Adenosine receptors as therapeutic targets for the treatment of myocardial infarction and its complications. Part I. Myocardial infarction

Receptory adenozynowe jako cele terapeutyczne w leczeniu zawału mięśnia sercowego i jego powikłań. Część I. Zawał mięśnia sercowego

Kamila Puchałowicz, Krzysztof Safranow, Monika Rać, Dariusz Chlubek, Violetta Dziedziejko

Pomorski Uniwersytet Medyczny w Szczecinie, Katedra Biochemii i Chemii Medycznej, al. Powstańców Wlkp. 72, 70-111 Szczecin
Pomeranian Medical University in Szczecin, Department of Biochemistry and Medical Chemistry

viola@pum.edu.pl

ABSTRACT
Adenosine is an endogenous compound with cardioprotective properties that acts via A1, A2A, A2B and A3 plasma membrane receptors. Over the last several decades, the mechanisms underlying adenosine’s protective effects in the ischemic myocardium were investigated. They are the basis for ischemic preconditioning and postconditioning procedures that protect the heart from ischemia/reperfusion injury. The promising results of animal model studies have encouraged a large group of researchers to conduct clinical trials assessing the benefits of adenosine as an adjunct to reperfusion therapy in myocardial infarction. This review describes the mechanisms behind the cardioprotective effect of adenosine and presents current reports on its clinical utility in the treatment of myocardial infarction.

Keywords: cardioprotection; ischemia-reperfusion injury; reperfusion; adenosine; adenosine receptors; myocardial infarction.

INTRODUCTION
Myocardial infarction is an acute form of ischemic heart disease which poses a direct risk to the patient’s life. Although its prevalence and mortality rate are dropping in most countries, the future prospects are not optimistic. This is associated with the ageing and growing population, which contributes to the increasing global burden of disease. Patients with a history of myocardial infarction stand a lower chance of long-term survival and carry a higher risk of cardiovascular events (particularly during the first 12 months after infarction) compared to the general population [1].

In the course of myocardial infarction, a part of the myocardium becomes necrotic due to occlusion of the coronary artery that supplies it. The subsequent damage results from 2 processes, namely ischemia and subsequent reperfusion (and is thus referred to as ischemia-reperfusion injury). Achieving reperfusion as quickly as possible is currently the main method for treating myocardial infarction. The development of mechanical and pharmacological techniques for restoring coronary circulation has contributed to limiting ischemic injury and considerably reducing infarction-related mortality. In this way, the number of patients suffering from complications of myocardial infarction, such as chronic heart failure or ventricular arrhythmias, has increased [2]. Therefore, prevention of the consequences of infarction by limiting reperfusion injury has been assumed as the main therapeutic objective.

An opportunity to reach this objective has been seen in, inter alia, modulation of the signaling pathways involving adenosine and its receptors. Adenosine is a common signaling molecule...
that plays a significant role in the functioning of the cardiovascular system. It operates by binding with P1 adenosine receptors: A1, A2B, A2A, and A3, a class of G protein-coupled receptors. Expression of all subtypes of adenosine receptors has been observed in cardiovascular system tissues, where they help regulate coronary perfusion and heart rate and are responsible for the cardioprotective properties of adenosine and its ability to modulate inflammatory response and tissue remodeling. Given the array of favorable functions of adenosine, adenosine receptors can be perceived as a promising target for pharmacological interventions [3]. Attempts are being made to utilize adenosine and synthetic adenosine receptor agonists and antagonists in cardiovascular therapies [4]. This paper reviews current reports on their utilization in the treatment of myocardial infarction.

