

EXPERIMENTAL PHARMACOLOGY



UDC: 616-092.9:599.323.4:616.12

DOI: 10.18413/2500-235X-2017-3-1-3-9

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STUDY OF DOSE-DEPENDENT EFFECT OF 2-ETHYL-6-METHYL-3 HYDROXYPYRIDINE SUCCINATE ON THE CONTRACTILE FUNCTION OF ISOLATED RAT HEART

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Abstract

In experiments on the isolated rat heart there were studied the effects of different doses (21.43 mg/kg/day and 85.72 mg/kg/day) 2-ethyl-6-methyl-3 hydroxypyridine succinate ("EkoPharmInvest", Russia), on the contractile function of isolated hearts subjected to prior doxorubicin model (20 mg/kg, intraperitoneal) of pathology. The dynamic of the power mechanisms of ion transport was evaluated by imposing high heart rate (480 BPM) and increase concentration of Ca^{2+} to 5 mmol in perfusate. The results indicate that Mexicor at the dose of 21.43 mg/kg/day does not provide a cardioprotective effect in this model of pathology. Mexicor has the cardioprotective effect at the dose of 85.72 mg/kg/day in relation to the isolated heart, that is resulted in the recovery of the contractile function of the heart and reducing the "diastole defect" (S_{TD}).

Key words: 2-ethyl-6-methyl-3 hydroxypyridine succinate, doxorubicin, reperfusion, isolated heart, antioxidant.

Introduction

One of the mechanisms of pathogenesis and progression of ischaemic alterations of the myocardium is oxidative stress in the myocardium, leading to damage of cardiomyocytes and related violations of the functional properties of the cardiac muscle [1-7]. The result of these complex changes is the left ventricular (LV) remodeling with subsequent progression to systolic and diastolic dysfunction, the development of reinfarctions, embolic stroke, sudden death [8-10].

In the present cardiology practice, along with antianginal, hypolipidemic, anticoagulant and antiplatelet drugs, patients with CHF and acute coronary syndrome are prescribed cardioprotective medications. The antioxidants are of particular interest as one of the promising groups of cardioprotective drugs, allowing to save viable myocardium, to limit damage and accelerate recovery of contractile activity of the myocardium [11-19].

Main part

The research objective of this study was to study the effect of 2-ethyl-6-methyl-3 hydroxypyridine

succinate in different doses on the contractile function of isolated heart in model of doxorubicin-induced cardiomyopathy.

Materials and methods. The study was performed in isolated hearts of Wistar rats weighing 300 ± 20 g. The experiment was performed with the requirements and principles of humane treatment of experimental animals. All rats were divided into 4 experimental groups of 8 animals. The first group, the control was injected intraperitoneally with physiological solution. The second group was injected intraperitoneally with doxorubicin (Teva) at a cumulative dose of 20 mg/kg once. The third was administrated doxorubicin and Mexicor (ZAO "MiraxBioPharma") at the dose of 21.43 mg/kg/day. The fourth group was administrated doxorubicin and Mexicor at the dose of 85.72 mg/kg/day. The dose of Mexicor was calculated taking into account the coefficient of interspecies transfer of human doses on the rat. Mexicor was administrated daily 1 time per day. Animals were taken out from experiment after 48 hours. Heart recovery from animals had been

performed under anesthesia by Zoletil (30 mg/kg), than hearts were placed in ice (2-4°C) solution of Krebs Henseleit the following composition (mmol): NaCl – 118.5; KCl – 4.7; MgSO₄/7H₂O – 1.2; KH₂PO₄ – 1.2; CaCl₂ – 1.5; glucose – 11.1; NaHCO₃ – 25.0. The pH of the solution during the whole experiment was 7.4. After asystolism aorta was isolated and connective tissue was separated. Then the aorta was cannulated and there was performed a retrograde perfusion of the heart using Langendorff method in the mode of flow perfusion for 20 min with a solution of Krebs-Henseleit, saturated with Carbogen (95 % O₂ + 5% CO₂) at 37°C and at a pressure of 100 mm Hg and perfusate speed of 10 ml/min. Contractile function of the heart was recorded using inserted into the cavity of the left ventricle a latex balloon connected to a pressure sensor in assessment equipment MP150 ("BiopacSystems, Inc" (California, USA)). The balloon was filled with distilled water, the volume of which was sufficient to keep left ventricular end diastolic pressure at the level of 3-5 mm Hg. Using the original software program AcqKnowledge ("BiopacSystems, Inc" (California, USA)), all rats there were recorded indices of contractility: left ventricle pressure (LVP, mm Hg), heart rate (HR, BPM), maximum rate of myocardial contraction (+dP/dtmax, mm Hg/sec), the maximum rate of myocardial relaxation (-dP/dtmax, mm Hg/sec). Then to determine the dynamics of diastolic tension of the heart there was used the method of HR increasing to 480 BPM in concentration of Ca²⁺ of 5 mmol/l. To create a high HR (480 BPM) the metallic cannula there was attached a ground connector of electrical stimulator, and left atrial appendage was attached a

