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USE OF PEROXIREDOXIN FOR PRECONDITIONING OF HETEROTOPIC HEART TRANSPLANTATION IN A RAT

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Peroxiredoxin 6 (Prdx6) is an antioxidant enzyme in the human body that performs a number of important functions in the cell. Prdx6 restores a wide range of peroxide substrates, thus playing a leading role in maintaining redox homeostasis in mammalian cells. In addition to peroxidase activity, Prdx6 has an activity of phospholipase A2, thus taking part in membrane phospholipid metabolism. Due to its peroxidase and phospholipase activity, Prdx6 participates in intracellular and intercellular signal transmission, thereby facilitating the initiation of regenerative processes in the cell, suppression of apoptosis and activation of cell proliferation. Given the functions performed, Prdx6 can effectively deal with oxidative stress caused by various factors, including ischemia-reperfusion injury. On an animal model of rat heterotopic heart transplantation, we showed the cardioprotective potential of exogenous recombinant Prdx6, introduced before transplantation and subsequent reperfusion injury of the heart. It has been demonstrated that exogenous Prdx6 effectively alleviates the severity of ischemia-reperfusion injury of the heart by 2–3 times, providing normalization of its structural and functional state during heterotopic transplantation. The use of recombinant Prdx6 can be an effective approach in preventing/alleviating ischemia-reperfusion injury of the heart, as well as in maintaining an isolated heart during transplantation.

Keywords: ischemia-reperfusion injury, peroxiredoxin, heterotopic heart transplantation.

INTRODUCTION

One of the key problems of cardiac surgery and transplantology is ischemia-reperfusion myocardial injury [13, 14, 16, 18]. Disruption of normal blood flow and the inadequacy of oxygen demand and delivery to tissues trigger a cascade of pathological ischemic processes leading to the formation of reactive oxygen species (ROS) and disruption of the structural and functional integrity of metabolic active tissues. Restoration of oxygenated blood flow (reperfusion) to ischemic tissues leads to an even greater ROS increase, the development of oxidative stress and aggravates the damage to myocardial tissues [16, 17]. This formidable complication occurs almost always, and only the level of damage can vary. Today, the number of proven and effective approaches to reducing the damaging effect of reperfusion is extremely small [12, 15].

Since the pathogenesis of ischemia-reperfusion injury (IRI) is associated with oxidative stress, the main direction in therapy may lie in reducing ROS concentration in the affected tissues using antioxidant drugs [1, 4].

Among many antioxidant enzymes, the most attractive is the family of peroxiredoxins (Prx) [5]. Prx play an important role in maintaining redox homeostasis in mammals. As a rule, their level increases with oxidative stress contributing to the normalization of ROS level in ischemic tissues. Among the family of peroxiredoxins, Prx6 is featured by the widest range of neutralized peroxide substrates of organic and inorganic nature including alkyl hydroperoxides, phospholipid peroxides, long-lived protein radicals, peroxynitrite, etc. [7]. Given the role of Prx6 in tissue protection against adverse effects, the use of Prx6 in transplantation should be explored to improve the safety of donor organs.

Purpose. To assess the possibility of using peroxiredoxin (Prx6) as a means to increase the resistance (preconditioning) of the myocardium to IRI.

TASKS

With a biological model of heterotopic rat heart transplantation, to compare the degree of damage to the donor heart in terms of troponin I concentration, rhythm disturbances and myocardial contractility, and to assess myocardial morphology in the group of animals receiving Prx6 and without Prx6.

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MATERIALS AND METHODS

The experiments used diclinous Wistar rats (250 g weight). The experimental program was approved by the Committee on Biological Safety and Bioethics. The experiments were performed in compliance with the rules of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes and Directive 2010/63/EU.

All rats were divided into 2 groups of 20 animals each. In group 1 (control group), 20 hearts of donor animals were transplanted into recipient animals by heterotopic transplantation. In group 2, the recipient animals underwent heterotopic heart transplantation from 20 donor animals with Prx6 introduced at the stage of reperfusion.

