Doxorubicin-induced cardiomyopathy: New approaches to the assessment of a cardioprotective activity

Lyudmila M. Danilenko*, Michael V. Pokrovskii, Tatyana V. Avtina, Alyona S. Timokhina, Anatoly V. Khavansky

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 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] noninvasive evaluation of systolic function 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.

ABSTRACT

Introduction: Despite the continuous improvement of methodical approaches to the assessment of the cardioprotective activity of pharmacological agents in an experiment, it should be noted the insufficient level of elaboration of this problem. Research Tasks: Development of the methodical approaches for the evaluation of the cardioprotective activity of pharmacological agents to objectively assess the degree of the cardioprotective activity of commonly used cardioprotectors in cancer patients. Methods: Simulation of the cardiomyopathy was performed by intraperitoneal administration of doxorubicin at a dose of 20 mg/kg. After 48 h, we assessed indices of a left ventricular contractility under conditions of high heart rate (HR) 480 bpm for 15 s on the background of increased concentration of Ca²⁺ in perfusate to 5 mmol in Langendorff heart of rats. As an additional index of the assessment of the cardioprotective action of pharmacological agents, there was used S_{TTI} coefficient, reflecting the diastole defect, that is, an area under the curve of the buildup of an end diastolic pressure. To evaluate the myocardial damage, there were determined the isoenzyme creatine phosphokinase and lactate dehydrogenase in perfusate flowing from the isolated hearts. For a comprehensive confirmation of the development of the simulated pathological processes, there was performed a morphological study of the hearts. As drugs, there were used enalaprilat (KRKA Slovenia) at the dose of 5 mg/kg intraperitoneally every 12 h, carvedilol (Teva, Israel) per os 1 times a day, verapamil (JSC Alkaloid, Macedonia) at the dose of 5 mg/kg intraperitoneally 1 time a day. Results: In a control group with the doxorubicin-induced cardiomyopathy under the conditions of submaximal stimulation frequencies (480 bpm) and increased concentration of Ca²⁺ in perfusate (5 mmol/l), we observed the diastolic defect which numerically was S_{TTI} = 8.3 ± 0.3 c.u. that shows significant damage and the failure of the calcium pumps of cardiac myocytes. In an intact group, S_{TTI} coefficient was 1.4 ± 0.1 c.u., that is, 8 times less than in the control group. The results of biochemical and morphological studies confirmed the degree of myocardial damage. In order of activity cardioprotectors located in following sequence: Enalaprilat (5 mg/kg), carvedilol (30 mg/kg), and 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 and increased concentration of Ca²⁺ in perfusate (5 mmol/l) in the intact group and the control group on the background of doxorubicin administration, naturally led to the necessity of introducing S_{TTI} coefficient, which is quite revealing and informative. The obtained results allow to use S_{TTI} at the screening of innovative molecules.

KEY WORDS: Diastolic defect, Doxorubicin-induced cardiomyopathy, Langendorff heart of rats, S_{TTI}

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Among pharmacological models of cardiomyopathy, features doxorubicin-induced cardiomyopathy are a progressive decline in the contractility of the left ventricular myocardium and the development of the diastolic defect.\textsuperscript{10} It is possible to overcome the cardiotoxicity of different ways.\textsuperscript{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” (Directive2010/63/EU). All the experiments were approved by the local Ethics Committee (Protocol No. 7-2016 October 11, 2016).

All rats were divided into five experimental groups of 10 animals. The first group (n = 10) control, which was administrated intraperitoneally with physiological solution. The second group (n = 10) was administrated intraperitoneally with doxorubicin (Teva) in a cumulative dose of 20 mg/kg, once. In three other groups on the background of administration of doxorubicin (20 mg/kg), there were administered cardioprotectors which are most frequently used in cancer patients: 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, hearts were removed from animals under Zoletil anesthesia (30 mg/kg) and placed in ice (2-4°C) Krebs–Henseleit solution the following composition (mmol): NaCl - 118.5; KCl - 4.7; MgSO\textsubscript{4}/7H\textsubscript{2}O - 1.2; KH\textsubscript{2}PO\textsubscript{4} - 1.2; CaCl\textsubscript{2} - 1.5; glucose - 11.1; and NaHCO\textsubscript{3} - 25.0. The pH of the solution during the whole experiment was 7.4. After 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, saturated with carbogen (95% O\textsubscript{2} + 5% CO\textsubscript{2}) in the mode of flow perfusion for 20 min, at 37°C and at a pressure of 100 mmHg and perfusate speed 10 ml/min. Heart contractile function was recorded with the help of the inserted into the cavity of the left ventricle a latex balloon connected to a pressure sensor of the device for physiological experiments MP150 of “Biopac Systems, Inc.” company (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 “Biopac Systems, Inc.” company (California, USA), all rats were performed the check of the contractility indices: Left ventricular pressure (mmHg), HR (bpm), the maximum contraction rate (+dp/dt\textsubscript{max}, mmHg/sec), and the maximum relaxation rate (−dp/dt\textsubscript{min}, mmHg/sec). To a high HR stimulation (480 bpm), 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 with the solution with the high concentration of Ca\textsuperscript{2+} (5 mmol/l), the heart was subjected to electrical stimulation pulses using the STM 200-1 device of the “Biopac Systems, Inc.” company (California, USA) for 15 s.