positive connector. After 20 minutes of perfusion with a solution with a high concentration of Ca²⁺ (5 mmol/l), the heart was subjected to electrical stimulation pulses for 15 seconds using the equipment STM 200-1 ("BiopacSystems, Inc" (California, USA)).

To assess the spare capacity of the myocardium there was used Tension-Time Index (TTI), it is the index of the change in mechanical stress of the myocardium, calculated by the dynamic curve of intraventricular pressure by the planimetry method (i.e. measurement the area under the curve). The area under the curve was calculated by adding areas of trapezoids, which is equal to the product of its height on the middle line. The index of diastolic dysfunction or "diastole defect" (S_{TTI}) was expressed in conditional unit (cu). About the intensity of cardioprotective effect of Mexicor at the doses of 21.43 mg/kg/day and 85.72 mg/kg/day we went by the influence of the drug on the S_{TTI}.

A statistical significance of the changes of the absolute measures were determined by a difference method of variation statistics, calculating the average workshift values, the arithmetical mean probability of a possible error (p) by Student tables. The differences between the results were considered statistically significant when p<0.05.

Results and evaluation

Indices of contractile function of the hearts of all experimental groups at the initial state and with increasing the concentration of Ca²⁺ to 5 mmol/l are presented in table 1 and table 2.

Studies have shown that in condition of this disease changes of contractility are characterized by a negative inotropic effect (table 1).

Table 1

**Indices of contractile function of the rat hearts (M±m; n=8)
The concentration of Ca²⁺ in perfusate is 2.5 mmol**

Animal experimental groups	LVP, mm Hg	+dP/dt, mm Hg/sec	-dP/dt, mm Hg/sec	HR, BPM
Intact animals	87.3±9.2**	1423±162.2**	-1265.2±173.2**	248±32.1
Control doxorubicin 48 hours before (20 mg/kg)	64.5±11.2*	1025.7±154.3*	-1031.1±159.4*	247±29.4
Doxorubicin +Mexicor 21.4 mg/kg/day	71.4±6.3*	1117±179.6*	-1108±89.3*	221±34.9
Doxorubicin +Mexicor 85.72 mg/kg/day	86.3±10.4**	1276±189.4**	-1259.2±149.3**	228±22.9

Comment: * – p<0.05 in comparison with intact animals; ** – p<0.05 in comparison with control group.

Changes in most hemodynamic parameters are cardiodepressant. When the concentration of Ca²⁺ 2.5 mmol/l, the contractile function of the myocardium is most affected, that evidenced by the decrease in

systolic pressure by 26% in comparison with group of intact hearts, and the maximum rates of contraction and relaxation (table 1) reduced by 28% and 19%, respectively. HR changed little.

A further stage of our experiments was to run up the concentration of Ca^{2+} to 5 mmol/l in the perfusion solution. After 20 minutes of perfusion with a solution with a high concentration of Ca^{2+} (5 mmol/l), the heart was subjected to stimulation by electrical impulses for 15 seconds. Perfusion of intact hearts and the hearts of the control group by the

solution with a high concentration of calcium ions within the 1 min was shown positive inotropic effect, that was reflected in the increase in systolic blood pressure and its rate characteristics. Thus, end diastolic pressure increased to 20-25 mm Hg and formed the "diastole defect" (figure 1a).

Table 2

Indices of contractile function of the rat hearts ($M \pm m$; $n=8$)
The concentration of Ca^{2+} in perfusate is 5 mmol, stimulation by electrical impulses (480 BPM)

Animal experimental groups	LVP, mm Hg	+dP/dt, mm Hg/sec	-dP/dt, mm Hg/sec	HR, BPM
Intact animals	127±12.7**	987.4±92.9**	-1012.6±113.4**	240±24.1
Control doxorubicin 48 hours before (20 mg/kg)	155±21.2*	533.4±154.3*	-561.6±119.2*	273±30.44*
Doxorubicin +Mexicor 21.4 mg/kg/day	135±19.9*	689.5±108.2*	-611.9±151.4*	243±27.9*
Doxorubicin +Mexicor 85.72 mg/kg/day	140±15.9**	821±132.4**	-778.6±163.9**	254±20.5**

