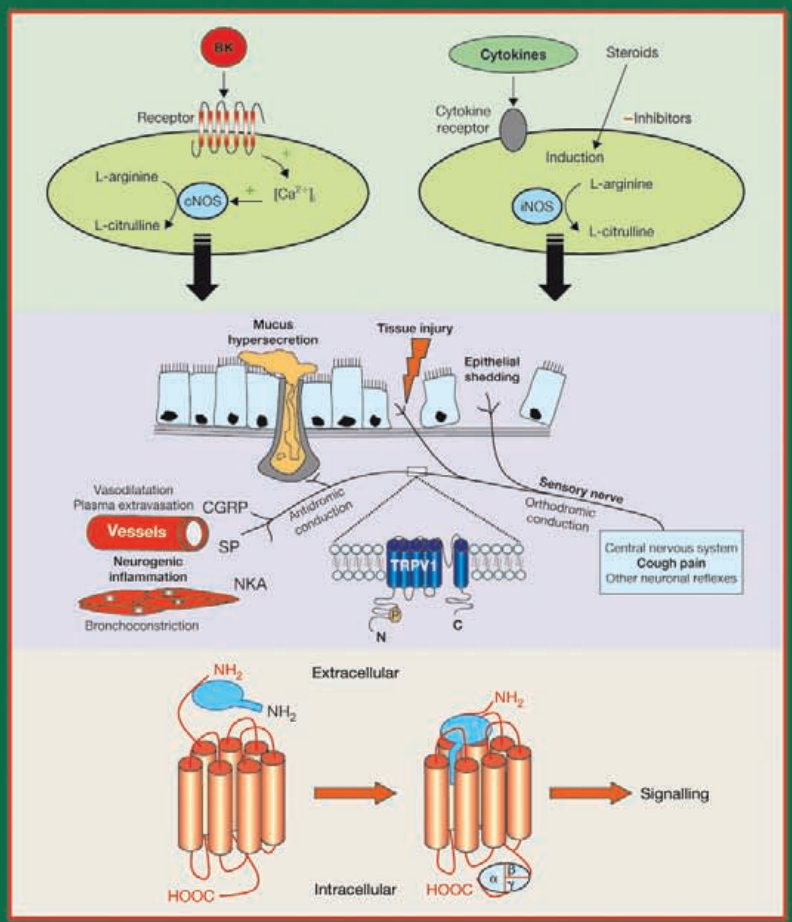
Therapeutic Strategies

ASTHMA

Modern Therapeutic Targets

R. Polosa · S. T. Holgate



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ASTHMA: MODERN THERAPEUTIC TARGETS

Edited by

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Contents

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Preface

The discovery of adrenergic agonists and corticosteroids at the start of the 20th century has provided the basis for much of the treatment of asthma. The last 50 years has witnessed major advances in our understanding of asthma and significant improvement in these therapeutic agents with respect to safety, efficacy and duration of action. Inhaled corticosteroids (ICS), short and long acting β_2-agonists (SABAs and LABAs) are now the mainstay of asthma treatment as advocated by disease management guidelines. When used regularly, ICS reduce both morbidity and the addition of LABAs to the management plan appears to improve control of moderate-to-severe asthma. Yet, despite the undoubted efficacy of this combination for most patients, there remains ~10% of the asthmatic population in whom symptoms persist with considerable impact on quality of life and disproportionate use of health care resources.

While ICS are highly effective in suppressing airway inflammation in asthma, they do not influence the natural history of the disease even when started in early childhood and are largely ineffective in virus-induced exacerbations and in those asthmatics who smoke. There is also heterogeneous group of asthma patients who are genuinely refractory to corticosteroids. A few additional therapies are available and include methlyxanthines, anticholinergics, cromones and leukotriene modifiers, but these are of variable efficacy. The introduction of a monoclonal antibody that is able to block IgE effects in severe allergic asthma is a breakthrough in asthma management but only for a limited number of patients.It should also be remembered that 'reagin', the biological activity of IgE was first discovered in 1922 by Prausnitz and Kustner and the biological activity of the leukotrienes, slow reacting substance (SRS), was recognised by Trethewie and Kellaway in 1938 and yet for both of these "activities" a further 40–45 years elapsed before their molecular basis was discovered and a another 15–40 years before the development of therapies that target these. One could legitimately ask why progress has been so slow in the development of new therapeutic agents in this field. Part of the difficulty may be in the high dependency that the pharmaceutical and biotechnology industries have placed on antigen challenge models both in animals and humans to screen for anti-asthma activity whereas allergen/antigen driven responses represent only part of the asthmatic paradigm: diet, air pollutants, tobacco smoke, drugs and viruses are all known to impact on the origins and progression of asthma. Much of the testing of novel chemical activities has also been undertaken on "acute" models, whereas asthma is often a chronic, albeit relapsing disease that often spreads across a lifetime. Some of the therapeutic targets identified in these models such as neuropeptide antagonists, PAF antagonists, bradykinin inhibitors, adhesion molecule antagonists, mast cell "stabilising" agents and some cytokine blockers (e.g. anti-IL5) have all shown great promise in animal models but have failed when tested in humans with asthma. The time has therefore arrived to take a fresh look at asthma and at the novel therapeutic agents that are appearing on the horizon, including biologicals that have proven so successful in other chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel diseases and psoriasis.

