Adenosine Receptors as Targets for Therapeutic Intervention

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ABSTRACT

Adenosine receptors are major targets of caffeine, the most commonly consumed drug in the world. There is growing evidence that they could also be promising therapeutic targets in a wide range of conditions, including cerebral and cardiac ischaemic diseases, sleep disorders, immune and inflammatory disorders and cancer. After more than three decades of medicinal chemistry research, a considerable number of selective agonists and antagonists of adenosine receptors have been discovered, and some have been clinically evaluated, although none has yet received regulatory approval. However, recent advances in the understanding of the roles of the various adenosine receptor subtypes, and in the development of selective and potent ligands, as discussed in this review, have brought the goal of therapeutic application of adenosine receptor modulators considerably closer.

KEYWORDS

Adenosine, asthma, ischaemia/reperfusion injury, Parkinson's disease, refractory primary pulmonary hypertension

INTRODUCTION

Adenosine is prevalent throughout the body. Adenosine may be important in the function of normal nerve cells, in controlling cell proliferation, and as a signal of inflammation. Adenosine is a metabolite of adenosine triphosphate (ATP), having a very short half-life of 1.5sec due to its rapid metabolism. It accumulates in the area where ATP is utilised but not reformed for e.g., during ischemia and possibly during sepsis.[1] Levels rise rapidly in ischaemic tissue due to adenosine kinase inhibition, and mediate ischaemic pre-conditioning, where a prior, brief episode of organ ischemia protects against subsequent ischemia. Inflamed tissues release adenine nucleotides which are converted to adenosine. Cells that release these nucleotides include platelets, mast cells, nerves and the endothelium ecto-nucleotidases (CD39, CD73) then turn the nucleotides into adenosine.[2] Adenosine receptors are found on the endothelial surface, on lymphocytes and on langerhans cells. CD39 is known as ATPDase or ENTPD2.[3] It converts ATP and ADP to AMP. CD39 may be both pro and anticoagulant due to its complex effects on platelets.

Adenosine is an intermediate metabolite in many biochemical pathways and has been shown to play a role in the regulation of coronary and systemic vascular tone, platelet function and lipolysis in adipocytes.[4,5]

Adenosine exerts its effects via P1 purinergic receptors which are A1, A2 (A2A & A2B) and A3 which detect local changes in adenosine concentration. They are seven-spanning proteins coupled to various G-proteins. A2 receptors work on Gs but A1 and A3 interact with Gi and Go. In addition, it mediates other important functions like induction of sleep, antioxidant and antiseizure effects, neuroprotection etc.[6]

Adenosine Receptors[7]

1) Stimulation of A1 receptors inhibits nerve cells, and these receptors mediate the profound effects of adenosine on the heart. A1 receptors are responsible for the important process of `pre-conditioning'.

By lowering heart rate, and, especially, slowing AV nodal conduction, adenosine causes `pharmacological cardio version', of particular use in AV nodal re-entrant tachycardia, but with other anti-arrhythmic uses too. In the basal forebrain accumulation of adenosine (seen with prolonged wakefulness) is thought to inhibit cholinergic cells and induce sleep. A1 receptors also promote vasoconstriction. A1 receptors in the pre-glomerular vessels and tubules regulate renal fluid balance. Antagonists to A1 receptors cause diuresis and natriuresis without major changes in GFR. A1 antagonists decrease afferent arteriolar pressure.

2) A2A stimulation is anti-inflammatory - the receptors are used to sense excessive tissue inflammation. These receptors enhance neural communication, promote coronary vasodilatation, and have anti-platelet effects. CNS effects may be favourable in patients with Huntington's chorea, and agonists may also inhibit psychosis. A2A agonists cause profound vasodilatation, with a corresponding increase in plasma renin activity.

A2B: similar to A2A, but not identical, these are perhaps the most poorly characterised of the adenosine receptors. Signalling pathways may differ substantially. A2B is found on the human mast cell - this may be particularly relevant to the management of asthma --- but A2B receptors are widespread throughout the body. Like A2A receptors, A2B promote vasodilatation.

