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اصول تنظیم قراردادها
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Effect of HEMADO on Level of CK-MB and LDH Enzymes after Ischemia/Reperfusion Injury in Isolated Rat Heart

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Introduction

Acute myocardial infarction (AMI) is a general trouble that threatens human’s health. AMI is often induced by the occlusion of coronary arteries and can cause ischemia.1 Ischemia in the heart is characterized by an imbalance between heart oxygen demand and supply due to occlusion of the coronary artery and then reduction of blood flow.2 Ischemic heart diseases are anticipated to become the primary reason of death worldwide and it is going to precede infectious diseases by 2020.3 Ischemia without consecutive reperfusion in the end leads to cell failure.4 Urgent reperfusion is a definite treatment to rescue ischemic myocardium from an expected death.1 Several studies have shown that reperfusion has the potential to extend the degree of myocardial injury, the so-called “reperfusion injury”.3,5,6 Short periods of ischemia before long ischemia reduce cardiac injury and this cardioprotective effect is called ischemic preconditioning. Several pharmacological agents are able to imitate the cardioprotective effects of ischemic preconditioning.7 Although the triggers and intermediaries of preconditioning are not still well understood, adenosine is one of the considerable pharmacological agents in last two decades. Adenosine is a nucleoside that plays several roles in different tissues such as heart, nervous system and endocrine system. The release of adenosine usually increases in ischemic situation with ATP breakdown that includes related acts through its several receptors.8

A recently known adenosine receptor, the A3 subtype, is expressed in the heart, and its activation protects the heart ischemia.9 Studying the A3 receptors is done using various agonists and antagonists and different mechanisms are suggested for it.10 It has been revealed that mitoKATP has a better operation on cardioprotection afforded by preconditioning.7,11 In this method, application of 5HD, as a definite mitoKATP channel blocker, prevents cardioprotective effect of ischemic

ABSTRACT

Introduction: Ischemia/Reperfusion (IR) injury mainly causes the increase of enzymes involved in myocytes injury including CK-MB (creatine kinase-MB) isoenzyme and LDH (lactate dehydrogenase). Leakage of CK-MB isoenzyme and LDH from myocardial tissues to blood is indicator of acute myocardial infarction. The aim of this study was to assess the effect of HEMADO on IR injury and its relationship with mitochondrial ATP-sensitive K+ channels (mitoKATP) in rat heart. Methods: Twenty eight male Wistar rats (250-300g) were divided into four groups (seven members in each group): control (without ischemia), I/R (with ischemia+without HEMADO), ischemia received HEMADO (HEMADO), ischemia received HEMADO and 5-HD (5-hydroxydecanoate, specific mitoKATP channel blocker) (HEMADO+5-HD). The animals were anesthetized and the hearts were quickly removed and mounted on Langendorff apparatus and perfused by Krebs-Henseleit solution under constant pressure and temperature of 37ºC. After 20 minutes of stabilization, ischemic groups were exposed to 40 minutes of global ischemia and consecutive 90 minutes of reperfusion. Results: IR injury increased the level of LDH and CK-MB in the collected coronary flow during 5 minutes since start of reperfusion. HEMADO reduced the enzymes' levels and using 5-HD abolished the effect of HEMADO. Conclusion: Our findings indicated that HEMADO could protect the heart against ischemia-reperfusion injury by decreasing the CK-MB and LDH levels. The cardioprotective effect of HEMADO may be mediated in part by mitoKATP.

Keywords: HEMADO Lactate Dehydrogenase Creatine Kinase Ischemia/Reperfusion Injury mitoKATP

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preconditioning.\textsuperscript{12} mitoKATP channels are most important signaling routes for A3 receptor’s action. A number of similar A3 adenosine receptor (AR) agonists including the IB-MECA, CI-IB-MECA, CB-MECA, and CP-532,903 have been exposed to be useful in protecting against myocardial IR injury in animal models of myocardial Ischemia.\textsuperscript{13}

The aim of this study was to evaluate the effect of HEMADO (2-(1-Hexynyl)-N-methyladenosine), a High affinity and selective adenosine A3 receptor agonist, on the level of CK-MB and LDH enzymes following cardiac IR injury.

