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The Role of Adenosine Signaling in Sickle Cell Therapeutics

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Abstract

Recent data suggest a role for adenosine signaling in the pathogenesis of sickle cell disease (SCD). Signaling through the adenosine A_{2A} receptor ($A_{2A}R$) has demonstrated beneficial effects in SCD. Activation of $A_{2A}Rs$ decreases inflammation in mice and patients with SCD largely by blocking activation of invariant NKT cells. Decreased inflammation may reduce the severity of vaso-occlusive crises. In contrast, adenosine signaling through the A_{2B} receptor ($A_{2B}R$) may be detrimental for patients with SCD. Priapism and the formation of sickle erythrocytes may be a consequence of $A_{2B}R$ activation on corpus cavernosal cells and erythrocytes, respectively. Whether adenosine signaling predominantly occurs through $A_{2A}Rs$ or $A_{2B}Rs$ may depend on differing levels of adenosine and disease state (steady state versus crisis). There may be opportunities to develop novel therapeutic approaches targeting $A_{2A}Rs$ and/or $A_{2B}Rs$ for patients with SCD.

Keywords

sickle cell disease; adenosine; adenosine A_{2A} receptor; adenosine A_{2B} receptor; NKT cells

Introduction

Sickle cell disease (SCD) is characterized by rigid, sickle-shaped erythrocytes, microvascular occlusion and tissue ischemia[1]. Sickle erythrocytes initiate the development of vaso-occlusion that ultimately leads to tissue ischemia in a complex multi-cellular

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process. Tissue ischemia promotes an inflammatory response that is further amplified by ischemia re-perfusion injury (IRI) [2]. Thereafter, a vicious cycle of vaso-occlusion, tissue injury and inflammation is set into motion promoting further erythrocyte sickling[1,2]. The consequences of vaso-occlusion are pain, end-organ damage and often premature death[3,4].

Therapies to prevent or treat sickle cell vaso-occlusion are limited. Broadly, treatments either prevent the formation of sickle erythrocytes (e.g., hydroxyurea) or interrupt the cellular interactions that follow red cell sickling and lead to vaso-occlusion[5]. Hydroxyurea is an anti-mitotic agent that disrupts the polymerization of sickle hemoglobin and, to date, is the only FDA-approved therapy for the prevention of painful vaso-occlusive crises (pVOC)[6]. Patient and provider barriers have limited the widespread use of hydroxyurea in patients with SCD, affecting the impact of the drug[7]. Hematopoietic stem cell transplantation is a potential cure; however, it is only an option for a few patients with SCD[8].

In this review, we examine the role of adenosine signaling in SCD pathogenesis. There are opportunities to modulate adenosine pathways using therapies to prevent or treat SCD complications. As evidence has emerged about the importance of adenosine in SCD, separate lines of investigation have demonstrated protective and detrimental effects of adenosine in regards to disease severity. Recent data suggest that actions of adenosine mediated through the adenosine A_{2A} receptor ($A_{2A}R$) decrease inflammation, largely by selectively inhibiting the activation of a subset of lymphocytes called invariant NKT (iNKT) cells[9–11]. In contrast, other studies have shown that adenosine signaling through the adenosine A_{2B} receptor ($A_{2B}R$) may contribute to the adverse processes of erythrocyte sickling[12] and priapism[13–15]. Although much additional work is needed to fully elucidate the roles of adenosine signaling in SCD, targeting these pathways may produce novel therapeutic approaches.

Adenosine signaling pathway

Adenosine physiology

Adenosine signaling protects tissues by promoting vaso-dilation as well as decreasing heart rate and inflammation[16]. During periods of cellular hypoxia or stress, adenosine is released from cells along with the adenine nucleotides, ATP, ADP and AMP, which are converted to adenosine by ecto-nucleotidases. Binding of adenosine to four receptor subtypes, A_1, A_{2A}, A_{2B}, or A_3, elicits responses that are dependent upon the receptor subtypes found in various tissues (Table 1). Adenosine receptors are 7-transmembrane, G-coupled receptors that signal through adenylyl cyclase, affecting the production of cyclic AMP (cAMP), calcium, or the conductance of ion channels. A_1 and A_3 receptors couple to inhibitory G receptors (Gi) and decrease adenylyl cyclase activity, whereas A_{2A} and A_{2B} increase adenylyl cyclase activity by coupling to stimulatory G receptors (Gs or Go)[16]. The affinity for adenosine also differs among the receptor subtypes, affecting the concentration of adenosine necessary for activation. A_1 and A_{2A} are high affinity receptors, activating at lower concentrations of adenosine (~0.01 μM to 1 μM). A_{2B} is a low affinity receptor requiring 10 to 1000-fold higher levels of adenosine (~10 μM) for activation[17]. Downstream from adenylyl cyclase and cAMP, adenosine signaling modifies the activity of nuclear factor kappa-B (NF-κB),

