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REVIEW ARTICLE

Pathological overproduction: the bad side of adenosine

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Adenosine is an endogenous ubiquitous purine nucleoside, which is increased by hypoxia, ischaemia and tissue damage and mediates a number of physiopathological effects by interacting with four GPCRs, identified as A_1, A_{2A}, A_{2B} and A_3. Physiological and acutely increased adenosine is mostly associated with beneficial effects that include vasodilatation and a decrease in inflammation. In contrast, chronic overproduction of adenosine occurs in important pathological states, where long-lasting increases in the nucleoside levels are responsible for the bad side of adenosine associated with chronic inflammation, fibrosis and organ damage. In this review, we describe and critically discuss the pathological overproduction of adenosine and analyse when, where and how adenosine exerts its detrimental effects throughout the body.

Abbreviations

AD, Alzheimer's disease; ADK, adenosine kinase; Aβ, amyloid-β; BBB, blood–brain barrier; CD39, apyrase; CD73, 5′-nucleotidase; COPD, chronic obstructive pulmonary disease; EAE, experimental autoimmune encephalomyelitis; HD, Huntington's disease; HIF, hypoxia-inducible factor; IBD, inflammatory bowel diseases; KO, knockout; L-DOPA, levodopa; MDSC, myeloid-derived suppressor cells; MS, multiple sclerosis; NTs, nucleoside transporters; PD, Parkinson's disease; RLS, restless legs syndrome; αSyn, α-synuclein

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Tables of Links

TARGETS		
GPCRs[a]	**Enzymes**[b]	**Transporters**[c]
A_1 receptor	5′-nucleotidase	Glutamate transporter 1
A_{2A} receptor	Adenosine deaminase	SLC 28 and 29 nucleoside transporters
A_{2B} receptor	Adenosine kinase	
A_3 receptor	Apyrase (CD39)	
D_1 receptor	Caspase 1	
D_2 receptor	ERK1	
	ERK2	
	JNK	
	P38	
	PLC	
	PKA	

LIGANDS
Adenosine
ADP
ATP
cAMP
CXCL8
Dopamine
IL-2
IL-6
IL-13
IL-17A
VEGF-A

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c]Alexander *et al.*, 2015a,b,c).

Introduction

Adenosine is an endogenous ubiquitous autacoid regulating the function of every tissue and organ in the body (Borea *et al.*, 2016). It is produced both intra- and extracellularly following the activity of specific enzymes, and its extracellular concentration is tightly regulated within a range of 30–200 nM (Fredholm *et al.*, 2011). In particular, the main extracellular source of adenosine is the degradation of ATP and AMP by the ectoenzymes apyrase (CD39) and 5′-nucleotidase (CD73), respectively, whilst for the intracellular level, its generation depends on AMP hydrolysis by an intracellular CD73 or S-adenosylhomocysteine hydrolase (Zimmermann, 2000; Eltzschig, 2009). Adenosine concentration is maintained in equilibrium through equilibrative solute carrier family 29 (SLC 29) or concentrative solute carrier family 28 (SLC 28) nucleoside transporters (NTs) present in the cell membrane or through its phosphorylation operated by an intracellular adenosine kinase (ADK) (Alexander *et al.*, 2015c). In addition, adenosine signalling can be arrested following adenosine deaminase activity, which is present both inside and outside the cell, transforming adenosine to inosine. Extracellular adenosine increases up to μM levels in hypoxia/ischaemia due to the up- and down-regulation of CD73 and ADK, respectively; this increase in adenosine is characteristic of several important pathological conditions as it is involved in the regulation of energy supply/demand (Fredholm, 2014). Due to its rapid metabolism, adenosine behaves like an autocrine/paracrine rather than a systemic mediator (Figure 1).

Adenosine interacts with four adenosine receptors named A_1, A_{2A}, A_{2B} and A_3 belonging to the family of GPCRs (Borea *et al.*, 2015). All of them affect cAMP levels, A_1 and A_3 receptors being coupled to inhibitory Gi and A_{2A} and A_{2B} receptors to stimulatory Gs proteins

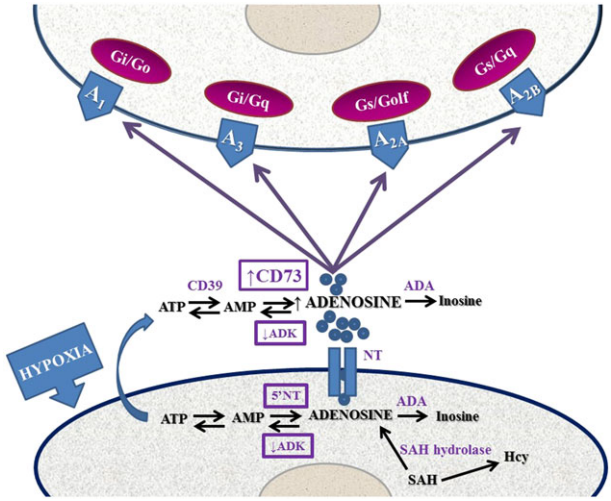

Figure 1

Schematic representation of the adenosinergic system from adenosine formation and release to signalling and removal from the extracellular space. Alterations occurring during hypoxia are shown. 5′NT, 5′-nucleotidase; ADA, adenosine deaminase; SAH, S-adenosylhomocysteine.

respectively. Furthermore, A_3 and A_{2B} receptors are also coupled to Gq proteins, leading to the activation of PLC and an increase in calcium levels. Upon activation of A_1 and A_3 receptors, K_{ATP} channels are opened. Furthermore, all adenosine receptors stimulate MAPKs, triggering a variety of intracellular signalling pathways relevant for a number of cell functions including growth and proliferation, apoptosis, necrosis and inflammation (Schulte and Fredholm, 2003).

The wide distribution of adenosine receptors in almost all cells of the organism makes them an attracting target for drug development in several diseases, even though this ubiquity makes the development of selective adenosine receptor agonists and antagonists difficult to realize without the occurrence of side effects (Chen *et al.*, 2013). Recently, we have published a review about the role of adenosine as a guardian angel against cellular damage, highlighting where, when and how adenosine exerts its protective mission under both physiological and pathophysiological conditions, in response to stress, both in the brain and in the periphery (Borea *et al.*, 2016). However, with regard to the 'guardian angel' metaphor, we should not forget that there are instances in which a chronic overproduction of adenosine is pathological, particularly in Parkinson's disease (PD), fibrosis, hepatic steatosis, colitis, asthma, diabetes and cancer.

