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Emerging adenosine receptor agonists

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Adenosine receptors (ARs) are a four-member subfamily of G protein-coupled receptors and are major targets of caffeine and theophylline. There are four subtypes of ARs, designated as A_1, A_{2A}, A_{2B} and A_3. Selective agonists are now available for all four subtypes. Over a dozen of these selective agonists are now in clinical trials for various conditions, although none has received regulatory approval except for the endogenous AR agonist adenosine itself. A_1AR agonists are in clinical trials for cardiac arrhythmias and neuropathic pain. A_{2A}AR agonists are now in trials for myocardial perfusion imaging and as anti-inflammatory agents. A_{2B}AR agonists are under preclinical scrutiny for potential treatment of cardiac ischemia. A_3AR agonists are in clinical trials for the treatment of rheumatoid arthritis and colorectal cancer. The present review will mainly cover the agonists that are presently in clinical trials for various conditions and only a brief introduction will be given to major chemical classes of AR agonists presently under investigation.

Keywords: adenosine receptor, agonist, cardiac arrhythmia, cardiac perfusion imaging, colon cancer, G protein-coupled receptor, inflammation, nucleoside, pain, rheumatoid arthritis

1. Background

Adenosine receptors (AR) are major targets of caffeine, theophylline and other methylxanthines, which are natural antagonists of ARs. Adenosine is the major endogenous agonist, although its metabolite, inosine, can also activate some subtypes of ARs.

The biologic action of adenosine was first identified in the cardiovascular system by Drury and Szent-Gyorgyi [1], who reported the effect of adenosine on heart rate, atrioventricular conduction and blood pressure. Since then adenosine has been reported to play a role in numerous tissues mainly through four subtypes of ARs (A_1, A_{2A}, A_{2B} and A_3), which are cell surface receptors containing seven transmembrane domains and couple to intracellular GTP binding proteins. Evidence of the role of adenosine in the brain was mainly postulated pharmacologically based on the effects of methylxanthines, such as caffeine and theophylline [2-4].

AR signaling has been extensively studied [5-7]. A_1 and A_3 ARs primarily couple to the $G_{i/o}$ proteins, which mediate the inhibition of adenylyl cyclase, and A_{2A} and A_{2B} ARs couple to G_S proteins, which activate adenylyl cyclase. In addition, the A_{2B}AR couples to $G_{q/11}$ proteins, which stimulate phospholipase C activity and subsequently the protein kinase C activity and intracellular calcium mobilization. In addition to the activation of Gα subunits, ARs also stimulate the activity of G$\beta\gamma$ subunits, which induce the activation of numerous signaling pathways, such as phospholipase C, PKC, phosphoinositide 3-kinases, MAPKs and ion channels. All four subtypes of ARs may have a role in cell growth, death and differentiation [7].

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ARs are widely distributed both in the brain and in peripheral systems [8,9]. In the brain, the A_1AR is widely expressed in many areas. Pre- and postsynaptic activation of the A_1AR inhibits synaptic transmission, in part by suppressing the release of excitatory transmitters [10]. The $A_{2A}AR$ is less widely expressed, with greatest density in the striatum, nucleus accumbens and olfactory tubercle. The $A_{2B}AR$ and A_3AR are expressed at low density in most brain regions and are implicated in purinergic signaling in neuronal–glial interactions. ARs are also widely distributed in the autonomic and enteric nervous systems. Distribution of neural ARs in the human intestine has been investigated. Four subtypes of AR mRNAs are differentially expressed in neural and non-neural layers of the jejunum, ileum, colon and cecum [11]. In the bladder, A_1ARs are prominently localized to the apical membrane of the umbrella cell layer, whereas A_{2A}, A_{2B} and A_3 ARs are localized on the basolateral membrane of umbrella cells and the plasma membrane of the underlying cell layers. A_1, A_{2A} and A_{2B} ARs are all expressed on normal human airway smooth muscle cells, and both A_1 and A_{2A} ARs are expressed in vagal pulmonary C fibers. A_1 and A_{2A} ARs are highly expressed in gastric mucosa. $A_{2A}ARs$ are also widely distributed in the immune cells, such as T cells and macrophages. A_3 ARs are abundantly expressed in various blood cells, such as neutrophils and eosinophils. Various subtypes of ARs have also been detected in the heart, blood vessels, kidney and other organs throughout the body [8,9]. The wide and abundant distribution of these receptors suggests that they potentially play a critical role in the body and, thus, potentially useful therapeutic agents may be developed based on the AR distribution and AR function [12]. For example, the abundance of A_1ARs in the atrioventricular node of the heart is the basis for clinical development of selective A_1AR agonists as antiarrythmic agents. The abundant $A_{2A}ARs$ in coronary blood vessels is the basis for the development of $A_{2A}AR$ selective agonists for cardiac perfusion imaging. The high-level expression of the A_3AR in neutrophils and eosinophils may give it a role in inflammation [13,14]. It should be noted that the distribution of ARs may be species dependent. For example, A_3ARs are rich in rat, but not human testes [8,15,16].

Genetic deletion of all four subtypes of ARs has been carried out and the resulting knockout mice are viable and not highly impaired in function [17-20]. The pharmacologic profile indicates that the peripheral analgesic effect of adenosine is mediated by the A_1AR, and analgesia is lost in mice in which the A_1AR has been genetically eliminated [17]. Genetic knockout of the A_1AR in mice removes the discriminative-stimulus effects, but not the arousal effect of caffeine [21]. Genetic knockout of the A_1AR also increases anxiety and hyperalgesia [17]. Study of $A_{2A}AR$ knockout mice reveals functional interaction between the spinal opioid receptors and peripheral ARs. A_1AR knockout mice demonstrate a decreased, whereas $A_{2A}AR$ null mice demonstrate an increased, thermal threshold to noxious heat stimulation, supporting an A_1AR-mediated inhibitory and $A_{2A}AR$-mediated excitatory

effect on pain transduction pathways [21]. Knockout of the $A_{2A}AR$, but not the A_1AR, eliminates the arousal effect of caffeine [18,22]. Genetic knockout of the $A_{2A}AR$ also suggests a link to increased anxiety and protection against the damaging effects of ischemia and the straital toxin 3-nitropropionic acid. Studies on $A_{2B}AR$ knockout mice suggested that the $A_{2B}AR$ may have both pro- and anti-inflammatory roles [19,23,24]. The importance of $A_{2B}AR$ in the brain still awaits future investigation. Genetic knockout of the A_3AR leads to increased neuronal damage in a model of carbon monoxide-induced brain injury [25]. Neutrophils lacking A_3ARs show correct directionality, but diminished speed of chemotaxis [13].