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JAK-STAT and ERK pathways, regulating transcription and ultimately cellular functions[18]. Adenosine that accumulates during cellular stress is removed by uptake into cells and converted to AMP or inosine by adenosine kinase and adenosine deaminase (ADA), respectively. In patients with SCD, tissue injury may increase levels of plasma adenosine suggesting that adenosine pathways may influence SCD pathogenesis[12].

Current therapeutic uses of adenosine and adenosine derivatives

Drugs that target adenosine receptors are part of current standard practice. Adenosine, dipyridamole and adenosine A_{2A}R agonists (e.g., regadenoson) are used clinically to induce cardiac hyperemia during myocardial stress testing via activation of coronary artery A_{2A}Rs. Adenosine-mediated activation of A_1 receptors in the heart is a treatment for tachyarrhythmias. Theophylline, either alone or in complex with ethylenediame (aminophylline), non-selectively blocks A_1, A_{2A} and A_{2B} receptors and is used as a therapy for asthma[16]. A limitation of these therapies is lack of selectivity for the adenosine receptor subtypes, sometimes resulting in unwanted and potentially dangerous side effects. These include hypotension and tachycardia from A_{2A}R activation, bradycardia or heart-block from A_1 activation and bronchospasm from A_{2B}R activation in patients with asthma. Newer generation adenosine agonists and antagonists have greater receptor subtype selectivity thereby minimizing toxicities[16]. There is emerging cellular, animal and human data suggesting a role for the A_{2A} and A_{2B} receptors in the pathogenesis of SCD (Table 2). Adenosine-based therapies are currently being examined in patients with SCD (Clinicaltrials.gov #01788631).

Role of A_{2A}R in sickle cell disease

A_{2A}R

A_{2A}R activation is well-known for producing vaso-dilation due to effects on vascular smooth muscle and some endothelial cells. In addition, A_{2A}R has a central role in the regulation of inflammation and immunity[17]. Ubiquitously expressed on neutrophils, monocytes, macrophages, T cells, NK cells and iNKT cells, adenosine signaling through the A_{2A}R has been shown to suppress key inflammatory and immune responses, including leukocyte activation, recruitment and cytokine production[18]. These immune suppressive effects of A_{2A}R activation are mediated by cAMP and protein kinase A (PKA)[18]. PKA signaling can inhibit other signaling pathways that activate inflammation mediated by NF-κB or the JAK-STAT pathway and serves to decrease transcription of key inflammatory genes[19]. The NF-κB pathway deserves special attention as it has been used as a marker of iNKT cell activity in clinical trials of the A_{2A}R agonist, regadenoson, in patients with SCD[11].

NF-κB is a critically important transcription factor that generally enhances inflammation[20]. Comprised of a dimer of transcription factors from the RelA family of proteins (p50 or p52 and p65), NF-κB resides in the cytoplasm of cells bound to the inhibitory protein IκB. Upon activation of the NF-κB by numerous inflammatory mediators including tumor necrosis factor-α or interleukin-1, IκB is phosphorylated by IκB kinase, ubiquinated and degraded, thus liberating NF-κB to translocate into the nucleus and promote the transcription of pro-

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inflammatory genes. When NF-κB is released from IκB, the 65 kDa subunit (p65) can be phosphorylated on several sites including Ser536. The phosphorylation of p65 (phospho-p65) serves as a marker of NF-κB activity used in flow cytometry assays[11]. In the case of $A_{2A}R$ activation, *in vitro* data suggest that agonists of the $A_{2A}R$ reduce IκB degradation, decreasing the ability of NF-κB to promote a pro-inflammatory cellular response[21]. NF-κB has also been shown to mediate the up-regulation of $A_{2A}R$ in iNKT cells following activation[19].

