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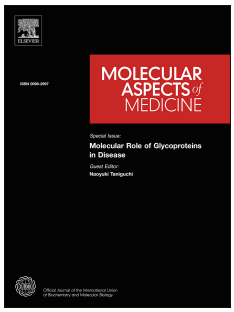

Pharmacological targeting of adenosine receptor signaling

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Title: *Pharmacological targeting of adenosine receptor signaling*

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Abstract

Adenosine receptor signaling plays important roles in normal physiology, but is also known to modulate the development or progression of several different diseases. The design of new, efficient, and safe pharmacological approaches to target the adenosine system may have considerable therapeutic potential, but is also associated with many challenges. This review summarizes the main challenges of adenosine receptor targeted treatment including tolerance, disease stage, cell type-specific effects, caffeine intake, adenosine level assessment and receptor distribution *in vivo*. Moreover, we discuss several potential ways to overcome these obstacles (*i.e.,* the use of partial agonists, indirect receptor targeting, allosteric enhancers, prodrugs, non-receptor-mediated effects, neoreceptors, conditional knockouts). It is important to address these concerns during development of new and successful therapeutic approaches targeting the adenosine system.

Key Words: Adenosine; adenosine receptor; drug target, drug discovery; pharmacology; disease

Abbreviations: T1D, type 1 diabetes; T2D, type 2 diabetes; A_1AR, A_1 adenosine receptor; A_{2A}AR, A_{2A} adenosine receptor; A_{2B}AR, A_{2B} adenosine receptor; A_3AR, A_3 adenosine receptor

Contents

1. Introduction

Since adenosine receptor-mediated signaling plays a role in modulating the progression of various pathological disorders, the creation of efficient and safe pharmacological ligands has considerable therapeutic potential, but is fraught with difficulty (Chen et al 2013). Efforts in medicinal and organic chemistry have been fruitful and numerous adenosine analogues with high affinity and selectivity have been generated (Fredholm et al., 2001; Fredholm et al., 2011). Therefore, the lack of selective ligands is not the major problem. The biggest challenge to overcome is the widespread expression of adenosine receptors and the redundancy of adenosine signaling.

2. Challenges associated with pharmacological targeting of adenosine signaling

2.1. Widespread distribution of adenosine receptors

Adenosine receptors are present on most cells. This means that a given type of adenosine receptor is going to be present not only on the target cell(s) involved in a disease process, but also on cells that are involved in very diverse physiological processes. This problem is accentuated with promiscuous agonists, which activate all receptors, than with antagonists selective to cells with substantial ongoing activation (Chen et al., 2013).

2.2. Tolerance

Tolerance can develop after repeated or chronic ligand exposure desensitizing receptor activation or reducing the signaling response of the targeted receptor over time. This can be due to reduced receptor expression, receptor internalization, or other mechanisms reducing the end effect of a specific dose of ligand. Tolerance has been reported for A_1AR agonists and A_{2A}AR agonists (Burgueno et al., 2003; de Mendonca et al., 2000; Jacobson et al., 1996), but it seems likely to occur also for A_{2B} and A_3 agonists. Use of receptor antagonists decreases the risk of tolerance, since this would require that the endogenous agonist occupies enough receptors as to cause desensitization. Indeed, even for A_{2A}AR which is very abundant in the basal ganglia, constantly occupied antagonists do not cause tolerance, making such drugs promising as therapeutic agents (Halldner et al., 2000; Pinna et al., 2001).

2.3. Developmental or disease stage

Many times the blockade of a specific adenosine receptor has almost opposite effects depending on the developmental stage of the tested animals. This has already been reported in relation to the role of A_1AR or A_{2A}AR in brain injury (Aden et al., 2003; Chen et al., 1999; Cunha, 2005; Turner et al., 2003) and in relation to metabolic abnormalities, contradictory findings related to the A_1 and A_{2B} receptors could be attributed to the different developmental stages (Csoka et al., 2014; Figler et al., 2011; Johansson et al., 2007; Peleli et al., 2015; Yang et al., 2015). Moreover, in various disease models both protective and detrimental effects of adenosine receptor activation have been observed depending on the stage of the disorder confirming the high complexity of the adenosine receptor-mediated

signaling (Chen et al., 2013). This also has implications in the conclusions one can draw from single adenosine receptor knockout mice on the usefulness of adenosine receptor antagonists.

2.4. Distinct effects on different cell types

The distinct effects of adenosine on different cell types become evident in the case of metabolic disorders. For example, diabetes mellitus involve the participation of many different organs such as pancreas, liver, skeletal muscle, and adipose tissue. Moreover, low-grade inflammation together with oxidative stress has been shown to be important in the progression of metabolic abnormalities. Considering that all the adenosine receptors are expressed on the different metabolic organs and various immune cells it becomes evident that the administration of a molecule that targets a specific adenosine receptor will have many and potentially opposing outcomes. For example, as nicely reviewed by Eisenstein et al. (Eisenstein et al., 2015) there are many opposing findings regarding the role of A_{2B}AR in metabolic pathologies since this receptor simultaneously and differently affects acute and chronic inflammation (macrophages), adipogenesis (adipose tissue), insulin release (pancreas) and gluconeogenesis as well as glycogenolysis (liver). Additionally, A_{2A}AR receptors seem essential in developing or maintaining endothelial dysfunction in the fetoplacental vasculature in diseases of pregnancy such as gestational diabetes mellitus or preeclampsia (Salsoso et al., 2015; Sobrevia et al., 2016). However, the A_1AR is required for the effect of insulin correcting the gestational diabetes mellitus-enhanced L-arginine transport in this vascular bed (Guzman-Gutierrez et al., 2016).