Due to the wide distribution and extensive and complex signaling coupling nature in the body, ARs are involved in a large variety of physiologic and pathophysiologic events. Numerous agonists of ARs are under development for potential therapeutic applications and some are already in the late phases of clinical trials [9,12,26], although none has yet received regulatory approval except for adenosine itself. The present review will mainly describe various agonists under clinical trials for various diseases. Major chemical classes of AR agonists presently under investigation will only be introduced briefly.

2. Medical need and existing treatment

Over a dozen AR agonists are now in clinical trials for various diseases. These agonists are primarily being developed for the following conditions: cardiac arrhythmias, neuropathic pain, myocardial perfusion imaging, inflammatory diseases and colon cancer [12,26]. The rest of this section will briefly discuss the medical need and major existing treatment of these conditions.

2.1 Cardiac arrhythmias

In clinical practice, among the most frequently encountered cardiac arrhythmias are atrial fibrillation (AF), supraventricular arrhythmias, paroxysmal supraventricular tachycardia (PSVT) and atrial flutter. Approximately 6 million people worldwide experience AF, a condition in which the heart beats at 2 – 3-times its normal rate [27]. Although AF is the most common of these arrhythmias, PSVT is also a relatively frequent occurrence with ~ 89,000 new cases being diagnosed on an annual basis [28]. Overall, it is estimated that PSVT is present in 570,000 individuals and accounts for ~ 30,000 hospitalizations in the US [28].

At present, available therapeutic agents for PSVT include adenosine, non-dihydropyridine calcium channel blockers (e.g., diltiazem or verapamil) or β-blockers. Adenosine is preferred over the other atrioventricular nodal blockers primarily because of its more rapid onset, shorter half-life and lack of negative inotropic effects [29]. Adenosine is similarly potent at A_1, A_{2A} and A_3, but weak at the A_{2B} AR. Its therapeutic effects in terminating PSVT are mediated by its actions at the A_1 AR, whereas its adverse effects most

likely result from its non-selective activation of the other ARs [30]. Despite its efficacy in the treatment of PSVT, the use of adenosine can be limited by adverse effects such as flushing, dyspnea, chest discomfort and hypotension [31]. In addition, adenosine has been shown to promote the development of AF in ≤ 15% of patients [32]. Although these adverse effects are usually transient in duration because of the short half-life of adenosine, there has been interest in developing a selective A$_1$AR agonist that would have minimal side effects [33].

2.2 Neuropathic pain

There are already three recent reviews in this journal specifically describing the medical need and the present treatment of neuropathic pain [34-36], thus, only a brief introduction will be given here. Unlike inflammatory pain, which can be adequately managed in the clinic, neuropathic pain is typically unresponsive to conventional analgesics, such as opioids, COX-2 inhibitors and NSAIDs. The presently available treatments for neuropathic pain include: antidepressants, anticonvulsants and opioids [35,36]. Drugs from the above-mentioned classes only showed moderate relief in < 50% of the treated patients. In addition, undesirable side effects of these drugs are common. Thus, there remains a clear medical need for novel classes of drugs with higher efficacy and reduced side effects.

2.3 Myocardial perfusion imaging

Myocardial perfusion studies use either physical exercise or pharmacologic vasodilator stress to induce maximum myocardial hyperemia. At present, pharmacologic stress is being performed with increasing frequency over exercise stress. Although exercise remains the preferred modality of stress testing, many patients are limited from completing a maximum stress test for a variety of reasons. Presently, almost 50% of myocardial performance index (MPI) performed in the US is done with pharmacologic stress testing [37]. At present, there are two classes of pharmacologic stressing agents used in the clinic: coronary vasodilating agents such as adenosine and dipyridamole; and positive inotropic and chronotropic agents that increase cardiac workload and potentially cause myocardial ischemia, such as catecholamines (dobutamine). Dipyridamole and adenosine have a high side effect profile, provide higher than needed coronary artery flow rates and use a relatively complicated method of administration. Adenosine not only activates A$_{2A}$ARs, but also non-selectively activates A$_1$ and A$_3$ ARs, which contributes to the many common and undesirable side effects of adenosine vasodilator stress (e.g., chest pain, dyspnea, severe bronchoconstriction and atrioventricular conduction abnormalities). Thus, there is a clear need to develop newer pharmacologic stressor agents that are simpler to administer, have an improved side effect profile and offer greater accessibility to patients who have contraindications to other agents [38].

2.4 Inflammation

The inflammation process involves a series of events that can be elicited by numerous stimuli, such as infectious agents and ischemia. Each type of stimulus provokes a characteristic pattern of response representing a relatively minor variation on a theme. The response is generally accompanied by the familiar clinical signs of erythema, edema, tenderness and pain. Uncontrolled inflammation plays an important role in the pathogenesis of major diseases including cancer, heart disease, atherosclerosis and sepsis [39,40]. Drugs used to control symptoms and signs of inflammation are generally classified as steroidal and NSAIDs. Steroidal drugs are known to be effective in many cases but they also produce considerable side effects especially during long-term application. Conventional NSAIDs showing therapeutic effects are often preferable. However, the effects of these drugs are often uncertain and they may also produce severe side effects.