Looking into the future, ***Asthma – Modern Therapeutic Targets*** provides readers with an overview of possible new therapeutics in a field in need of innovation. The book is divided

into four sections, each covering a particular theme. The first section provides a series of contributions on a number of approaches targeted towards specific autocoid mediators (including newly identified mediators such as adenosine), and transcription factors. Increasingly, tissue injury and disordered repair is being recognised as important in asthma, with the airways behaving like a 'chronic wound'. Thus, the second section of the book focuses on proteases and their inhibitors as novel therapeutic targets. Although simple neuropeptide receptor antagonists have been proven to lack efficacy, the next section underscores the fact that there is a resurgence of interest in modulating neural pathways. Given that asthma is an inflammatory disorder with a strong immunological basis, the book ends with a section focusing on some exciting new immunological molecular targets, including cytokines (with a particular focus on a newly identified target in corticosteroid refractory asthma – TNFα), chemokines, and IgE.

The range of subjects covered and the level of imagination required to make each section a stimulating and educational read has called for remarkable commitment from a large number of leading experts from the pharmaceutical industry and academic world. We would like to acknowledge their considerable contributions to this book without whose help, this collection of informative and up-to-date reviews would not have been possible.

We hope that you will find this book interesting and helpful, and that it will give as much enjoyment to you, the reader, as we have had in its design and editing. Finally, and most importantly of all, our hope is that this new publication shows that the field of novel asthma therapies has a most promising future and that it may be of assistance in the process of finding better therapies for our patients with asthma both now and in the future.

Riccardo Polosa
Stephen T. Holgate

Section I

Autocoids and their receptors in airway diseases

1

Adenosine receptors: novel molecular targets in asthma

D. Zeng, R. Polosa, I. Biaggioni, L. Belardinelli

INTRODUCTION

Adenosine is proposed to play a pro-inflammatory and immunomodulatory role in the pathogenic mechanisms of chronic inflammatory disorders of the airways such as asthma and chronic obstructive pulmonary disease (COPD) [1, 2]. Elevated levels of adenosine are present in chronically inflamed airways [3, 4]. Inhaled adenosine causes dose-dependent bronchoconstriction in subjects with asthma [5] and COPD [6]. Mice with genetic deletion of the adenosine deaminase (ADA) gene [7] or over-expression of interleukin (IL)-13 cytokine [8] in the lung develop features of pulmonary inflammation and airway remodelling with concurrent increases in tissue levels of adenosine in the lung. This and other evidence summarized in this article suggest that adenosine plays an important role in the initiation and progression of inflammatory disorders of the airways. Because adenosine exerts its multiple biological activities by activating four adenosine receptor subtypes, selective activation or blockade of these receptors may lead to therapeutic benefit in the management of pulmonary diseases. Several agonists and antagonists to the adenosine receptors are currently in pre-clinical and clinical development for the treatment of asthma and COPD. In this chapter, we review the rationale of targeting adenosine receptors and the current status of adenosine ligands in development.

ROLE OF ADENOSINE IN PULMONARY DISEASES

Adenosine modulates numerous cardiovascular functions [9] and is currently used clinically as a rapid intravenous bolus for the acute termination of re-entrant supraventricular tachyarrhythmias (Adenocard) and used with radionuclide imaging of the heart to detect under-perfused areas of myocardium as a diagnostic test to detect coronary artery disease in patients unable to exercise (Adenoscan). In the last two decades, it has been recognized that adenosine may also play a critical role in the pathogenesis of chronic inflammatory disorders of the airways such as asthma and COPD. Elevated levels of adenosine are present in chronically inflamed airways; they have been observed both in the bronchoalveolar

