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Effect of IQ-1 on the Infarct Size and the Parameters of Cardiodynamic Indicators in the Acute Period after Myocardial Ischemia/Reperfusion in Rats

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The effect of a new JNK inhibitor IQ-1 (11*H*-indeno[1,2-*b*]quinoxalin-11-one oxime) was studied in male Wistar rats in a model of acute myocardial ischemia/reperfusion. Area at risk and myocardial infarct zones were studied in two series of experiments: 16 h after a single dose of IQ-1 (25 mg/kg intraperitoneally during cardiac ischemia) and on day 5 after its course administration (25 mg/kg intraperitoneally during cardiac ischemia and daily over 4 days). On day 5 after ischemia/reperfusion, cardiodynamic indicators were also studied: systolic, end-diastolic, and minimum pressure in the left ventricle, stress–time index, as well as the maximum rates of pressure rise and fall in the left ventricle ($+dP/dt_{max}$ and $-dP/dt_{max}$). In 16 h after ischemia/reperfusion, the infarct area in the control was 24±2% of the total area of the sections, while after administration of IQ-1 this parameter was 14±1% ($p<0.05$). On day 5, the infarct area in the control group was 25±1% of the total area of myocardial sections. A course of IQ-1 administration led to a significant reduction in the infarct area to 10±2% of the total area of myocardial slices. Course administration of IQ-1 led to improvement in contractile function and weakening of the diastolic dysfunction of the left ventricle: systolic pressure in the left ventricle increased by 20%, $+dP/dt_{max}$ by 23%, voltage–time index by 12%, $-dP/dt_{max}$ by 43%, and the minimum pressure in the left ventricle decreased by 3.4 times.

Key Words: *myocardial ischemia/reperfusion; cardiac hemodynamic; c-Jun N-terminal kinase inhibitor; 11H-indeno[1,2-b]quinoxalin-11-one oxime; rats*

Coronary heart disease is the leading cause of global mortality from various cardiovascular diseases [1]. The most effective treatment for coronary heart disease is timely reperfusion, including primary percutaneous coronary intervention and thrombolytic therapy. However, reperfusion leads to oxidative stress, arrhythmias, microvascular obstruction, contractile dysfunction, apoptosis and death of cardiomyocytes [2,3], and this phenomenon is called myocardial ischemia/reperfusion injury [4]. Studies have shown that the intracellular signaling pathway c-Jun N-terminal kinase (JNK) is involved in ischemia/reperfusion injury [5]. Oxidative stress leads to activation of JNK [6], resulting in apoptosis and necrosis of cardiomyocytes [7]. Pharmacological inhibition of JNK by various synthetic

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inhibitors reduces myocardial infarct size and reduces cardiomyocyte apoptosis early after ischemia/reperfusion injury [8-11]. However, the effect of various JNK inhibitors on the contractile function of the heart at the early stages after ischemia/reperfusion is ambiguous [12,13].

The search for highly selective JNK inhibitors suitable for therapeutic purposes is in progress. 11*H*-indeno[1,2-*b*]quinoxalin-11-one oxime (IQ-1) is a new JNK inhibitor with a selective effect on JNK3 [13,14], which is an important feature of this compound, because JNK3 is expressed exclusively in the heart, brain, and testicles and plays a unique role in the myocardium [15].

The aim of this work is to investigate the effect of single and course administration of IQ-1 on the myocardial infarct size in rats after acute myocardial ischemia/reperfusion, as well as the effect of course administration of IQ-1 on myocardial contractile function.

MATERIALS AND METHODS

The study was conducted in accordance with Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes) and was approved by the Commission for the Control of the Care and Use of Laboratory Animals of the E. D. Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center (Protocol No. 130092017 of September 8, 2017).

The experiments were performed on mature male Wistar rats (n=62) weighing 250-280 g. The rats were housed in groups of 5 animals per cage (57×36×20 cm) under standard laboratory conditions (22±2°C, relative humidity 60%, and 12/12-h light/dark cycle) in cages with sawdust bedding and had unrestricted access to water and standard rodent feed (PK-120-1, Laboratorsnab).

IQ-1 (M314 series) was synthesized as described previously [16]. The chemical structure of IQ-1 was confirmed by mass spectrometry and NMR; sample purity 99.9%. IQ-1 suspension for intraperitoneal administration was prepared under aseptic conditions with the addition of 20 μl of Tween-80 and 2 ml of sterile saline NaCl solution.