MYOCARDIAL INFARCTION AND ISCHEMIA-REPERFUSION INJURY

Over the last several decades, numerous studies have explored the potential cardioprotective properties of a number of compounds preventing reperfusion injury and thus contributing to reducing the size of the infarct and limiting complications. The reperfusion injury concept was introduced in 1960 by Jennings et al. [5]. It claims that restoration of blood flow to a previously ischemic vessel exacerbates the ischemia-induced damage. Such harmful effects of reperfusion result from the activation of complex multifactorial mechanisms, among them oxidative stress, pH restoration as a result of hydrogen ion (H+) washout from the acidified myocardium, and Ca2+ accumulation in the cells. Each of these processes is characterized by its own mechanism of toxicity, but the central and integratory role in all of them is played by the mitochondrial permeability transition pore (mPTP). Mitochondrial permeability transition pore activation secondary to reperfusion injury leads to the formation of a non-selective channel in the inner mitochondrial membrane, an uncontrolled flow of ions, inter alia Ca2+ and H+, and disturbance of the structure of myocardial cells, which is followed by their necrosis and apoptosis. Another important mechanism in ischemia-reperfusion injury is related to the inflammatory process. The activity of reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to the production and release of cytokines, chemokines and other pro-inflammatory agents, which are responsible for the migration and accumulation of neutrophils and other leukocytes in the myocardial tissue. Neutrophils are believed to be the effector cells of ischemia-reperfusion injury [6].

A breakthrough in the prevention of reperfusion injury came with the publishing in 1986 of a study by Murry et al. [7], in which ischemic preconditioning (IPC) was described. Ischemic preconditioning is a non-pharmacological intervention that, by way of short ischemia-reperfusion episodes that precede myocardial ischemia, exerts cardioprotective effects, thus reducing the size of the infarct considerably. It was found much later that the effects of IPC are largely thanks to the limitation of reperfusion injury [8]. Almost 20 years later, Zhao et al. [9] showed that a similar procedure (with short ischemia-reperfusion incidents) performed immediately after restoring blood supply had a substantial limiting impact on the size of the infarct as well. They named the phenomenon ischemic postconditioning (POC). According to contemporary reports, the cardioprotective effects of ischemic pre- and postconditioning result from the prevention of mPTP activation [9]. The finding that IPC and POC improve cardiac function by mobilizing molecular and cellular mechanisms that limit reperfusion injury has greatly contributed to the development of pharmacological interventions in myocardial infarction therapy, and attempts at introducing cardioprotective medications into clinical practice are currently a very active field of research.

THE ROLE OF ADENOSINE IN PREVENTING MYOCARDIAL ISCHEMIA-REPERFUSION INJURY

Adenosine has proven cardioprotective properties. In 1985, Ely et al. [11] showed that adenosine limited myocardial injury caused by ischemia. The early 1990s saw the publishing of more papers claiming that the protective effect of adenosine on the myocardium in the course of ischemia- and reperfusion-induced injury was the result of activation of adenosine receptors [12, 13, 14]. Studies performed on various animal models utilizing synthetic P1 receptor agonists have shown that the activation of A1 [12, 15, 16] and A2 [14, 17, 18, 19, 20, 21, 22, 23, 24] receptors prior to the ischemic incident limits myocardial injury caused by ischemia-reperfusion and reduces the size of the infarct. There have also been reports pointing to the favorable effects of activation of A1 [16, 25] and A2 [21, 26, 27] receptors prior to or during reperfusion. Auchampach et al. [21] have studied whether the time of administration (prior to ischemia or prior to and during reperfusion) of an A2 receptor agonist has an impact on its cardioprotective activity. In their research, the reduction in the size of the infarct was similar in both these cases. On this basis, the authors concluded that the cardioprotective effect stemmed chiefly from the ability to limit reperfusion injury rather than ischemic injury. Perhaps a similar mechanism is at play in the case of the A3 receptor. Also, the participation of both the A3 receptors in preventing reperfusion injury has been confirmed [25, 28, 29, 30, 31, 32, 33, 34]. More papers on the receptor mechanism have shown that for full cardioprotective activity to occur more than one receptor needs to be activated. It has been found that the reduction of infarct size in response to A3 receptor activation requires the activation of both the A2 receptors [35, 36, 37]. Cooperation between the A2A and A2B receptors has been described [33, 34]. Both these receptors are coupled with a G protein and are probably responsible for activating common signaling pathways. This is of essential importance for cardioprotective effects to occur where A2 receptor expression is low, where the activation of a single receptor class is insufficient [33]. However, Methner et al. [34] have shown that the A2A and A2B receptors activate different signaling pathways. Further research is required in order to explain the mechanism responsible for the cardioprotective
effect occurring only when the A2 receptors are activated simultaneously. For the A1 receptor, its interaction with the A1 [38] and A2a [39] receptors, but not with the A2b [32] receptor, has been demonstrated to play an essential role in triggering the cardioprotective effect. The occurrence of interactions between the PI receptors may explain the lack of cardioprotective effect of the A1 receptor agonist GR79236 in the Adenosine A1 agonist at reperfusion trial (AART) study, where no reduction in the size of the infarct and no decreased risk of lethal myocardial injury were shown to occur in rabbits in response to A1 receptor activation at the beginning of reperfusion [40].