In this review, we focus on the pathological overproduction of adenosine describing when, where and how it exerts its detrimental effects throughout the body.

Pathological role of adenosine in cerebral ischaemia

In the CNS, both neurons and glial cells produce adenosine and express adenosine receptors that affect several homeostatic functions ranging from sleep to arousal, learning, memory and cerebral blood circulation. The principal adenosine receptor subtypes located in the CNS are A_1 and A_{2A} receptors, which are crucial in the regulation of neurotransmitter release, neuronal excitability, synaptic plasticity, vasodilatation and neuroinflammation (Sebastiao and Ribeiro, 2009). In general, the role of A_1 receptors emerges as neuroprotective being an inhibitor of excitatory transmission, while that of the A_{2A} receptor is detrimental, even though evidence has been obtained showing that its activation has beneficial effects (Melani *et al.*, 2014; Pedata *et al.*, 2016). The molecular bases of the adverse effects of A_{2A} receptor stimulation are attributed to its modulation of neuronal glutamate release, potentiation of NMDA-mediated effects, central inflammatory processes, glial reactivity, blood–brain barrier (BBB) permeability and infiltration of peripheral immune cells, which are associated with excitotoxicity and the pathogenesis of several brain diseases (Yu *et al.*, 2004, 2008; Chen *et al.*, 2008a,b; Azdad *et al.*, 2009; Carta *et al.*, 2009; Gui *et al.*, 2009; Melani *et al.*, 2009; Dai *et al.*, 2010). Serendipitous pioneering studies about the involvement of A_{2A} receptors in ischaemia found that its antagonism protected the brain against ischaemic insults (Gao and Phillis, 1994; Lubitz *et al.*, 1995; Phillis, 1995; Monopoli *et al.*, 1998). Later, this finding was confirmed in A_{2A} receptor knockout (KO) mice and/or following pharmacological inhibition of the receptor, where a reduction in infarcted area was observed following ischaemic damage (Chen *et al.*, 1999; 2007; Chen and Pedata, 2008). It has been demonstrated that after ischaemia, excitotoxicity is the first phenomenon occurring in the brain in the first 4 h, when the A_{2A} receptor is responsible for the increase in glutamate levels, through both the release of glutamate from glutamatergic terminals and the inhibition of the glutamate-1 transporter (GLT-1) in astrocytes. Indeed, it has been shown that A_{2A} receptor blockade protects against excitotoxicity (Matos *et al.*, 2012b, 2013). Furthermore, A_{2A} receptor activation reduces the affinity of agonists for A_1 receptors in A_1–A_{2A} receptor heteromers, which exert a presynaptic control of striatal glutamate release, resulting in the fine-tuning of the modulatory effect on striatal glutamatergic neurotransmission (Ciruela *et al.*, 2006).

At the intracellular level, the inhibition of p38 MAPK in microglia and JNK in oligodendrocytes may be responsible for the protective effect mediated by A_{2A} receptor antagonists in ischaemia (Melani *et al.*, 2006, 2009). However, it is also known that later, hours and days after insult, there is a massive infiltration of blood cells and neuroinflammation that may be inhibited by activation of A_{2A} receptors located on blood cells. Accordingly, the chronic administration of an A_{2A} receptor antagonist for 7 days after transient focal ischaemia failed to protect the brain (Melani *et al.*, 2015), suggesting that A_{2A} receptor antagonists should be administered for a limited time window of about 4 h after stroke (Pedata *et al.*, 2016). However, it is important to mention that chronic enhanced levels of adenosine may lead to seizures, through A_{2A} receptor activation and neurotrophins, suggesting that in this context A_{2A} receptor antagonists could be useful clinically (Sandau *et al.*, 2016). Interestingly, recent evidence adds a new pathological aspect to adenosine overproduction related to the development of pain behaviour. This effect seems to be due to the activation A_{2B} receptors on myeloid cells, which are able to transactivate nociceptors of sensory neurons, and induce hypersensitive neurons and chronic pain through a pathway involving IL-6. Hence, the A_{2B} receptor may play a major role in chronic pain by promoting immune–neuronal interactions (Hu *et al.*, 2016) (Figure 2).

Pathological role of adenosine in neurodegenerative diseases

There is a well-established allosteric relationship between striatal A_{2A} receptors and dopamine D_2 receptors, supported by evidence that A_2 receptor activation is responsible for the decreased affinity of agonist binding to D_2 receptors (Ferrè *et al.*, 1991). This represented the first proof of concept for the therapeutic utility of A_{2A} receptor antagonists in PD (Armentero *et al.*, 2011; Preti *et al.*, 2015; Ferré *et al.*, 2016) (Figure 2). Interestingly, the well-known epidemiological consideration that caffeine protects against PD has been confirmed in an animal model of PD and attributed to A_{2A} receptor antagonism (Ross *et al.*, 2000; Ascherio *et al.*, 2001, 2004; Chen *et al.*, 2001; Yu *et al.*, 2008; Xu *et al.*, 2016). A_{2A} receptors and D_2 receptor heteroceptor complexes have been detected in cellular models and in the striatum (Hillion *et al.*, 2002; Canals *et al.*, 2003; Azdad *et al.*, 2009; Borroto-Escuela *et al.*, 2010, 2011, 2013; Varani *et al.*, 2010; Trifilieff *et al.*, 2011; Bonaventura *et al.*, 2015). As the receptors show antagonistic interactions at both membrane and intracellular signalling levels, dopamine depletion may be responsible for an overactivation of A_{2A} receptors and the consequent symptoms of PD (Peterson *et al.*, 2012). Therefore, antagonists of A_{2A} receptors are beneficial for the improvement in motor function in different animal models of PD, as they attenuate