Rheumatoid arthritis (RA) is a chronic, destructive inflammatory joint disease [41]. It leads to functional impairment in most patients and to severe disability in many. Prognosis has improved in recent years because of the availability of new biologic therapies and management strategies. The plethora of available treatments provides a challenge for rheumatologists to choose the most appropriate therapy for each individual patient. The efficacy of a treatment regimen is the most important factor to consider, as only in those regimens with convincing efficacy are considerations of safety and cost meaningful. The primary question in clinical practice will, therefore, be the likelihood and degree of clinical response to different therapies.

Although the pathogenesis of RA is largely unknown, it appears to be an autoimmune disease driven primarily by activated T cells, giving rise to T-cell-derived cytokines, such as IL-1 and TNF. Many cytokines, including IL-1 and TNF, have been found in the rheumatoid synovium. The drugs available at present include steroids, which are known to interfere with the synthesis and/or actions of cytokines, and COX inhibitors such as aspirin, ibuprofen and diclofenac, which presumably inhibit the synthesis of prostaglandins. However, the therapeutic effects of these drugs are often uncertain and side effects such as gastric and intestinal ulceration are common. Thus, there is still a clear need to develop novel and more effective NSAIDs.

2.5 Colorectal cancer

Colorectal cancer is one of the world's most common cancers. It is the second leading cause of death due to cancer in the US with a 6% personal lifetime risk, > 130,000 case/year and > 48,000 deaths expected in 2001 [42]. Colorectal cancer incidence and mortality rates increase with age, sharply so after age 60. Diagnostic stage determines survival: patients with lesions that are confined to the colonic wall have a 75% probability of surviving for 5 years, whereas those with distant metastases have only a 5 – 10% probability of living for 5 years. Unfortunately, most colorectal cancers are

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Box 1. List of chemical names in full.

CCPA: 2-Chloro-N^6-cyclopentyladenosine

CGS21680: 2-p-(2-Carboxyethyl)phenethylamino-5′-N-ethylcarboxamidoadenosine

CHA: N^6-Cyclohexyladenosine

Cl-IB-MECA: 2-Chloro-N^6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide

CPA: N^6-Cyclopentyladenosine

DPMA: N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine

DU124183: N-phenyl-2-cyclopentyl-1H-imidazo[4,5-c]quinolin-4-amine

IB-MECA: N^6-(3-iodobenzyl)-adenosine-5′-N-methyluronamide

LUF6000: N-(3,4-dichloro-phenyl)-2-cyclohexyl-1H-imidazo[4,5-c]quinolin-4-amine

NECA: 5′-(N-ethylcarboxamido)adenosine

PD81,723: (2-Amino-4,5-dimethyl-3-thienyl)-[3-(trifluoromethyl)phenyl]methanone

(S)-ENBA: (2S)-N^6-[2-endo-Norbornyl]adenosine

T62: 2-Amino-3-(4-chlorobenzoyl)-5,6,7,8-tetrahydrobenzothiophene

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recognized after regional or distant metastasis has occurred. There are limited treatment options for advanced disease, but favorable survival chances for early disease, so in addition to detection at an early stage, the prospects for control of colorectal cancer rest in early surgery and the development of novel and more effective therapeutic agents.

3. Adenosine receptor agonist class review

Most AR agonists have been adenosine derivatives thus far. Adenosine derivatives have been developed as both non-selective AR agonists and selective agonists for A_1, A_{2A} and A_3 ARs [9,12,26]. Generally, these agonists are substituted at 2-,5′- or N^6- positions of adenosine. However, it was found that, in addition to the 5′-substitution, the ribose moiety could also be replaced with a methanocarba and the 4′-oxygen could be replaced with a thio group [43,44]. Adenosine derivatives selective for A_1, A_{2A} and A_3 ARs have been extensively used as pharmacologic tolls and many of them are commercially available. Selective agonists for the A_1AR include: CPA, CCPA, CHA and (S)-ENBA. CPA, CCPA and CHA are very selective at the murine A_1AR. For all drug names in full, see **Box 1**. At human A_1AR, they only show a modest selectivity (10 – 30-fold over A_3AR) [45,46]. (S)-ENBA is selective for the A_1AR in both humans and rats [45]. CGS21680 and DPMA are selective at the murine, but not the human, A_{2A}AR, and they are also fairly potent at the human A_3AR [45]. NECA has been used to study the A_{2B}AR, although it is non-selective. A series of NECA derivatives has recently been reported to be modestly A_{2B}AR selective [47] as measured with a cyclic AMP assay at the A_{2B}AR and binding assays at other AR subtypes. However, the selectivity has not been determined with a similar assay system at all four AR subtypes. IB-MECA and Cl-IB-MECA are potent at

both human and rat A_3ARs [45,48], but a certain degree of species-dependent selectivity may also be found.

In addition to adenosine, inosine has also been reported to be an agonist of ARs, although shown to be weaker than adenosine in rats. Inosine binds to the A_3AR and stimulates mast cell degranulation. Inosine lowers cyclic AMP in HEK-293 cells expressing rat A_3ARs with an EC_{50} of 12 μM and stimulates rat basophilic leukemia (RBL)-2H3 rat mast-like cell degranulation with an EC_{50} of 2.3 μM [49]. It was found later that inosine is more potent for human A_3AR, although it only shows partial maximal agonist efficacy [9]. The potency, maximal agonist efficacy and selectivity of inosine derivatives as agonists for the ARs have not been extensively explored.

Several classes of non-nucleoside agonists have also been reported recently. The first highly potent agonist for the A_{2B}AR is actually a non-nucleoside agonist [50,101,102]. Based on the structure–activity relationship studies, the first selective A_{2B}AR agonist, BAY-60-6583 [51-53], has been developed. This class of compounds was also to display a remarkable agonistic–antagonistic profile at the A_1AR [54]. Selective agonists for A_{2A} and A_3 ARs from this class of compounds have not been reported so far.