2.5. Widespread use of caffeine

Although often underestimated, a very large part of the adult population consumes coffee on a daily basis. Two cups of coffee leads to an almost 50% blockade of the A_1AR and A_{2A}AR (Fredholm et al., 1999). Therefore, any new drug on the market that inhibits adenosine receptors should be able to exert a larger and more obvious effect compared to the one already existing from the low-cost caffeine. In agreement to this concept, any new clinical trial affecting adenosine receptors must carefully calculate the caffeine intake of the study's participants and interpret any results with caution.

2.6. Measuring adenosine levels or the number-distribution of adenosine receptors in vivo

The reliable measurement of adenosine and its receptors on a specific tissue *in vivo* is crucial for understanding its biology and pharmacology. However, adenosine's concentration varies a lot over time and current methodological approaches destroy some cells locally, potentially leading to higher false positive measurements (Chen et al., 2013). Interestingly, the adenosine plasma concentration in the human umbilical vein at birth is higher in gestational diabetes mellitus compared with normal pregnancies (Westermeier et al., 2015), and maternal plasma concentration of this nucleoside is elevated at early stages of pregnancy in women that later develop preeclampsia (Escudero and Sobrevia, 2008; Espinoza et al.,

2011). Moreover, we are still lacking knowledge of how the different receptors are distributed in patients with various diseases. The latter problem could be potentially solved by taking advantage of the newest *in vivo* imaging methods already in practice with A_{2A}AR ligands in patients with Parkinson's disease (Mishina et al., 2011; Ramlackhansingh et al., 2011). Therefore, the advancement in analytical methods for assessing adenosine and its receptors would be of great aid for the use of adenosine receptor drugs in therapy only when adenosine receptors alterations are observed as an example of personalized medicine.

3. Potential approaches to target adenosine receptor signaling

There are several potential ways to overcome some of the abovementioned obstacles, including:

3.1. Partial agonists

Partial agonists are drugs that bind to and activate a receptor, but they have only partial efficacy compared to the full agonist. In practice this means that a partial agonist acts predominantly as an antagonist when there is substantial endogenous signaling of the targeted receptor (Lambert, 2004). Indeed, many commonly used "antagonists" are in fact partial agonists. A high expression levels of a receptor are positively correlated to the receptor reserve phenomenon across different tissues (Kenakin, 2009). Receptor reserve, which means that stimulation of only a fraction of the receptors is sufficient to elicit the maximum response in case of a full agonist, is very sensitive to an agonist's intrinsic efficacy and therefore. This implies that a full agonist can exert strong effects even at tissues where there is relatively low expression of a receptor. This simultaneous action of a full agonist on many target tissues could lead potentially to many side effects. However, this is not the case when a partial agonist is administrated and therefore many of the side effects on other tissues are avoided. For example, the adipocytes highly express A_1AR and therefore partial A_1AR agonists can more selectively target those receptors instead of others (Dhalla et al., 2003).

3.2. Indirect receptor targeting

An alternative approach that could lead to less side effects is to increase the local endogenous adenosine concentration and therefore activate the surrounding adenosine receptors. This approach could provide some degree of tissue specificity, but more studies are warranted in order to establish this hypothesis. Drugs that could be used include adenosine deaminase inhibitors (*e.g.* deoxycoformycin) and adenosine uptake inhibitors, including dipyridamole and ticagrelor. Both deoxycoformycin and ticagrelor have already been used in clinical trials with T1D or acute coronary syndrome patients, showing some beneficial effects related to renovascular diabetic complications, *e.g.* reduced albuminuria and platelet activation (Aizawa et al., 1990; Bonello et al., 2014; Laine et al., 2014). In addition to diabetes and cardiovascular disease, alternative strategies for increasing locally adenosine levels are tested in epilepsy. In particular, adenosine kinase (ADK) seems to be a

key regulator of adenosine's clearance in the brain and over-activation of ADK results in adenosine deficiency and seizures (Boison, 2013). Therefore, efforts have been made in targeting and inactivating ADK in specific brain areas, which could potentially reduce the frequency of epileptic episodes. For example, gene therapy directed to ADK through an antisense oligonucleotide is being explored as a means of conserving adenosine by reducing ADK expression in animal models of epilepsy (Boison, 2010). Promisingly, adenosine has also been delivered directly to the brain ventricles of epileptic rats, thereby reducing DNA methylation and slowing disease progression (Borea et al., 2016).