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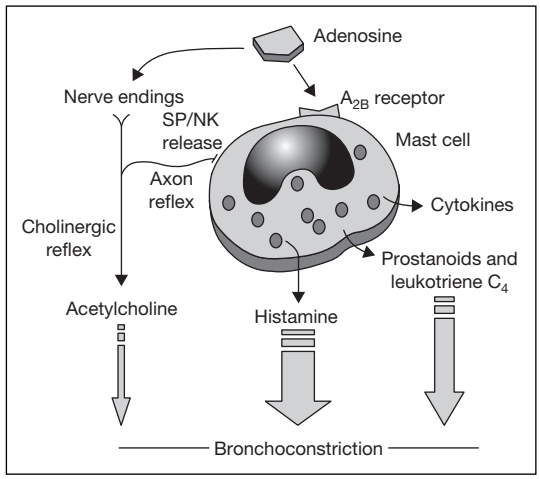

Figure 1.1 Proposed mechanisms for adenosine-induced bronchoconstriction. Stimulation of specific adenosine A_{2B} receptors by adenosine activates airway mast cells to release pro-inflammatory mediators some of which (e.g. histamine, prostaglandins and leukotrienes) act as potent bronchoconstrictors *in vivo* (modified from reference [79]). Note that mast-cell derived mediators are largely implicated in the airway response to adenosine (largest arrow), whereas the role of neural pathways is negligible (smallest arrow). NK = neurokinins; SP = substance P.

lavage fluid (BALF) [3] and the exhaled breath condensate [4] of patients with asthma. Adenosine levels are also increased after allergen exposure [10] and during exercise in atopic individuals [11]. The observed increases in adenosine concentrations suggest that adenosine signalling may regulate aspects of acute and chronic airway disease.

The acute effect of adenosine on bronchoconstriction is well-established by now. Adenosine administration by inhalation was shown to elicit concentration-dependent bronchoconstriction in subjects with asthma whereas the nucleoside had no discernible effect on airway calibre of normal individuals [5]. Since this initial observation, a considerable effort has been directed at revealing the cellular and molecular mechanisms of adenosine-induced bronchoconstriction [1, 2]. One of the proposed mechanisms involves an interaction between adenosine and activated airway mast cells with subsequent release of preformed and newly formed mediators [12] (Figure 1.1). In addition to mast cells, other cells may also play a role in adenosine-mediated bronchial hyperresponsiveness (BHR). The observation that adenosine-mediated BHR is reduced but persistent in mast cell-deficient mice supports the existence of a mast cell independent mechanism [13].

Consistent with the hypothesis of adenosine playing a critical role in the pathogenesis of chronic inflammatory disorders, mice deficient in ADA develop features of severe pulmonary inflammation and airway remodelling in association with increases in adenosine concentrations in the lung [7]. Features of the pulmonary phenotype noted include the following: (1) the accumulation of eosinophils and activated macrophages in the airways, (2) mast cell degranulation, (3) mucus metaplasia in the bronchial airways, and (4) emphysema-like injury of the lung parenchyma. Although the histological observation in ADA-deficient mice does not completely resemble that of human asthma due to the lack of epithelial shedding, subepithelial fibrosis, or muscle/submucosal gland hypertrophy, the ADA-deficient mouse model is a useful tool to study the pathogenic role of adenosine in chronic airway inflammation. The central role of adenosine in chronic lung inflammation is also supported by studies carried out in mice that have increased levels of IL-13 in the lung. These mice develop inflammation, fibrosis, and alveolar destruction concurrently with increases in adenosine concentrations in the lung [8]. Treatment with polyethylene glycol

adenosine deaminase (PEG-ADA) to prevent the increases in adenosine concentrations result in a marked decrease in the pulmonary phenotypes suggesting that adenosine mediates IL-13-induced inflammation and tissue remodelling in this experimental model.

An important clinical development in this research area is the use of an adenosine (or AMP) inhalation challenge as a diagnostic test for asthma and COPD [14, 15]. Unlike BHR to methacholine, which is related to the changes in airway calibre, BHR to inhaled AMP seems to be more sensitive to treatment with inhaled corticosteroids (ICS) [16]. In addition, AMP provocation also increases the release of serum neutrophil chemotactic factor [17] and induces sputum eosinophilia [18]. Moreover, inhalation challenge with AMP appears to be useful at establishing the appropriate dose of ICS needed to control airway inflammation, or at predicting safe dose reductions of ICS in patients with mild-to-moderate asthma [19]. The growing body of evidence supports the hypothesis that BHR to inhaled AMP may reflect the inflammatory status of allergic patients and could be useful in evaluating the effectiveness of different treatment regimens with ICS and monitoring corticosteroid requirements and dose selection in asthma treatment [20].