Recombinant Prx6 was obtained at the laboratory of reception mechanisms of the Institute of Cell Biophysics of the Russian Academy of Sciences by the previously described technique [19].

The model of a heterotopic rat heart transplant comprised the stages of donor anesthesia, heart explantation, heart storage in Custodiol solution, anesthesia of the recipient, transplantation of the donor heart to the recipient's abdominal aorta, wound closure, and the recipient eduction. The operated animals were placed in a vivarium for 24 hours under observation. The rats were kept in standard cages with a heating pad and *adlibitum* water supply under 12-hour day/night cycle. 24 hours after the operation, the animals were euthanized for histological examination of the state of myocardial tissues.

Heart explantation stage. After treating the surgical site with an antiseptic solution, a complete midline laparotomy was performed, the inferior vena cava (IVC) and abdominal aorta were isolated from the surrounding tissues below the renal arteries. Heparin solution (20 U) was injected into the IVC, then a micro-clip was applied to the injection site to prevent bleeding. Next, the aorta was cannulated with a 22G catheter, and continuous perfusion with 100 ml Custodiol cardioplegic solution was initiated through the infusion pump for 7 min. To decompress the right and left sections, the IVC and left pulmonary veins were transected. After the onset of cardioplegia, a median sternotomy was performed, and the edges of the wound were diluted with a dilator. The donor's heart was covered with ice. At the end of cardioplegia, cardiac explantation was started. To do this, step by step, the inferior and superior vena cava, then the aorta and the pulmonary trunk were isolated and ligated. The aorta was transected at the level of the origin of the brachiocephalic trunk, and the pulmonary trunk at the level of the bifurcation. The pulmonary veins were ligated in a single block. The inferior and superior vena cava were ligated separately.

After explantation, the donor heart was placed in a sterile container with a Custodiol solution. The container was covered with ice and stored at +4 °C for 4 hours until implantation. The total ischemia time was 5 hours.

The recipient was *anesthetized* in the same way as the donor.

Transplantation of a donor heart into the recipient's abdominal aorta. After anesthesia, a complete midline laparotomy was performed, the small intestine loops were removed to the left in relation to the operating wound and covered with a damp gauze wad to prevent drying. The wound edges were parted with a retractor, and the aorta and inferior vena cava were exposed in the infrarenal section. The lumbar branches, 3–4 permanent branches on average, were sutured. After lumbar vein ligation and vascular mobilization, 20 U heparin solution was injected into the inferior vena cava, a micro clip was applied to the injection site. A few minutes later, a vascular clamp was applied to the inferior vena cava and aorta in the proximal and distal directions. The recipient's aorta was cut longitudinally, and the aortic lumen was washed with heparinized saline to remove blood from the lumen. The heart graft was placed into the abdominal cavity, an end-to-side anastomosis was applied with a 10/0 atraumatic needle on a piercing needle. Then the recipient vena cava was opened longitudinally, and an end-to-side anastomosis was applied between the recipient's inferior vena cava and the donor pulmonary artery with a similar suture material. After performing the anastomoses, Antegrade Prx6 injection was performed at a calculated dose of 3 mg, the distal clamp was removed, filling the donor heart with blood. The donor's aorta was punctured with a 10/0 needle to prevent air embolism. Then the proximal forceps were removed. The restoration of cardiac activity occurred spontaneously. In case of disturbances in the implanted heart rhythm, electrical stimulation (ES) was used with an ЭКСН-4М pacemaker with a heart rate of 110 bpm and 6 mA amplitude. After controlling hemostasis, the loops of the small intestine were returned to the abdominal cavity, and 6/0 silk sutures were placed on the anterior abdominal wall (separately on the aponeurosis). The skin was sutured with a continuous 5/0 PET suture and antiseptic-treated. After the cessation of the inhalation anesthetic, the animal was taken out of anesthesia for 5 minutes. Then the recipient was placed in a standard cage with a heating pad and access to water.