3.3. Allosteric enhancers

Allosteric enhancers are molecules that bind to the adenosine receptors on a different site compared to the agonist, and stabilize the tertiary complex between agonist, receptor and the G-protein. This action can for example stabilize the binding of endogenous adenosine and enhance its effects when its concentration increases locally. This magnifies the effects of adenosine in an event-responsive and temporally specific manner, thus minimizing the side effects. So far, allosteric enhancers have successfully been used for the A_1AR and A_3AR, enhancing adenosine's anti-lipolytic and anti-ischemic action, respectively (Goblyos and Ijzerman, 2011).

3.4. Prodrugs

Another approach for a more specific tissue targeting of an adenosine receptor ligand is the administration of a molecule in an inactive form (*i.e.* prodrug), which is subsequently activated at the desired tissue by a locally expressed enzyme. To this end, prodrugs have been generated, acting as A_{2A}AR agonist after selective cleavage by the enzyme ecto-5'-nucleotidase or CD73 in inflamed tissues (Flogel et al., 2012).

3.5. Multiple targets of adenosine signaling (not only at the receptor level)

The use of multi-target drugs that act not only at the receptor level, but also influence the biosynthesis or metabolism of adenosine, can potentially enhance its effects since they act simultaneously on many different levels. For example drugs have been synthesized that combine a dual action of an A_{2A}AR agonist together with adenosine transporter or monoamine oxidase B inhibition (Chen et al., 2002; Huang et al., 2011). These drugs have demonstrated improved efficacy in neurodegenerative disorders such as Parkinson's or Huntington's disease.

3.6. Design of neoceptors

The term neoceptor refers to reengineered adenosine receptors that recognize selectively uniquely modified ligands (neoligands), which are unable to bind to and activate the endogenous receptors. This approach gives spatial and temporal specificity since the neoceptor can be selectively targeted to a specific tissue and the neoligand can be administrated only when needed (Jacobson et al., 2007). So far, neoceptors and respective

ligands have been designed for A_1AR, A_{2A}AR and A_3AR (Gao et al., 2006; Jacobson et al., 2001; Jacobson et al., 2005; Palaniappan et al., 2007). This technology is undoubtedly promising, but many more *in vivo* studies are warranted in order to evaluate its practical efficacy. Potential difficulties could result to the use of genetic targeting techniques in man.

3.7. Use of conditional knockouts (Cre-Lox recombination) and shRNA approaches

These approaches would provide us with a better understanding of the spatial and temporal changes of the adenosine-mediated receptor signaling and would hopefully allow us to synthesize more efficient and selective drugs that target the adenosine receptors. So far most of the studies have used global knockouts or adenosine receptor ligands that could act simultaneously on many different target tissues and the results have often been conflicting. Therefore, the use-creation of conditional knockout mice or shRNA approaches for selective gene silencing is imperative. So far conditional knockouts for the A_{2A}AR and A_1AR for specific brain areas have been created (Lazarus et al., 2011; Scammell et al., 2003).

4. Conclusions

There are many ongoing or already completed phase I-III clinical trials with adenosine receptor ligands for various diseases, but for all the above mentioned reasons few of them may reach the clinic. So far, the clinical applications of adenosine itself, A_1AR, A_{2A}AR and A_3AR agonists, caffeine, A_1AR and A_{2A}AR antagonists have been tested against several pathologies such as liver ischemia and liver cancer, treatment after stenting, blood flow in T1D, wound healing after foot ulcers in T1D and T2D, psoriasis, rheumatoid arthritis, acute heart failure and Parkinson's disease (Avni et al., 2010; Chen et al., 2013; Fernandez et al., 2010; Fishman et al., 2012; Massie et al., 2010; Pinna, 2009; Powers et al., 2008; Schmidt et al., 2006, 2007; Silverman et al., 2008). Some of the findings from these clinical trials are promising, but in many cases the tested drugs seem to be ineffective indicating the importance of overcoming the above mentioned obstacles. For example, there are several studies showing that non-selective adenosine receptor inhibitors improve hypoglycemia unawareness in T1D or insulin secretion in T2D (Arias et al., 2001; de Galan et al., 2002). Moreover, pharmacological agents that lead to higher extracellular concentration of adenosine decrease albuminuria in diabetic patients (Aizawa et al., 1990). However, so far there are no data from large-scale clinical trials or from more selective adenosine receptor ligands. In addition, a large-scale trial where adenosine was administrated as an adjunct to reperfusion in the treatment of acute myocardial infarction concluded that adenosine did not prevent the development of heart failure but was able to significantly reduce the infract size (Ross et al., 2005). Parkinson's disease is also another example of pathology where targeting of the adenosine receptors seem to have a therapeutic potential (Lopes et al., 2011). Despite encouraging findings of the administration of A_{2A}AR antagonists in rodent and primate models of Parkinson's disease, these effects have proven difficult to demonstrate on a consistent basis in humans (Morelli et al., 2009). Therefore, the design of more targeted proper clinical studies is a necessity to establish the therapeutic value. According to the current bibliographic literature, and everything mentioned above, more selective targeting of A_1AR, A_{2A}AR, A_{2B}AR and A_3AR would be a very promising approach provided that issues, such as age of the individuals, main targeted tissue, stage and type of the disease, are carefully taken into consideration.

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Conflict(s) of Interest

None

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