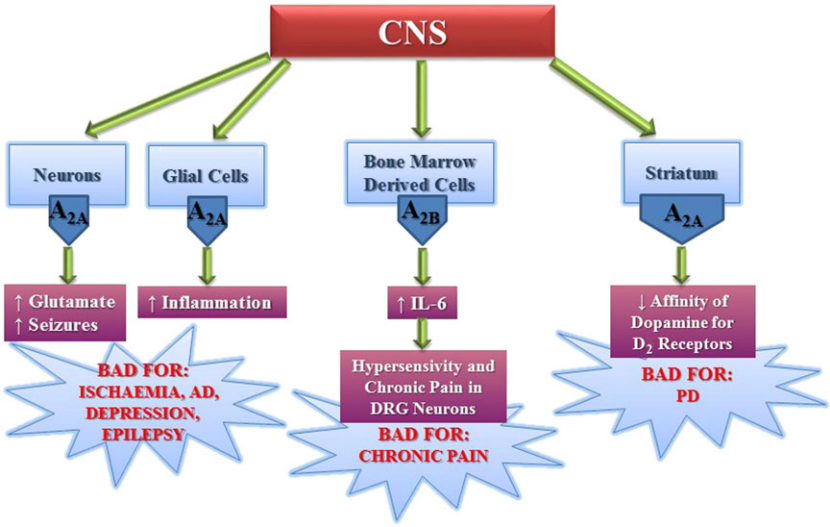

Figure 2

Deleterious effects of adenosine mediated through A_{2A} and A_{2B} receptors expressed in different areas of the CNS. DRG, dorsal root ganglion.

the inhibition exerted via A_{2A} receptors on the effects of D_2 receptors in the gabaergic striato-pallidal neurons (Fuxe *et al.*, 2015). Recently, pharmacological data have suggested a new model that indicates A_{2A} receptors and D_2 receptor heteromers form heterotetramers, which consist of A_{2A} receptors and D_2 receptor homodimers. This model supports the evidence that, at high concentrations, A_{2A} receptor antagonists exert the same effects as A_{2A} receptor agonists, thus reducing D_2 receptor-mediated activity in neurons; these findings could be important from a clinical point of view, when the use of A_{2A} receptor antagonists is being considered for therapy (Bonaventura *et al.*, 2015). Even though the development of A_{2A} receptor antagonists as new drugs for PD treatment has encountered several obstacles in the clinic, istradefylline has recently been registered in Japan, as it has been found to decrease the "off time" when used in combination with levodopa (L-DOPA). However, it has not receive approval by the American Food and Drug Administration, due to the lack of significant improvement in the phenomenon of "wearing off" in comparison with L-DOPA. Other potential new drugs like preladenant and vipadenant have failed in phase III clinical studies for PD (Navarro *et al.*, 2016; Oertel and Schulz 2016). The reasons for which these drugs failed were the lack of significant benefits of preladenant combined with L-DOPA when compared to L-DOPA alone, while vipadenant was found to have toxic effects. It has been hypothesized that administration of this class of compounds should start as early as possible to avoid a structural change in the A_{2A} receptor–D_2 receptor heteroceptor complexes that may induce dyskinesias and the onset of "off time" caused by L-DOPA (Fuxe *et al.*, 2015). Another pharmacological approach for PD treatment is suggested from the results obtained with bivalent drugs acting at A_{2A} receptor–D_2 receptor complex (Soriano *et al.*, 2009), with integrated dual acting ligands having an improved efficacy in preliminary BBB permeability tests (Jörg *et al.*, 2015). Recently, a novel protective effect of A_{2A} receptor antagonists has been attributed to the modulation of α-synuclein (αSyn) effects, as α-synuclein-induced damage to striatal neurons was clearly reduced in A_{2A} receptor KO mice and A_{2A} receptor antagonists prevented the neurotoxicity related to α-synuclein aggregation. These results provide additional evidence in support of antagonists as effective drugs for the treatment of PD and related synucleinopathies (Kachroo and Schwarzschild, 2012; Ferreira *et al.*, 2015). The use of A_{2A} receptor antagonists has been found useful in other pathologies involving neuronal dysfunction including Huntington's (HD) and Alzheimer's (AD) diseases as well as major depression and schizophrenia, epilepsy, acute and chronic stress, restless legs syndrome (RLS) and memory fear (Cunha, 2005, 2016; Canas *et al.*, 2009; Batalha *et al.*, 2013; Krügel, 2016; Laurent *et al.*, 2016; Quiroz *et al.*, 2016; Simões *et al.*, 2016) (Figure 2). Different studies have reported an up-regulation of A_{2A} receptors in animal models of HD (Varani *et al.*, 2001; Tarditi *et al.*, 2006), and recently, it has been shown that A_{2A} receptor antagonists block working memory deficits at early stages of HD models (Li *et al.*, 2015). Hyperactivation of both D_1 and A_{2A} receptors has been found in HD striatum where D_1 plus A_{2A} receptor antagonists reduced PKA activity, which is involved in hippocampal-dependent cognitive dysfunction in HD, further supporting a therapy based on A_{2A} receptor blockade. Nevertheless, evidence in favour of A_{2A} receptor activation as a strategy for HD treatment has also been reported (Gomes *et al.*, 2011; Martire *et al.*, 2013; Tyebji *et al.*, 2015). In addition, a positive action of A_{2A} receptors in the early phases of neurodegeneration has been attributed to the presynaptic facilitation of GABA transmission exerted by A_{2A} receptor agonists, involving brain-derived neurotrophic factor–tropomyosin receptor kinase B in the hippocampus (Colino-Oliveira *et al.*, 2016).

A change in the pattern of adenosine receptors with a decrease in A_1 and an increase in A_{2A} receptors has been also detected in AD. As a consequence A_{2A} receptor antagonists have been found to prevent neuronal death and the decrease in astrocytic glutamate uptake, caused by an amyloid-β (Aβ)

fragment, that may be responsible for excitotoxicity in AD (Dall'Igna *et al.*, 2003; Canas *et al.*, 2009; Matos *et al.*, 2012a). Importantly, it has been shown that humans with AD have increased levels of A_{2A} receptors in astrocytes. Also, in young and aging mice, their genetic removal increased long-term memory and increased the levels of an immediate-early gene necessary for memory (Orr *et al.*, 2015). Furthermore, deletion of A_{2A} receptors has been shown to have a protective effect from spatial memory and hippocampal long-term depression caused by Tau pathology (Laurent *et al.*, 2016; Müller *et al.*, 2016). In addition, A_{2A} receptors are up-regulated in CA3 synapses at early stages of AD and their antagonism reversed the block of long-term synaptic potentiation in a mouse model of AD amyloidosis (Viana da Silva *et al.*, 2016). Caffeine has been found to have a protective effect against cognitive impairment in both human and animal studies (Maia and de Mendonça, 2002; Ritchie *et al.*, 2007; Smith, 2009; Santos *et al.*, 2010). Furthermore, caffeine reduced plasma and brain Aβ levels in an animal model of AD and prevented memory deficits caused by Aβ administration (Dall'Igna *et al.*, 2007; Cao *et al.*, 2009). Interestingly, the initial findings of a case–control study were the first to demonstrate that caffeine/coffee consumption is associated with a reduced risk, or delayed onset, of dementia (Cao *et al.*, 2012). Therefore, it seems that caffeine, the most popular and widely used drug in the world, by antagonizing the effects of adenosine, retains a big potential to counteract different neurodegenerative diseases (Woods *et al.*, 2016).

