Effect of Nadcin on Energy Supply System and Apoptosis in Ischemia-Reperfusion Injury to the Myocardium

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Nadcin in a dose of 90 mg/kg administered to dogs with subacute stage of ischemia-reperfusion myocardial injury immediately after blood flow resumption normalized redox potential of cardiomyocytes and mitochondria and restored the total content of adenyl and pyridine nucleotides. The decrease in the synthesis of ATP and pyridine nucleotides and reduction of the redox potential of the energy supply system were inversely related to the increase in poly-(ADP-ribose)-polymerase activity in the ischemic area and nonischemic region. Nadcin abolished the increase in poly-(ADP-ribose)-polymerase activity in the ischemic area of the right ventricle, nonischemic region, and ischemic area of the left ventricle (by 2.4, 2.9, and 1.52 times, respectively) and normalized bioenergetic activity of cardiomyocytes during ischemia-reperfusion myocardial injury.

Key Words: *ischemia; reperfusion; Nadcin; energy supply system; apoptosis*

Systemic inflammatory response and hyperactivation of free radical processes during ischemia/reperfusion, inflammation, and shock are followed by a decrease in the cytosolic and mitochondrial redox potential of cardiomyocytes, induction of apoptosis, and increase in activity of poly-(ADP-ribose)-polymerase (PARP) [5,7,11,14]. Exhaustion of energy reserves in the neuronal cell inhibits induction of apoptosis and promotes necrosis [8,10]. These data were confirmed after identification of ATP as the major component of apoptosomes (complex formation from protease-activating factor (APAF-1), cytochrome C, and procaspase-9) [5,7,9]. The adaptive mechanisms in tissue cells are directed towards the increase in the synthesis of NAD and ATP. However, the decrease in the reserve capacities for adenyl and pyridine nucleotide synthesis is followed by the progressive necrotic changes and cardiomyocyte death [11,13,15]. The PARP-mediated pathway of cell death was described for immunostimulated macrophages and peroxynitrite-induced or hydrogen peroxide-induced dysfunction or death of thymocytes, macrophages, endothelial cells, neuronal cells, and fibroblasts [15]. The inhibition of PARP with drugs or the absence of this activity in animals prevents the progression of inflammatory and ischemic injury to the brain and heart, diabetes, and diabetic endothelial dysfunction [4,6,8,10,15].

This work was designed to perform a comparative placebo-controlled randomized study of the effect of various antihypoxic and antiischemic agents on the energy supply system and activation of PARP in dogs with chronic ischemia-reperfusion myocardial injury.

MATERIALS AND METHODS

Experiments were performed on male and female dogs (n=35) weighing 15-25 kg and maintained in a special vivarium. The animals were quarantined before the start of study. The animals were subjected to 20-min stenosis of the posterior descending coronary artery for 4 days. These dogs were randomized into 4 groups. Group 1 consisted of 10

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animals with modeled stenosis. Group 2 animals with stenosis (n=7) received NAD in a dose of 0.5 mg/kg. Group 3 animals (n=7) received inosine in a dose of 80 mg/kg. Group 4 animals (n=7) received Nadcin (lyophilized preparation of 0.5 mg NAD and 80 mg inosine) in a dose of 90.5 mg/kg. The therapy was started immediately after the first 20-min ligation of the artery and continued for 14 days. All drugs were dissolved in 20 ml physiological saline and injected intravenously in a dropwise manner for 20-30 min. The control group included 7 healthy dogs that were subjected to thoracotomy under similar conditions. An equivalent volume of physiological saline was injected intravenously to control animals.

The technique of preoperative treatment, study of intracardiac hemodynamics, and method of coronary artery stenosis were described elsewhere [3]. The treated and control animals were killed on day 14 after coronary artery occlusion.

We measured the contents of adenyl and pyridine nucleotides and creatine phosphate (CP). The results were analyzed statistically [1-3]. PARP activity was estimated with Amersham Pharmacia Biotech kits [12].