To assess the effectiveness of Prx6 as a means for increasing myocardial resistance to IRI, the following parameters were analyzed:

– time to spontaneous restoration of the rhythm, intensity of cardiac activity, and myocardial kinetics;
– TnI concentration in blood.

A histological examination was also performed including Masson's and hematoxylin/eosin staining of myocardial preparations.

The spontaneous recovery of the heart rhythm was assessed in terms of the time from the moment of removal

of the proximal and distal clamps and the start of blood flow at the site of implantation of the donor heart to the appearance of electrical activity of the heart and visual signs of myocardial contraction, as well as the duration of the required temporary pacemaker. To confirm the data, 1-lead ECG was performed. Four electrodes were applied on the surrounding tissue around the graft. In addition to objective methods of control, the intensity of cardiac activity and myocardial kinetics were determined by intraoperative palpation assessment of the strength of heart contractions on the left ventricle (high/low) and the total filling of the graft chambers (high/low).

TnI concentration was determined in 60 minutes after the start of reperfusion and 24 hours after transplantation and restoration of blood circulation. TnI concentration was recorded by i-Stat System (Abbott Point of Care, USA) analyzer with TnI assay cartridges (Abbott Point of Care, USA). The initial TnI concentration did not exceed 0.01 ng/ml which corresponds to the norm.

The graft was retrieved for histology in 24 hours after the transplantation. For histological studies, myocardial samples were fixed in 10% formaldehyde solution. Images of histological sections were obtained using a Carl Zeiss Axio lab A1 microscope.

Table

Baseline characteristic of procedure and intraoperative parameters

	Group 1, n = 20	Group 2, n = 20	p
Animal weight, g	250 ± 7	250 ± 8	1
Duration of surgery, min	62.4 ± 5.2	71.4 ± 5	0.003
Duration of explantation, min	13.8 ± 1.5	14.3 ± 1.7	0.5
Duration of graft ischemia, min	305.3 ± 3.6	304.9 ± 2.7	0.8
Duration of graft perfusion with Custodiol, min	7	7	1
Custodiol volume for graft perfusion, ml	100	100	–
Myocardial contractile activity, % high low низкая	 0 30 70	 90 0 10	 0.0001

Note. Group 1 – rats after heterotopic heart transplantation, group 2 – rats after heterotopic heart transplantation with Prx6 administration 3 mg during reperfusion. There is standard deviation after "±".

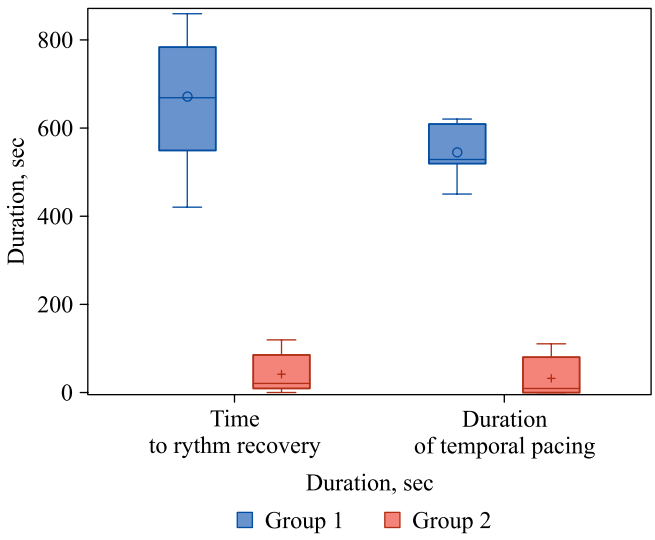

Fig. 1. Time to rhythm recovery of transplant and duration of temporal transplant pacing. Group 1 – rats after heterotopic heart transplantation, group 2 – rats after heterotopic heart transplantation with Prx6 administration 3 mg during reperfusion. There are statistically significant differences between groups: time to rhythm recovery of, p = 0,002; duration of temporal transplant pacing, p = 0,0001