ADENOSINE RECEPTOR SUBTYPES

Extracellular adenosine elicits its biological effects by interacting with four cell surface G protein-coupled receptors designated as A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors [21]. The genes for these receptors have been cloned from human and several animal species. Tissue distributions of these receptors have been determined at the mRNA level using Northern blot or *in situ* hybridization techniques, or at the protein level using subtype-selective radioligands or antibodies. In general, these receptors are widely expressed. For example, high levels of A_1 receptors are found in brain, adipose tissue and atria, whereas high levels of A_{2A} receptors are found in spleen, thymus, striatum, and blood vessels [22, 23]. In addition, these receptor subtypes are often found to co-express in the same tissues or even on the same cell types. The relative expression levels of these receptors have been found to be modulated by physiological and/or pathological tissue environments [24–29]. It remains challenging to attribute the actions of adenosine to specific receptor subtypes based on the distribution of these receptors.

The four adenosine receptors also differ in their coupling to G proteins and the intracellular signalling pathways they activate [21]. In most cells, A_1 and A_3 receptors couple to $G_{i/o}$ and inhibit the adenylate cyclase (AC), whereas A_{2A} and A_{2B} receptors couple to G_s proteins and increase AC activity and intracellular cyclic AMP (cAMP) levels (Figure 1.2). While the AC–cAMP-protein kinase A axis is the most well-studied second messenger system involved in adenosine receptor function, it is clear that adenosine receptors utilize other signalling pathways as well. These include members of the mitogen-activated protein kinase (MAPk) family, such as p38, p42/p44 (ERK 1/2), and c-Jun terminal kinase, as well as various phospholipases, protein phosphatases, and ion channels.

Although adenosine is the natural agonist for these four receptor subtypes, its ability to activate these receptor subtypes varies. In many tissues, A_1 and A_{2A} receptors have relatively higher receptor reserves for adenosine, and can be activated by the physiological levels of adenosine, and thus mediate the tonic actions of adenosine [27–29]. On the other hand, A_{2B} and A_3 receptors appear to have relatively lower affinities and/or receptor reserves for adenosine and require higher concentrations of adenosine for their activation. However, it is hypothesized that the tissue adenosine levels in many pathological conditions are increased to sufficiently high levels to activate the A_{2B} and A_3 receptors.

Numerous subtype-selective agonists and antagonists of adenosine receptors have been synthesized and are used as pharmacological tools [21]. Although these ligands were classified as selective ligands based on their differential binding affinities for the four adenosine receptors, these compounds are often not well characterized functionally in biological sys-

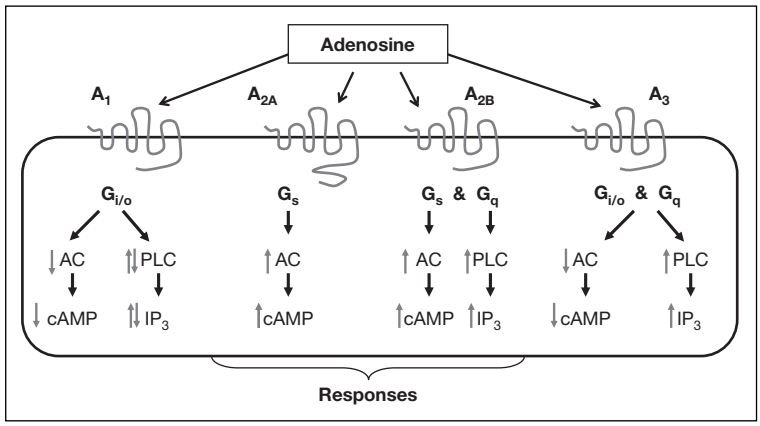

Figure 1.2 Adenosine signalling pathways. In most cell systems, adenosine binding to A_1 and A_3 receptors results in the inhibition of AC with overall reduction of cAMP levels. On the other hand, the canonical signalling mechanism of adenosine A_{2A} and A_{2B} receptors is the stimulation of AC with increase in cAMP levels *via* coupling to stimulatory G proteins (G_s). However, adenosine A_{2B} and A_3 receptors can, in addition, elevate inositol (1,4,5)-trisphosphate (IP_3) levels *via* G_q activation.

tems and many have been found not to be as selective as originally suggested. There are pharmacological reasons for the lack of functional selectivity. For example, potencies of an agonist for a given receptor subtype could vary from one tissue (or cell) to another depending on receptor reserve, receptor expression levels and coupling efficiencies. As mentioned above, adenosine receptors are widely distributed and their expression levels are often modified during disease processes. This certainly adds complexity in predicting functional selectivity of agonists. In the case of antagonists, the functional blocking effects of competitive antagonists are related to the tissue adenosine levels. If the adenosine levels are too low to activate a given receptor, antagonists for this receptor would not have any functional effects, regardless of their binding affinities. In addition to these pharmacological issues, the pharmacokinetic properties of these compounds need to be considered when using them as 'selective' ligands *in vivo*. In many cases, the half-life and tissue distributions of these compounds are poorly understood, making it difficult to draw conclusions on the role of receptor subtypes based on the absence of effects of 'selective ligands' in animal models (Table 1.1). In spite of these limitations, selective agonists and antagonists are commonly utilized to establish the functions mediated by adenosine receptor subtypes.

