endings.

It has long been recognised that mediator release from human mast cells contributes to the airflow limitation and accompanying symptoms of asthma.[18] In active disease, immunohistochemical and altered structural analysis of submucosal and epithelial mast cells reveals that many of them are actively degranulating.[18 19] The role of the mast cell in the pathogenesis of COPD is more speculative. Increased levels of histamine have been found in the sputum of patients with obstructive bronchitis[20] and Postma *et al*[21] have reported an increase in the urinary excretion of the N-methyl metabolite of histamine in the urine of patients with COPD. Lamb *et al*[22] have also reported a greater number of mast cells in the respiratory epithelium in the distal airways of smokers than in non-smokers. Immunohistochemical analysis of bronchial mucosa obtained from patients with COPD reveals that larger numbers of mast cells are present in the bronchiolar epithelium than in the airways of smokers without airway obstruction.[23] Likewise, Pesci *et al*, who studied mast cell infiltration in bronchial biopsy specimens of subjects with chronic bronchitis, observed higher numbers of mast cells both in the epithelium and in the bronchial glands than in control subjects.[24] Relevant to this are the findings of de Boer *et al*[25] who have recently shown that the number of mast cells in the bronchiolar epithelium of COPD patients is strongly associated with the increased level of expression for epithelial transforming growth factor (TGF)β_1, a well known chemotactic factor for mast cells.[26] Mediator secretion released from mast cells during the active "inflammatory" phase of COPD may therefore contribute to its airway pathophysiology. Indeed, mast cells may release chemotactic factors for neutrophils and secrete proteases—for example, tryptase, chymase, elastase—which are able to induce tissue injury,[27] airway smooth muscle hyperresponsiveness,[28] and airway mucus secretion.[29]

Mast cells are likely to play a critical role in the bronchoconstrictor response to inhaled adenosine as indicated by in vitro studies in which adenosine markedly enhances the release of histamine and other preformed mediators from immunologically primed rodent mast cells.[30] The timing of adenosine addition seems quite critical to the effect produced, and pharmacological manipulations have suggested involvement of the A_{2B} receptor.[31] A series of studies in human dispersed lung mast cells by Church *et al*[32] and Peachell *et al*[33] have shown similar effects, including a small potentiation of leukotriene C_4 release in the latter study. Feoktistov and Biaggioni have since shown that stimulation of the A_{2B} receptor in a human mast cell line in vitro produces cellular activation, and that phosphoinositide hydrolysis and intracellular calcium mobilisation are involved in this process.[34] Most of the above mentioned studies refer to mast cells obtained by either mechanical dispersion or enzymatic digestion of whole lung. In a recent study Forsythe *et al*[35] have produced evidence that adenosine can directly stimulate histamine release from human mast cells obtained by bronchoalveolar lavage.

There is also abundant evidence in vivo to indicate that the mast cell may be involved in the bronchoconstrictor response to inhaled adenosine, principally via release of granule derived preformed mediators. Premedication with the potent H_1 histamine receptor antagonists terfenadine and astemizole have been shown to inhibit the acute bronchoconstrictor response to inhaled AMP in asthmatic and COPD patients.[36–39] These initial studies provided strong support for the concept that mast cell derived mediators are implicated in the bronchoconstrictor response to inhaled adenosine in both asthma and COPD. More direct evidence that histamine released from airway mast cells is critical for adenosine induced responses has come from a study in which venous plasma histamine levels were measured after bronchial provocation with inhaled allergen and AMP in a group of atopic subjects. A small but significant increase in histamine levels was observed after AMP challenge.[40] Direct instillation of AMP into asthmatic bronchi[41] or into the nose of patients with allergic rhinitis[42] resulted in significant increases in the concentration of histamine and tryptase in their lavage fluid. However, in addition to histamine, a role for other mast cell derived mediators has to be considered. A role for prostanoids in the response to AMP is supported by the demonstration that potent cyclooxygenase inhibitors such as indomethacin and flurbiprofen attenuate the constrictor effect of the nucleotide.[43 44] In addition, lysine-aspirin administered by inhalation causes some attenuation of the AMP response.[45] More direct evidence for a role for newly generated mediators has come from the study by Polosa *et al*.[41] In addition to the rise in histamine and tryptase levels in the bronchoalveolar lavage fluid, an even greater increase in concentrations of PGD_2 were found. Recently, premedication with ABT-761,[46] a potent 5-lipoxygenase inhibitor, and the selective cysteinyl leukotriene (Cys LT_1) receptor antagonist montelukast[47] has been shown to attenuate the acute bronchoconstrictor response to inhaled AMP, thus suggesting a role for spasmogenic leukotrienes.