Müller and colleagues also reported another two classes of non-adenosine compounds as agonists for ARs, although they only showed micromolar potency. Lignans isolated from Valerian have been reported to be partial agonists for the A_1AR [55]. Some amino acid derivatives are reported to be weak partial agonists of A_1 and A_3 ARs [56].

In addition to directly acting agonists, allosteric modulators for ARs have also been developed. PD81,723 and T62 are allosteric enhancers of the A_1AR function [57]. DU124,183 and LUF6000 are allosteric enhancers of the A_3AR function [58].

4. Current research goals

Briefly, the major research goals currently are to understand more about the functions of the AR subtypes in the body and to develop more potent and selective agonists for an individual AR subtype independent of species. With the availability of knockout animals and selective agonists for all AR subtypes, especially the recent availability of the $A_{2B}AR$ knockout animals and $A_{2B}AR$ selective agonists [19,52], it is expected that many ambiguous roles of individual AR subtypes will be clarified in the near future. This will further help the rational clinical development of AR agonists.

5. Scientific rationale and the development of adenosine receptor agonists for various conditions

5.1 Cardiac arrhythmia

One of the original findings of the biologic functions of adenosine by Drury and Szent-Gyorgyi [1] is its effect on heart rate. Thus, the potential therapeutic effect of adenosine for cardiac arrhythmias has been a long-term area of investigation. Intravenous infusion of adenosine has been found to restore normal heart rhythm in patients with paroxysmal supraventricular tachycardia (PSVT) [12,26]. Although adenosine is preferred over other atrioventricular nodal blockers due to its more rapid onset, shorter half-life and lack of negative inotropic effects, the use of adenosine can be limited by side effects such as flushing, dyspnea, chest discomfort and hypotension [31]. It is now known that the A_1AR is abundant in the atrioventricular node of the heart and the effect of adenosine on heart rhythm is mainly through activation of the A_1AR [33]. Thus, selective A_1AR agonists are needed to avoid side effects related to other AR subtypes.

Tecadenoson (CVT-510) is a novel A_1AR agonist under development for the treatment of PSVT, AF and atrial flutter [33]. In Phase III clinic trials it effectively terminated PSVT. Compared with other antiarrhythmic agents on the market, it appeared to have minimal cardiac side effects, such as blood pressure and atrioventricular nodal conduction during sinus rhythm, at low doses. However, at higher doses, it also caused high-degree atrioventricular block. Thus, the use of full agonist tecadenoson as antiarrhythmic agents may be limited by their ability to cause high-degree atrioventricular block and profound bradycardia and AF. Thus, the partial agonist for the A_1AR, CVT-2759, has been developed, which in guinea-pig heart appears to be useful to slow atrioventricular nodal conduction and thereby ventricular rate without causing second-degree atrioventricular block, bradycardia, atrial arrhythmias or vasodilation [59,60]. However, the clinical trial data of CVT-2759 has not been available so far. N^6-cyclopentyl-5′-(N-ethyl)carboxamidoadenosine (selodenoson) is a selective A_1AR agonist whose Phase II clinical trials has been completed for patients with AF [201]. Although several A_1AR agonists are in development, it is suggested that seledenoson may be the only one that can effectively be formulated for intravenous administration to control heart rate during acute attacks and for oral administration for the chronic management of AF [104]. The pharmaceutical formulation for controlled release of selodenoson has been reported in 2005 [104].

5.2 Neuropathic pain

A_1AR agonists reduce pain signaling in the spinal cord where the receptors are highly expressed. The A_1AR activation produces inhibitory effects on pain in a number of preclinical models and is a focus of attention. In humans, intravenous infusions of adenosine reduce some aspects of neuropathic pain and can reduce postoperative pain mainly through the A_1AR. It has been shown that mice lacking A_1ARs exhibited moderate hyperalgesia to heat stimulation [17], whereas mice lacking $A_{2A}AR$ were shown to be hypoalgesic, probably reflecting a peripheral pronociceptive function [18]. Increased nociceptive response in mice lacking the A_1AR has been observed [61]. Thus, it is suggested that selective A_1AR agonists may be tested clinically as analgesics, particularly under conditions of neuropathic pain.

In addition to directly acting A_1AR agonists, allosteric enhancers of the A_1AR function are also of potential interest for the treatment of neuropathic pain. It has been shown in a GTPγS saturation analysis that an allosteric enhancer T62 increased the number of G proteins activated by agonist, but had no effect on the affinity of activated G proteins for GTPγS. [^{35}S]GTPγS autoradiography showed that the neuroanatomical localization of T62-stimulated [^{35}S]GTPγS binding was identical to that of A_1AR agonist phenylisopropyladenosane (PIA)-stimulated activity. The increase in PIA-stimulated activity by T62 varied between brain regions, with areas of lower A_1AR activation producing the largest per cent modulation by T62. These results suggest a mechanism of allosteric modulators to increase the number of activated G proteins per receptor and provide a neuroanatomical basis for understanding potential therapeutic effects of such drugs [62].

A selective A_1AR agonist, GR-79236, has been in clinical trial for the treatment of pain, however, it was discontinued probably due to cardiovascular side effects. Phase II clinical trials of another A_1AR agonist, GW-493838 (structure not disclosed), for the treatment of neuropathic pain has been completed [202]. However, it seems that the further development of GW-493838 has also been discontinued. The A_1AR-selective allosteric enhancer T-62 was also shown to reduce hypersensitivity in carageenin-inflamed rats by a CNS mechanism. Phase I clinical trials of T62 have been completed as a treatment for neuropathic pain. The $A_{2A}AR$ agonist BVT.115959 (structure not disclosed) is now under Phase II clinical trials for diabetic neuropathic pain [105,203]. A_1 and A_{2A} ARs are widely distributed in the brain and spinal cord and represent a non-opiate target for pain management. Activated spinal A_1ARs inhibit sensory transmission by

inhibiting the slow ventral root potential, which is the C-fiber-evoked excitatory response associated with nociception. Additionally, adenosine is also being tested in Phase II clinical trials for perioperative pain [204].