RATIONALES OF TARGETING ADENOSINE RECEPTOR SUBTYPES

Asthma and COPD are complex diseases sharing clinical heterogeneity and a number of pathogenic traits, which include variable degree of airflow obstruction, BHR, and chronic airway inflammation [30–32]. Many cell types that play important roles in the pathogenesis of chronic inflammatory airway diseases are known to express adenosine receptors. These cell types include various inflammatory cells, such as mast cells, eosinophils, lymphocytes, neutrophils, and macrophages, and the structural cells in the lung, such as bronchial epithelial cells, smooth muscle cells, lung fibroblasts, and endothelial cells. In addition, numerous animal models have been used to assess the contribution of adenosine and its receptor subtypes in the pathology of pulmonary diseases. The most commonly used models are allergic animal models and genetically modified models including receptor knockout mouse models and the ADA-deficient mouse model.

Table 1.1 Effect of adenosine ligands in animal models

Class of compounds	*Compounds (route of administration)*	*Animal model*	*Biological effects*	*Reference*
A_1 antagonist	L-97-1 (intragastric)	Allergic rabbit model	Blocked BHR to allergen or adenosine	[36]
A_{2A} agonist	CGS21680 (intratracheal)	Ovalbumin-sensitized Brown Norway rat	Reduced eosinophils and neutrophils and inflammatory markers in BALF	[48]
A_{2B} antagonist	CVT-6883 (i.p.)	Allergic mouse model	Inhibited adenosine-induced BHR	[75]
		ADA KO	Inhibited pulmonary inflammation, fibrosis, airway enlargement	[76]
A_3 antagonist	MRS 1523 (osmotic pump)	ADA KO	Reduced eosinophils and mucus production	[66]
A_1, A_{2B}, A_3 antagonist and p38 α, β and PDE4D inhibitor	CGH2466 (intranasal or oral)	Allergic mouse challenged by ovalbumin	Reduced eosinophils in BALF	[78]
		LPS-induced neutrophilic lung inflammation	Reduced neutrophils in BALF	

BALF = bronchoalveolar lavage fluid; KO = Knockout; LPS = lipopolysaccharide

A_1 ADENOSINE RECEPTOR

The early evidence suggesting a role of the A_1 receptor in asthma came from experimental work using an allergic rabbit model [33]. In this model, aerosolized adenosine caused dose-dependent bronchoconstriction in rabbits sensitized with allergens but not in non-immunized animals. In addition, adenosine produced contractions of tracheal and bronchial smooth muscles isolated from the sensitized animals [34]. Pharmacological studies using selective agonists and antagonists revealed that this effect of adenosine was mediated by the A_1 subtype [33]. Furthermore, the expression of the A_1 receptor was increased in smooth muscles of the allergic rabbits suggesting that the acute bronchoconstriction effect of adenosine in this model was mediated by the A_1 receptors on bronchial smooth muscles. Two potential therapeutic agents were tested in this allergic rabbit model; EPI-2010, which is a 21-mer antisense oligodeoxynucleotide targeting the adenosine A_1 receptor [35], and L-97-1, which is a small molecule A_1 receptor antagonist [36]. As expected, both agents blocked the BHR to allergen or adenosine. The relevance of these observations to human asthma has been questioned due to the fundamental mechanistic difference between adenosine-induced bronchoconstriction in allergic rabbits, which appears to be due to activation of A_1 receptor on the bronchial smooth muscle, and that in man, which appears to be dependent on activation of mast cells [37].

The A_1 receptor has also been implicated in both pro- and anti-inflammatory aspects of disease processes. For example, it has been shown that activation of A_1 receptor promotes activation of human neutrophils and enhances neutrophil adhesion to the endothelium suggesting a pro-inflammatory role of the A_1 receptor [38, 39]. In contrast, in ADA/A_1

double knockout mice, the lack of A_1 receptors results in enhanced pulmonary inflammation, mucus metaplasia, alveolar destruction and earlier death possibly due to the respiratory distress [40]. These later findings suggest that A_1 receptors may play an anti-inflammatory and/or tissue-protective role in the regulation of pulmonary disorders triggered by adenosine.