3) A3: This is the Janus of the adenosine receptors. A variety of effects have been claimed, but other reports then allege completely opposite effects! Many of these conflicting reports seem to be explained by use of different concentrations of agonists, or cells at different stages of their lifespan. A3 is a key receptor in both stimulation and inhibition of cell growth (stimulates many normal cells in micromolar concentrations, induces apoptosis at higher concentrations in both normal and tumour cells). Low concentrations may have antiproliferative effects on tumour cells despite stimulating bone marrow cells. Others claim that adenosine may have many bad effects, promoting tumour growth and angiogenesis. A3 receptor stimulation (at various concentrations and over various time-spans) may be harmful or beneficial in cerebral ischemia. There is some evidence that, like the A1 receptor, the A3 receptor may contribute to pre-conditioning.

Newer potential therapeutic role of adenosine and its receptor

Cardiovascular effects

The role of adenosine in treating supraventricular tachyarrhythmia is now well accepted. Due to the inhibitory effect of adenosine on the AV node, adenosine is the drug of choice for AV nodal re-entrant tachycardia. A1 receptors are responsible for the important process of "pre-conditioning".

1) Ischemia/reperfusion (I/R) Injury

Ischaemic preconditioning (IPC) refers to the mechanism whereby brief periods of ischemia/reperfusion render a tissue relatively resistant to the harmful effects of subsequent prolonged periods of ischemia/reperfusion .The exact mechanism of IPC may vary in different tissues and species where adenosine has an important role.[8-11] This 'adenosine theory' is supported by three facts:

• Interstitial adenosine concentration doubles after five min of cardiac ischemia.[12]

• Adenosine antagonists reduce the effect of cardiac IPC.[9,10]

•Adenoreceptor stimulation reduces myocardial damage following ischemia/reperfusion and during cardiopulmonary bypass.[13,14]

Adenosine may attenuate ischemia/reperfusion injury by a number of possible mechanisms, including purine salvaging, improved tissue perfusion, anti-inflammatory action and a direct intracellular initiator/effector mechanism.[15-19]

2) Refractory primary pulmonary hypertension (RPPH)

Primary pulmonary hypertension of the newborn (PPHN) is a serious disease in which the pulmonary vascular resistance remains elevated during the neonatal period. It is a clinical syndrome that may occur in association with diverse neonatal cardiorespiratory disorders.[20,21] The pathophysiologic hypothesis is supported by the fact that pulmonary vasodilation is achieved by two known pathways. Nitric oxide acts by elevating intracellular cyclic guanosine monophosphate levels resulting in smooth muscle relaxation with a specific potent vasodilator effect.[22] On the other hand, adenosine causes potent selective pulmonary vasodilation by acting at adenosine receptors (A2) on vascular smooth muscle to increase intracellular cyclic adenosine 3'5' monophosphate (AMP), resulting in smooth muscle relaxation and improvement in systemic and myocardial oxygen delivery.[23]

Central nervous system

Parkinson's disease

Although current medication treatment of Parkinson's disease (PD) provides good benefit for number of years, long-term treatment remains inadequate. Continued neuronal degeneration can lead to the emergence of dementia or imbalance, problems that can cause substantial disability. Due to these limitations of current therapy, an intense search for new medications to treat PD is ongoing. There is a need for medications that can slow the underlying progression of degeneration, improve PD symptoms in early disease without inducing dyskinesia and improve motor fluctuations and 'off' time in advanced disease. Much interest has focused on non-dopaminergic therapies, especially adenosine A2a receptor antagonists. Istradefylline (KW-6002) is an adenosine A2a receptor antagonist that is now in phase III clinical trials for PD.[24] Expression of A2A receptors in the brain is predominantly in the basal ganglia, especially the striatum. At a receptor level, there appears to be antagonism between A2A and

D2 dopaminergic receptors, and also between A1 and D1 receptors. This is important, because dopamine's effect seems to be in allowing initiation of movement. Adenosine receptor stimulation antagonises this effect. Two sets of pathways are notable:

• GABAergic Striatopallidal neurones which rely on A2A/D2, and

• Striatonigral and Striatoentopeduncular neurones, which use A1/D1.

