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Myocardial Protection against Ischemic and Reperfusion Injuries (Experimental Study)

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The effects of hypoxic, hyperoxic, and hypoxic-hyperoxic preconditioning were examined in the prospective study on narcotized and artificially ventilated rabbits. Under artificial circulation, acute myocardial ischemia was modeled by ligation of anterior descending coronary artery, which was followed by reperfusion. The degree of ventricular arrhythmias was assessed, and the ischemic area was evaluated in percent of the area at risk. Microscopic characterization of the myocardium was employed to assess the cardioprotective effect of hypoxic and/or hyperoxic preconditioning. According to Kruskal—Wallis test, the greatest resistance of the myocardium to ischemic and reperfusion injury was observed after hypoxic-hyperoxic preconditioning (H=42.459; p=0.009). The rabbits subjected to this type of preconditioning demonstrated the least damaged myocardium in comparison with nonconditioned controls.

Key Words: *hypoxic preconditioning; hyperoxic preconditioning; myocardium; ischemic and reperfusion injury; artificial circulation*

Cardiosurgery interventions under conditions of artificial circulation are sometimes complicated by functional disturbances of vital organs. Preconditioning with moderate hypo- (HypPC) or hyperoxia (HyperPC) is an effective non-pharmacological way to enhance resistance of the organism to injuries and damaging interventions [1,8,11]. It is an accepted view that HypPC exerts a pronounced myocardial infarct size-limiting action [3,5]. According to modern data, HyperPC performed 24-48 h prior to the critical event significantly diminishes the infarct size by 20% and decreases the number of arrhythmic episodes [2]. Comparison of hyperoxic gas mixtures with various oxygen concentrations showed that mixture with 80% O_2 diminished the infarct region most effectively [6,10].

Our aim was to examine effectiveness of HypPC and HyperPC in protection of myocardium against ischemic and reperfusion injuries in modeled myocardium infarction under artificial circulation.

MATERIALS AND METHODS

A prospective study was conducted on male grey giant rabbits weighing 4.0-4.5 kg (n=30). In series I, the rabbits (n=20) were subdivided into 4 equal groups and were either not conditioned or subjected to HypPC, HyperPC, or HHPC.

In series II, the animals (n=10) were randomized into 2 equal groups (nonconditioned control and HHPC) to examine the myocardium microscopically.

The rabbits were maintained under standard vivarium conditions. All painful procedures were carried out on narcotized animals in strict adherence to Directive No. 199n (April 1, 2016) of Ministry of Health of the Russian Federation "On Establishing the Rules of Good Laboratory Practice", GOST 33044-2014 "Principles of Good Laboratory Prac-

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tice", and Guide for the Care and Use of Laboratory Animals [4].

The induction narcosis was performed with sevoflurane administered via anesthetic mask, thereupon the trachea was intubated with endotracheal tube No. 2.5 and connected to a Puritan Bennett 760 (Covidien) ventilator set at 50-55 min^{-1} respiratory rate and 30-40 ml tidal volume. Anesthesia was maintained with sevoflurane (1.2-1.5 v/v) supplied by a Vapor 2000 (Dräger) vaporizer. AP was measured in femoral artery via an Arteriofix 20G (BBraun) catheter. Another catheter of the same type was passed into femoral vein for infusion and drawing the blood samples. A temperature sensor was inserted into esophagus. The basic hemodynamic parameters, ECG, body temperature, and the pulse oximetry data were recorded using a Siemens 7000 (Siemens) point-of-care monitor.

In series I, HypPC was modeled by 2 cycles of 10-min hypoxemia produced by inhaling the gas mixture with low (10%) oxygen, the intercycle time being 5 min. The inhaled gas mixture was composed by graduated inflow of nitrogen into the closed breathing system of the ventilator to establish the necessary oxygen concentration. HyperPC was produced with hyperoxic gas mixture (75-80% O_2) inhaled for 30 min. HHPC was modeled by alternating use of a 10-min hypoxic and a 30-min hyperoxic gas mixtures. No preconditioning was conducted in the nonconditioned control group. Arterial and venous oxygen saturation parameters (SaO_2 and SvO_2) as well as blood lactate were monitored continuously. The oxygen extraction index (OEI) was calculated, and the acid-base balance was controlled. The blood gases were measured using a Stat Profile 5 (Nova Biomedical) analyzer.

To reveal activation of anaerobic metabolism, we used the ratio $dP_{v—a}CO_2/C_{a—v}O_2$, where $dP_{v—a}CO_2$ is the difference between venous and arterial partial carbogen pressure, whereas $C_{a—v}O_2$ is the difference between arterial and venous oxygen concentration. Tissue hypoxia was diagnosticated when this ratio surpassed 1.4.

After preconditioning followed by sternotomy, the rabbits were connected to an artificial circulation system consisted of an NPM-1 roller pump and a KidsD100 neonatal oxygenator (Dideco) incorporated into the aorta-right atrial appendage contour. Perfusion was performed at volumetric flow rate of 1.5 l/min/m^2, assuming the body surface area of 0.25 m^2. The left coronary artery was ligated for 45 min; then reperfusion was performed during 120 min against the background artificial circulation.