The mechanism responsible for the cardioprotective effect of the conditioning procedures is very complex, and proceeds according to the following sequence of events: (i) the occurrence of the triggering stimulus, i.e. an ischemia-reperfusion episode, which is accompanied by the release of endogenous cardioprotective compounds, (ii) activation of cytoplasmic signaling pathways and (iii) activation of the effectors of cardioprotective activity [10]. Adenosine is one of the compounds released by myocardial and endothelial cells in response to the conditioning procedure applied and mediates the procedure’s protective effect. The early phase of IPCs protective effect begins immediately after the conditioning stimulus initiation, lasts for 2–3 h and is dependent on the activation of the so-called early protection protein kinases [10, 41]. In experimental animal models, adenosine has been found to trigger the early protection phase by activating the A1 [12] or A2 [14] receptor, or both receptors simultaneously [42, 43]. Moreover, the A2 receptor-dependent mechanism has been indicated as associated with the activation of protein kinase C (PKC) [17, 44]. According to Kuno et al. [45], PKC activation contributes to increasing myocardial sensitivity to endogenous adenosine, which allows for the activation of the A2 receptor that mediates the cardioprotective effect through phosphoinositide-dependent kinase (PI3K) and extracellular signal-regulated kinase (ERK). This pathway is associated with protein kinase G, which activates PKC through the mediation of ROS produced in response to the opening of mitochondrial ATP-sensitive potassium channels (mitoK-ATPs) [46]. Mitochondrial ATP-sensitive potassium channels are the common element between the pathways of early- and late-phase IPC. Additionally, the aforementioned PI3K and ERK kinases are components of the reperfusion injury salvage kinase (RISK) pathway, which is activated at the beginning of reperfusion and plays an essential role in the functioning of IPC, POC and cardioprotective compounds in murine models. The pathway’s end-product is glycogen synthase kinase 3β (GSK-3β), which prevents mPTPs from opening [41]. The RISK pathway has been seen activated, inter alia, following the activation of the A2 receptor [47]. The late conditioning phase (of a lower protective effect) commences 12–24 h after the occurrence of the trigger and lasts for 48–72 h. It is caused by the activation of the ‘late protection genes’, which results in the synthesis of effector proteins, among them inducible nitric oxide synthase (iNOS) and superoxide dismutase [10, 41]. Similarly to the early phase, the late protective effect of adenosine is dependent on A1 and A2 receptors that initiate signaling pathways associated with mitoK-ATP opening [48, 49]. For both receptors, iNOS production is an indirect effect of the activation of the channels [48, 49], although for the A1 receptor the presence of a pathway independent of this effector has also been described [48]. Mitochondrial ATP-sensitive potassium channels activation by the A3 receptor is supposed to involve PKC- and tyrosine kinase-dependent pathways, which are further mediated by p38 mitogen-activated protein kinases (MAPK p38) and heat shock protein 27 (Hsp27), which demonstrate cytoprotective properties [50]. The mechanism responsible for IPC also involves activating the hypoxia-inducible factor 1 (HIF-1) transcription factor, which provides an important contribution to adaptation to hypoxia/ischemia. The hypoxia-inducible factor 1 indirectly stimulates the production of adenosine from adenine nucleotides hydrolyzed by ectonucleotidases and induces A2 receptor expression [53]. This favors the A2 receptor-dependent activation of transcription factors, and the subsequent increase in the expression and stabilization of the Period2 (PER2) protein. As a result of HIF-1 and PER2 activation, glycolytic enzyme expression is increased, which helps the heart to adapt to anaerobic conditions [52]. The mechanism behind adenosine’s function in POC is poorly understood. The available papers point to the role of the A2 and/or A2b receptors associated with PKC [30], PI3K and ERK [36, 55] kinases, which indicates that the RISK pathway is involved. The aforementioned molecular mechanisms lie at the root of the cardioprotective function of adenosine, protecting the mitochondria from the effects of ischemia-reperfusion and facilitating adaptation of cardiomyocyte metabolism to cope with anaerobic conditions, thereby preventing the latter’s necrosis and apoptosis, and thus contributing to the reduction of the size of the infarct and the maintenance of cardiac function.