$A_{2A}R$ agonist decreases inflammation following ischemia-reperfusion injury by interfering with iNKT cell activation

Murine models of liver and kidney transplant demonstrated that activation of $A_{2A}Rs$ by adenosine analogues administered during or after IRI markedly inhibit inflammation and secondary injury[22]. An investigation of the cell type primarily responsible for the protective effect of $A_{2A}R$ activation implicated the iNKT cell as the primary target[22]. Although iNKT cells normally constitute < 1% of the lymphocyte population, iNKT cells can rapidly release large amounts of pro-inflammatory cytokines giving them a critical role in inflammation, despite representing a only small proportion of lymphocytes[23]. Similar to B and T cells that produce adaptive immune responses, iNKT cell activation requires the engagement of an antigen presented on an antigen presenting cell[24]. Unlike B and T cells, which undergo genetic recombination to generate diverse receptors that recognize various peptides, iNKT cells express a semi-invariant T cell receptor that non-specifically binds to lipid antigens presented on CD1d, a MHC class I-like molecule. Different lipids (glycolipids, phospholipids) have been shown to activate iNKT cells[24,25]. The activation of iNKT cells is enhanced by cytokines produced by antigen presenting cells in response to Toll-like-receptor activation[26]. Thus, the activation of iNKT cells is facilitated by innate immune responses stimulated by pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). Upon CD1d-restricted activation, iNKT cells rapidly make mRNAs and release large quantities of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2) and interleukin-4 (IL-4)[27]. IFN-γ stimulates the production in many cells of interferon-inducible CXCR3 chemokines, CXCL9, CXCL10 and CXCL11[28]. IL-2 is known to induce CXCR3 receptors on lymphocytes[28]. Thus, through rapid activation and generation of copious amounts of cytokines and chemokines, iNKT cells stimulate a pro-inflammatory cascade that may promote and sustain vaso-occlusion[9,10]. Activation of $A_{2A}Rs$, abundantly expressed on activated iNKT cells, reduces this inflammatory response and are critical to modulating the immune functions of iNKT cells[10,11].

$A_{2A}R$ agonists decrease iNKT cell activation and reduce inflammation in SCD mice

In a series of experiments in an NY1DD mouse model of SCD, Linden and colleagues generated several lines of evidence implicating iNKT cells as critical to the process of sickle cell vaso-occlusion[9,10]. Lung inflammation and injury were reduced when: 1) iNKT cells were antibody depleted or genetically knocked out, 2) activation or chemotaxis was inhibited and 3) upon administration of $A_{2A}R$ agonists[9]. NY1DD mice treated with a continuous subcutaneous infusion of the $A_{2A}R$ agonist ATL146e demonstrated a maximal improvement in lung function, histology and inflammatory cell infiltrate in 3 days at an infusion rate of 10 ng/kg/minute. The improvement was sustained up to the end of infusion

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at 7 days[10]. The infused dose of ATL146e only achieved plasma concentrations about 1 nM, and the mice did not demonstrate cardiovascular toxicities[10]. The absence of toxicity is in accord with prior studies demonstrating that the anti-inflammatory effects of $A_{2A}R$ agonists occur at 10–100 fold lower concentrations compared to the cardiovascular effects[22]. Blockade or depletion of iNKT cells mitigated the beneficial effects of the $A_{2A}R$ agonist, providing evidence that the anti-inflammatory actions of $A_{2A}R$ activation are mediated largely through iNKT cells[9,10].

In the plasma of adult patients with SCD, circulating iNKT cells were also more likely to be activated and expanded compared to healthy controls[9]. There is selective expansion of iNKT cells among lymphocytes, from < 1% in control blood to an average of about 5% in the blood of SCD patients whose iNKT cells were also more like to express the activation markers CD69, intracellular IFN-γ, and CXCR3[9].

Phase 1 study of the $A_{2A}R$ agonist regadenoson in patients with SCD: study design and rationale

Based on the promising data from mice and patients with SCD suggesting that $A_{2A}R$ agonists may interrupt activation of iNKT cells and potentially decrease sickle cell complications, a phase 1 trial was conducted of the $A_{2A}R$ agonist regadenoson[29]. FDA-approved for inducing cardiac hyperemia during myocardial imaging, regadenoson is a selective $A_{2A}R$ agonist with 10-fold greater affinity for A_{2A} versus A_1 and few if any effects on A_{2B} or A_3 [30,31]. When used for myocardial imaging regadenoson is administered as a 400 μg bolus over 10 seconds[30]. Bolus injection induces vaso-dilation and hyperemia in a time frame appropriate for capturing images before blood flow reverts to normal[31]. If the goal of administering regadenoson is to dampen the severity of a pVOC over several days, a continuous infusion would be necessary given its terminal half-life of 2 hours. When designing the study, three relatively low doses of regadenoson were selected based on data extrapolated from animal models[29]. All of these doses produced the desired anti-inflammatory effects while avoiding cardiovascular toxicities[29]. Using a traditional 3+3 study design, the dose levels were examined during a 12 hour infusion of regadenoson while patients with SCD were at steady state[29]. Once the highest dose of infusional regadenoson (1.44 mcg/kg/hour) was found to be safe, SCD subjects were examined during a 24 or 48 hour infusion at steady state and then during a pVOC[29].