Although activation of both cholinergic[48] and peptidergic neural pathways[49] may contribute to the contractile airway response to adenosine in asthma, the role of the neural pathway in adenosine induced bronchoconstriction in patients with COPD has not been fully addressed. In a recent study Reutgers *et al*[36] found no significant effect on AMP responsiveness after inhaled ipratropium bromide in patients with COPD, implying that vagal nerve activation does not play a role. This is at variance with the findings in asthmatic patients, where ipratropium bromide caused a significant increase in PC_{20}AMP.[48] It is possible that in asthma AMP stimulates mast cells to release histamine which causes an additive effect via vagal nerve stimulation. In COPD, histamine release may be smaller and inadequate to stimulate vagal nerve endings during AMP challenge.

CLINICAL VALUE OF AIRWAY RESPONSIVENESS TO ADENOSINE IN ASTHMA AND COPD

Airway or "bronchial" hyperresponsiveness (BHR) is best defined as airways that narrow too much to a provoking stimulus.[50] Although BHR is well established as a hallmark of asthma, the clinical and diagnostic relevance of airway responsiveness as currently defined is still unclear. BHR is neither sensitive nor specific for asthma,[51] as it is also detected in approximately two thirds of smokers with COPD[52] and in various other inflammatory airway diseases such as cystic fibrosis,[53] bronchiectasis,[54] and Sjogren's syndrome.[55 56] Despite this lack of specificity and sensitivity, it remains an important physiological marker in diagnosing and determining asthma severity.

The provoking stimuli can be classified into two categories: (1) those that act predominantly directly on airway smooth muscle such as histamine and methacholine; and (2) those that act indirectly through the release of inflammatory mediators or stimulation of neural pathways such as adenosine. Airway hyperresponsiveness is often linked to the degree of airway inflammation and this is reflected by the number and state of activation of various inflammatory cells.[57–59] At present there is no single effective marker of the underlying inflammatory process in the lungs, but various surrogate markers have been used to reflect the severity of airway inflammation. The non-invasive technique of measuring BHR

using either a direct or indirect stimuli may provide us with more information about the inflammatory process and enable us to differentiate between different disease processes.

As illustrated previously, adenosine is an indirect stimulus that exerts its effect primarily on inflammatory cells, subsequently leading to smooth muscle contraction. This distinctive feature of adenosine suggests that AMP responsiveness may correlate better than other stimuli with airway inflammation, enabling superior diagnostic discrimination between asthma and COPD and allowing better monitoring of disease activity and progression. It should also be noted that AMP challenge testing would be useful in evaluating response to treatment.

Although there is not enough information to establish clear indications for AMP challenge testing and a standardised population based cut off PC_{20} AMP value needs to be delineated, airway responsiveness to AMP may be used to differentiate asthma from COPD when traditional diagnostic methods have not established a clear diagnosis. In addition, it appears that bronchoprovocation with AMP 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 and has a greater probability than methacholine for the diagnosis of asthma.[8] AMP challenge may also be used as a practical tool in determining bronchial hyperresponsiveness in epidemiological surveys as described by De Meer and colleagues using the short dosimeter protocol method of AMP challenge.[60]

Atopy is the single most important determinant of enhanced adenosine induced responses in vivo. Phillips *et al*[40] have shown that atopic subjects are more responsive than non-atopic controls 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.[33 61] Increased adenosine responsiveness in the form of heightened histamine release has also been shown in sensitised mice compared with non-sensitised controls.[62] Moreover, nasal challenge with AMP elicits rhinitic symptoms and a rapid increase in histamine levels in the lavage fluid with a greater increase occurring in atopic than in non-atopic individuals.[36] Adenosine induced bronchoconstriction in asthmatic and atopic subjects may therefore be used as an index of mast cell priming in vivo. The role of atopy in adenosine induced bronchoconstriction is also emphasised in a recent study by van Daele *et al*.[63] These authors compared histamine and AMP bronchial challenges in preschool children with recurrent wheeze to identify atopic mechanisms for their wheezing and found that all non-atopic children with wheeze had a negative adenosine provocation test. Adenosine bronchoprovocation testing is therefore more specific than histamine in establishing allergic factors in preschool children with wheeze.