RESULTS

The dogs with coronary artery stenosis were examined on day 14 after 4-fold myocardial ischemia/reperfusion for 20 min. ATP content in the ischemic area of the right (RV) and left ventricles (LV) and nonischemic region of RV remained below the normal by 35.5, 26.7, and 32.4%, respectively (Tables 1-3). The total content of adenyl nucleotides and ATP/ADP ratio decreased by 19 and 27%, respectively. CP concentration in the ischemic area of RV and LV and nonischemic region of RV remained below normal by 59, 56, and 52%, respectively. The cardiomyocyte redox potential and NAD content in the ischemic area of RV and LV and nonischemic region of RV did not return to normal (Table 1-3). The NAD/NADH ratio in cardiomyocytes of the ischemic area in LV and RV was lower than in healthy animals (by 2.8 and 1.59 times, respectively). Hence, energy deficit due to acute myocardial ischemia persists for 18 days.

The decrease in ATP synthesis of was inversely related to an increase in PARP activity (r=0.87, p<0.001) [3]. On day 14, activity of PARP in the ischemic area of RV and LV and nonischemic region of RV surpassed the control by 5.86, 2.3, and 2.0 times, respectively (Tables 1-3).

NAD treatment (0.5 mg/kg) was started 20 min after the first ligation of the artery and continued for 14 days. The content of adenyl nucleotides and ATP/ADP ratio in animals of the NAD group practically returned to normal during the subacute stage of ischemia-reperfusion. However, CP concentration differed from the control.

Injection of inosine in a dose of 80 mg/kg was followed by an increase in the amount of ATP, total

TABLE 1. Effect of Nadcin on the Pool of Adenyl and Pyridine Nucleotides, Concentration of CP, and Activity of PARP in Myocardial Cardiomyocytes of the Ischemic Area in LV after Four Sessions of 20-min Stenosis and 2-week Reperfusion ($M\pm m$)

Parameter	Group				
	control	1	2	3	4
ATP	5.55±0.13	4.07±0.15*	5.13±0.23^{+}	4.68±0.19^{+}	5.17±0.15*
ADP	1.60±0.06	1.63±0.07	1.64±0.08	1.83±0.08	1.58±0.06
AMP	0.65±0.03	0.70±0.05	0.45±0.05*$^{+}$	0.65±0.05*$^{+}$	0.62±0.04
Total content of AN	7.80±0.25	6.30±0.14*	7.22±0.18^{+}	7.16±0.13^{+}	7.37±0.16*
ATP/ADP	3.47±0.10	2.53±0.09*	3.13±0.08^{+}	2.55±0.06^{+}	3.27±0.07*
ADP/AMP	2.46±0.10	2.32±0.09	3.64±0.10*$^{+}$	2.82±0.08*$^{+}$	2.55±0.06
NAD	5.45±0.12	3.69±0.10*	4.77±0.14	4.05±0.08	5.09±0.10*
NADH	1.67±0.09	1.80±0.06*	1.60±0.10	1.73±0.09	1.65±0.08*
NAD/NADH	3.26±0.07	2.05±0.06**	3.0±0.1^{+}	2.34±0.09^{++}	3.08±0.09^{++}
CP	6.49±0.17	2.83±0.12*	4.16±0.13*$^{+}$	4.05±0.09*$^{+}$	4.83±0.12*
PARP	0.043±0.008	0.099±0.009*	0.065±0.007*$^{+}$	0.060±0.007*$^{+}$	0.049±0.005*

Note. Here and in Tables 2 and 3: AN, adenyl nucleotides. The contents of adenyl nucleotides and CP are expressed in μmol/g wet tissue. PARP activity is expressed in pmol/mg protein. *p<0.05 and **p<0.01 compared to the control; ^{+}p<0.05 and ^{++}p<0.01 compared to group 1.