At least 50 new molecular entities have reached clinical development for neuropathic pain, which include glutamate receptor antagonists, cytokine inhibitors, opioids, cannabinoids, COX inhibitors and AR agonists. At present, eight drugs are in Phase III clinical trials for neuropathic pain [36].

5.3 Cardiovascular imaging

In addition to the effect of adenosine on heart rate, another prominent cardiovascular effect is its vasodilatory effect. Adenosine and dipyridamole are presently used in clinic as pharmacologic stress agents for cardiovascular imaging [37,63]. It is now known that this vasodilatory effect is mainly through the $A_{2A}AR$, which is abundant in coronary blood vessels. Thus, AR agonists selective at the $A_{2A}AR$ are needed for this purpose.

Based on preclinical animal work, three selective $A_{2A}AR$ agonists, regadenoson, binodenoson and apadenoson, have been in Phase III studies as pharmacologic stress agents. For single-photon emission computed tomography imaging, binodenoson and regadenoson were concordant with adenosine images for detection and quantitation of ischemia. Despite the high $A_{2A}AR$ selectivity of binodenoson and regadenoson in preclinical studies, subjective side effects attributable to other AR subtypes were still observed in human studies and although they are slightly lower than adenosine. Apadenoson was reported to be more selective than the other two agonists that entered Phase III trials earlier. It remains to be seen whether or not approval of regadenoson and binodenoson by the FDA will translate into first-to-market advantages. It is reported that a comparison study of apadenoson and adenosine with treadmill exercise stress has been terminated. A study to examine MPI single photon emission computed tomography (SPECT) imaging with apadenoson and adenosine compared with coronary angiography has also been terminated [205]. The Phase II optimization study of apadenoson and sestamibi planar imaging has been completed [205].

5.4 Inflammation

Adenosine is a ubiquitous molecule that influences every physiologic system studied thus far. Endogenous adenosine exerts a significant proportion of its anti-inflammatory actions by means of binding to $A_{2A}ARs$ found in almost all immune cells, including lymphocytes, monocytes, macrophages, human natural killer T cells and dendritic cells [64-66].

Adenosine acts non-selectively by reducing expression of adhesion molecules and release of pro-inflammatory mediators (e.g., reactive oxygen species, elastase and TNF-α). Selective $A_{2A}AR$ agonists have been developed [67,68] and shown to inhibit multiple manifestations of inflammatory cell activation including production of superoxide, nitric oxide, TNF-α, IL-12, IL-10 and VEGF [69]. $A_{2A}AR$ agonists are also

vasodilators, but the inhibition of inflammation occurs at low doses that produce few or no cardiovascular side effects. Through $A_{2A}AR$ activation, adenosine can inhibit T-cell activation, proliferation, and production of inflammatory cytokines and enhance the production of anti-inflammatory cytokines. Therefore, the selective $A_{2A}AR$ agonists hold significant potential in the treatment of inflammation.

Unlike the anti-inflammatory role of the $A_{2A}AR$, the role of the A_{2B} and A_3 ARs as to pro-inflammatory or anti-inflammatory is still controversial (e.g., agonists have been found to be pro-inflammatory and anti-inflammatory, and there is evidence for an anti-inflammatory effect of A_{2B} and A_3AR antagonists [20,23]). Adenosine augments IL-10 production by macrophages through an $A_{2B}AR$-mediated post-transcriptional mechanism [70]. Adenosine inhibits TNF-α release from mouse peritoneal macrophages by means of A_{2A} and A_{2B}, but not the A_3AR [71]. It is suggested that the pro- or anti-inflammatory roles of A_{2B} and A_3ARs might depend on tissues, inflammatory stages and types.

Both A_{2A} and A_3 AR agonists have been proposed for the treatment of rheumatoid arthritis. The rationale for this is likely at least in part due to their modulatory effects on TNF-α and NF-κB. It is known that TNF inhibitors have changed the therapeutic standard of treatment for patients with rheumatoid arthritis. The activation of A_{2A}, A_{2B} and A_3ARs are involved in the inhibition of TNF and NF-κB, which could be a potential mechanism for the anti-inflammatory, especially the antirheumatic, effect of theses ARs. However, the exact scientific or clinical rationale has not been clear so far. It has been suggested that both A_{2A} and A_3 ARs are required for inhibition of inflammation by methotrexate and its analog MX-68 [72], and stimulation of AR subtypes A_3 and A_{2A} may be a strategy worthy of further evaluation for the abrogation of acute or chronic inflammatory disorders [73]. Additionally, it is suggested that NF-κB is involved in regulating the level of A_3ARs overexpressed in peripheral blood mononuclear cells in a RA model [74]. Anti-inflammatory effect of A_3AR agonists have also been demonstrated in murine autoimmune arthritis models [75].

An $A_{2A}AR$ agonist, GW328267, designed for intranasal administration, has been in Phase II clinical trials for upper respiratory inflammatory disease, chronic obstructive pulmonary disease and asthma; however, the results at the dose used were negative and the compound has been withdrawn from clinical testing. MRE-0094 is in Phase II clinical trials for chronic, neuropathic and diabetic foot ulcers, due to the anti-inflammatory and wound healing effects of $A_{2A}AR$ agonists [76,206]. Clinical trails of ATL146e as an anti-inflamatory agent have also been initiated.