Due to their modulation of glutamatergic and monoaminergic transmission in the striatum, A_{2A} receptors may also be involved in depression (Krügel, 2016). In behavioural animal models, A_{2A} receptor antagonists produced antidepressant effects (El Yacoubi *et al.*, 2003; Hodgson *et al.*, 2009). However, istradefylline was found to exert them independently from monoaminergic transmission (Yamada *et al.*, 2014a,b). Furthermore, caffeine or selective A_{2A} receptor antagonists reversed the performance deficits in reserpine-treated rats and prevented mood and memory dysfunctions induced by chronic stress (Batalha *et al.*, 2013; Kaster *et al.*, 2015; Minor and Hanff, 2015). In addition, in a broad-based model of depression mediated by an increase in hippocampal A_{2A} receptors, which control synaptic glutamatergic function, caffeine was able to prevent memory, but not mood, deficits (Machado *et al.*, 2016).

Overall, based on the progress obtained in clarifying the molecular mechanisms underlying CNS diseases, adenosine reveals its bad side essentially through the activation of A_{2A} receptors, which may be considered as targets of therapeutic strategies and may represent a promising future in the battle against a wide spectrum of unmet central diseases.

Pathological role of adenosine in autoimmune diseases

Immune cells express all adenosine receptors, which means adenosine can affect inflammatory and immune events (Cekic and Linden, 2016). In particular, it was found that adenosine modulates immune function by exerting inhibitory effects on neutrophils, lymphocytes, monocytes/macrophages and dendritic cells (Mills *et al.*, 2012). Therefore, a possible area of investigation for novel therapeutic strategies through adenosine receptor modulation is the study of adenosine's role in the immunopathogenesis of multiple sclerosis (MS).

Interestingly, it has been observed that mice with CD73 knocked-down, the critical enzyme for generating extracellular adenosine in various cells including T cells, are protected against experimental autoimmune encephalomyelitis (EAE, an animal model for MS) and lack the CNS lymphocyte infiltrates associated with disease progression (Mills *et al.*, 2008).

Moreover, caffeine decreases the incidence of EAE and attenuates it at the behavioural, histological and neurochemical levels in mice and rats (Tsutsui *et al.*, 2004; Chen *et al.*, 2010). Furthermore, it shifted the status of the cytokine levels from Th1, pro-inflammatory to Th2, anti-inflammatory in EAE, then ameliorated inflammatory injury in the brain and spinal cord, suggesting it has a neuroprotective effect via its role as an immunomodulator (Chen *et al.*, 2010).

Surprisingly, even though the A_{2A} receptor is recognized as a major mediator of anti-inflammatory responses, it has been reported that the pharmacological blockade of this subtype attenuates EAE pathology in CD73 KO mice (Mills *et al.*, 2008). Furthermore, A_{2A} receptor blockade prevented oligodendrocyte damage through a reduction of JNK/MAPK signalling in these cells (Melani *et al.*, 2009). However, to explain the beneficial effects of A_{2A} receptor antagonists in EAE, it has been suggested that although the A_{2A} receptor present on lymphocytes is anti-inflammatory, that expressed in the CNS plays a pro-inflammatory role and appears to be essential for EAE development (Mills *et al.*, 2012). However, an A_{2A} receptor agonist worsened experimental autoimmune neuritis through an inhibitory effect of T-cell proliferation and IL-2 secretion (Zhang *et al.*, 2016). The role of A_{2B} receptors in the pathogenesis of MS has also been investigated by knocking out this subtype or blocking it with selective antagonists. A_{2B} receptors have been found to be up-regulated in peripheral blood leukocytes of MS patients and the peripheral lymphoid tissues of EAE mice. Furthermore, pharmacological blockade of the A_{2B} receptor ameliorated the clinical symptoms of EAE and reduced immune damage in the CNS. Importantly in A_{2B} receptor KO mice, EAE was less severe. The mechanism involved was suggested to be the inhibition of Th17 cell differentiation by the blockade of IL-6 production from dendritic cells, via the PLCβ–PKC and p38 MAPK pathways (Wei *et al.*, 2013). Accordingly, A_{2B} receptor stimulation in dendritic cells greatly increased the development of experimental autoimmune uveitis (EAU) by enhancing the Th17 autoimmune responses (Chen *et al.*, 2015). In conclusion, adenosine exerts a complex role, acting at A_{2A} and A_{2B} receptors to increase inflammation, EAE and EAU.

Pathological role of adenosine in inflammatory conditions

Inflammation is a hallmark of important diseases such as cardiovascular, metabolic, intestinal and pulmonary

disorders (Hotamisligil, 2006; Libby *et al.*, 2011; Barnes, 2016; Karin and Clevers, 2016). As for the detrimental signalling of adenosine in inflammatory conditions, we have to focus on A_{2B} receptors as mediators of the effects of autacoids because this subtype is activated by μM concentrations of adenosine, occurring in inflammation, hypoxia and cell injury (Fredholm, 2007; Bartels *et al.*, 2013). In particular, the expression of A_{2B} receptors is increased in hypoxia in response to hypoxia-inducible factors (HIFs) (Eckle *et al.*, 2014). Indeed, it has been demonstrated that A_{2B} receptors have a role in the inflammatory environment typically present in asthma, chronic obstructive pulmonary disease (COPD), kidney pathologies and inflammatory bowel diseases (IBD) (Kolachala *et al.*, 2005; Haskó *et al.*, 2008). Likewise, A_3 receptors expressed in the human lung, mast cells and in macrophages enriched with lipids, known as foam cells (FC), play a role in the regulation of the inflammation present in pulmonary and atherosclerotic diseases (Hua *et al.*, 2008; Gessi *et al.*, 2010; Borea *et al.*, 2015).

