ORIGINAL ARTICLE

Experimental Approaches to the Assessment of Potential Cardioprotective Means with Doxorubicin-Associated Cardiomyopathy

Lyudmila M. Danilenko, Mikhail V. Pokrovskiy, Alla S. Kotelnikova, Alyona S. Timokhina, Elena B. Artyushkova, Nina I. Zhernakova, Vladimir V. Gureev

Department of Pharmacology and Clinical Pharmacology, Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia

Abstract

Introduction: At present, doxorubicin (DOX) is the most popular anthracycline antibiotic in oncology. The search for substances with a cardiotropic effect in DOX-associated cardiomyopathy is conducted among various classes of chemical and pharmacological groups. **Research Tasks:** Development of methodological approaches for evaluation of cardioprotective activity of drugs in doxorubicin cardiomyopathy. **Materials and Methods:** Simulation of the cardiomyopathy was performed by intraperitoneal administration of DOX at a dose of 20 mg/kg 1 time per day. After 48 h, we assessed indices of a left ventricular contractility under conditions of high heart rate 480 bmp for 15 s on an isolated Langendorff heart of rats. As an additional index of the assessment of the cardioprotective action of pharmacological agents, S_{tTTI} coefficient was used, reflecting the diastole defect, which is an area under the curve of the buildup of an end diastolic pressure. To evaluate the myocardial damage, the isoenzyme creatine phosphokinase-MB and lactate dehydrogenase (LDH) in perfusate flowing from the isolated hearts were determined. For a comprehensive confirmation of the development of the simulated pathological processes, a morphological study of the hearts was performed. As drugs, enalaprilat (KRKA Slovenia) at the dose of 5 mg/kg intraperitoneally every 12 h, carvedilol (Teva, Israel) per os 1 time a day, and verapamil (JSC Alkaloid, Macedonia) at the dose of 5 mg/kg intraperitoneally 1 time a day were used. **Results:** In a control group with the DOX-induced cardiomyopathy under the conditions of submaximal stimulation frequencies (480 bpm), we observed the diastolic defect which numerically was $S_{tTTI} = 8.3 \pm 0.3$ c.u. which shows significant damage and the failure of the calcium pumps of cardiac myocytes. In an intact group, S_{tTTI} coefficient was 1.4 ± 0.1 c.u. which is 8 times less than in the control group. The results of biochemical and morphological studies confirmed the degree of myocardial damage. In the comparative analysis of cardioprotective activity of drugs in doxorubicin cardiomyopathy, the studied compounds were located in the following sequence: Enalaprilat (5 mg/kg), carvedilol (30 mg/kg), verapamil (5 mg/kg). **Conclusion:** The fundamental difference in the area under the curve of the buildup of the end diastolic pressure under the conditions of submaximal stimulation frequencies (480 bpm) for 15 s in the intact group and the control group on the background of DOX administration naturally led to the necessity of introducing S_{tTTI} coefficient, which is quite revealing and informative. The obtained results allow to use S_{tTTI} at the screening of innovative molecules.

Key words: Cardiomyopathy, coefficient S_{tTTI}, diastolic defect, doxorubicin, rats

INTRODUCTION

The search for innovative molecules[1,2] with cardioprotective activity is an important task of pharmacology. In this case, their study should be conducted on pharmacological targets, which provides a targeted search for the creation of new drugs.[3-5] Today, different methods are proposed for the assessment of the cardioprotective activity of pharmacological agents: Check the absorption of calcium ions by isolated heart of rats,[6] the tests for the load resistance,[7,8] non-invasive evaluation of systolic function

Address for correspondence:
Lyudmila M. Danilenko, Department of Pharmacology, Belgorod State University, 85, Pobedy St., Belgorod, 308015, Russia. E-mail: Danilenko_L@bsu.edu.ru

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of the heart by permanent wave Doppler echocardiography in dogs with mitral regurgitation,[9] and others. However, the performance, information, and reliability of these methods are insufficient. The introduction of new methods of assessment of the cardioprotective actions is an actual task. Among pharmacological models of cardiomyopathy, features of doxorubicin (DOX)-induced cardiomyopathy are a progressive decline in the contractility of the left ventricular myocardium and the development of the diastolic defect.[10] It is possible to overcome the cardiotoxicity of different ways.[11-17]

MATERIALS AND METHODS

The experiments were performed on 50 adult Wistar rats weighing 220 ± 20 g. All manipulations with animals were performed in compliance with the "European Convention for the protection of vertebrate animals used for experimental or other scientific purposes" (Derective2010/63/EU). All the experiments were approved by the local ethics committee (Protocol No. 7-2017 October 11, 2017).