The $A_{2A}AR$ has long been proposed to be effective in the treatment of arthritis, however, clinical trial data have not been available so far. The A_3AR agonist IB-MECA is an oral drug that has been successfully used in animal models and human Phase II RA trials to test the concept of targeting the A_3AR for the treatment of inflammatory diseases. Phase I

studies in healthy volunteers, as well as the Phase IIa clinical studies in RA human patients have demonstrated this drug to have a favorable safety profile. Recent interim analysis of Phase IIa pilot study data indicates that CF101 has disease-modifying anti-inflammatory activity in RA patients failing methotrexate therapy. Can-Fite is now completing Phase IIb clinical trials of CF101 in RA. CF502, a more selective A_3AR agonist, is in preclinical development for RA [97,98]. Additionally, Can-Fite is about to initiate a Phase II clinical trial with CF101 for the treatment of psoriasis.

In the US, six biologic agents have been approved for the treatment of patients with RA [77]. However, it is not clear about the data on the comparative efficacies of the biologics, the appropriate sequencing of agents, the best cotherapies or the possible combined or staggered use of biologics marketed by different companies.

5.5 Cancer

There are several lines of evidence to show that AR agonists may induce, as well as protect, cell death depending on cell types. There has been evidence to suggest that some adenosine analogs may be potentially used for cancer therapy. Cladribine and cordycepin are used in the clinic for the treatment of leukemia, although presumably not through AR activation.

Kohno et al. [78] found that two synthetic adenosine analogs IB-MECA (30 and 60 μM) and Cl-IB-MECA (10 – 30 μM), but not CPA, CGS21680 and NECA ≤ 30 μM), induced apoptosis in HL-60 human promyelocytic leukemia cells. However, it was found later that low concentrations of the A_3AR agonist Cl-IB-MECA (10 nM or 1 μM) actually protected against apoptosis in HL-60 cells induced by a selective A_3AR antagonist (MRS1191), suggesting that apoptosis in HL-60 cells induced by adenosine analogs are not through the A_3AR or opposing effects can occur at different levels of receptor stimulation. This is consistent with the results from Gao et al. [79] with RBL-2H3 mast-like cells. The selective A_3AR agonist, IB-MECA (10 nM and 1 μM), but not CPA and CGS21680 (100 nM), significantly protects apoptosis caused by exposure of RBL-2H3 cells to UV light. Lu et al. [80] also found that the adenosine analog, IB-MECA, indeed downregulates estrogen receptor α and suppresses human breast cancer cell proliferation, but not through an A_3AR-mediated mechanism, as low concentrations of IB-MECA did not inhibit cell death and selective A_3AR antagonists did not affect the effect of IB-MECA. Kim et al. [81] found that Cl-IB-MECA at the concentration of 30 μM, but not lower, induced apoptosis in HL-60 cells, but a corresponding (N)-methanocarba agonist, known to be equipotent to Cl-IB-MECA at the A_3AR, did not. Lee et al. [82] have shown that a novel adenosine analog, thio-Cl-IB-MECA, induces G_0/G_1 cell cycle arrest and apoptosis in human promyelocytic leukemia HL-60 cells also not by means of the A_3AR. The above results clearly suggested that inhibition of cell growth by high concentrations of A_3AR agonists is not through an A_3AR-mediated mechanism, but the A_3AR agonists induced protective effects are A_3AR-mediated. From this point of view, A_3AR antagonists are potentially useful for certain types of cancers, but this has to be tested in various animal models. A_3AR antagonists have been proposed to be used as both antitumor agents and for treating inflammatory disorders such as RA [103].

By studying the role of the A_3AR in various cell lines Borea and colleagues suggested that the A_3AR may mediate both cell growth and cell death [83]. For example, the A_3AR mediates a cell survival signal in A375 human melanoma cells [84], but it also inhibits cell proliferation by means of phosphatidylinositol 3-kinase/Akt-dependent inhibition of the extracellular signal-regulated kinase 1/2 phosphorylation in A375 human melanoma cells [85]. In colon cancer cell lines, endogenous adenosine, through the interaction with A_3ARs, mediates a tonic proliferative effect [86]. It has been found that, in patients with colorectal cancer, overexpression of ARs in tumors was reflected in peripheral blood cells, and thus, the A_3AR has been proposed to be potentially used as a diagnostic marker or a therapeutic target for colon cancer [87].

Fishman et al. [88] found that adenosine and other low molecular weight factors released by muscle cells inhibit tumor cell growth. It was found in a later study that at a relatively low concentration only IB-MECA, but not CCPA and DPMA, induced inhibition of the growth of Nb2-11C rat lymphoma cell line [89]. In addition, the inhibitor effect of 25 μM adenosine can be blocked by an AR antagonist MRS1220 (0.1 μM) [89]. However, it should be noted that MRS1220 does not block the rat A_3AR at 0.1 μM, although it can block the rat $A_{2A}AR$ at this concentration.

Mechanistic studies of the A_3AR agonists, IB-MECA (CF-101) and Cl-IB-MECA (CF-102) have also been performed in various cell types. Evidence has been presented for the involvement of Wnt signaling pathway in IB-MECA-mediated suppression of melanoma cells [90]. Inhibition of primary colon carcinoma growth and liver metastasis by the A_3AR agonist CF101 have been described [91]. It has also been shown that IB-MECA inhibits colon carcinoma growth in mice by means of modulation of GSK-3β and NF-κB [92]. Evidence has also been shown that CF101 enhances the chemotherapeutic effect of 5-fluorouracil in a colon carcinoma murine model [93] and induces G-CSF production by means of NF-κB activation to produce myeloprotective effect [94]. The A_3AR is shown to be highly expressed in tumor versus normal cells, suggesting a potential target for tumor growth inhibition [95]. However, it is not clear if the A_3AR overexpression is a reason for or a result of cancer.