There is mounting evidence that adenosine challenges could possibly be more exploited in differentiating asthma from COPD in subjects where the diagnosis is clinically uncertain. In adults, AMP and methacholine provocation both distinguish subjects with COPD from normal controls. However, only AMP could separate non-smoking COPD patients from asthmatic patients. In the COPD patients who smoked, AMP responsiveness was similar to that found in asthmatic patients, perhaps as a result of the additional inflammatory effect of cigarette smoking.[7] In children, bronchoprovocation tests with inhaled AMP appears to be considerably more specific and sensitive than methacholine at discriminating asthma from paediatric chronic obstructive lung disorders such as cystic fibrosis, bronchiolitis, pulmonary ciliary dyskinesia, and bronchiectasis.[64] The mechanism underlying bronchial hyperresponsiveness may very between asthma, COPD, and other diseases which also have a component of BHR. A study investigating the bronchial responsiveness profile produced by AMP, methacholine, and cold air in subjects with asthma and Sjogren's syndrome suggested that more than one challenge may be required to detect different aspects of bronchial responsiveness. Atopic asthmatic subjects were significantly more responsive to AMP than non-atopic subjects and patients with Sjogren's syndrome.[65] From a practical standpoint we speculate that AMP challenge becomes useful only when the diagnosis of asthma or COPD is clinically uncertain. However, it is clear that more population based epidemiological studies are needed to determine how valuable is adenosine responsiveness in differentiating asthma from COPD.

The view that adenosine responsiveness may be used as a specific marker of disease activity with a closer relationship to allergic airway inflammation than histamine or methacholine has been addressed in a number of clinical studies. This feature could be exploited in the clinical setting to differentiate better asthma from COPD when traditional diagnostic methods have not established a clear diagnosis. In subjects with active allergic rhinitis we have recently shown that airways responsiveness to AMP, but not methacholine, is strongly correlated to sputum eosinophilia.[66] Exhaled nitric oxide (eNO) is increasingly being used as a marker of airway inflammation and, in a study by van den Toorn *et al*, a significant correlation could be established between eNO and responsiveness to AMP, but not between eNO and responsiveness to methacholine.[67] A recent study by van den Berge and colleagues also supported previous findings that PC_{20} AMP is a better marker of airway inflammation than PC_{20} methacholine.[68] One hundred and twenty atopic asthmatics underwent bronchial provocation testing with methacholine and AMP, as well as sputum induction, blood samples, and measurement of NO in exhaled air. PC_{20} AMP provided a better reflection of airway inflammation than PC_{20} methacholine since the percentage of sputum eosinophils explained 25% of the variance in PC_{20} AMP while it was not a significant independent predictor for PC_{20} methacholine. In non-smoking patients with COPD hyperresponsiveness to AMP was also related to increased percentages of eosinophils in induced sputum and increased numbers of mucosal CD8+ cells in bronchial biopsy specimens, thereby reflecting the close association between AMP hyperresponsiveness and airway inflammation in COPD.[69]

A series of clinical studies have confirmed the potential usefulness of AMP in detecting inflammatory changes in adult and paediatric asthma. Various investigations have shown a pronounced improvement in AMP responsiveness compared with methacholine or histamine after allergen avoidance, suggesting reduced airway inflammation following avoidance of aeroallergens.[70–72] Doull *et al*[73] have shown that regular treatment of asthmatic children with the inhaled corticosteroid beclomethasone dipropionate results in a significant reduction in AMP but not methacholine or bradykinin responsiveness. This finding confirms earlier observations that regular treatment with inhaled budesonide resulted in greater attenuation of the airway response to AMP than to methacholine.[74–76] In asthma the ability of this test to discriminate changes in airway reactivity with anti-inflammatory treatment better than histamine or methacholine has also been validated with inhaled ciclesonide, mometasone, and fluticasone propionate,[77–80] as well as with oral prednisolone.[80] More recently, Ketchell *et al* have reported that sensitive prediction of the AMP response to inhaled corticosteroids is already apparent as early as 48 hours[81] and have reported significant attenuation of airway responsiveness to AMP within 2 hours of a single dose of fluticasone propionate.[82] In contrast, in patients with COPD adenosine appears to be as insensitive as methacholine in detecting changes in airway reactivity after treatment with high dose inhaled steroids.[83] This distinctive feature is of diagnostic interest as it may indicate an additional way by which adenosine challenge may be useful in discriminating asthma from "true" COPD.