A_{2A} ADENOSINE RECEPTOR

It is now well known that activation of A_{2A} receptors on lymphoid cells by adenosine causes inhibition of an inflammatory response and this response is largely due to its effect of inducing accumulation of intracellular cAMP in activated immune cells [41, 42]. The actions of A_{2A} receptors on these inflammatory cells are numerous. For example, in human neutrophils, stimulation of A_{2A} receptors reduces neutrophil adherence to the endothelium [39], inhibits formyl-Met-Leu-Phe (fMLP)-induced oxidative burst and inhibits superoxide anion generation [38]. In monocytes and macrophages, activation of A_{2A} receptors inhibits lipopolysaccharide (LPS)-induced tumour necrosis factor α (TNFα) expression [43, 44]. Therefore, A_{2A} agonist may have an anti-inflammatory effect in diseases such as COPD where neutrophil/monocyte-mediated tissue injury is implicated [32]. Activation of lymphocytes, which plays a key role in the recruitment of leukocytes to the lung in clinical asthma, is also suppressed by activation of A_{2A} receptors [45]. Thus, there are a multitude of mechanisms by which activation at A_{2A} receptors could suppress inflammation.

Results from studies in animal models have confirmed the anti-inflammatory effects of A_{2A} receptors. Perhaps the strongest evidence for the critical role of A_{2A} receptors in the regulation of inflammation *in vivo* comes from studies using mice deficient in A_{2A} receptors. In this model, the absence of the A_{2A} receptors resulted in enhanced tissue inflammation and damage [46] and increased levels of pro-inflammatory cytokines associated with enhanced activity of nuclear factor-κB (NF-κB) transcription factor [47] confirming an anti-inflammatory role of A_{2A} receptor. As for airway inflammation, the effect of a selective A_{2A} agonist, CGS 21680, on allergen-induced airway inflammation was tested in the ovalbumin-sensitized Brown Norway rat model [48]. CGS 21680 (administered intratracheally) significantly reduced the numbers of eosinophils and neutrophils, it also reduced the activities of myeloperoxidase and eosinophil peroxidase, and protein concentrations in BALF. These anti-inflammatory effects of CGS 21680 were comparable to the effect of budesonide in the same model. However, similar doses of CGS 21680 also caused marked decreases in blood pressure. Thus, it is difficult to separate the anti-inflammatory effect of CGS 21680 from its cardiovascular effects.

A_{2B} ADENOSINE RECEPTOR

The initial evidence for the role of A_{2B} receptors in asthma and COPD came from pharmacological studies of enprofylline, a methylxanthine structurally closely related to theophylline [49]. It was shown that enprofylline is a selective antagonist for the A_{2B} receptors whereas theophylline has similar binding affinities for A_1, A_{2A} and A_{2B} receptors. Importantly, the therapeutic concentrations of theophylline and enprofylline are in the range of their affinities for A_{2B} receptors. Thus, it was proposed that A_{2B} receptor might be the therapeutic target for the long-term clinical benefit achieved with relatively low doses of theophylline and enprofylline [50].

Recently, A_{2B} receptors have been shown to mediate several pro-inflammatory effects of adenosine in mast cells and lung structural cells. For example, functional human adenosine A_{2B} receptors have been identified in mast cells [49, 51–53], endothelial cells [24, 54, 55], bronchial smooth muscle cells [56, 57], lung fibroblasts [58], and bronchial epithelium. In these cells, adenosine, *via* activation of A_{2B} receptors, increases the release of various

inflammatory cytokines, which induce IgE synthesis from human B lymphocytes [53], and promote differentiation of lung fibroblasts into myofibroblasts [58]. Such findings provide support for the hypothesis that adenosine, *via* activation of A_{2B} receptors, could enhance the inflammatory responses associated with asthma. Thus, an A_{2B} antagonist could potentially be beneficial in the treatment of asthma and other pulmonary inflammatory diseases.

A_3 ADENOSINE RECEPTOR

The functional significance of the A_3 receptor in the pathogenesis of chronic inflammatory airway diseases remains controversial largely due to major species differences in the expression and function of A_3 receptor subtype [59]. Studies performed in rodents have revealed that the effects of adenosine on mast cell degranulation and/or enhancement of mast degranulation in response to allergen are dependent on the activation of A_3 receptor [60–62]. Perhaps the strongest evidence came from studies using genetic knockout mice. While the A_3 receptor knockout mouse appears to reproduce and develop well and has normal cardiovascular functions, it does exhibit altered mast cell functions [63, 64]. Unlike mast cells from wild type mice, adenosine could no longer potentiate antigen-induced release of hexosaminidase from bone marrow-derived mast cells [63] nor could adenosine induce histamine release from lung mast cells of A_3 knockout mice [65]. Interestingly, adenosine-induced airway hyperresponsiveness was markedly reduced but not completely blunted in the A_3 knockout mice (C57BL/6), suggesting the existence of both A_3-dependent and -independent mechanisms in mice [13].