The A_{2B} receptor is present in the intestinal epithelium where it exerts a controversial role in inflammation (Kolachala *et al.*, 2005). Indeed, different data have reported that A_{2B} receptors are able to protect from inflammation development in the intestine (Schingnitz *et al.*, 2010; Hart *et al.*, 2011; Aherne *et al.*, 2015). However, the expression of A_{2B} receptors in non-immune cells plays an important role in the occurrence of murine colitis. Indeed, colitis is attenuated in A_{2B} receptor KO mice and after of pharmacological blockade of A_{2B} receptors, indicating that A_{2B} receptor antagonism may be an effective treatment for acute inflammatory intestinal diseases, such as IBD (Kolachala *et al.*, 2008a,b; Ingersoll *et al.*, 2012).

Adenosine is responsible for the production of VEGF, IL-6 and IL-8 through interaction with all four adenosine receptors in inflamed tissues, in which the levels of these autacoids are increased (Cekic and Linden, 2016; Koszałka *et al.*, 2016). However, the A_{2B} and A_3 receptor subtypes are mainly involved in the modulatory effects of adenosine on wound healing processes, for example, angiogenesis and fibrosis (Zhou *et al.*, 2011) (Figure 3). The A_{2B} receptor plays a significant role in the chronic phase of wound healing after tissue injury and increases the adverse signalling responsible for the continuous tissue remodelling and fibrosis occurring

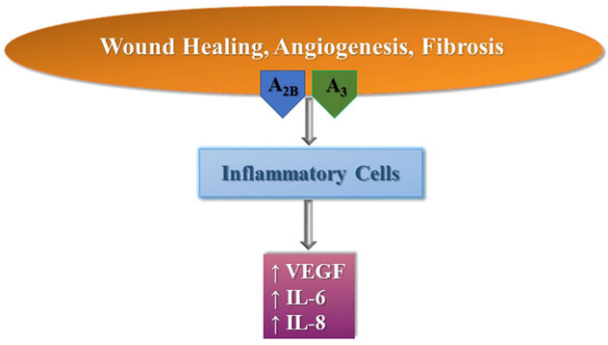

Figure 3

Modulation of wound healing, angiogenesis and fibrosis by A_{2B} and A_3 receptors in inflammatory cells.

in chronic inflammatory conditions (Feoktistov *et al.*, 2009). Pulmonary fibrosis is a harmful lung disease with limited therapeutic options. Adenosine has a role as a pro-inflammatory messenger in different chronic pulmonary inflammatory conditions, such as asthma and COPD (Cekic and Linden, 2016). Very recently, it has been reported that increased extracellular adenosine concentrations, by inducing the production of IL-6 and IL-17, are associated with the progression of experimental pulmonary fibrosis (Luo *et al.*, 2016). As the A_{2B} receptor promotes Th17 differentiation (Wilson *et al.*, 2011), it has been hypothesized that adenosine may induce IL-17 expression through its A_{2B} receptor subtype in chronic lung injury, thus contributing to lung fibrosis. Furthermore, adenosine promotes the differentiation of alternatively activated macrophages, a macrophage subtype that has been shown to contribute to pulmonary fibrosis, stimulated by Th2 cytokines (IL-4 and IL-13) (Karmouty-Quintana *et al.*, 2015). In addition, it has been reported that A_{2B} receptors are up-regulated in lung tissue from idiopathic pulmonary fibrosis patients (Zhou *et al.*, 2010) and that the pharmacological blockade of A_{2B} receptors in mice (Karmouty-Quintana *et al.*, 2012, 2013b; Sun *et al.*, 2006) or genetic removal of this subtype (Zhou *et al.*, 2011) are associated with decreased fibrosis. Therefore, it is important to verify and understand the association between extracellular adenosine levels and the progression of pulmonary fibrosis to develop new drugs for this pathology based on the structures of adenosine ligands binding to A_{2B} receptors. As for the role of adenosine in asthma and COPD, it is interesting to note that A_{2B} receptor signalling promotes the production of Th2-type cytokines and the recruitment and degranulation of eosinophils (Belikoff *et al.*, 2012; Karmouty-Quintana *et al.*, 2013a). Furthermore, it has been known for a long time that the secretion of IL-4 by human mast cells is mediated through A_{2B} receptors and this increases IgE production by B cells and as a consequence triggers allergic inflammation (Ryzhov *et al.*, 2006, 2008). In addition, there is unequivocal evidence from studies in rodent mast cells that the A_3 receptor is involved in the bronchoconstrictor response; however, this has not been verified in humans (Rudich *et al.*, 2012). On the one hand, in primary human lung mast cells, stimulation of A_3 receptors increases IgE-induced degranulation (Feoktistov *et al.*, 2003; Gomez *et al.*, 2011); and on the other, no effect was obtained in human umbilical cord blood-derived mast cells (Hua *et al.*, 2011) or in the LAD2 human mast cell line (Leung *et al.*, 2014), suggesting that this response depends on the tissue studied (Gomez *et al.*, 2011). Recently, it has been demonstrated that the primary function of the A_3 receptor is to prime the human mast cells towards tissue remodelling activity (Rudich *et al.*, 2015). Therefore, A_{2B} and A_3 receptor antagonists may also be useful for the treatment of asthma and COPD (Figure 4).