Statistical data processing

All studied parameters were checked for normality of distribution [11]. In the case of a normal distribution, the t-test was used to compare the groups; when it differed from normal, Mann–Whitney test was used [10]. Qualitative indicators were compared with exact chi-squared test. Statistical data processing and plotting were performed with the SAS Enterprise Guide 6.1 licensed software.

RESULTS

Baseline characteristic of procedure and intraoperative parameters are given in Table.

Contractive activity of the transplanted heart

The contractile activity and the parameters of the electrical activity of the myocardium were better in group 2 (Table, Fig. 1).

Biochemical analysis of myocardial damage level

Myocardial injury in group 2 was significantly less than in group 1 (Fig. 2).

Histology of transplanted heart

Histology of myocardial fragments in group 2 showed the greater tissue structure preservation compared with the control group (Fig. 3, 4).

DISCUSSION

To date, 6 types of Prx have been identified in mammals, which, according to the number of conserved cysteine residues in the active site and mechanisms of catalysis, are subdivided into typical 2-Cys (Prx1–4), atypical 2-Cys (Prx5) and 1-Cys (Prx6). Besides the ability to neutralize a wide range of ROS, Prx have a number

of other important functions, among them the initiation of regenerative processes in cells due to chaperone and signal-regulatory activities [2, 8]. Of particular interest among mammalian peroxiredoxins is Prx6 capable of neutralizing a wide range of peroxide substrates of both inorganic and organic nature, including alkyl hydroperoxides, phospholipid peroxides, long-lived protein radicals, peroxynitrite, etc. [7]. Besides its peroxidase activity, Prx6 exhibits the activity of Ca2+-independent phospholipase A2 (aiPLA2), which normally manifests itself only under acidic conditions (at pH 4–5) is important in the metabolism of phospholipids and the transmission of intracellular and intercellular signals [3]. Prx6-knockout animals are featured by increased sensitivity to oxidative stress [3, 9]. Exogenous Prx6 has been shown to realize its signal-regulatory function through the TLR4/NF-kB pathway [7]. Thus, Prx6 is a multifunctional enzyme that participates in many cell processes and plays an important key role in antioxidant protection. It should be noted that the amount of intrinsic endogenous Prx6 synthesized in ischemic tissues is insufficient to suppress the development of oxidative stress. At the same time, the introduction of exogenous recombinant human Prx6 changes the situation. Thus, a high therapeutic activity of exogenous Prx6 has been demonstrated in laboratory rats in vivo [6]. At the same time, no toxic effects were observed when high doses of recombinant Prx6 were introduced into the body of laboratory animals. The introduction of Prx6 before or after an adverse effect contributes to the preservation or rapid restoration of the morphofunctional state of tissues, which may indicate a high therapeutic efficiency of the protein [3].

Due to these features, Prx6 can be considered a potential agent in perfusion solutions for the preservation and subsequent transplantation of isolated organs.