Besides the effects on mast cells, A_3 receptors have been shown to play an important role in eosinophilia and mucus production in animal models [66]. The effects of a selective A_3 antagonist, MRS 1523, on pulmonary inflammation and remodelling were tested in ADA- deficient mice. While MRS 1523 had no significant effects in wild type mice, treatment with MRS 1523 reduced the numbers of eosinophils in BALF and mucus production by airway epithelium in the ADA-deficient mice. Consistent with this finding, in ADA/A_3 double knockout, lack of A_3 receptors resulted in marked reduction in eosinophils and mucus production suggesting an important role of A_3 receptors mediating the lung eosinophilia and mucus hyperplasia in the pulmonary disorders triggered by elevated adenosine levels.

In man, there is not yet convincing evidence to support the role of A_3 receptor in promoting degranulation of lung mast cells [67]. On the other hand, A_3 receptors are found in human eosinophils [67, 68] and transcript levels for the A_3 receptor are elevated in lung biopsies of patients with asthma or COPD [67]. Activation of A_3 receptors inhibited eosinophil chemotaxis and migration [67, 69], eosinophil degranulation, and superoxide anion (O^{2-}) release [70]. Because inflammation in asthmatic patients is characterized by extensive infiltration of the airways by activated eosinophils, it is possible that the elevated adenosine concentrations associated with asthma contribute to the inhibition of eosinophil activation *via* activation of A_3 receptors in human. If this is the case, A_3 agonists would potentially be useful in the management of asthma. Hence, there is conflicting evidence between animal and human data on the possible role(s) of A_3 receptors in the pathophysiology of asthma. Therefore, the role of A_3 receptor in the lung of asthmatics remains to be established.

ADENOSINE LIGANDS IN CLINICAL DEVELOPMENT FOR ASTHMA AND COPD

All four adenosine receptor subtypes are expressed in the lung and in inflammatory cells involved in asthma. It is not surprising, therefore, that selective agonists or antagonists to these receptor subtypes are being exploited by the pharmaceutical industry in an attempt to generate novel therapies for asthma and COPD (Table 1.2).

Table 1.2 Adenosine ligands in clinical development for asthma and/or COPD

Drug name	*Molecular targets*	*Proposed mechanisms of action*	*Status*
EPI-2010	Antisense oligonucleotide of A_1 receptor	Inhibition of BHR	Discontinued
UK-432097	A_{2A} agonist	Anti-inflammation	Phase 2
GW328267	A_{2A} agonist	Anti-inflammation	Phase 2
CVT-6883	A_{2B} antagonist	Inhibition of BHR and anti-inflammation	Phase 1

EPI-2010

EPI-2010 is a 21-mer antisense oligodeoxynucleotide of the A_1 receptor [35]. In an allergic rabbit model, it was shown that intratracheal administration of aerosolized EPI-2010 (twice daily for 2 days) inhibited the BHR triggered by either adenosine or allergen [35]. In the same model, EPI-2010 also caused approximately 75% reduction in the numbers of A_1 receptor in airway smooth muscle. In a placebo-controlled phase 2 trial in asthmatics with moderate-to-severe persistent disease who were already on ICS, inhaled EPI-2010 (once or twice weekly for 4 weeks) did not cause any significant improvement in indices of bronchoconstriction, such as baseline forced expiratory volume at one second (FEV_1), peak expiratory flow rate (PEFR), forced expiratory flow $(FEF)_{25–75}$, symptoms of nocturnal wakening, or rescue β-agonist use [71]. Based on this disappointing clinical result, the development of EPI-2010 has been reported to have been discontinued.

GW328267

GW328267 is an agonist to the A_{2A} receptor. In a randomized, double-blind, placebo-controlled three-way crossover study, the effects of inhaled GW328267 (25 μg, twice daily for 6 days and once on the seventh day), inhaled fluticasone propionate (FP, 250 μg, twice daily), or placebo on allergen-induced early and late asthmatic responses, sputum cell differential counts, inflammatory markers in sputum and blood, and exhaled nitrc oxide (NO) were compared in 14 asthmatics without concurrent steroid treatment. Inhaled fluticasone significantly inhibited both early and late asthmatic responses accompanied by inhibitory effects on sputum eosinophils, eosinophil cationic protein and exhaled NO. In contrast, no protective effect of GW328267 was found [72]. In addition, GW328267 did not cause significant changes in baseline FEV_1. This dose of GW328267 was chosen based on the findings in previous studies in healthy non-asthmatic subjects that higher doses of GW328267 may cause decreases in blood pressure and increases in heart rate.