Specific A2a receptor antagonists consistently reverse motor deficits or enhance dopaminergic treatments in animal models of PD. For example, in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the dopaminergic pathway, A2a receptor antagonists including KF17837, KW-6002 and MSX-3 potentiated the contralateral turning behaviour induced by Levodopa or a dopamine agonist.[25] Furthermore, the case for developing adenosine A2a receptor antagonists as anti-Parkinsonian therapy has been built on a solid foundation of preclinical evidence.[26]

Epilepsy

Current therapies of epilepsy largely rely on the suppression of spontaneous seizures by pharmacotherapy or surgical intervention; however, till date no effective prophylaxis or true pharmacotherapeutic cure is available. Epileptogenesis i.e. the process that leads to epilepsy and spontaneous seizures is thought to be triggered by an initial acute brain injury, e.g. status epilepticus, followed by progressive neuronal cell loss, mossy fibre sprouting and formation of an astrogliotic scar.[27] However, it is presently unclear why some brain injuries evolve into epilepsy while others do not. Therefore, the identification of diagnostic markers to predict epileptogenesis is of utmost importance. The identification of astrogliosis as a hallmark in brain of epileptics, and the identification of astrocytes as important modulators of neuronal activity imply that dysfunction of astrocytes might play a key role in the pathogenesis of epilepsy.[28] Adenosine is an inhibitory modulator of brain activity. By acting on its receptors, mainly by activation of A1 receptors in hippocampus, it exerts predominant inhibitory effects.[29] These inhibitory actions of adenosine can be used therapeutically to suppress seizures and are considered important for maintaining postictal depression and for restoring the metabolic equilibrium following seizures.[30-32] However, despite more than 20 years of research on the role of adenosine in experimentally induced seizures and the identification of adenosine as endogenous anticonvulsant of the brain,33 the pathogenic role of the adenosine system in epileptogenesis remains understudied.

Anaesthesia and intensive care medicine

Extracellular adenosine and adenosine triphosphate (ATP) are involved in biological processes including neurotransmission, muscle contraction, vasodilatation, signal transduction and secretion in a variety of cell types.[34] Recently established and potential clinical applications of adenosine, ATP in general and ATP-MgCl2 in intensive care medicine have been well documented.

Several double-blind, placebo-controlled, cross-over studies in healthy human subjects have shown pain-reducing effects of intravenous adenosine infusion at doses of 50-70 mg/kg/min.[35] In addition, the effectiveness of adenosine in reducing ischaemic pain (70 mg/kg/min IV for 30 min) is comparable to morphine (20 mg/kg/min IV for 5 min) or ketamine (20 mg/kg/min IV for 5 min). Furthermore, adenosine given in combination with morphine or ketamine has an additive effect on pain reduction.[36] A recent study suggested that, adenosine infusion (50-500mg/kg/min) during general anaesthesia for surgery provided good recovery from anaesthesia associated with pronounced and sustained postoperative pain relief.[37] This may suggest that adenosine could be useful in anaesthesia and intensive care medicine where the action is via nociceptive mechanism.

Pain

Adenosine receptor agonists might just become important in pain management. Intrathecal adenosine is a potential treatment for neuropathic pain (adenosine 0.5 or 2.0mg) by this route antagonises capsaicin-induced hyperalgesia and allodynia.[38]

Respiratory System

Bronchial asthma

The nucleotide adenosine monophosphate (AMP) induces bronchoconstriction in asthma, but not in normal airways. Following facts convince that adenosine plays a key role in pathophysiology of asthma and has an important function in acute bronchoconstrictor and airway inflammatory responses in humans.

• Adenosine levels are increased in broncho-alveolar-lavage fluid exhaled breath of patients with allergic asthma and in the plasma of patients with exercise-induced asthma.[39-41]

• The sensitivity of airways to adenosine and adenosine monophosphate (AMP), which is metabolized locally by the 5' nucleotidase to adenosine, more closely reflects an inflammatory process and the phenotype for allergic asthma.[42, 43]

• Adenosine induces hyper responsiveness in the airways of asthmatics, in vivo following inhalation and in vitro in small airways.[44, 45]

• Theophylline, a non-selective adenosine receptor antagonist and bamiphylline, a selective A1 adenosine receptor antagonist (which does not bind to human A_2 b and A_3 receptors), improve lung function and symptoms in humans with asthma.[46, 47]

•Adenosine elicits hyperactive airway response in humans with allergic asthma by acting on its receptors. All the four adenosine receptors, which have been cloned in

humans, are expressed in lung and all are targets for drug development for human asthma.[48]

Gastrointestinal system

Inflammatory bowel diseases (IBDs)