Moreover, adenosine receptors play an important role in reducing the size of the infarct by inhibiting cellular response during reperfusion. Neutrophils and platelets accumulate at the ischemic site, where they produce ROS and vasoconstrictive factors, thus contributing to endothelial dysfunction and myocardial injury. Activation of the A2 receptor in cells originating from the bone marrow helps reduce leukocyte infiltration into the infarct and limit the local immune response [27]. The interaction between neutrophils and the endothelium, and the release of myeloperoxidase, are weakened [26]. The inhibition of neutrophil infiltration and activity has also been observed after activation of the A2 receptors [28]. Activation of A2b results in a reduced release of TNF-α by mononuclear cells and a limited inflammatory response triggered by the ischemia-reperfusion injury [54]. The A2 receptor mediates inhibition of the activation of CD4⁺ T-lymphocytes and their accumulation within the infarct [31] and prevents mast cell degranulation in the heart [32]. The favorable effects of adenosine on microcirculation during reperfusion may not only be the result of neutrophil and platelet inhibition, but also its vasodilatory effect [55]. The signaling pathways responsible for inhibiting the cellular response have yet to be elucidated.

The presented results of animal model research indicate that all the PI receptors are involved in the cardioprotective activity.
of adenosine. Moreover, it is more and more often emphasized that the optimal protective effect requires the activation of more than one subtype of adenosine receptor. The observed decrease in the size of the infarct results from limitation of the ischemia-reperfusion injury that constitutes the sum of cardiomyocyte death and coronary dysfunction processes. Reducing the size of the infarct is an important therapeutic objective due to its association with mortality and heart failure.

ADENOSINE AS AN ADJUNCT TO REPERFUSION THERAPY IN MYOCARDIAL INFARCTION

Reports on the cardioprotective effects of adenosine and synthetic P1 receptor agonists in animal models have contributed to the commencement of clinical research aiming at assessing the safety and benefits of using adenosine as an adjunct to reperfusion therapy in myocardial infarction [56, 57]. Two adenosine administration techniques have been proposed, intravenous infusion or direct coronary infusion during percutaneous coronary interventions (PCI), both of which have been perceived as potentially able to limit the adverse effects of reperfusion. Description of the most important clinical trials is given in Table 1.