Although it has been suggested that A_3AR agonists may produce a dual effect on tumor and normal cell growth, these studies have not been performed in parallel with normal and tumor tissues. It is also not clear if the behavior of the A_3AR activation will be similar or different in vitro and in vivo,

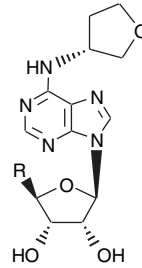**Emerging adenosine receptor agonists**

Native agonist
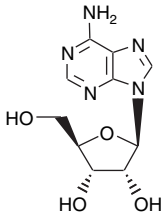
A₁ agonists and modulator
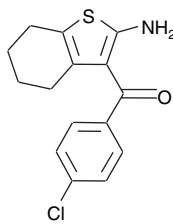
Adenosine
(Adenocard,
Adenoscan)
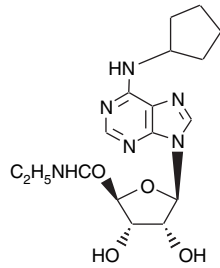
Selodenoson
(RG 14202)
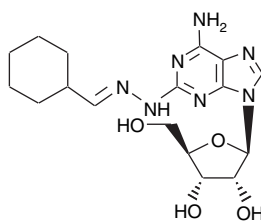
Tecadenoson
(CVT-510)   R = HOCH₂,
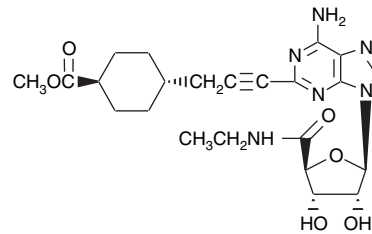CVT-2759   R = CH₃ NHCO₂ CH₂
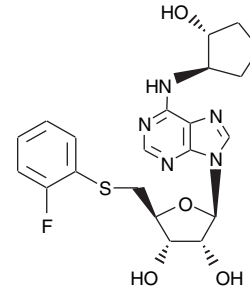
CVT-3619

A₁ modulator

T62

A₂A agonists
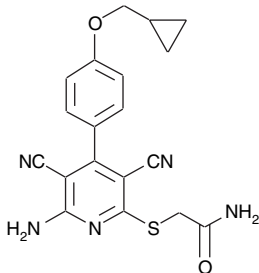
Binodenoson
(MRE0740)
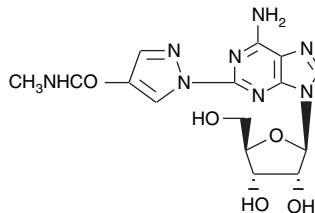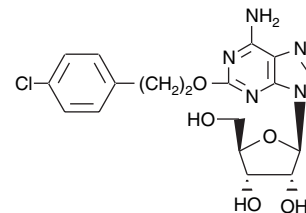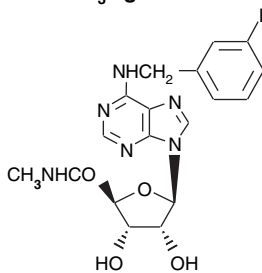
Apadenoson
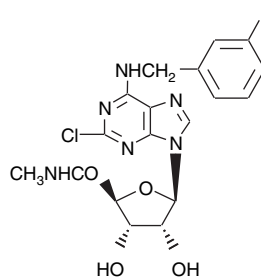(ATL-146e)

A₂B agonist

Bay 60-6583

Regadenoson
(CVT-3146)

MRE0094
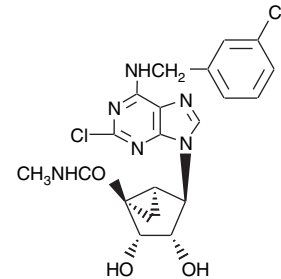
A₃ agonists

IB-MECA
(CF101)

Cl-IB-MECA
(CF102)

MRS3558
(CF502)

Figure 1. Structures of adenosine receptor agonists in clinical trials for various conditions*.
*Structures of GW493838 and BVT.115959 not disclosed.

Table 1. Binding affinity or functional potency of selected agonists in clinical trials at four subtypes of adenosine receptors (EC$_{50}$ and K$_i$, nM).

AR agonists	Potency at ARs				Diseases	Development stages
	A$_1$	A$_{2A}$	A$_{2B}$	A$_3$		
Adenosine (Adenocard, adenoscan)	310	700	24,000	290	PSVT; cardiac imaging	Marketed
Selodenoson (DTI0009)	1.1*	306*	ND	ND	AF	II
Tecadenoson (CVT-510)	6.5‡	2320‡	ND	ND	PSVT	III
CVT-2759	180*	ND	ND	ND	AF or PSVT	ND
Binodenoson (MRE0470)	48,000	270	430,000	903	Cardiac imaging	III
Apadenoson (ATL-146e)	77	0.5	ND	45	Cardiac imaging	III
Regadenoson (CVT-3146)	> 10,000	290	> 10,000	> 10,000	Cardiac imaging	III
MRE0094	> 10,000	59	> 10,000	ND	Diabetic foot ulcers	II
BAY-60-6583	> 10,000	> 10,000	~10	ND	Cardiac ischemia	ND
CF101 (IB-MECA)	51	2900	11,000	1.8	Rheumatoid arthritis	II
CF102 (Cl-IB-MECA)	220	5360	> 10,000	1.4	Colorectal cancer	ND
CF502 (MRS3558)	260	2300	> 10,000	0.29	Rheumatoid arthritis	ND

*Rat.
‡Pig.
Data for BVT.115959 and GW493838 are not available. An A$_{2A}$AR agonist BVT.115959 is in Phase II clinical trials for diabetic neuropathic pain. Phase II trials of GW493838 for the treatment of neuropathic pain has been completed. T62 is an allosteric enhancer of the A$_1$AR. For more information related to AR agonist potency and selectivity, see references [9,12,26,52,54].
AF: Atrial fibrillation; AR: Adenosine receptor; ND: Not determined or not disclosed; PSVT: Paroxysmal supraventricular tachycardia.

in humans and in animals. Given that the anticancer effect of A$_3$AR agonists is convincingly demonstrated, IB-MECA and Cl-IB-MECA (with highly oral bioavailability) may represent a novel family of anticancer agents.