To evaluate the effects of regadenoson on iNKT cell activation, various activation markers were examined. Phosphorylation of the p65 subunit of NF-κB (phospho-p65 NF-κB) was identified as the most promising marker of iNKT cell activation because, as opposed to cell surface markers or cytokines, changes in phosphorylation are pre-transcriptional and thus occur quickly.

Phase 1 study of the $A_{2A}R$ agonist regadenoson in patients with SCD: study results

Twenty-seven adult patients with SCD were administered regadenoson, 21 at steady state and 6 during pVOC[11]. Circulating iNKT cells from adults with SCD during pVOC showed increased phospho-p65 NF-κB activation compared to steady state or healthy controls. When adults with SCD were administered a 24 hour infusion of the $A_{2A}R$ agonist

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regadenoson during pVOC, the percentage of iNKT cells expressing increased phospho-p65 NF-κB decreased to levels similar to steady state patients and healthy controls. The effects of regadenoson were achieved at plasma concentrations that peaked at 2 ng/ml and were devoid of effects on heart rate or blood pressure. A large, randomized-controlled, phase 2 trial is in progress to evaluate the clinical efficacy of regadenoson during pVOC (Clinicaltrials.gov #01788631).

Role of $A_{2B}R$ in sickle cell disease

$A_{2B}R$

The $A_{2B}R$ is a lower affinity receptor that has well-described pro-inflammatory roles in the pathogenesis of asthma, chronic obstructive pulmonary disease and inflammatory bowel disease[32]. Higher levels of adenosine are necessary to activate the $A_{2B}R$ (10–100 fold > $A_{2A}R$) and therefore signaling through $A_{2B}R$ occurs selectively in stressed cells when adenosine is generated by ischemia, injury, or inflammation[17]. Although the distribution of the $A_{2B}R$ is widespread on cells and tissues including smooth muscle cells, endothelial cells and macrophages, pathogenic inflammation is most notably promoted by the actions of the $A_{2B}R$ on mast cells and intestinal epithelial cells[32]. Recent data suggest that activation of $A_{2B}R$ on mast cells results in the production of cytokines with important roles in asthma pathogenesis, such as IL-4 and IL-13, and stimulates B cells to produce IgE[33]. The role of $A_{2B}R$ in asthma is also supported by data demonstrating increased inflammation in asthma patients after inhalation of adenosine[34,35]. To this end, the non-selective adenosine receptor antagonist, theophylline, has a long-standing role in the management of asthma in part working through blockade of the $A_{2B}R$[36]; although, its lack of selectivity is associated with side effects. $A_{2B}R$ expression on intestinal epithelial cells is also important in disease pathogenesis promoting IL-6 production and resulting in intestinal inflammation, potentially contributing to the process of inflammatory bowel disease[37]. More recently, $A_{2B}R$ activation in the corpus cavernosum of the penis and erythrocytes has been shown to promote priapism[13–15] and red cell sickling[12], respectively, in a murine model of SCD.

Adenosine signaling through $A_{2B}R$ is implicated in priapism and penile fibrosis

Studies in non-SCD mouse models demonstrating that intracavernosal injections of adenosine provoked priapism provided preliminary evidence for a role for adenosine signaling in the pathogenesis of priapism[38,39]. More recent work has found that the actions of adenosine are mediated through $A_{2B}R$ signaling on corpus cavernosum smooth muscle cells within the penis[40]. In a series of experiments, investigators determined that ADA-deficient mice had higher intra-penile adenosine levels leading to cavernosal smooth muscle relaxation and priapism[15]. Administration of ADA or the $A_{2B}R$ antagonists, theophylline or MRS1706, antagonized the effects of excess adenosine and reversed priapism[14,15]. $ADA^{-/-}/A_{2B}R^{-/-}$ mice were also protected from priapic episodes[15]. Many of the same findings were recapitulated in a transgenic SCD mouse model. SCD mice had higher plasma levels of adenosine than controls and similarly their episodes of priapism were counteracted by decreasing adenosine levels with ADA or administration of an $A_{2B}R$ antagonist[15]. In SCD mice, there is also evidence that adenosine signaling through the $A_{2B}R$ contributes to penile fibrosis, a consequence of priapism episodes that contributes to erectile

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dysfunction[13]. Taken together, these data suggest that interruption of adenosine signaling through $A_{2B}R$ may have a role in the treatment of priapism and prevention of penile fibrosis in SCD.