In addition, more recently, it has been demonstrated that A_{2B} receptor blockade in hypoxia significantly decreased renal fibroblast proliferation and activation with a consequent reduction of profibrotic cytokine release, thus preventing the generation and development of renal fibrosis, the outcome of all types of chronic kidney disease (Tang *et al.*, 2015). Indeed, in a novel mouse model of renal and cardiovascular disease induced through uninephrectomy

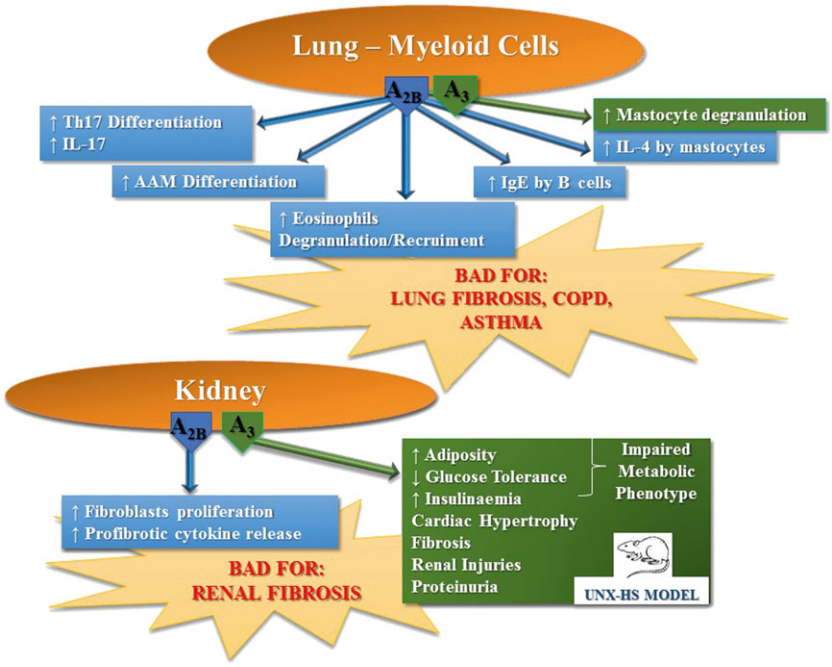

Figure 4

Deleterious effects of adenosine mediated through A_{2B} and A_3 receptors expressed in the lung and kidney. AAM, alternatively activated macrophages; HS, high salt; UNX, uninephrectomy.

and chronic high salt intake, the inhibition of A_3 receptor signalling prevented the development of hypertension and attenuated the cardiac hypertrophy and fibrosis together with renal injuries and proteinuria (Yang *et al.*, 2016) (Figure 4). This finding accords with that of a previously reported study showing that A_3 receptor antagonism reduces the progression of renal fibrosis (Lee *et al.*, 2013). Overall, these findings indicate that A_{2B} and A_3 receptor antagonists could be an interesting new therapeutic strategy for the development of new drugs to prevent tissue remodelling after tissue ischaemia and, consequently, for the treatment of fibrotic diseases including heart failure. Interestingly, it has been shown in a rat model that the blockade of A_{2B} receptors, beginning 1 week after myocardial infarction, reduces the pathology of this condition (Zhang *et al.*, 2014).

Many cardiovascular diseases have atherosclerosis as an aetiological factor. In particular, endothelial dysfunction with the accumulation and oxidation of LDL leads to an increase in foam cells (Eisenstein *et al.*, 2015). The role of adenosine receptors in the development of atherosclerosis has been investigated. Adenosine has been shown to increase the accumulation of HIF-1α through the stimulation of all of its receptors. This is important because hypoxia is characterized by atherosclerotic plaques and HIF-1 promotes intraplaque angiogenesis and the development of FCs (Gessi *et al.*, 2010). The signalling mediating this effect on HIF-1α induced by stimulation of A_1, A_{2A} and A_{2B} receptors involves ERK1/2, p38 MAPK and Akt phosphorylation, while only ERK1/2 is involved in the activation induced by A_3 receptors. In addition, A_{2B} and A_3 receptors stimulate VEGF production in an HIF-1α-dependent manner. Finally, adenosine-stimulated FC formation was decreased by the pharmacological blockade of A_3 and A_{2B} receptors and by the silencing of HIF-1α. This study indicated for the first time that A_3 and A_{2B} receptor antagonists may be useful for preventing the development of atherosclerotic plaques (Gessi *et al.*, 2010). In addition, A_{2B} receptor antagonists have been found to inhibit fatty liver formation in mice post alcohol assumption (Peng *et al.*, 2009). However, in contrast, in a different study, using an *in vivo* mouse model of atherosclerosis, the role of A_{2B} receptors in plaque formation was found to be protective (Koupenova *et al.*, 2012).

Pathological role of adenosine in diabetes

It is well recognized that adenosine regulates insulin secretion, glucose homeostasis and lipid metabolism, by stimulation its receptors. The stimulation of A_1 and A_{2A} receptor subtypes seems to promote an antidiabetic phenotype even though recently it has been shown that blockade of A_1 receptor activation offers protection from age-related oxidative stress and secretion of pro-inflammatory cytokines, thus improving insulin release and effect (Yang *et al.*, 2015). As for the A_{2B} receptor, its role is still controversial with a number of studies supporting a role for its agonists as a therapy for diabetes (Johnston-Cox *et al.*, 2012; 2014; Peleli *et al.*, 2015). However, this protective effect is challenged by a series of studies reporting the beneficial effects of A_{2B} receptor antagonists. It was firstly reported that A_{2B} receptor antagonists behave as hypoglycaemic agents in rat models of hepatic glucose production induced by adenosine (Harada *et al.*, 2001a,b). Furthermore, it was found

that A_{2B} receptor activation increases glucose production by affecting glycogenolysis and gluconeogenesis in the rat liver (Yasuda *et al.*, 2003). Following this line, A_{2B} receptor antagonists were shown to counteract the reduction in insulin levels induced by a non-selective adenosine receptor agonist in pancreatic cells and plasma from rats, even though this effect is not mediated through A_{2B} receptor activation (Rüsing *et al.*, 2006). A_{2B} receptor antagonists were also demonstrated to reduce the levels of IL-6 and other cytokines affecting glucose and fat metabolism in a diabetic mouse model, thus improving insulin resistance (Figler *et al.*, 2011). Furthermore, A_{2B} receptor blockade reduced the activation of the pro-inflammatory caspase-1 in rat retinal cells incubated in hyperglycaemic conditions (Trueblood *et al.*, 2011; Vindeirinho *et al.*, 2016). Interestingly, it has been found that high glucose levels and experimental diabetes increase the concentrations of adenosine in plasma by reducing the equilibrative NT in proximal tubule cells. This increase correlated with a marker of renal fibrosis in diabetic rats. Furthermore, the expression of profibrotic cell activation markers α-smooth muscle actin and fibronectin was increased by stimulation of A_3 receptors (Kretschmar *et al.*, 2016) (Figure 5).