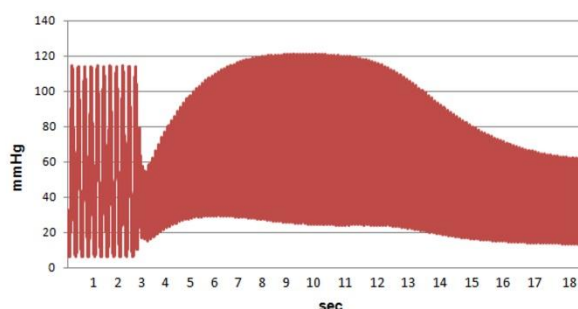
Comment: * – $p < 0.05$ in comparison with intact animals; ** – $p < 0.05$ in comparison with control group.

S_{TI} coefficient for intact group was 1.4 ± 0.1 cu (figure 3).

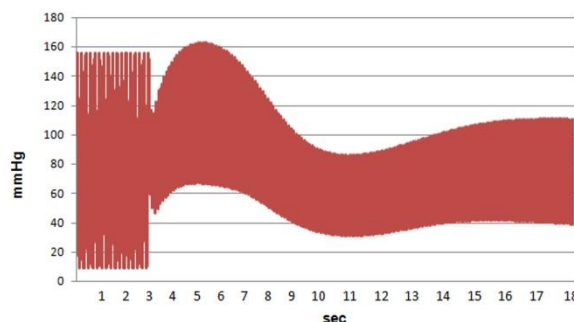
In the control group with doxorubicin-induced cardiomyopathy in the cardiac stimulation with submaximal rate (480 BPM) during the first minute of perfusion of the solution with a high concentration of calcium ions there was detected positive inotropic effect (table 2). Then inotropic effect was neutralized, there was observed a pronounced negative inotropic effect and increase in contracture heart contraction, that led to decrease in the power and rate characteristics of contractility decreased, there was an

increase in end diastolic pressure of 40-60 mm Hg (figure 1b), and the "diastole defect" S_{TI} was 8.3 ± 0.3 cu, that indicates a significant damage and failure of calcium pump of cardiomyocytes (figure 3).

The perfusion of the solution with a high concentration of calcium ions led to loss of the heart contraction rate by 46 % and relaxation rate more than 45% in control group (table 2). The data indicate that the perfusion of the solution with a high concentration of calcium ions and base solution of calcium affect the activity of isolated animal hearts varying degrees.



a



b

Figure 1. The pressure dynamic in the left ventricle: the hearts of the intact group (a) with doxorubicin 20 mg/kg (b) in the cardiac stimulation with submaximal rate (480 BPM). The concentration of Ca^{2+} is 5 mmol/l.

The absence of a pronounced positive inotropic effect in the control group and the group injected with Mexicor at the dose of 21.4 mg/kg/day, suggest the combination dysfunction each of the heart contraction processes and the mechanisms responsible for the relaxation, the net effect of which

is the failure of the calcium pumps of myolemma and a sarcoplasmic reticulum (table 2).

In the experimental groups injected with Mexicor, we see a cardioprotective effect of it at the dose of 85.72 mg/kg/day (table 2).

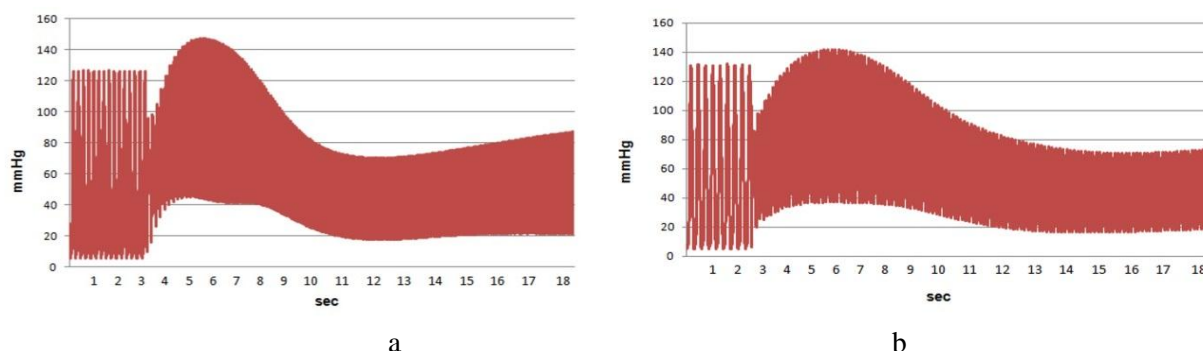


Figure 2. The influence of Mexicor the reaction of the heart, caused by the cardiac stimulation with submaximal rate (480 BPM). The concentration of Ca^{2+} is 5 mmol.
a – Mexicor at the dose of 21.43 mg/kg/day; b – Mexicor at the dose of 85.72 mg/kg/day

Mexicor therapy at the dose of 85.7 mg/kg/day resulted in a significant reduction of the "diastole defect" to 5.3 ± 0.3 , that goes to prove a marked

cardioprotective effect of the drug at this dose (figure 2b).