In the search for novel therapeutic options, increasing attention is being paid to the adenosine system and its involvement in the pathophysiology of IBDs. The expression of adenosine receptor subtypes in the gastrointestinal tract has been investigated in humans and evidence has been obtained for their localization, both in small and large bowel. Once released at sites of inflammation, adenosine plays prominent roles in maintaining tissue integrity by modulation of immune functions, down-regulation of phlogistic reactions, interference with the biosynthesis of proinflammatory cytokines and inhibition of neutrophil adhesion, degranulation and anti-oxidant activity.[49] It has been proposed that the purinergic system may act as a sensor apparatus, which provides the immune system with essential information about tissue health, thus contributing to the resolution of inflammation. In gastrointestinal tract, adenosine also contributes to the control of enteric neurotransmission and smooth muscle contractility, thus participating in physiological regulation of gut motor functions. The involvement of the adenosine system in the anti-inflammatory action has been recognized since the early 1990s. In recent years, its being focused on the search for drugs that act via a direct stimulation of adenosine receptor subtypes, in particular A2a and A3 or through an increase in local adenosine concentration and could offer novel therapeutic options for treatment of IBDs. Evidence supporting the prominent role played by A2a receptors in the anti-inflammatory actions of adenosine has prompted the synthesis of drugs acting as selective agonists of this receptor subtype and their testing in models of intestinal inflammation. In a study by Odashima et al the potential anti-inflammatory effect of ATL-146e, a selective A2a receptor agonist, was investigated on the acute and chronic model of colitis evoked by formalin-immune complex in rabbits, as well as in a model of spontaneous ileitis in SAMP1/YitFc mice.[50] The stimulation of A2a receptors resulted in amelioration of inflammation in the intestinal mucosa, with a reduction of leucocyte infiltration and inhibition of proinflammatory cytokine levels (TNF-α, IFN-γ and IL-4). However, it has been recently observed that the selective A2a receptor agonist CGS21680 was ineffective in ameliorating various inflammatory parameters of colitis induced by dextran sodium sulphate (DSS) in mice. Overall, the actual significance of A2a receptors in the pathophysiology of intestinal inflammation remains undetermined, and further investigations are required to establish the therapeutic relevance of A2a agonists in IBD. Adenosine A3 receptors are also emerging as possible targets for treatment of bowel inflammation.[51]

Renal effects

A1 antagonists act as potassium-sparing diuretics and may protect against contrast-induced injury. A1 receptors are an absolute requirement for normal tubuloglomerular feedback (where increases in NaCl delivery at the macula densa heighten afferent arteriolar tone). It seems that A1 antagonists protect against decline in renal function seen with diuretic therapy, while augmenting the diuresis! Increased adenosine sensitivity (with increased vasoconstriction) may be important in the pathogenesis of contrast-induced nephrotoxicity.[52]

Blood:

Platelets effects

Effects have been well reviewed by Gessi et al Platelets are rich in A2A receptors, and adenosine appears to have an anti-aggregatory effect when it stimulates these receptors.[53] Study of these receptors on platelets is made difficult due to the presence of adenotin, a non-receptor protein that also binds A2 agonists, but A2A appears to be high-affinity. A2A knockout mice show increased platelet aggregation. Anti-coagulant effects of exogenously administered adenosine will necessarily be very brief. Ecto-nucleotidases on endothelial cells may limit propagation of clot, preventing its extension over normal endothelium. They could do this by converting pro-aggregant ADP to adenosine, which inhibits platelet function by acting at A2A receptors.

Immune Implications

Adenosine accumulation and stimulation of (?A2) receptors has been implicated in the immunosuppression seen in critical illness. A3 receptor stimulation may inhibit tumour growth, perhaps melanomas, colon or prostate carcinoma, and lymphomas. Peripheral blood monocytes produce G-CSF when stimulated by adenosine.[54]

SUMMARY

Recently specific adenosine receptor therapeutics or gene targeted mice deficient in extracellular adenosine production became available. These models enabled physicians and the scientists to learn more about the biologic functions of extracellular nucleotide metabolism and adenosine signalling. Functions include specific signalling effects through adenosine receptors expressed by many mammalian tissues; for example vascular endothelia, myocytes, hepatocytes, intestinal epithelia or immune cells. Currently pharmacological approaches to modulate extracellular adenosine signalling are evaluated for the potential use in preoperative medicine, including attenuation of acute lung injury, renal, intestinal and myocardial ischemia. Almost all the modulators are under clinical trial, but could be a very useful therapeutic tool in handling a variety of clinical conditions.

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