Therefore, before establishing a role for A_{2B} receptor agonists or A_{2B}/A_3 receptor antagonists in diabetic therapy, when looking at the evidence it is important to accurately examine the experimental conditions associated with glucose and insulin regulation including the method used for inhibiting the receptors (pharmacological vs. genetic) and the cell types or model system used (Antonioli *et al.*, 2015; Merighi *et al.*, 2015).

Pathological role of adenosine in cancer

Adenosine plays a role in promoting cancer development by evoking immunosuppressive effects and directly affecting the growth, metastasis and angiogenesis of tumour cells (Antonioli *et al.*, 2014; Allard *et al.*, 2016; Di Virgilio and Adinolfi, 2016; Ohta, 2016) (Figure 6). There is a strong relationship between cancer, hypoxia and adenosine metabolism resulting in increased levels of the autacoids in hypoxic tumours. This effect is essentially a consequence of specific alterations in the enzymes involved in adenosine production, for example, the overexpression of CD73 and the down-regulation of adenosine kinase (ADK) are both increased by hypoxia (Ohta, 2016). Indeed, clinical studies have reported that the expression of CD73 is associated with a poor prognosis in different types of cancer (Antonioli *et al.*, 2016), including breast (Loi *et al.*, 2013), ovarian (Turcotte *et al.*, 2015), prostate (Leclerc *et al.*, 2016), brain (Quezada *et al.*, 2013) and leukaemia (Coustan-Smith *et al.*, 2011; Serra *et al.*, 2011). It has been reported that antibody- or genetic silencing-mediated inactivation of CD73 inhibited breast, prostate, fibrosarcoma and melanoma tumour growth (Zhi *et al.*, 2007; Stagg *et al.*, 2010; 2011; 2012; Terp *et al.*, 2013). The effects of CD73 on cancer development have been attributed to the immunosuppressive effects of A_{2A} and A_{2B} receptor stimulation (Beavis *et al.*, 2013; Young *et al.*, 2016). In particular, adenosine induces an immune-tolerant micro-environment around tumours affecting the functions of immune and inflammatory cells like T- and natural killer (NK) cells, macrophages and dendritic and myeloid-derived suppressor cells (MDSC). Treg cells express high levels of

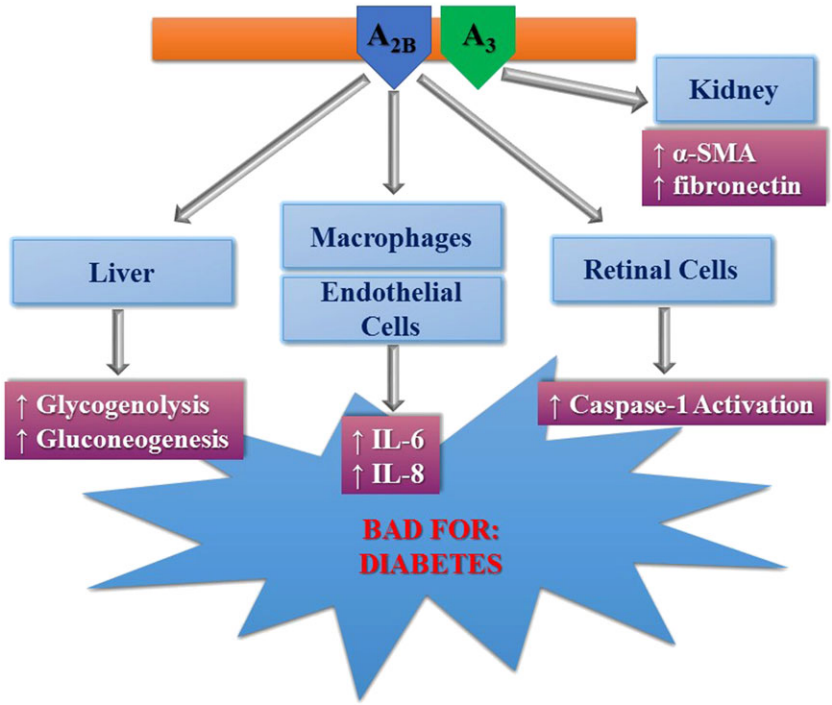

Figure 5
Deleterious effects of adenosine mediated through A_{2B} and A_3 receptors in diabetes. α-SMA, α-smooth muscle actin.

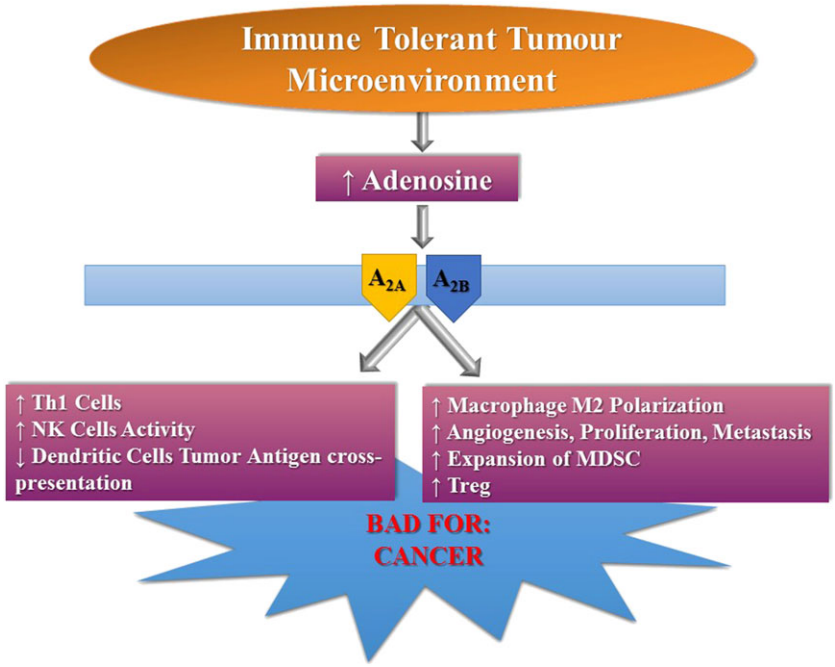

Figure 6
Deleterious effects of adenosine mediated through A_{2A} and A_{2B} receptors in cancer.