UK-432,097

UK-432,097 is a potent and selective agonist to the A_{2A} receptor. The selectivity of UK-432,097 for the recombinant adenosine receptors was determined using cAMP assays, and the potencies (EC_{50} values) for stimulation of cAMP mediated by A_{2A} and A_{2B} receptors were 0.46 and 67.5 nM, respectively, whereas the potencies (IC_{50} values) for the inhibition of cAMP mediated by A_1 and A_3 receptors were >300 and 66.5 nM, respectively [73]. UK-432,097 inhibited the fMLP-induced release of elastase, superoxide and LTB4 in human neutrophil and also inhibited the LPS-induced release of MIP1β and TNFα in human peripheral blood mononuclear cell (PBMC) with IC_{50} values of approximately 2–3 nM [74]. In a randomized, double-blind, placebo-controlled two-way crossover study, the effect of inhaled UK-432,097 or placebo on lung functions were compared in 16 non-smoking, mild asthmatic

subjects [73]. Inhaled UK-432,097 had no effect on baseline FEV_1/FVC and PC_{20}-AMP, suggesting that adenosine-induced bronchoconstriction in humans is unlikely to be mediated by the A_{2A} receptor. It would need to be determined whether chronic use of this A_{2A} agonist would provide a beneficial anti-inflammatory effect in the lung without eliciting cardiovascular side-effects.

CVT-6883

CVT-6883 is an antagonist to the A_{2B} receptors. In an allergic mouse model, CVT-6883 (1 mg/kg, i.p.) inhibited AMP-induced airway hyperreactivity [75]. In the ADA-deficient mouse model, CVT-6883 attenuated pulmonary inflammation, fibrosis, airway enlargement, production of cytokines and chemokines in the lung tissues [76]. CVT-6883 is currently being developed as an oral treatment for asthma.

OTHER APPROACHES AND CHALLENGES

NON-SELECTIVE ADENOSINE LIGANDS

Several compounds that have multiple mechanisms of action have been described in the literature [77, 78]. Among these, CGH2466 has combined activities for multiple targets of asthma. CGH2466 is not only an antagonist for A_1, A_{2B} and A_3 adenosine receptors but also an inhibitor of the p38 mitogen-activated protein (MAP) kinase α and β and PDE4D isoenzyme [78]. In human neutrophils and monocytes, CGH2466 is a more potent anti-inflammatory compound than selective inhibitors of MAP kinase or PDE4, or non-selective adenosine antagonist alone. In two mouse models of lung inflammation, CGH2466 (administered intranasally or orally) inhibited the allergen-induced increase in eosinophils and LPS-induced increases in neutrophils. Thus, CGH2466 is a powerful anti-inflammatory agent due to the multiple mechanisms of action. However, it is possible that CGH2466 would be more likely to have side-effects due to its effects on multiple targets.

CURRENT CHALLENGES IN THE DEVELOPMENT OF THERAPEUTIC AGENTS TARGETING ADENOSINE RECEPTORS

One of the most formidable challenges is the lack of animal models that mimic the clinical features of asthma and predict the therapeutic efficacy in human. The endpoints measured in animal models are often different from those in the clinical studies. While monitoring the changes in lung physiology in humans is a routine test in clinics, measuring bronchoconstriction in animal models is neither easy nor routinely done. On the other hand, while invasive procedures such as BAL collection and histological examination of the lung to monitor pulmonary inflammation and remodelling are routinely performed in animal models, these procedures could not easily be carried out in large-scale clinical studies. Thus, it remains challenging to translate pre-clinical results into the clinical setting.

Given that adenosine receptors are widely distributed in different organs, another critical challenge is to develop agonists or antagonists to adenosine receptors that are devoid of side-effects due to the possible actions of adenosine receptors in other organ systems. Disturbances in cardiac and renal functions, in metabolic homeostasis and in activities of the central nervous system may be potential problems especially for systemically-delivered agents targeting A_1 and A_{2A} receptors.

SUMMARY

It has been 20 years since the first demonstration that adenosine is a bronchoconstrictor in asthmatics [5]. Since then, a large body of literature supports the hypothesis that adenosine

plays an important role in airway hyperresponsiveness. In addition, BHR to adenosine has been shown to correlate well with the inflammatory status of the lungs of asthmatic patients. While adenosine has been convincingly shown to be implicated in the inflammatory and remodelling processes of the lungs in numerous animal models, the exact role of adenosine in the inflammatory processes of asthmatic patients is yet to be clearly defined. Due to the multiple and sometimes opposing functions of adenosine receptor subtypes, selective antagonists for the A_1, A_{2B}, A_3 receptors as well as A_{2A} agonist have been proposed to inhibit bronchial hyperresponsiveness and/or airway inflammation. A number of compounds targeting adenosine receptors have been in pre-clinical and clinical development in recent years. We eagerly await proof of the efficacy of these compounds in clinical asthma and other pulmonary diseases.

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