Two series of experiments were carried out. Rats in the control and experimental groups in both series underwent temporary ligation of the left coronary artery, rats in the sham-operated groups received only surgical intervention without coronary artery ligation. The coronary artery occlusion model has been previously described in detail [17]. Ligation of the left coronary artery was performed without disturbing the topography of the heart in the chest in anesthetized rats (propofol intravenously, 10 mg/kg/h) after thoracotomy and pericardiotomy. The duration of occlusion was 45 min. The study of the risk and myocardial infarct zones was carried out in two series of experiments: in series I — 16 h after a single injection of IQ-1 (25 mg/kg at the 30th minute of myocardial ischemia) and in series II — on day 5 after course administration of IQ-1 (25 mg/kg at the 30th minute of myocardial ischemia, then daily for 4 days). Rats of the control groups received 2 ml of solvent intraperitoneally according to the same scheme.

In series II, the parameters of cardiac contractile function were also studied. Three hours after the last injection of IQ-1, the animals were placed under general anesthesia by diethyl ether inhalation, then a catheter was implanted into the right femoral vein, and the animals anesthetized with sodium thiopental (35 mg/kg/h). To measure cardiac contractile function, a microsensor TSD282 of an intracardiac pressure device (OpSens) was inserted through the right common carotid artery into the left ventricular (LV) cavity and connected to an MP150 high-speed data acquisition and processing system (Biopac Systems, Inc.) The pressure curve in the LV cavity was used to determine systolic pressure (LVSP, mm Hg), end-diastolic pressure (LVEDP, mm Hg), minimum pressure (LVP_{min}, mm Hg), stress—time index (STI, mm Hg), and maximum rates of rise and fall of intraventricular pressure ($+dP/dt_{max}$ and $-dP/dt_{max}$).

To identify the risk zone, in rats anesthetized with sodium thiopental, the ligature on the coronary artery was again tightened, 0.2 ml of 5% solution of patent blue violet dye (Sigma) was injected as a bolus into the femoral vein, and the heart was removed after 10-20 sec. The atria, right heart, and great vessels were removed. The heart was frozen for 1 h and 1-mm sections were prepared using a RBM2000C tissue matrix. The sections were covered with glass slides and scanned on an HP Scanjet 3770. Then the sections were washed and stained with a 1% buffer solution of 2,3,5-triphenyltetrazolium chloride (pH 7.4) at 37°C for 10 min to identify the infarct zone by the level of dehydrogenase activity; the stained side of the sections was scanned again. The resulting images were processed using Adobe Photoshop 6.0. The areas of risk and myocardial infarct zones and the entire LV were calculated. The areas of risk zones and myocardial infarct zones were expressed as a percentage of the area of the LV myocardium.

Statistical analysis was performed using Statistica 8.0 (StatSoft, Inc.). The data were expressed as M±SEM. Group variability was assessed using the Kruskal—Wallis test. Significant differences between the groups were determined using the Fisher's exact test (animal mortality) and the Mann—Whitney U test (other parameters) at p<0.05.

RESULTS

In the groups of sham-operated animals, no deaths were observed. In series I (16 h after ischemia/reperfusion), 5 (38%) of 13 rats died in the control group and 2 (22%) of 9 in the experimental group. In series II (5 days after ischemia/reperfusion), rat mortality in the control and experimental groups was 43% (6 out of 14 rats died: 5 rats during the first day and 1 rat on the third day) and 38% (5 out of 13 rats died during the first day), respectively. Mortality in the control and the experimental groups did not differ significantly.

In series I, the size of myocardial infarct was ~24% of the section area in rats of the control group and ~14% in rats receiving single injection of IQ-1. In series II, the myocardial infarct zone in animals of the control group was ~25% of the total area of myocardial sections. The size of the myocardial infarct zone in the group of rats treated with IQ-1 was significantly lower than in the control: ~10% of the total area of myocardial sections (Table 1; Fig. 1). Risk zones in all studied groups did not differ significantly (Table 1).

TABLE 1. Effect of IQ-1 on the Risk and Myocardial Infarct Zones in Rats 16 h (Series I) and on Day 5 (Series II) after Myocardial Ischemia/Reperfusion (*M*±*SEM*)

Group		Risk zone, %	Myocardial infarct zone, %
Series I	control (*n*=8)	35±1	24±2
	IQ-1 (*n*=7)	36±1	14±1*
Series II	control (*n*=8)	36±2	25±1
	IQ-1 (*n*=8)	36±1	10±2*

Note. *p<0.05 in comparison with the control.

In 5 days after ischemia/reperfusion, control animals showed impaired myocardial contractile function and a statistically significant decrease in LVSP (by 31%), $+dP/dt_{max}$ (by 36%), and STI (by 18%) relative to the values in sham-operated rats. Disturbance in the processes of myocardial relaxation were detected in control animals: a decrease in $-dP/dt_{max}$ (by 47%) and an increase in LVEDP (by 139%). The differences in LVP_{min} in sham-operated and control rats were qualitative (Table 2). These results are in good agreement with the data of similar studies conducted on rats at similar terms after ischemia/reperfusion [18,19].