All rats were divided into five experimental groups of 10 animals. The first group (n = 10), control, were administrated intraperitoneally with physiological solution. The second group (n = 10) were administrated intraperitoneally with DOX (Teva) in a cumulative dose of 20 mg/kg, once. In three other groups on the background of administration of DOX (20 mg/kg), cardioprotectors which are most frequently used in cancer patients were administered: Enalaprilat (KRKA Slovenia) 5 mg/kg intraperitoneally every 12 h; verapamil (JSC Alkaloid, Macedonia) 5 mg/kg intraperitoneally once a day; and carvedilol (Teva, Israel) per os once a day. After 48 h, the hearts were removed from animals under Zoletil anesthesia (30 mg/kg). After the arrest of autonomic heart contractions, we isolated aorta and separated connective tissue. Then, the aorta was cannulated and we performed a retrograde perfusion of the heart by the Langendorff method with the Krebs-Henseleit solution. The contractile function of the heart was recorded with the help of a latex balloon inserted into the left ventricle cavity, connected to a pressure sensor, built into the apparatus for physiological studies of MP150 of BiopacSystems, Inc. (California, USA) and the original application program of the company "BiopacSystems, Inc." (California, USA). The balloon was filled with distilled water, the volume of which was sufficient to create end-diastolic pressure in the left ventricle at the level of 3–5 mmHg. Using the original software program AcqKnowledge of the "BiopacSystems, Inc." company (California, USA), all rats were performed the check of the contractility indices: Left ventricular pressure (LVP, mmHg), heart rate (HR, bpm), maximum contraction rate (+dp/dt_{max}, mmHg/s), and maximum relaxation rate (-dp/dt_{max}, mmHg/s). To a high HR stimulation (480 bmp), the metallic cannula was attached to a ground connector of an electrical stimulator and a left atrial was attached to a positive connector. After 20 min of perfusion, the heart was subjected to electrical stimulation pulses using the STM 200-1 device of the "BiopacSystems, Inc." company (California, USA) for 15 s.

To assess the myocardium functional capacity, we used a diastolic dysfunction coefficient or "diastolic defect" (S_{tTTI}) calculated from the dynamic curve of the intraventricular pressure. The area under the curve was calculated by adding of trapezoids areas, which is equal to the product of its height on the middle line. The S_{tTTI} coefficient was expressed in c.u.[10]

Biochemical markers of damage were evaluated by standard methods.[18]

The study of microscope slides, photography, and morphometry was performed using LeicaDM 4000 B microscope equipped with a video recording system and software for archiving and image analysis, Leica Application Suite Version 3.8.0. A measurement of the diameters of cardiomyocytes in the middle part on a strictly longitudinal sections was conducted. The use of a single set of equipment for preparatory and analytical stages with simultaneous processing of all the material provided the standardization of obtained morphological data.

RESULTS AND DISCUSSION

The DOX-induced cardiomyopathy was characterized by the decline of myocardial contractility.

Functional tests with high-rate stimulation revealed the development of "diastolic defect" [Figure 1], and S_{tTTI} increased to 8.3 ± 0.3 c.u., compared to the intact animals 1.4 ± 0.1 c.u., i.e., 8-fold [Figures 1 and 2].

The ability of DOX to damage of cell membranes was assessed by the change in the activity of creatine phosphokinase (CPK-MB) and lactate dehydrogenase (LDH) in perfusate [Figures 3 and 4].

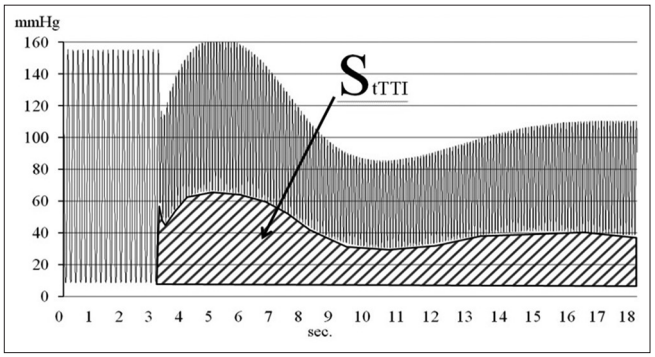

Figure 1: Stress test with submaximal electrical stimulation in Langendorff heart of rats with DOX-induced cardiomyopathy. Dynamic of pressure in the left ventricle (mm Hg) with high-rate stimulation of the heart (480 bmp) for 15 s. DOX (20 mg/kg) was administrated once 48 h before the experiment