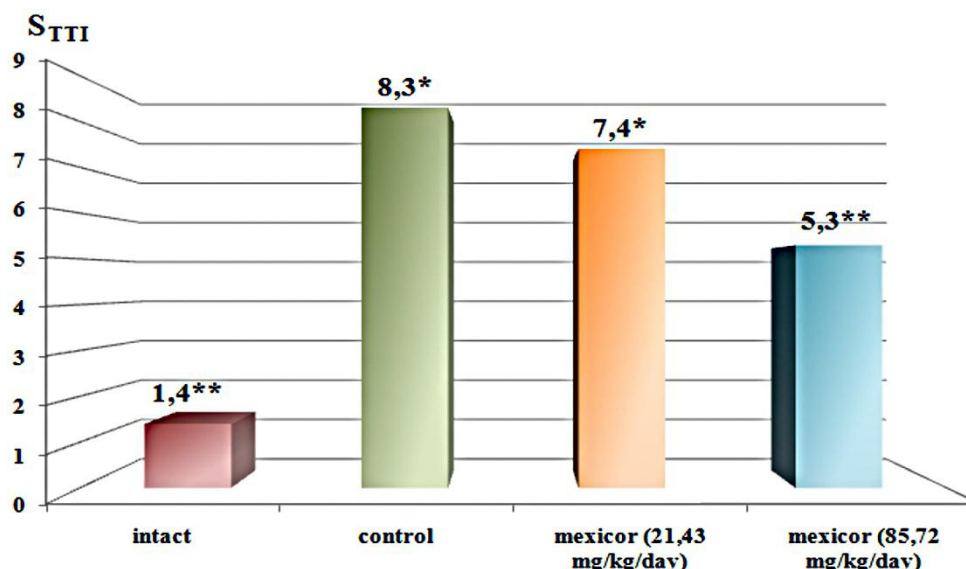


Figure 3. The influence of Mexicor (21.43 mg/kg/day, 85.7 mg/kg/day) on the S_{TTI} , cu (under doxorubicin-induced cardiomyopathy).

Comment: * – $p < 0.05$ in comparison with intact animals; ** – $p < 0.05$ in comparison with control group.

It should be noted that S_{TTI} in the group injected with Mexicor at the dose of 21.43 mg/kg/day did not decrease significantly and amounted to 7.4 ± 0.1 (figure 2a).

Thus, the comparative dynamic analysis of the indices of contractile function of the hearts of the experimental groups has allowed to establish that Mexicor at the dose of 85.7 mg/kg/day contributed the maximum increase of stability of the contractile apparatus of cardiac muscle, that proves demonstratively a pronounced cardioprotective effect of it.

A comparative analysis of the cardioprotective effect of this drug has revealed that low dose of Mexicor (21.43 mg/kg/day) does not have cardioprotective effect in this model of pathology.

Free radical formation on the background of reducing the amount of antioxidants leads to increase of oxidative stress, that can be the direct cause of cardiomyopathy and heart failure in doxorubicin-induced cardiomyopathy [20, 21, 22].

It is known that different reactive oxygen species (O^{2-} , H_2O_2 , OH) have different capacities to initiate the subsequent free radical reactions. A superoxide anion (O^{2-}) has the lowest activity, and a

hydroxyl radical (OH) has the maximum activity. OH is formed in the Haber-Weiss reaction, with the participation of superoxide dismutase and ions of ferrous iron (Fe^{2+} ions). One of the alleged reasons of doxorubicin-induced cardiomyopathy is associated with effect on iron metabolism: anthracyclines bind with Fe^{2+} ions, that leads to the formation of hydroxyl radical and promotes the release of Fe^{2+} from ferritin, further exacerbating oxidative stress [23-27].