Materials and methods

Animals

Twenty eight male Wistar rats (12-week old and initial body mass of 250-300 g) were obtained from laboratory animal house of Tabriz University of Medical Sciences. They were housed in an animal room at 22-24\textdegree C and given free access to commercial rat chow and tap water. The animals were adapted to an inverse 12: 12 h light. All the experimental procedures employed, as well as rat care and handling, were in accordance with guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and use Committee of Tabriz University of Medical Sciences.

Rats were divided into four groups as: control (without ischemia), I/R (with ischemia+without HEMADO), ischemia received HEMADO (HEMADO), and ischemia received HEMADO+5-HD (HEMADO+5-HD). Each group was composed of seven members (n=7). Minimum dose of HEMADO was 0.1 \mu M/L; hence the work was on three different groups which were obtained for this study. In I/R group after 20 minutes of stabilization, animals were exposed to 40 minutes of global ischemia and consecutive 90 minutes of reperfusion. HEMADO was purchased from Tocris Bioscience company and 5-HD obtained from Sigma (St. Louis, USA). Remaining materials were purchased from Pars Azmoon (Tehran, Iran).

Experimental preparation

Male Wistar rats were anesthetized with ketamine and xylazine and given sodium heparin intravenously. The heart was quickly removed following thoracotomy and arrested in ice-cold perfusion buffer. The hearts were cannulated via the aorta and perfused by the Langendorff method using Krebs-Henseleit buffer containing (in mM) NaCl 118, NaHCO3 25, KCl 4.7, MgCl2 1.2, CaCl2 2.5, KH2PO4 1.2, and glucose 11. The perfusate was oxygenated with 95\% O and 2.5\% CO2.

Experimental protocol

All hearts were perfused on Langendorff apparatus and were stabilized for about 20 minutes in order to obtain the baseline measurements. After stabilization, pretreatment was performed with HEMADO for 25 minutes and mitoKATP channel blocker 5-HD for 5 minutes in each group. Blocking the perfusion to the hearts and global ischemia were induced for 40 minutes and followed by 90 minutes perfusion.

At the start of reperfusion, samples were collected (coronary flow collected in 5 minutes of reperfusion) to measure the myocardial enzyme leakage, including CK-MB and lactate LDH. LDH activity in the coronary effluent was estimated by LDH monitoring kit (Pars Azmoon, Tehran, Iran) using an auto analyzer apparatus. The level of CK-MB was calculated in coronary effluent samples at 5 min of reperfusion with a specific CK-MB Kit (Pars Azmoon Tehran, Iran), using an auto analyzer.

Statistical tests

Data were expressed as means ± SEM. Statistical comparison of means between groups was made by one-way ANOVA and a subsequent Tukey test. Significant differences were determined as P<0.05.

Results

Effect of HEMADO on CK-MB, LDH: CK-MB, and LDH was significantly higher in I/R group than that in the control group (P<0.05), and we found that HEMADO significantly reduced the release of CK-MB and LDH from I/R Myocardium. These protective effects of HEMADO were completely abolished by 5-HD (Fig. 1, 2).

![Fig. 1. Effect of HEMADO on CK: level of CK-MB in coronary effluent in 5 min after reperfusion in control, ischemia/reperfusion (IR), HEMADO, HEMADO+5-HD.](image1)

![Fig. 2. Effect of HEMADO on LDH: level of LDH in coronary effluent in 5 min after reperfusion in control, ischemia/reperfusion (IR), HEMADO, HEMADO+5-HD.](image2)
Effect of HEMADO on the level of cardiac enzymes

Discussion
This study shows that HEMADO induces cardioprotection in the ischemic rat heart by a significant decrease in CK-MB and LDH release from coronary effluent. These protective effects of HEMADO were inhibited when 5HD was applied before ischemia. Therefore, we demonstrated that HEMADO conferred cardioprotection from IR injury by activation of the mitoKATP channels. The amount of LDH and CK-MB level is an index for identifying the cell injury and membrane integrity in current research. When ischemia destructs cell membrane, these enzymes are leaked out of cells. So the level shows the injury rate and cell necrosis.