The first major clinical trial to assess the utility of intravenous infusions of adenosine in acute myocardial infarction was Acute Myocardial Infarction Study of ADenosine (AMIS-TAD). It showed that a 3 h adenosine infusion (70 μg/kg/min) beginning prior to the introduction of thrombolytic therapy contributed to reduction of the size of the infarct, but did not bring clinical benefits in the form of a reduced risk of death, repeated myocardial infarction, congestive heart failure or cardiogenic shock [58]. The research was later continued as AMIS-TAD II, which used a larger patient pool, and where adenosine (in doses of 50 or 70 μg/kg/min for 3 h) was administered as an adjunct to thrombolytic therapy or PCI. The study confirmed the earlier observation that adenosine infusion did reduce the size of the infarct, but was not accompanied by clinical benefits [59]. This left the clinical benefits of adenosine administration inconclusive, therefore further and more focused research with a larger group of patients was needed [60]. In accordance with the results of previous reports, further studies have proven that the size of the infarct diminishes at 3 h post adenosine infusion (in doses of 50 or 70 μg/kg/min) commenced prior to or during PCI, but have also shown that coronary flow improves and the no-reflow phenomenon is restricted [61, 62]. No-reflow is a complication of PCI, where the flow in a coronary vessel stops despite effective revascularization. Myocardial infarction complicated by no-reflow is difficult to treat and is associated with a worse prognosis for the patient [63]. In their papers, these authors stressed that the shortest possible time before restoration of coronary artery patency was the factor that determined whether the aforementioned benefits were achieved or not. The favorable effect, as described by Zhang et al. [62], of a higher dose of adenosine could have been the result of the inclusion of patients within 12 h after symptom onset in the study group. Contrary to Zhang et al., Micari et al. [61, 62] observed a positive effect of a lower dose of adenosine if administered within 6 h after symptom onset.

Another group of studies has focused on intracoronary administration of adenosine during PCI. The result of a small study carried out by Marzilli et al. [64] showed that adenosine (4 mg) administered intracoronarily during PCI (≤3 h after symptom onset) was well tolerated, improved coronary flow and ventricular function, prevented no-reflow and was associated with a more favorable clinical outcome. However, the results of larger clinical studies have not been so optimistic. No clear answer has been provided as to whether adenosine should be introduced in the guidelines for management of acute myocardial infarction. Among its favorable effects is improvement of angiographic and electrocardiographic parameters [65]. However, adenosine administered intracoronarily either within 4 h after symptom onset or later has not been found to expand the rescued area of myocardium or reduce microvascular obstruction (MVO) [66]. In their study, Garcia-Dorado et al. [67] did not show a reduced size of infarct among patients receiving adenosine, although where the intervention was carried out quickly (with an ischemia time of less than 200 min) there was an improvement in the left ventricular ejection fraction (LVEF). Meta-analysis results have confirmed the safety of intracoronary adenosine administration, as well as improved electrocardiographic parameters [68] and LVEF, with a lower prevalence of heart failure [69], but have not shown adenosine to decrease mortality due to major adverse cardiac events, heart failure or other cardiovascular causes. Disturbingly, the results of the multi-center REperfusion Facilitated by L0cal adjunctive therapy in ST-Elevation Myocardial Infarction (REFLO-STEMI) trial published in 2016 [70] did not confirm the favorable effects of adenosine (1 mg x 2) and sodium nitroprusside on coronary flow and infarct size. What the trial did show, however, was that high adenosine doses demonstrated cardiotoxicity and had an adverse impact on further clinical course. Patients receiving adenosine were observed to have a significantly higher prevalence of heart failure as compared to the control group. These results question the validity of the procedure of administering repeated adenosine doses to patients diagnosed with MVO, which was entered into the guidelines on the basis of previous research results.

The results of 3 meta-analyses published in 2015–2016 [55, 63, 71] agreed that adenosine improved myocardial perfusion and prevented no-reflow, although Bulluck et al. [7] indicated that this effect was observed following intracoronary administration rather than intravenous infusion. Moreover, adenosine decreased the prevalence of heart failure and improved LVEF [63]. Nevertheless, no clinical benefits were observed, such as a reduced risk of death [55, 63] or subsequent infarction [63]. Administration of adenosine is associated with the occurrence of short-term adverse events, such as bradycardia, hypotension and atrioventricular block [55]. The authors of these papers pointed out that the available results did not allow for unequivocal conclusions about the clinical utility of adenosine as an adjunct to reperfusion treatment of ST-elevation myocardial infarction. Further studies are needed to determine the optimal adenosine dose and to confirm its clinical benefits.
TABLE 1. Description of the route, dose and time of administration for the clinical trials presented