CD39 resulting in an increased production of adenosine, which then inhibits the antitumour immune response; this effect of adenosine is mediated through inhibition of NK effector lymphocytes that lose their ability to recognize neoplastic cells. This effect was initially attributed to A_3 receptor activation, but is now thought to be mediated by A_{2A} receptors (MacKenzie *et al.*, 1994; Ohta *et al.*, 2012; Antonioli *et al.*, 2013). As for tumour-associated macrophages, stimulation of A_{2A} and A_{2B} receptors induces an enhanced expression of the M2 phenotype, which contributes to the angiogenesis, proliferation and metastasis of tumour cells (Csóka *et al.*, 2012). In addition, A_{2B} receptors mediate the increased production of VEGF by dendritic cells; VEGF is essential for tumour angiogenesis and growth, and favours the activity of MDSC that are able to suppress immune surveillance (Ryzhov *et al.*, 2011). Overall, these results have led to the development of phase I clinical trials that are now underway to consider the safety and the efficacy of CD73 and A_{2A} receptor inhibitors in cancer patients (Allard *et al.*, 2016).

The adenosine machinery also mediates its effects directly in tumour cells by affecting their proliferation and cell death through recruitment of different receptors. In particular, opposite effects on cell growth and motility have been observed, with A_1, A_{2A} and A_{2B} receptors being promoters whilst A_3 receptors are inhibitors of cell proliferation (Merighi *et al.*, 2002; 2005; Gessi *et al.*, 2011a; Antonioli *et al.*, 2013; Borea *et al.*, 2015; Ledderose *et al.*, 2016). Indeed, it is important to note that clinical trials are undergoing for A_3 receptor agonists, based on their antiproliferative effects, representing an opportunity for new anticancer drugs (Borea *et al.*, 2016).

Nevertheless, it has been reported that A_{2B} receptors have a prometastatic and prosurvival effect and that stimulation of A_1, A_{2A} and A_3 receptors increases melanoma growth, neovascularization, angiogenesis and macrophage infiltration in CD73 KO mice (Linden, 2013; Ntantie *et al.*, 2013; Koszałka *et al.*, 2016) (Figure 5).

Interestingly, an overexpression of A_3 receptors has been reported in different tumours including colon, breast, hepatocellular and mesothelioma (Madi *et al.*, 2004; Gessi *et al.*, 2004; 2011b; Varani *et al.*, 2011; Borea *et al.*, 2015). Furthermore, an up-regulation of A_{2B} receptors has also been observed in colorectal cancer (Ma *et al.*, 2010). Therefore, we can conclude that the important effects adenosine exerts on the progression and development cancer depend on the subtype of adenosine receptor expressed in each tumour.

The negative actions of long-lasting increases in the extracellular adenosine concentration lead to overactivation of membrane receptors, thus opening the way for the clinical development of specific antagonists. First of all, starting with the CNS injuries, A_{2A} selective antagonists may have a role in the therapy of ischaemia, where a careful attention to administration time and to the dose of A_{2A} receptor antagonist has to be considered for a novel therapeutic strategy. PD and other neurodegenerative diseases such as HD and AD, major depression, schizophrenia, epilepsy, acute and chronic stress, RLS and memory fear are pathologies for which A_{2A} selective inhibitors could became new drugs. Even though istradefylline, in combination with L-DOPA, is now approved in Japan for reducing the "off time" in PD, to date no disease-modifying treatment is available. Therefore, the research to identify new therapeutics for PD should be increased and the reasons why the translation of basic

research to disease-modifying therapies has been unsuccessful so far should be investigated. The new elegant evidence regarding the modulation of heterotetrameric structure of the A_{2A}/D_2 receptor complex and the similarity between A_{2A} receptor agonist and antagonist effect on the affinity and intrinsic efficacy of D_2 receptor ligands offers a novel model that can provide new clues about how to adjust the clinical use of these drugs (Ferré *et al.*, 2016).

In MS, an autoimmune disease of central origin, both A_{2A} and A_{2B} receptor antagonists attenuated the increased inflammation and then the development of its pathology. Hence, the blockade of these receptors might be a new strategy for the development of new drugs for MS therapy.

Understanding the effect of A_{2B} receptor modulation in intestinal inflammation, glucose and lipid regulation is necessary to clarify whether this subtype is protective or injurious in both colitis, diabetes and atherosclerosis, respectively, with the aim of adequately recognizing its therapeutic potential.

Furthermore, A_{2B} and A_3 receptor antagonists may represent a starting point for the development of novel drugs to hamper remodelling occurring after tissue ischaemia and for the therapy of consequent fibrotic diseases arising in the lung, kidney and heart.

The pioneering research by Sitkowsky and co-workers on tumours and hypoxia also opens the way for the use of A_{2A} receptor antagonists in the therapy of cancer. Due to their immunosuppressive effects and tumour tolerant behaviour, now both A_{2A} an A_{2B} receptors are considered important and promising targets for the development of a novel class of antitumoural drugs able to weaken the hypoxia-adenosine mediated signalling pathways. It is important to note that existing A_{2A} receptor antagonists tested in human clinical studies for PD have already demonstrated their safety profile. However, in the future, it will be important to consider the development of new A_{2A} receptor blockers unable to cross the BBB, thus avoiding the occurrence of potential neurological side effects in cancer patients with non-cerebral tumours (Hatfield and Sitkovsky, 2016). This task may be supported by important advances in the structure-based design of A_{2A} receptor antagonists that take advantage of the recently revealed molecular basis of this adenosine subtype (Carpenter *et al.*, 2016; Ye *et al.*, 2016; Jazayeri *et al.*, 2017).

Conclusions and perspectives

It is well known that adenosine is beneficial, adopting the role of guardian angel when present at physiological levels or when increased in acute situations (Borea *et al.*, 2016). However, we have to remember that there are instances in which its chronic overproduction is pathological. In this review, we have presented evidence for its bad side, particularly in association with ischaemia, neurodegenerative diseases, fibrosis, hepatic steatosis, colitis, asthma, diabetes and cancer, while focusing on the potentiality of this system as a new therapeutic weapon in the battle against important unmet diseases. Indeed, A_{2A}, A_{2B} and A_3 receptor antagonists may find a specific/unique place in different clinical applications. Overall, this is an exciting period for scientists involved in the adenosinergic field seeking to harness the information for new therapeutic applications.

Conflict of interest

The authors declare no conflicts of interest.

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