The recent work by van den Berge *et al*[84] showing greater improvement in BHR for AMP than for methacholine after treatment with corticosteroids underscores the view that airway responsiveness to AMP may also be used as a sensitive marker to monitor the effects of steroid therapy in asthma. Perhaps a limitation of this study is that airway responsiveness was recorded at a single time point after only 2 weeks of treatment with corticosteroids. Only longitudinal studies can better define a role for AMP challenge testing in the assessment of anti-inflammatory therapy in asthma. We have recently examined the time course of change in sputum cellularity and in bronchial reactivity to inhaled AMP and methacholine after administration of inhaled budesonide (800 μg/day) in 10 asthmatic patients.[85] Treatment with budesonide significantly reduced the airway responsiveness to AMP as early as by the first week of treatment, whereas changes in methacholine airway responsiveness and in sputum cellularity could be observed only by the fourth week of treatment. These findings emphasise the superior sensitivity profile of AMP challenge testing in evaluating airways response to anti-inflammatory therapy. However, this should be discussed against the evidence that other non-invasive putative markers (such as exhaled NO) may be as sensitive as AMP challenge testing in monitoring glucocorticoid responsiveness in asthma.

There is substantial evidence that adenosine is a better marker of airway allergic inflammation than the direct stimuli histamine or methacholine, but whether it is closely related to disease severity needs to be further explored. Avital *et al*[86] compared exercise, methacholine, and AMP in 135 children and young adults. They concluded that the sensitivities of AMP and methacholine challenges in the detection of bronchial hyperreactivity were very similar, but that methacholine was better at discriminating between mild and moderate asthma than AMP. This finding was also confirmed in a retrospective analysis of 487 adult asthmatic patients.[87] Methacholine and AMP challenges were compared as screening tools and any relationships between BHR and disease severity markers identified. The results suggested that methacholine was a more appropriate screening tool for BHR than AMP in their population and was related to asthma severity. From these two studies adenosine does not seem to be a good indicator of disease severity but further clinical trials are needed to confirm this. However, since airway response to direct stimuli is more strictly related to the actual degree of airway constriction than inflammation, it is not surprising that AMP does not serve as a valuable tool for monitoring disease severity.

CONCLUDING REMARKS

The mechanism of airway hyperresponsiveness to adenosine/AMP has now been largely elucidated, although some questions remain. The available evidence clearly indicates that AMP challenge has a distinctive ability to probe immunological as well as non-specific responsiveness in asthma and COPD and, in this regard, can be expected to yield important and clinically relevant results in the future. Moreover, bronchoprovocation testing with adenosine offers substantial advantages (especially in term of sensitivity) over other non-invasive tests including induced sputum. The premise for this is that adenosine elicits bronchoconstriction by stimulating the release of bronchoconstrictor mediators from cells/nerves within the airway and thus may be sensitive to the underlying inflammatory state of the airway. However, BHR to direct stimuli such as methacholine remains an exceptionally sensitive diagnostic test and, as such, it serves well to exclude disease. By contrast, because of its superior specificity, BHR to inhaled AMP may be preferred to confirm a diagnosis of asthma.

- Inhaled adenosine causes concentration related bronchoconstriction in asthma and COPD.
- Airway hyperresponsiveness to adenosine may be valuable in differentiating asthma from COPD.
- The response to adenosine is likely to assess disease activity in relation to allergic airway inflammation.
- Serial measurements of airway hyperresponsiveness to adenosine may be useful in monitoring the anti-inflammatory effects of topical steroids.

Current GINA guidelines recommend careful monitoring of asthma symptoms and pulmonary function and recognise the need for "developing noninvasive test(s) of airway inflammation for use in diagnosis, monitoring the disorder's activity, and evaluating treatments". Based on the emerging evidence, adenosine bronchoprovocation testing can be put forward as being useful in differentiating allergic asthma from COPD and for monitoring airway inflammatory changes in adult and paediatric asthma. In particular, serial measurements of adenosine airway responsiveness may, in future, become of increased value in monitoring anti-inflammatory effects of asthma treatment. However, well planned and well conducted large clinical trials are needed to show that information gained from this test will lead to improved patient management.

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