<table>
<thead>
<tr>
<th>Trial and year of publication</th>
<th>Route</th>
<th>Dose</th>
<th>Time of administration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahaffey et al. [58] 1999 AMISTAD I</td>
<td>IV</td>
<td>70 μg/kg/min for 3 h</td>
<td>prior to commencement of thrombolytic therapy (≥6 h after symptom onset)</td>
<td>reduction of infarct size, no clinical benefits</td>
</tr>
<tr>
<td>Marzilli et al. [64] 2000</td>
<td>IC</td>
<td>1 mg × 2 during PCI, 1st dose after reaching the thrombus (≤3 h after symptom onset)</td>
<td>improved coronary flow and ventricular function, reduction of no-reflow</td>
<td></td>
</tr>
<tr>
<td>Ross et al. [59] 2005 AMISTAD II</td>
<td>IV</td>
<td>50 or 70 μg/kg/min for 3 h</td>
<td>commenced ≤15 min prior to thrombolytic therapy or PCI (≤6 h after symptom onset)</td>
<td>reduction of infarct size (70 μg/kg/min), no clinical benefits</td>
</tr>
<tr>
<td>Micardi et al. [61] 2005</td>
<td>IV</td>
<td>50 or 70 μg/kg/min for 3 h</td>
<td>commenced ≤15 min prior to PCI (≤6 h after symptom onset)</td>
<td>reduction of infarct size, improved coronary flow, reduction of no-reflow</td>
</tr>
<tr>
<td>Desmet et al. [66] 2011</td>
<td>IC</td>
<td>4 mg</td>
<td>during PCI, prior to balloon inflation (≤4 h and &gt;4 h after symptom onset)</td>
<td>no effect on infarct size and coronary flow</td>
</tr>
<tr>
<td>Grygier et al. [65] 2011</td>
<td>IC</td>
<td>1-2 mg × 2 during PCI, first dose after reaching the thrombus, second dose after balloon inflation (≤6 h after symptom onset)</td>
<td>improved angiographic and electrocardiographic parameters</td>
<td></td>
</tr>
<tr>
<td>Zhang et al. [62] 2012</td>
<td>IV</td>
<td>50 or 70 μg/kg/min for 3 h</td>
<td>during PCI (≤12 h after symptom onset)</td>
<td>reduction of infarct size (70 μg/kg/min), improved left ventricular function and coronary flow and reduction of no-reflow (50 and 70 μg/kg/min)</td>
</tr>
<tr>
<td>Nazir et al. [70] 2016</td>
<td>IC</td>
<td>1 mg × 2 during PCI, 1st dose after removal of the thrombus, 2nd dose after placing the stent (≤6 h after symptom onset)</td>
<td>no effect on infarct size and coronary flow, adverse effect on further clinical course (higher prevalence of heart failure than in control group)</td>
<td></td>
</tr>
</tbody>
</table>

IC – intracoronary; IV – intravenous; PCI – percutaneous coronary intervention

myocardial infarction. Moreover, such results cast considerable doubt on the cardioprotective properties of adenosine in humans and the possibility of using it in the treatment of myocardial infarction. The difficulties in providing an unambiguous assessment of the benefits of using adenosine in myocardial infarction treatment stem from the numerous limitations of the clinical trials conducted thus far: their small number, different patient selection criteria (i.a. different times from infarction symptom onset at the time of intervention), adenosine administration procedures (route, dose and time) and definitions of the endpoints. Therefore, there is a need for further clinical research, particularly large randomized controlled trials (RCTs).

SUMMARY AND DIRECTIONS OF FUTURE RESEARCH

Research on the use of adenosine as an adjunct in myocardial infarction therapy conducted thus far does not provide an unequivocal answer as to whether its application has any clinical benefits. This is disappointing, given the very promising outcomes of animal model studies. These discrepancies could be the result of the fact that most animal models are rodents, rather than large mammals that would closer resemble human physiology. Moreover, research is mostly performed on young animals and the possibility of using it in the treatment of myocardial infarction. The difficulties in providing an unambiguous assessment of the clinical utility of adenosine is of paramount importance [72]. Planning large randomized clinical trials requires an ability to explain the existing failures and to implement the conclusions drawn from them for an unequivocal assessment of the clinical utility of adenosine in treating myocardial infarction patients.

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