TABLE 1. Effect of HHPC on Oxygen Balance (Me, 25Q-75Q)

Parameter	Initial value	Hypoxic phase (*n*=5)	Hyperoxic phase (*n*=5)		
		10 min	10 min	20 min	30 min
Partial pressure of oxygen, mm Hg					
arterial blood	142 (108; 193.5)	52* (47.5; 60.5)	340* (264.5; 391)	326* (238.5; 357.5)	342* (284.5; 391)
venous blood	39 (38; 46)	37 (32; 38.5)	44 (35.5; 48.5)	53 (45; 56)	61 (52.5; 67)
$dP_{v—a}CO_2/C_{a—v}O_2$	0.9 (0.8; 1.0)	0.8 (0.7; 0.9)	1.1 (0.9; 1.2)	1 (0.8; 1.1)	1.1 (0.9; 1.2)
Oxygen saturation, %					
arterial blood	98 (96; 99)	82* (76.75; 85)	99 (98.5; 99)	99 (98.5; 99.5)	98 (97.5; 100)
venous blood	55 (49; 64.5)	51 (48.5; 53.05)	53 (48; 57.5)	56 (50; 64)	57 (52.5; 63)
OEI	43.8 (34.8; 48.9)	37.8 (36.8; 7.6)	46.4 (41.9; 1.2)	43.4 (35.7; 9.2)	41.8 (37; 46.1)
Glucose, mmol/liter	7 (5.5; 7.5)	5.5 (4; 5.5)	7 (5; 8.5)	6 (5; 7.5)	4 (3; 6.5)
Lactate, mmol/liter	4 (3.65; 4.5)	4 (3; 4.5)	5 (3.5; 6.5)	4 (4; 6.5)	7 (5; 8)

Note. *$p<0.05$ in comparison with the initial value (Wilcoxon's test).

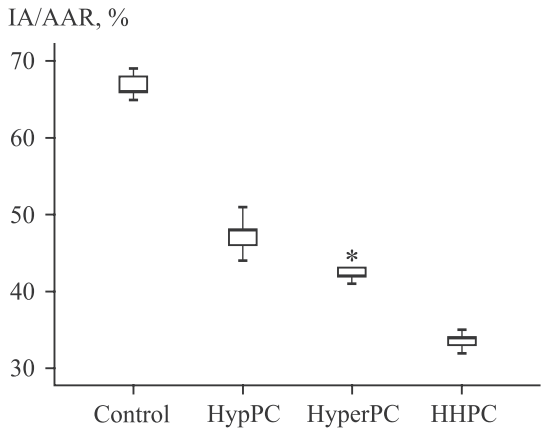

Fig. 1. Effect of preconditioning types on IA/AAR index. Asterisk indicates an outlier in the corresponding group.

The ratio of ischemic area (IA) to hypoperfused area at risk (AAR) was determined by a modified method [7]. To assess AAR, the ligature was tightened again, and the heart was stained with 5% potassium permanganate solution injected via aortic cannula. The rabbits were sacrificed by intravenous 10% KCl. The heart was excised, and the right ventricle was isolated to prepare 1-mm slices cut strictly perpendicular to its long axis. The necrotic tissues were delineated in the hypoperfused region. AAR and IA were determined by a computerized planimetric method employing original software. The IA/AAR ratio was expressed in percentage.

ECG was analyzed during entire (45 min) ischemic period and during the first 10 min of reperfusion. Under coronary occlusion, the arrhythmic episodes were assessed during the first 10 minutes (phase 1a) and during the following 35 minutes (phase 1b), because the mechanisms provoking arrhythmias in these periods significantly differ [1,9].

In series II, the experiments were carried out according to the same protocol except for sacrificing the rabbits after the end of a 120-min reperfusion period. The heart was excised for biopsy in ischemic, hibernating, and intact left ventricular regions determined visually according to the changes of myocardium color during ischemia and reperfusion.

The data were analyzed statistically using SPSS 23.00 software (IBM). Multiple comparison analysis of the quantitative data was performed by Kruskal—Wallis *H* test followed by analysis of the paired data by Mann-Whitney *U* test and Bonferroni correction. The intergroup difference between the ventricular arrhythmia incidences was analyzed by χ^2 test. Significance was assessed at $p<0.05$. The quantitative data were summarized as median (Me) with lower (Q25) and upper (Q75) quartiles.

RESULTS

In all compared rabbits, the acid-base composition of the blood was stable during entire experiment with normal levels of pH, buffer bases, $dP_{v—a}CO_2/C_{a—v}O_2$, lactate, glucose, potassium, and calcium. During 10-min HypPC, 30-min HyperPC, and 40-min HHPC, the expected changes in blood oxygen were observed attesting to adequate choice (10 or 75-80%) of oxygen concentration in the inhaled gas mixture (Table 1).