Table 1: The effect of enalaprilat, carvedilol, and verapamil on the S_{tITT} (c.u.), CPK-MB (IU/l), LDH (IU/l), and cardiomyocyte diameter (μm) on the background of the DOX-induced cardiomyopathy (20 mg/kg once in 48 h) (M±m; *n*=10)

Group	S_{tITT} (c.u.)	CPK-MB (IU/l)	LDH (IU/l)	Cardiomyocyte diameter (μm)
Intact animals	1.4±0.1*	98.0±11.8*	263.0±24.9*	8.1±0.3*
DOX (20 mg/kg) control group	8.3±0.3**	740.0±13.6**	1583.0±30.6**	17.3±0.4**
Enalaprilat (5 mg/kg)	4.1±0.1*	449.1±10.1*	987.5±26.6*	12.6±0.2*
Carvedilol (30 mg/kg)	4.6±0.2*	564.3±12.7*	1027±21.3*	13.8±0.3*
Verapamil (5 mg/kg)	5.7±0.3*	603.4±9.8*	1216.8±19.4*	14.2±0.4*

**P<0.05 in comparison with the group of intact animals, *P<0.05 in comparison with the control group, CPK-MB: Creatine phosphokinase, LDH: Lactate dehydrogenase, DOX: Doxorubicin

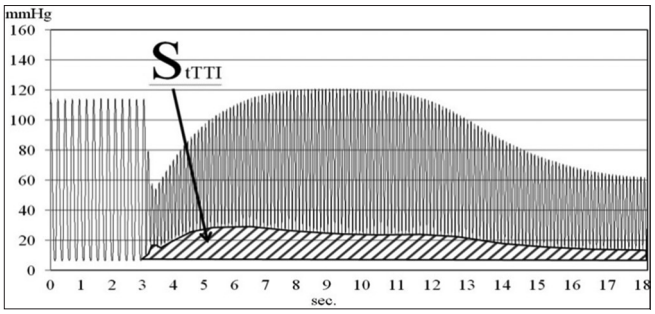

Figure 2: Stress test with submaximal electrical stimulation in Langendorff heart of rats with DOX-induced cardiomyopathy. Dynamic of pressure in the left ventricle (mmHg) with high-rate stimulation of the heart (480 bmp) for 15 s. The intact group

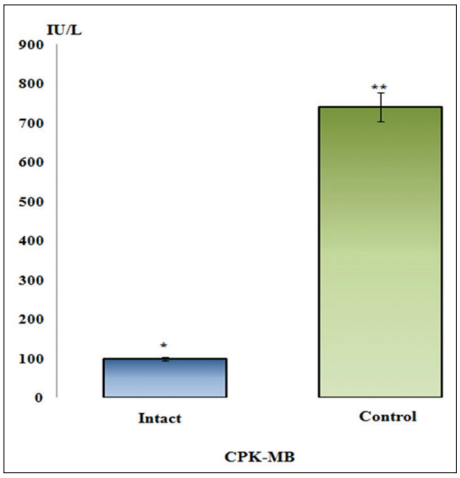

Figure 3: The concentration of the creatine phosphokinase (CPK-MB) in perfusate. *P < 0.05 in comparison with the control group, **P < 0.05 in comparison with the group of intact animals

The administration of DOX contributed to increase in the levels of CPK-MB and LDH in 7.1 and 8 times, respectively, in comparison with the intact group [Figures 3 and 4].

To confirm the functional and biochemical parameters, reflecting the development of cardiomyopathy on the background of DOX, the morphological studies were performed.

