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 chronic obstructive pulmonary disease (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.

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, dipryridamole, to enhance adenosine induced effects. On the basis of molecular cloning and ligand affinity data, adenosine receptors are currently classified into four subtypes—A1, A2A, A2B, and A3—each with their unique patterns of tissue distribution and signal transduction.

In 1983 Cushley et al. 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. Ten years later Oosterhoff et al. 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. 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, the action of adenosine on airway smooth muscle in vitro is conflicting, varying between species and even within 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 A2 receptor mechanism whereas constriction occurs when the preparation is maintained at intrinsic tone. In isolated human airway preparations the predominant effect of the nucleoside is contractile, although the effect is weak. However, bronchial preparations obtained from asthmatic subjects were more sensitive to the contractile effects of adenosine than those obtained from non-asthmatic controls, and when inhaled by asthmatics adenosine provoked bronchoconstriction that was not elicited in normal controls.

The adenosine nucleotides AMP and ADP are equipotent with the parent nucleoside. As neither has any effect on adenosine receptors, 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 adeno-
sine mediates bronchoconstriction in asthma and COPD. Although no adenosine antagonists have acceptance for use in
humans, alternative pharmacological approaches have sug-
gested 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 recognized that mediator release from
human mast cells contributes to the airflow limitation and
accompanying symptoms of asthma. In active disease, inmunohistochemical and altered structural analysis of sub-
mucosal mast cells revealed that many of these cells are
actively degranulating. 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 and Postma et al. reported an increase
in the urinary excretion of the N-methyl metabolite of
histamine in the urine of patients with COPD. Lamb et al. have also reported a greater number of mast cells in the resi-
piratory 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 airflow
obstruction. 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. Relevant to this are the findings of de Boer et al. who have recently shown that the number of mast cells in the
bronchiolar epithelium of COPD patients is strongly associ-
ated with the increased level of expression for epithelial
transforming growth factor (TGF)-β, a well known chemotac-
tic factor for mast cells. Mediatory 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,
airway smooth muscle hyperresponsiveness, and airway mucus secretion.

 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. The timing of
adenosine addition seems quite critical to the effect produced,
and pharmacological manipulations have suggested involve-
ment of the A1 receptor. A series of studies in human
dispersed lung mast cells by Church et al. and Peachell et al. have shown similar effects, including a small potentiation of
leukotriene C4 release in the latter study. Fekotisov and Biag-
gionti have since shown that stimulation of the A1 receptor in
a human mast cell line in vitro promotes cellular activation,
and that phosphoinositol hydrolysis and intracellular cal-
cium mobilisation are involved in this process. 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. 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 H1 hist-
amine receptor antagonists terfenadine and astemizole have
been shown to inhibit the acute bronchoconstrictor response
to inhaled AMP in asthmatic and COPD patients. These
initial studies provided strong support for the concept that

**Clinically Relevant Conclusion:**

**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. 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, as it is also detected
in approximately two thirds of smokers with COPD and in
various other inflammatory airway diseases such as cystic
fibrosis, bronchiectasis, and Sjogren’s syndrome. 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 adenos-
ine. 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. 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
Adenosine in asthma and COPD

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 with 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 PC20 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. AMP challenge may also be used as a practical tool in determining bronchial hyperresponsiveness in epidemiological surveys as described by De Meur and colleagues using the short dosimeter protocol method of AMP challenge.8

Atopy is the single most important determinant of enhanced adenosine induced responses in vivo. Phillips et al73 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.44 45 Increased adenosine responsiveness in the form of heightened histamine release has also been shown in sensitised mice compared with non-sensitised controls.46 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.47 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 Dael et al.67 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.74 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.48 The mechanism underlying bronchial hyperresponsiveness may vary 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 Sjögren’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 Sjögren’s syndrome.75 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.68 Exhaled nitric oxide (eNO) is increasingly being used as a marker of airway inflammation and, in a study by van den Torn et al, a significant correlation could be established between eNO and responsiveness to AMP, but not between eNO and responsiveness to methacholine.76 A recent study by van den Berge and colleagues also supported previous findings that PC20 AMP is a better marker of airway inflammation than PC20 methacholine.77 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. PC20 AMP provided a better reflection of airway inflammation than PC20 methacholine since the percentage of sputum eosinophils explained 25% of the variance in PC20 AMP while it was not a significant independent predictor for PC20 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.

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.78 79 Doull et al80 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.81 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,82 83 as well as with oral prednisolone.84 More recently, Ketchell et al have reported that sensitive prediction of the AMP response to inhaled corticosteroids is already apparent as early as 48 hours85 and have reported significant attenuation of airway responsiveness to AMP within 2 hours of a single dose of fluticasone propionate.86 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.87 This distinctive feature is of diagnostic importance as it indicates 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. 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 only 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. 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. Aival et al. 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. 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, broncho-constricting provocation testing with adenosine offers substantial advantages (especially in terms 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.

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 treatment". 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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