TABLE 2. Effect of Nadcin on the Pool of Adenyl and Pyridine Nucleotides, Concentration of CP, and Activity of PARP in Myocardial Cardiomyocytes of the Ischemic Area in RV after Four Sessions of 20-min Stenosis and 2-week Reperfusion (*M*±*m*)

Parameter	Group				
	control	1	2	3	4
ATP	5.43±0.15	3.50±0.10*	4.89±0.11*+	3.93±0.11*	5.37±0.12*+
ADP	1.66±0.07	1.60±0.08	1.72±0.06	1.70±0.09	1.46±0.06
AMP	0.58±0.05	0.84±0.04*	0.52±0.04	0.82±0.06*	0.69±0.05+
Total content of AN	7.66±0.18	6.03±0.11*	7.13±0.10*+	6.45±0.09*	7.52±0.12+
CP	5.83±0.23	2.43±0.11*	4.78±0.16*+	3.76±0.12*	5.19±0.12*+
NAD	5.27±0.07	3.67±0.09*	5.05±0.07*+	3.67±0.05*	5.16±0.06*+
NADH	1.66±0.12	2.98±0.09*	1.98±0.14*+	2.15±0.09*	1.56±0.12*+
NAD/NADH	3.17±0.08	1.23±0.07**	2.55±0.09	1.71±0.06	3.32±0.10
PARP	0.035±0.006	0.205±0.016*	0.085±0.009*+	0.139±0.010*	0.056±0.006*+

TABLE 3. Effect of Nadcin on the Pool of Adenyl and Pyridine Nucleotides, Concentration of CP, and Activity of PARP in Myocardial Cardiomyocytes of the Nonischemic Area in RV after Four Sessions of 20-min Stenosis and 2-week Reperfusion (*M*±*m*)

Parameter	Group				
	control	1	2	3	4
ATP	5.43±0.15	3.67±0.08*	5.30±0.15*+	4.45±0.11*	5.47±0.13+
ADP	1.66±0.07	1.72±0.06	1.72±0.05	1.67±0.09	1.54±0.08
AMP	0.58±0.05	0.84±0.04*	0.68±0.05	0.80±0.05*	0.60±0.06+
Total content of AN	7.66±0.18	6.23±0.11*	7.70±0.10*+	6.92±0.10*+	7.61±0.13+
CP	5.83±0.23	2.78±0.10*	4.78±0.16*+	4.21±0.11*	5.28±0.12*+
NAD	5.27±0.07	3.67±0.09*	5.05±0.07*+	4.46±0.09*	5.45±0.09*+
NADH	1.66±0.12	2.98±0.09*	1.98±0.14*+	2.89±0.11*	1.81±0.12*+
NAD/NADH	3.17±0.12	1.23±0.06	2.55±0.08	1.54±0.08	3.01±0.07**
PARP	0.035±0.006	0.205±0.016*	0.085±0.009*+	0.109±0.011*	0.049±0.005*+

content of adenyl nucleotides, and concentration of CP (by 12, 7, and 54.7%, respectively, compared to group 1 animals). Although the content of oxidized NAD did not increase, the redox potential of the energy supply system was elevated by 28%. Similar changes in the ischemic area of the LV myocardium and nonischemic region of RV were observed after inosine treatment (Tables 1-3).

Injection of Nadcin in a dose of 90 mg/kg for 14 days had a normalizing effect on ATP content in the ischemic area of the LV and RV myocardium. However, the relative content of ADP decreased by 12% in the ischemic area of RV. The total content of adenyl nucleotides increased to the control level (as differentiated from animals of the NAD or inosine group). CP concentration in cardiomyocytes in the ischemic area of RV, nonischemic region of RV, and ischemic area of LV increased by 114, 90, and 71%, respectively. It should be emphasized that CP concentration in dogs of the Nadcin group was higher than in animals receiving NAD or inosine. As distinct from NAD, Nadcin had a normalizing effect on the redox potential (NAD/NADH ratio) of the energy supply system in the ischemic area of RV and LV. Normalization of the redox potential and ATP content probably contributes to a decrease in PARP activity, restoration of apoptosis, and reduction of necrotic changes. Nadcin surpassed NAD in the ability to inhibit PARP in the ischemic area of RV and LV and nonischemic region of RV (by 1.50, 1.33, and 1.24 times, respectively; Tables 1-3). Inosine was less potent than PARP in decreasing activity of PARP in the ischemic area of RV and LV and nonischemic region of RV (by 2.5, 1.2, and 1.45 times, respectively). Our results indicate that Nadcin has a sy-

nergistic normalizing effect on apoptotic processes and induces the signal mechanisms to interrupt the vicious circle of transition from apoptosis into necrosis. It is related to normalization of the redox potential and ATP content and inhibition of PARP.

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