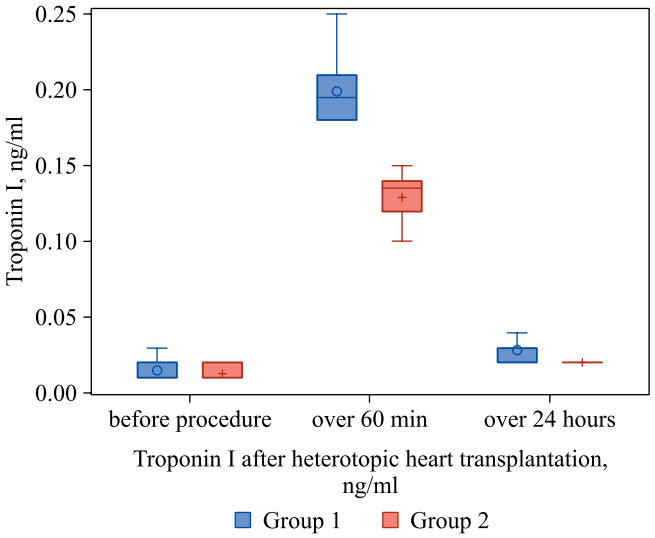

Fig. 2. Dynamic of troponin I level after heterotopic heart transplantation. Group 1 – rats after heterotopic heart transplantation, group 2 – rats after heterotopic heart transplantation with Prx6 administration 3 mg during reperfusion. Upper reference limit of troponin I is 0,05 ng/ml

Based on the data obtained, it can be assumed that Prx6 mitigates myocardial reperfusion injury after prolonged ischemia. This is supported by a lower increase in troponin I concentration, as well as better myocardial contractility in group 2. However, it should be noted that along with objective methods for assessing myocardial damage in the work performed, myocardial contractility was assessed subjectively, by a manual method, which is a limitation of the study. It is possible that the study of Prx6 in experiments on an isolated heart using a Langendorf device will help clarify the prospects for using Prx6 in transplantation.

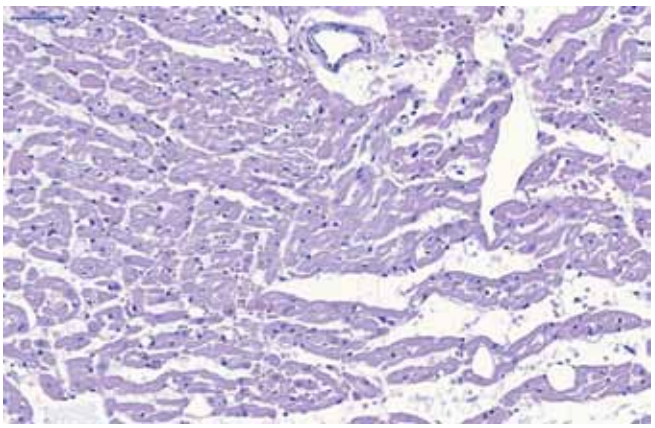

Fig. 3. Morphology of transplant with Prx6 cardioprotection. There are light myocardial sclerosis, activated endothelium and edema as well. Mild myocardial ischemic-reperfusion injury is presented

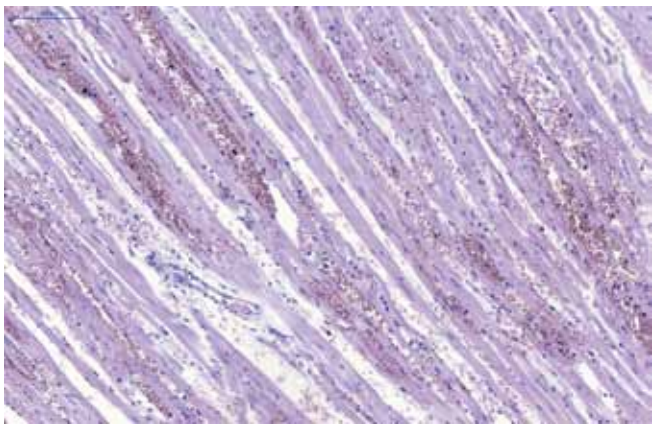

Fig. 4. Morphology of transplant in control group. There are hemorrhage with fibrin debris, activated endothelium and necrosis of cardiomyocytes with edema. Sever myocardial ischemic-reperfusion injury is presented

CONCLUSION

The experimental studies have shown that Prx6 can be considered a potential agent in perfusion solutions to protect the explanted organs. However, further experimental studies are required to clarify and quantify the protective effect of Prx6.

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The authors declare no conflict of interest.

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