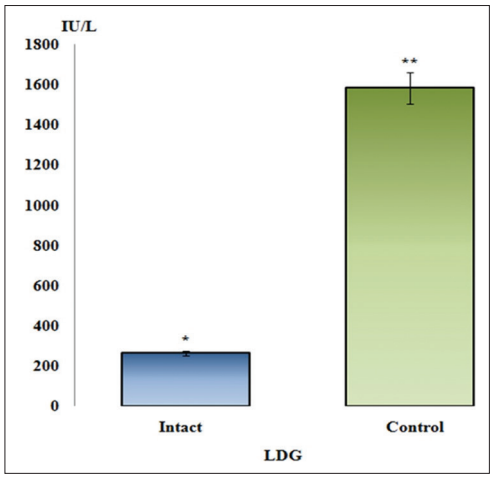

Figure 4: The concentration of the lactate dehydrogenase in perfusate. *P < 0.05 in comparison with the control group, **P < 0.05 in comparison with the group of intact animals

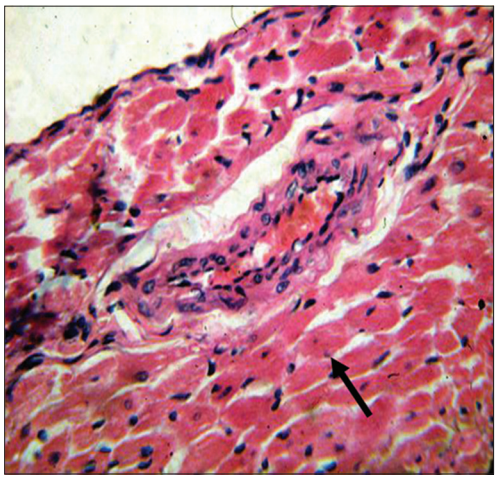

Figure 5: The myocardium of rat on the background of doxorubicin (20 mg/kg)

During the experiment in the hearts of animals of the control group, hypertrophy of cardiomyocytes of the left ventricle and increase in the size of nuclei and diameter in comparison with intact animals were observed [Figures 5 and 6].

A symptom of cardiomyopathy is hypertrophy of arterial wall of the heart, the thickness of which was 26.5 ± 0.5 μ

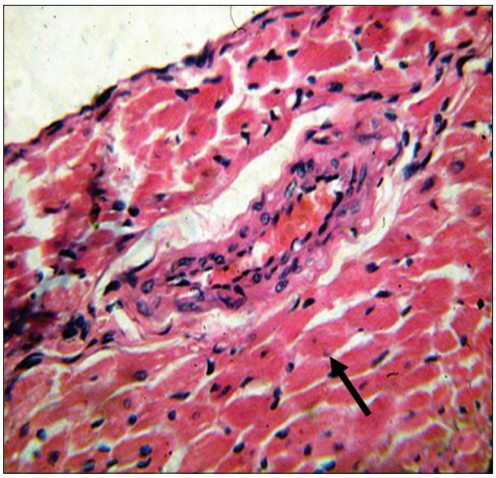

Figure 6: The myocardium of the intact rat

(compared to thickness of the vessel walls of the intact animals 10.3 ± 0.8 μm). Often hypertrophied vessels underwent morphological changes, indicating the development of cardiomyopathy. During the experiment, hypertrophy of left ventricular cardiomyocytes and an increase in the size of nuclei were found in the hearts of animals of the control group compared with intact animals of 17.3 ± 0.4 μm control, and 8.1 ± 0.3 μm intact group. Therefore, the results of morphological studies found that the DOX-induced cardiomyopathy on the 2nd day leads to the severe hypertrophy of cardiomyocytes and increasing diameter of cardiomyocytes [Table 1].

Thus, the functional, biochemical, and morphological changes demonstrate the development of pathology on the background of DOX (20 mg/kg) for 48 h, as expressed in the development of the diastolic defect when the high rate stimulation of the Langendorff heart of rats and the increase in the StTTI coefficient increased the levels of the damage markers CPK-MB and LDH in perfusate and hypertrophy of cardiomyocytes. The most informative indicator was the S_{tTTI} coefficient, reflecting the dynamics of the area under the curve of the intraventricular pressure. This coefficient allows to estimate the degree of myocardial damage with the simulation it in various ways, as well as cardioprotective effects of various cardiovascular drugs.

In the comparative analysis of cardioprotective activity of drugs in doxorubicin cardiomyopathy, the studied compounds were located in the following sequence: Enalaprilat (5 mg/kg), carvedilol (30 mg/kg), verapamil (5 mg/kg).

The obtained results allow to use S_{tTTI} at the screening of innovative molecules.

CONCLUSION

The fundamental difference in the area under the curve of the buildup of the end diastolic pressure under the conditions of submaximal stimulation frequencies (480 bpm) for 15 s in the intact group and the control group on the background of DOX administration naturally led to the necessity of introducing S_{tTTI} coefficient, which is quite revealing and informative. The obtained results allow to use S_{tTTI} at the screening of innovative molecules.

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