Sickle erythrocyte formation promoted through $A_{2B}R$

Recently, another detrimental effect of $A_{2B}R$ activation was described when Zhang and colleagues reported a novel mechanism of erythrocyte sickling in transgenic SCD mice caused by high plasma adenosine and activation of $A_{2B}Rs$ expressed on red cells[12]. These investigators first discovered that in transgenic SCD mice a reduction in circulating adenosine levels following pegylated (PEG)-ADA administration was associated with a decreased number of sickle erythrocytes. To identify the adenosine receptor subtype responsible for promoting sickle cell formation, red cells from mice genetically deficient in one of the four receptor subtypes were activated with an adenosine analogue. The deleterious effects of adenosine were found to be mediated through the $A_{2B}R$ due to an increase in intra-erythrocyte 2,3-DPG levels and a decreased hemoglobin-oxygen affinity. The resulting increased formation of deoxy-hemoglobin provides an explanation for the increased erythrocyte sickling. Translating these findings to humans, levels of plasma adenosine were found to be higher in patients with SCD versus healthy controls as were intra-erythrocyte 2,3-DPG levels (the latter more likely due to the effects of anemia). *In vitro* treatment of erythrocytes from patients with SCD with PEG-ADA or $A_{2B}R$ antagonists reduced red cell sickling, consistent with the findings from the murine model.

Can adenosine have both protective and deleterious roles in SCD?

Recent data describing the role of adenosine signaling in SCD suggest discrepant effects on morbidity when the actions of adenosine are medicated through the $A_{2A}R$ versus $A_{2B}R$[41]. On one hand, data show that activation of $A_{2A}R$ decreases inflammation, largely through the inhibition of iNKT cell activation, potentially dampening the severity of pVOC[10,11]. On the other hand, a separate line of research suggests that adenosine signaling through $A_{2B}R$ promotes priapism[13–15] and erythrocyte sickling[12]. Although further studies are needed to fully understand the seemingly conflicting roles of adenosine signaling in SCD, differences in adenosine receptor expression and affinity along with a better understanding of *in vivo* levels of adenosine in patients with SCD may help to reconcile the confusion.

Effects of adenosine levels and receptor density on $A_{2A}R$ versus $A_{2B}R$ signaling in SCD

Under steady-state conditions and potentially during pVOC, levels of adenosine may be sufficient to signal through the $A_{2A}R$, yet not high enough to trigger $A_{2B}R$ signaling. In comparison to $A_{2B}R$, affinity of adenosine for $A_{2A}R$ is 10 to 1000 times greater than $A_{2B}R$[17]. Moreover, the effects of adenosine are further potentiated in cells or tissue densely expressing adenosine receptors, as is the case for $A_{2A}R$ expression on activated iNKT cells. Compared to CD4+ T cells, iNKT cells express 10 times more $A_{2A}R$ and receptor expression increases further (100-fold) during pVOC[10,19]. Thus, iNKT cells are exquisitely sensitive to inhibition through adenosine signaling due to dense expression of the high affinity receptor, $A_{2A}R$. Based on receptor affinity and density, a model for adenosine signaling emerges whereby differing levels of adenosine in a localized area may produce

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actions through $A_{2A}R$ and/or $A_{2B}R$. Potentially, under conditions where levels of adenosine in SCD are not extremely high, the effects of adenosine are mediated through the $A_{2A}R$ and only during the more extreme conditions of pVOC would the deleterious effects of the $A_{2B}R$ activation be observed (Figure 1).