To assess the myocardium functional capacity, we used a diastolic dysfunction coefficient or “diastolic defect” (S\textsubscript{TTE}) 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\textsubscript{TTE} coefficient was expressed in c.u.\textsuperscript{10}

Biochemical markers of damage were evaluated by standard methods.\textsuperscript{18}

The study of microscope slides, and morphometry was performed using Leica DM4000B microscope equipped with a video recording system and software for archiving and image analysis, Leica Application Suite Version 3.8.0. There was conducted a measurement of the diameters of cardiomyocytes in the middle part on strictly longitudinal sections. 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. Morphological studies were performed in the laboratory of the scientific-educational center “Applied Immunomorphology and Cytogenetics” of the Belgorod State National Research University.

**RESULTS AND DISCUSSION**

The doxorubicin-induced cardiomyopathy was characterized by decline of myocardial contractility (Table 1).

Functional tests with high-rate stimulation revealed the development of “diastolic defect” (Figure 1), and S\textsubscript{TTE} increased to 8.3 ± 0.3 c.u., compared to the intact animals 1.4 ± 0.1 c.u., i.e. 8-fold (Figure 2).

The ability of doxorubicin 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).

The administration of doxorubicin contributed to increase in 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
on the intact group. Background of doxorubicin, there were performed the morphological studies.

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

Asymptom of cardiomyopathy is hypertrophy of arterial wall of the heart, the thickness of which was 26.5 ± 0.5 µ (compared to thickness of the vessel walls of the intact animals 10.3 ± 0.8 µ). Often, hypertrophied vessels underwent morphological changes indicating the development of cardiomyopathy. During the experiment in the hearts of animals of the control group was observed hypertrophy of cardiomyocytes of the left ventricle and increase in the size of nuclei compared with intact animals: 17.3 ± 0.4 µm in the control, and 8.1 ± 0.3 µm in the intact group. Therefore, the results of morphological studies found that the doxorubicin-induced cardiomyopathy on the second day leads to the severe hypertrophy of cardiomyocytes and increasing diameter of cardiomyocytes (Table 2).

**CONCLUSION**

The functional, biochemical, and morphological changes demonstrate the development of pathology on the background of doxorubicin (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 $S_{ITT}$ coefficient, increased levels of the damage markers CPK-MB and LDH in perfusate, hypertrophy of cardiomyocytes. The most informative indicator was the $S_{ITT}$ 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 order of activity cardioprotectors located in following sequence: Enalaprilat (5 mg/kg), carvedilol (30 mg/kg), and verapamil (5 mg/kg).

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

**REFERENCES**


Table 2: The effect of enalaprilat, carvedilol, and verapamil on the $S_{ITT}$ (c.u.), CPK-MB (IU/l), LDH (IU/l), and cardiomyocyte diameter (µm) on the background of the doxorubicin-induced cardiomyopathy (20 mg/kg once in 48 h) (M±m; n=10)

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>$S_{ITT}$ (c.u.)</th>
<th>CPK-MB (IU/l)</th>
<th>LDH (IU/l)</th>
<th>Cardiomyocyte diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact animals</td>
<td>1.4±0.1</td>
<td>98.0±11.8*</td>
<td>263.0±24.9*</td>
<td>8.1±0.3*</td>
</tr>
<tr>
<td>Doxorubicin (20 mg/kg) control group</td>
<td>8.3±0.3</td>
<td>740.0±13.6**</td>
<td>1583.0±30.6**</td>
<td>12.6±0.4**</td>
</tr>
<tr>
<td>Enalaprilat (5 mg/kg)</td>
<td>4.1±0.1</td>
<td>449.1±10.1*</td>
<td>987.5±26.6*</td>
<td>12.6±0.2*</td>
</tr>
<tr>
<td>Carvedilol (30 mg/kg)</td>
<td>4.6±0.2</td>
<td>564.3±12.7*</td>
<td>1027±21.3*</td>
<td>13.8±0.3*</td>
</tr>
<tr>
<td>Verapamil (5 mg/kg)</td>
<td>5.7±0.3</td>
<td>603.4±9.8*</td>
<td>1216.8±19.4</td>
<td>14.2±0.4*</td>
</tr>
</tbody>
</table>

**P<0.005 in comparison with the group of intact animals; *P<0.005 in comparison with the control group. CPK: Creatine phosphokinase, LDH: Lactate dehydrogenase

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

Figure 6: The myocardium of the intact rat