In the group of rats with course administration of IQ-1, a significant improvement in myocardial contractile function was noted on day 5 after ischemia/reperfusion in comparison with the control group. LVSP, $+dP/dt_{max}$, and STI increased by 20, 23, and 12%, respectively. There was an improvement in the processes of myocardial relaxation: $-dP/dt_{max}$ significantly increased (by 43%) and LVP_{min} decreased (by 3.4 times) (Table 2).

The protective effects of JNK inhibitors have been demonstrated *in vitro* in cell lines, *ex vivo* on the isolated hearts, and *in vivo* in myocardial ischemia/reperfusion models [8-13]. Studies of the cardioprotective activity of JNK inhibitors conducted *in vivo* on a model of myocardial ischemia/reperfusion are limited to short periods (3-24 h) [8,9,11]. At the same time, the ischemia/reperfusion damage to the myocardium appear at later terms, after 5-7 days [18-20]. In our

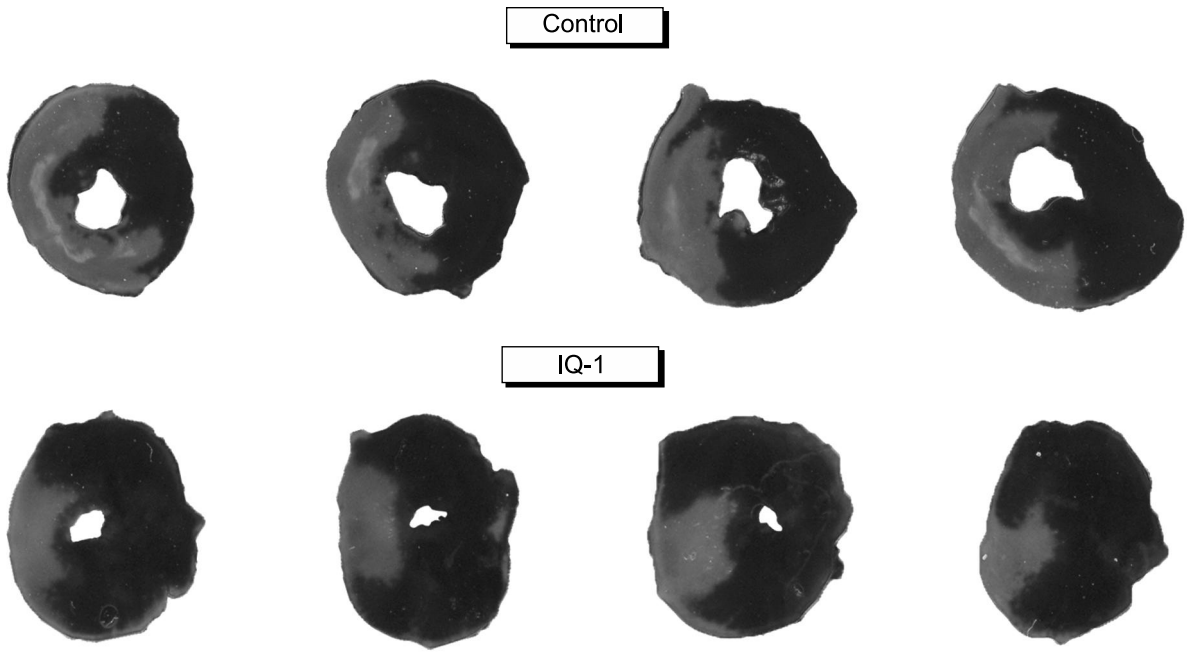

Fig. 1. Sections of the LV of the rat heart (series II). The light zones of the sections are the infarct zones.

TABLE 2. Effect of IQ-1 on Parameters of Contractile Function of the Heart of Rats on Day 5 after Myocardial Ischemia/Reperfusion (*M*±*SEM*)

Parameter	Sham-operated (*n*=8)	Control (*n*=8)	IQ-1 (*n*=8)
LVSP, mm Hg	150±7	103±3*	124±4^{+}
+dP/dt$_{max}$, mm Hg/sec	9254±308	5936±204*	7283±236^{+}
-dP/dt$_{max}$, mm Hg/sec	8351±307	4459±261*	6374±391^{+}
LVEDP, mm Hg	4.4±1.1	10.5±1.6*	6.3±1.1^{+}
LVP$_{min}$, mm Hg	-0.5±0.8	6.4±1.6*	1.9±11^{+}
STI, mm Hg	9.9±0.6	8.1±0.3*	9.1±0.3^{+}

Note. $p<0.05$ in comparison with *sham-operated, $^{+}$control.

experiments, a course intraperitoneal administration of IQ-1 to rats led to a decrease in the infarct area, improvement in LV contractile function, and weakening of diastolic dysfunction in 5 days after myocardial ischemia/reperfusion. IQ-1 may be a promising compound for the development of a drug based on it for the treatment of myocardial ischemia/reperfusion injury.

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