Adenosine measurements have limitations

Levels of adenosine in patients with SCD have been shown to be higher than healthy controls and, likely, levels will rise further during pVOC[12]. Adenosine measurements in the extracellular space reflect the sum of adenosine's formation, transport and degradation[42]. During periods of microvascular occlusion and tissue ischemia, extracellular concentrations of adenosine may increase and activate adenosine signaling pathways to counteract further tissue damage by increasing blood flow and reducing heart rate and inflammation. Unfortunately, rapid cellular uptake and degradation cause adenosine to have a half life of approximately 5 seconds creating challenges to obtaining accurate measurements[42]. Further compounding errors in measurement is the compartmentalization of adenosine between the intravascular and interstitial spaces and the restricted, tissue-specific rise that may occur in adenosine levels following ischemia and injury[42]. Thus, measurement of adenosine levels in blood from the right arm may not accurately reflect the physiologic effects of adenosine in a patient who is experiencing ongoing vaso-occlusion in the vasculature of the left leg. A final consideration in the interpretation of adenosine levels in the preceding studies are the differences in adenosine biology between people and mice. Adenosine's half-life is longer in mice than in people, and therefore blood levels are also higher in mice[41]. Under these conditions, adenosine signaling through $A_{2B}R$ may be more pronounced in mouse models than in patients. A better understanding of adenosine levels in patients with SCD may clarify the role of $A_{2A}R$ and $A_{2B}R$ signaling under varied conditions and the contribution of this signaling to SCD morbidities.

Limitations of adenosine therapeutics in SCD

The main challenges for using adenosine analogues to treat and prevent vaso-occlusion are the need for a continuous intravenous infusion and unwanted side effects. The short half-life of regadenoson necessitates a continuous infusion, which requires stable intravenous access, and limits the role of regadenoson to the treatment of acute crises. Regadenoson has multi-phasic pharmacokinetics, but the terminal half-life is still only 2 hours[30]. Current conceptual models of SCD describe ongoing vaso-occlusion with inflammation and end organ damage that is punctuated by severe pVOC that result in a hospitalization. Treatments aimed to shorten the duration of hospitalizations, such as regadenoson, have value as there is a higher risk of death during these acute events[4], however, they will not affect the continuing daily damage that culminates in organ dysfunction. Preventing a major crisis is a better strategy to decease morbidity and mortality than treating one. To this end, a humanized monoclonal antibody that targets iNKT cells independently of the adenosine signaling pathways is currently under investigation in a phase 1 study (Clinicaltrials.gov #01783691). This investigational agent could potentially deplete iNKT cells on a longer-term basis than the effects of regadenoson and prevent crises. Another challenge to the use of adenosine signaling in the treatment of SCD is unwanted side effects due to a lack of adenosine

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receptor selectivity. Adenosine and dipyridamole cause activation of all adenosine receptor subtypes and are associated with severe side effects such as heart-block and hypotension. The $A_{2A}R$ agonist regadenoson has been administered to patients with SCD without toxicity due to a high degree of selectivity for the $A_{2A}R$, along with the fact that lower concentrations of the drug are still able to achieve anti-inflammatory effects[11].

Future directions: combined $A_{2A}R$ and $A_{2B}R$ therapies for SCD?

Modulating the adenosine signaling pathway holds promise as a treatment for patients with SCD. Independent lines of investigation have provided evidence that the $A_{2A}R$ and $A_{2B}R$ are respectively protective and deleterious in the pathogenesis of vaso-occlusion. The combination of an $A_{2A}R$ agonist with an $A_{2B}R$ antagonist may be an ideal treatment in SCD. Thus far, the $A_{2A}R$ agonist regadenoson is the only adenosine-based therapeutic that has been studied in patients with SCD[11]. Another approach that has been suggested is PEG-ADA infusions to lower circulating adenosine levels and minimize signaling through the $A_{2B}R$. ADA is an FDA-approved therapy for patients with ADA deficiency[43]. The possible shortcoming of this approach is that positive effects from adenosine signaling through the $A_{2A}R$ might be negated with ADA therapy. A more novel and potentially effective approach to prevent and treat vaso-occlusion is dual therapy with $A_{2B}R$ antagonists to prevent erythrocyte sickling and $A_{2A}R$ agonists to decrease inflammation and dampen the severity of pVOC (Figure 1)[44]. Highly selective $A_{2B}R$ antagonists are currently in development for maintenance treatment of asthma[16,44]. Clearly, rigorously-designed clinical trials demonstrating the clinical efficacy of $A_{2A}R$ agonists and/or $A_{2B}R$ antagonists need to be conducted prior to considering combination therapy, however, innovative approaches are needed in the treatment of SCD and multi-modal therapies may be necessary to demonstrate clinical benefit.