IB-MECA has been in Phase II clinical trials for colorectal cancer. Cl-IB-MECA (CF102), a similar compound in its biologic properties to IB-MECA, is planned for use in cancer therapy in combination with chemotherapeutic drugs. Clinical studies carried out with CF101 that include some pilot studies in cancer patients, as well as extensive preclinical work, provide a rationale for use of CF102 in cancer therapy in combination with chemotherapy.

The structures of AR agonists presently in clinical trials are shown in **Figure 1**. The potencies of the AR agonists for four subtypes, their development stages and disease targets are listed in **Table 1**.

6. Potential development issues

Although selective agonists are available for all four subtypes, selectivity of some compounds for certain AR subtypes are still limited. Thus, potential side effects due to activation of AR subtypes other than the intended target are still possible.

Species-dependence for AR agonists is still a problem for the clinical development. This includes problems arising from studies using small animals if receptor homologs differ in pharmacology or same receptor function related to different subtypes in different species. For example, human and rat A$_3$ARs only share 72% overall identity of amino acids and A$_3$ARs are abundant in the rat, but not human testis. Mast cell degranulation is induced by the A$_{2B}$AR in dogs and humans, but by the A$_3$AR in mice. Thus, it can be speculated that some of the functions observed with one animal model may not be the case in other animal models and in humans. Cross-species testing systems are necessary to validate the receptor function or effects of agonists.

Another issue that should be noted is the pharmacokinetics of these agonists. Most AR agonists contain a ribose, which make these drugs hydrophilic. Thus, the penetration of these drugs to the CNS through the blood–brain barrier can be problematic. Of course it can also be argued that this property is advantageous if peripherally targeted. Adenosine itself can be transported by means of adenosine transporters, it is not clear if these transporters are permissible to other AR agonists.

In the clinical development of adenosine derivatives as AR agonists, one important aspect to be considered is that these agonists are nucleosides, which may interfere with nucleoside metabolism in the cells. For example, some selective A$_1$AR agonists are 5′-unmodified adenosines, which could be phosphorylated by adenosine kinases and then by nucleotide kinases and subsequently be incorporated into DNA or become agonists of P2Y receptors, the activation of which may induce different pharmacologic consequences. Also, the

stability of some compounds, such as ATL-146e, which is an ester derivative, could also be a development issue.

Additionally, as caffeine and theophylline are natural antagonists of ARs and are commonly consumed, the simultaneous antagonism should be considered when AR agonists are in clinical trials.

7. Expert opinion

With the availability of both selective agonists and genetic knockout animals for all four AR subtypes, it is expected that numerous roles of ARs that used to be ambiguous will be clarified in the near future. Also, with the identification of non-adenosine AR agonists, more selective and specific agonists for a certain subtype of ARs will hopefully be available. These agonists may show improved chemical and physical properties as drugs.

Adenosine itself is used under the name of Adenocard for controlling PSVT by activating the A_1AR, and under the name of Adenoscan for myocardial perfusion imaging by activating the $A_{2A}AR$. Despite its non-selective action, adenosine only shows moderate side effects due to its extremely short half-life (within seconds). The development of selective agonists for these purposes should be encouraging as they may exhibit reduced side effects. However, it should also be noted that the half-life of these synthesized agonists may be significantly longer than that of adenosine. Also, the presently available A_1 and A_{2A} AR agonists in clinical trials are only relatively selective. Both the longer half-life and the lack of absolute selectivity may contribute to additional side effects.

The A_1AR could be a promising target for neuropathic pain. Phase II clinical trials of GW-493838 as an A_1AR agonist for neuropathic pain have been completed. However, the next generation of A_1AR agonists for neuropathic pain have not been reported. It is assumed that the non-adenosine A_1AR agonists could be further clinically developed for neuropathic pain and other conditions.

Several $A_{2A}AR$ agonists are in trials as anti-inflammatory agents and for myocardial perfusion imaging. Compounds showing uncertain stability and lack of high selectivity may be useful for myocardial perfusion imaging and for external applications, such as diabetic foot ulcer and skin wound healing, but may not be suitable for anti-inflammatory agents

administered orally or intravenously, as a long-term and lasting effect is needed. The $A_{2A}AR$ agonists have shown robust immunosuppressive effect and tissue protective action as well. Thus, selective $A_{2A}AR$ agonists could be promising agents for induction therapy in organ transplantation. Additionally, it is now known that both the sleep-promoting effect of adenosine and the arousal effect of caffeine are through the $A_{2A}AR$, thus, the development of an $A_{2A}AR$ selective agonist that could penetrate the blood–brain barrier and produce minimal peripheral side effects would be extremely attractive. However, it should be noted that although the sleep-promoting effect of adenosine in rodents appears to be due to the $A_{2A}AR$ activation, there is an ongoing debate as to whether or not in humans the A_1AR may be more important. A recent positron emission tomography study has shown that sleep deprivation increases A_1AR binding in the human brain [96].

There will be new momentum in the $A_{2B}AR$ field, as both selective agonists and knockout mice are only recently available. Thus, some of the ambiguous AR functions, such as pro- and anti-inflammatory effects, ischemic protection and vasodilation need to be further clarified with these tools. One of the potent and selective non-adenosine $A_{2B}AR$ agonists, BAY-60-6583, has been shown to be effective in reduction of the infarct size by application after the onset of cardiac ischemia in a rabbit infarct model. Selective agonists for A_{2A} and A_3ARs could be potentially developed from non-adenosine agonists, which may show less species difference than nucleoside analogs (unpublished observation).

The abundance of the A_3ARs in inflammation-related blood cells, such as neutrophils and eosinophils, may suggest a role for the A_3AR in inflammation. However, the pro- or anti-inflammatory role of the A_3AR has to be further clarified. Nevertheless, preliminary clinical trial data suggest that the A_3AR may be a new promising target for RA. Regarding the anticancer role of some nucleosides or the A_3AR, it is still to be further investigated.

Acknowledgements

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