HEMADO significantly decreased the CK-MB and LDH levels of coronary flow in HEMADO group. Another research on ischemic hearts of dogs reduced the CK-MB level by using IB-MECA that is consistent with our result. The heart cells were contracted by Reperfusion due to an increase in some factors. this situation is seen in severe or long ischemia which causes mechanical stiffness and tissue necrosis. Contracted cells put pressure on neighboring cells and cause the decomposition of them and development of contraction which causes widespread necrosis.

In this study capacity of balloon in left ventricle was fixed during ischemia and reperfusion, so increase in end diastolic pressure resulted from stiffness in left ventricle wall or ventricle contracture.

HEMADO prevented end diastolic pressure from rising in reperfusion period, so it could be said that HEMADO was reduced in contracture and accordingly, it significantly decreased the subsequent necrosis. This outcome is obtained indirectly for this research as using HEMADO caused the reduction in level of LDH and CK-MB. Based on several studies, mitochondria is one of the important locations for drug where it can work well and protect heart in ischemia duration. mitoKATP channels exist in the mitochondrial membrane of cardiomyocytes. It has been recently revealed that preconditioning induces activation and trafficking of mitoKATP channels, which, in turn, decreases the duration of membrane action potential and Ca influx, thus promoting cell survival during ischemia.

It was shown that pretreatment with diazoxide, a definite opener of the mitoKATP channel, induced useful cardioprotection against IR injuries and this effect was prevented by 5HD, a putative specific blocker of this channel. It has been found that other pharmacological agents such as bradykinin, acetylcholine, opioids and phenyphrine open mitoKATP channels and then activate preconditioning in the isolated rabbit heart. Our study also indicated that HEMADO can produce cardioprotection through opening of mitoKATP channel in the isolated rat heart. 5-HD has complex metabolic actions in cardiomycytes. It has been reported that 5-HD blocks sarcolemmal KATP channels and it seems that 5-HD contributes in preconditioning through diverse mechanisms and does not act only as inhibitor of mitoKATP channels.

Most drugs affect two parts of mitochondria: mitoKATP channels and mitochondrial permeability transition pore (MPTP). Activation of mitoKATP channels protected the heart against ischemia and activation of MPTP released apoptosis factors causing death of myocardial cells; so injury increased.

Generally it is imagined that calcium increases and ROS fulfills a main role in activation of MPTP having major role in tissue injury. Opening MPTP prevented ATP building; so the intercellular ATP decreased. This reduction resulted in ionic and metabolic Homeostasis perturbation and activation of destructive enzymes such as nucleases, proteases and phospholipases. All these variations resulted in not returnable cellular injury and its necrotic death. If mitoKATP channels were activated in this duration, they could prevent opening MPTP and decreasing IR injury by stopping ATP hydrolyze and reducing calcium entry to cell.

Observations in this study showed that protective effects of HEMADO after IR injury are reduced by mitoKATP channels’ blocker. These results stated that HEMADO caused opening the mitoKATP channels. This opening probably resulted in MPTP closing and also applying its protective effect indirectly.

Conclusion
This study indicated that HEMADO decreases level of CK-MB and LDH maybe through reduction in Leakage of CK-MB and LDH from myocardial tissues to blood and can have a cardioprotective effect after I/R injury. Possible mechanisms of this protection may be due to the effect of HEMADO on mitoKATP channels.

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Ethical issues
All the experimental procedures employed, as well as rat care and handling, were in accordance with guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and use Committee of Tabriz University of Medical Sciences.

Competing interests
The authors declare no conflict of interests.
References


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