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Key points

- Activation of adenosine A_{2A} receptor ($A_{2A}R$) on invariant NKT cells decreases inflammation in a transgenic mouse model of sickle cell disease (SCD). The effects of regadenoson, an $A_{2A}R$ agonist, are currently being examined in patients with SCD.
- The adenosine A_{2B} receptor ($A_{2B}R$) on red blood cells and corpus cavernosal cells of the penis has been implicated in the formation of sickle erythrocytes and priapism, respectively.
- These two independent lines of research examining the roles of $A_{2A}R$ and $A_{2B}R$ signaling in SCD may provide opportunities for new therapies.

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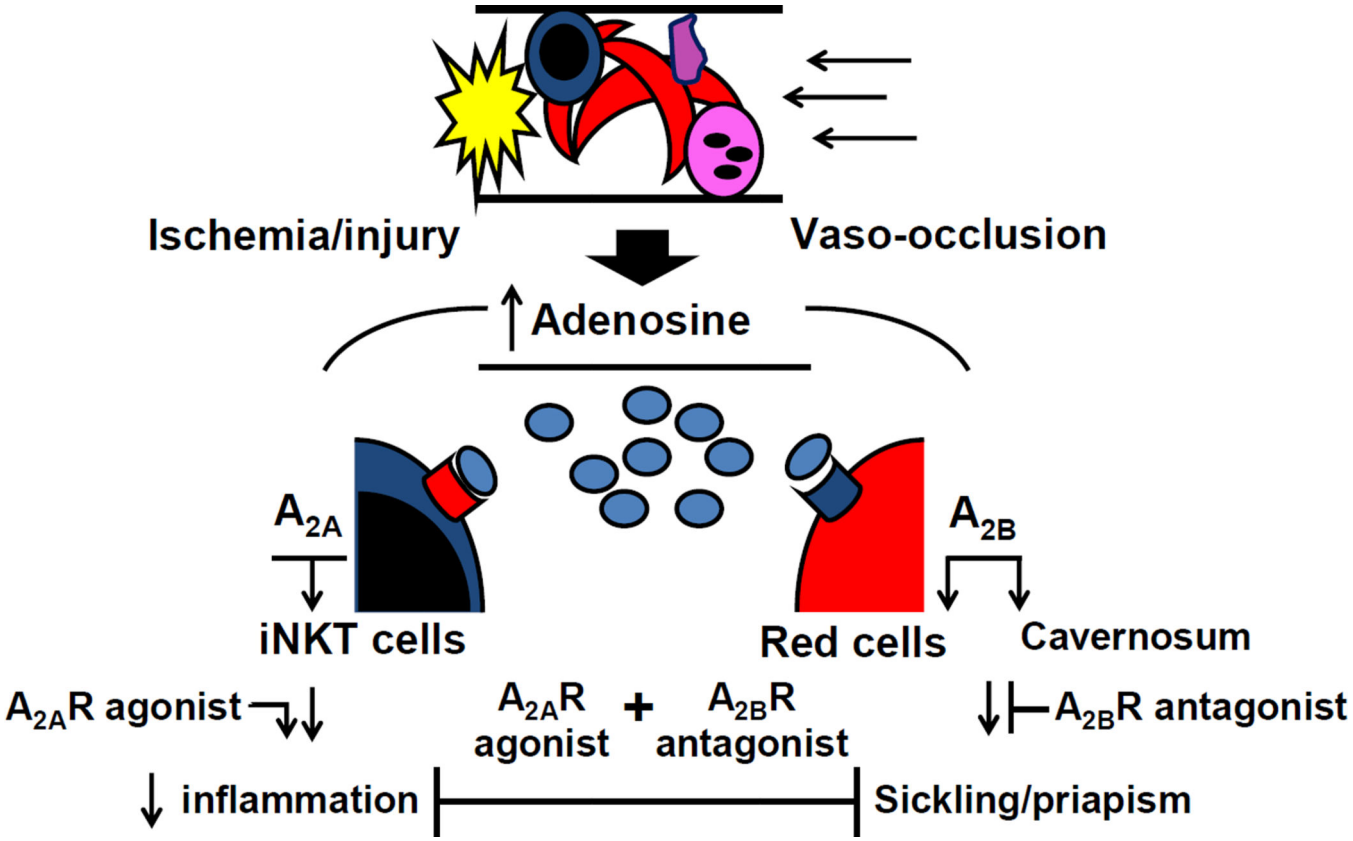

Figure 1. The proposed roles of the adenosine A_{2A} receptor ($A_{2A}R$) and the adenosine A_{2B} receptor ($A_{2B}R$) in the pathogenesis of sickle cell disease (SCD)