TABLE 2. Effect of Different Types of Preconditioning on Incidence of Ischemic (45 min) and Reperfusion (First 10 min) Arrhythmias

Arrhythmia type	HypPC	HyperPC	HHPC	Control
Myocardial ischemia (phase 1a)				
Arrhythmia-free or rare monotopic VES	2	4	5	2
Polytopic and polymorphic VES	2	1	—	2
Ventricular fibrillation	1	—	—	1
Myocardial ischemia (phase 1b)				
Arrhythmia-free or rare monotopic VES	5	4	5	3
Polytopic and polymorphic VES	—	—	—	1
Ventricular fibrillation	—	1	—	1
Reperfusion (first 10 min)				
Arrhythmia-free or rare monotopic VES	1	3	4	—
Polytopic and polymorphic VES	2	2	1	3
Ventricular fibrillation	2	—	—	2

Note. The cells show the number of rabbits. VES — ventricular extrasystoles.

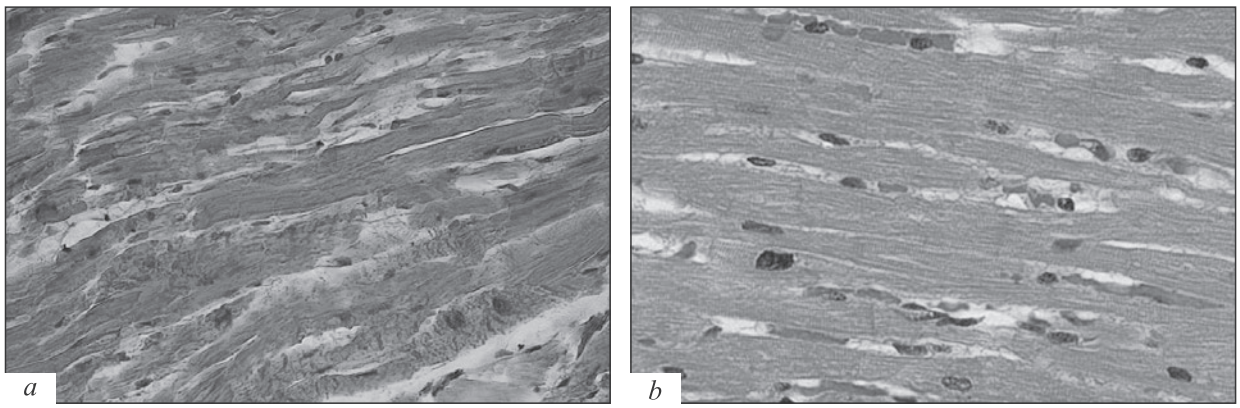

Fig. 2. Left ventricular myocardium (rabbit). Hematoxylin and eosin staining, ×200. *a*) Contracture-induced alterations of the myofibrils near infarction area in the nonconditioned control animal; *b*) capillary hyperemia in the left ventricular myocardium at the infarction area, and adequate integrity of the cardiomyocytes after HHPC.

In all groups, $dP_{v—a}CO_2/C_{a—v}O_2$ ratio as well as blood lactate and glucose did not significantly change during entire preconditioning procedure.

HypPC decreased IA/AAR index by 23% in comparison with nonconditioned controls (Fig. 1). Similarly, HyperPC significantly decreased this value by 26%. At this, HHPC produced the greatest benevolent action: it significantly diminished IA/AAR index by 32% in comparison with the controls according to Kruskal—Wallis test (H=42.459, p=0.0009).

During the first 10 min of myocardial ischemia (phase 1a), the incidence of arrhythmia was minimum in HyperPC and HHPC groups, where only rare monotopic ventricular extrasystoles were recorded (Table 2). The nonconditioned control rabbits demonstrated polytopic and polymorphic ventricular extrasystoles, while ventricular fibrillation was observed only in one animal. During ischemic phase 1b, any type of preconditioning enhanced the electrical stability of myocardium. Various types of ventricular arrhythmias were observed during reperfusion, although their numbers were smaller in HyperPC and HHPC groups (Table 2).

The study did not reveal significant differences between the effects of preconditioning on incidence of ischemic and reperfusion arrhythmias, which can be related to small sample size.

The microscopic examination of ischemic area in the left ventricle of nonconditioned control rabbits (series II) revealed capillary hyperemia and perivascular edema with occasional petechial hemorrhages. The nuclei of cardiomyocytes were characterized by peripheral condensation of chromatin and increased distance between the intercalated disks. We also observed contractures of type 1 and type 2 myofibrils. In HHPC group, these myocardial alterations were far less pronounced (Fig. 2).

The present study showed that hypoxic and hyperoxic preconditioning and their combination protect the myocardium. HHPC ensured maximum resistance of the heart to ischemic and reperfusion injuries. However, the antiarrhythmogenic effect of preconditioning was observed without significant intergroup differences. Thus, the effects of various preconditioning methods on myocardial resistance to ischemia/reperfusion injuries need further studies.

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