Sickle cell vaso-occlusion leads to tissue injury and liberation of adenosine. *Left side of schema:* expression of $A_{2A}R$ are increased on iNKT cells and activation of $A_{2A}R$ on iNKT cells leads to a reduction in pro-inflammatory mediators (IFN-γ, IL-4) and dampening in the severity of vaso-occlusion. $A_{2A}R$ agonists (e.g., regadenoson) may promote the anti-inflammatory effects of the $A_{2A}R$. *Right side of schema:* Activation of $A_{2B}R$ on erythrocytes results in increased levels of 2,3 DPG decreasing hemoglobin affinity for oxygen and contributing to red cell sickling. On corpus cavernosal cells of the penis, activation of $A_{2B}R$ promotes priapism. Antagonists to $A_{2B}R$ may decrease the formation of sickle erythrocytes and prevent priapic episodes. Potentially, dual therapy with $A_{2A}R$ agonist/$A_{2B}R$ antagonist would be an ideal for patients with SCD: anti-inflammatory, anti-sickling, anti-priapism.

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Table 1

Adenosine receptor subtypes[17,32]

Adenosine receptor subtypes				
Characteristics	**A_1**	**A_{2A}**	**A_{2B}**	**A_3**
Predominant tissue/cell expression	• Lung • Heart • Brain • Leukocytes	• Lung • Brain • Vasculature • Leukocytes	• Lung • Colon • Vasculature • Leukocytes • Erythrocytes • Penis	• Lung • Liver • Heart • Brain • Leukocytes
Actions	• Negative chronotropic • ↑inflammation	• Vasodilation • ↓inflammation	• ↑inflammation in lung and colon	• ↓inflammation
Affinity for adenosine	High	High	Low	High
Major disease associations	• Sepsis	• SCD • Ischemia • Arthritis • Wound healing	• SCD • Asthma • COPD • IBD	• Asthma • Arthritis

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Table 2

Comparison of $A_{2A}R$ and $A_{2B}R$ data in sickle cell disease

Reference	Type of study	Transgenic SCD model	# SCD Patients	Agents evaluated	Key findings
$A_{2A}R$					
VOC					
Wallace et al. Blood 2010[10]	Investigation of transgenic SCD mice	NY1DD	-	$A_{2A}R$ agonist ATL146e	• SCD causes induction of $A_{2A}R$ on iNKT cells • $A_{2A}R$ agonist ATL146e reverses pulmonary dysfunction in SCD mice
Field et al. Blood 2013[11]	Phase 1 clinical study	-	27	$A_{2A}R$ agonist regadenoson	• $A_{2A}R$ agonist regadenoson decreases activation of iNKT cells in patients with SCD during VOC • $A_{2A}R$ agonist regadenoson is safe in patients with SCD
Lin et al. PLosOne 2013[19]	Investigation of transgenic SCD mice	NY1DD	8	-	• SCD causes induction of $A_{2A}R$ on iNKT cells • $A_{2A}R$ expression on iNKT cells is mediated through NF-κB
$A_{2B}R$					
Red blood cell sickling					
Zhang et al. Nat Med 2011[12]	Investigation of transgenic SCD mice, cultured human RBCs and SCD patient blood samples	Berkley	12	PEG-ADA, theophylline, $A_{2B}R$ antagonist MRS1754	• Adenosine is elevated in the plasma of mice and patients with SCD • $A_{2B}R$ on erythrocytes mediates sickling via decreasing hemoglobin oxygen binding affinity
Priapism					
Mi et al. JCI 2008[15]	Investigation of ADA deficient and transgenic SCD mice	Berkley	-	PEG-ADA, theophylline, $A_{2B}R$ antagonist MRS1706	• Priapism is present in $ADA^{-/-}$ mice and corrected by PEG-ADA • Interruption of $A_{2B}R$ activity on corpus cavernosal cells decreases priapism
Wen et al. J Sex Med 2010[14]	Investigation of ADA deficient and transgenic SCD mice	Berkley	-	PEG-ADA	• PEG-ADA therapy prevented episodes of priapism in SCD mice
Wen et al. FASEB 2010[13]	Investigation of ADA deficient and transgenic SCD mice	Berkley	-	PEG-ADA, $A_{2B}R$ antagonist MRS1706	• PEG-ADA therapy prevented penile fibrosis in SCD mice

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Reference	Type of study	Transgenic SCD model	# SCD Patients	Agents evaluated	Key findings
					• Interruption of $A_{2B}R$ in corpus cavemosal fibroblast cells prevents penile fibrosis