So if in the cytoplasm of cells there are conditions for the chelation or oxidation of ferrous iron to the catalytically inactive ferric iron (Fe^{3+} ions) and thereby decreasing the effective concentration of hydroxyl radicals, it will create the conditions to achieve micro molar concentrations of reactive oxygen species in the cytoplasm of cells. Mexicor has a antioxidative activity and plays an important role in the regulation of free radical mechanisms [28, 29, 30]. As an example it can be suggested that mexicor at the dose of 85.7 mg/kg/day has the property of ferrous iron chelator and oxidate Fe^{2+} to Fe^{3+} [31]. In addition, monitoring the concentration of Fe^{2+} may have a role in the regulation of free radical reactions: lipid peroxidation, inactivation

of proteins and nucleic acids. It is well known that the activation of free radical reactions is observed in the initiation and development of a number of inflammatory diseases. This circumstance allowed to call this disease "free radical pathologies". Activation of the free radical reactions in development of free radical pathologies is due to two main reasons: the increase in the production of primary and secondary radical initiators and participants of the free radical reactions (the stage of initiation of the free radical reactions) and the appearance of the catalysts of the free radical reactions, of Fe^{2+} ions in the main (stage oxidation chain branching) [32, 33]. It follows that inhibition of free radical reactions can be performed by capturing free radicals and the elimination of catalytically active Fe^{2+} ions+ [34, 35, 36]. The latter is particularly important in pathologies which are characterized by violation of the integrity of blood vessels: stroke, gastric hemorrhage, wounds, etc., [21]. Mexicor, possessing the property of ferrous iron chelator and oxidation of Fe^{2+} to Fe^{3+} can inhibit the catalysis of free radical reactions and thus to inhibit free-radical oxidation [28] (figure 4).

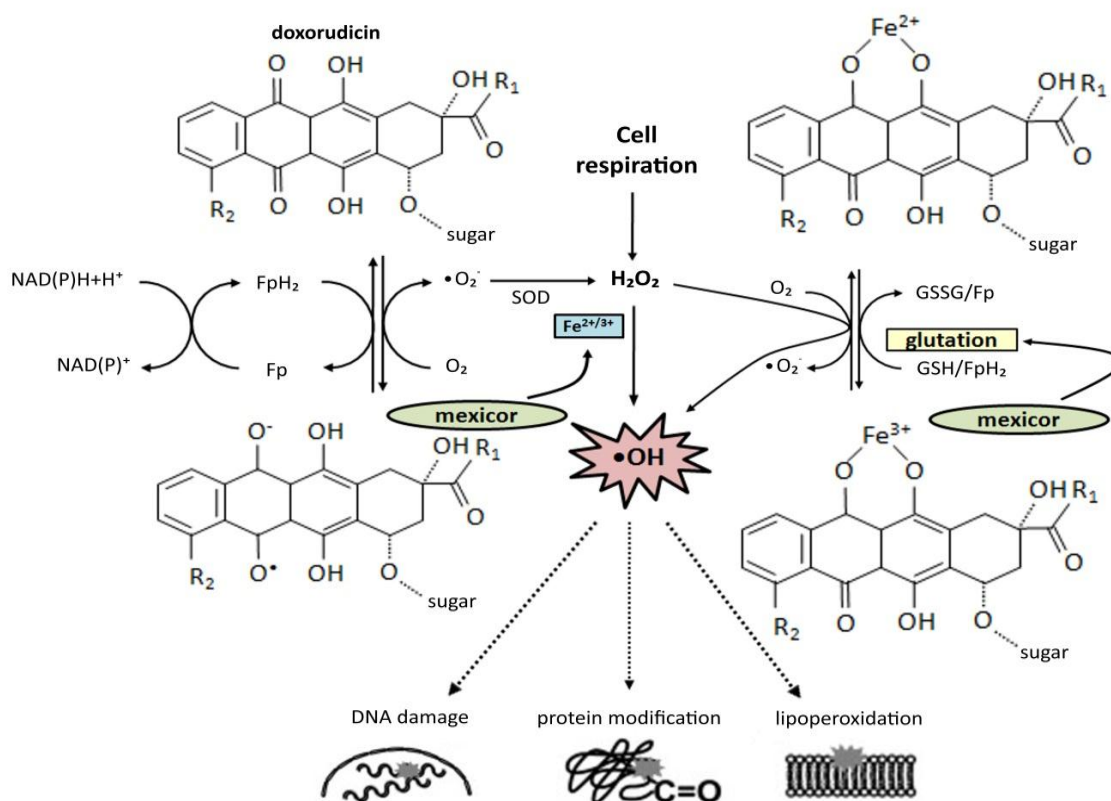


Figure 4. Diagramming of the main ways of action of doxorubicin and iron, which catalyzes the oxidative stress leading to cardiomyopathy and the application points of Mexicor

Comment: Fe – iron, FAD/FADH₂ – flavoprotein, GSH – reduced glutathione, GSSG – oxidized glutathione, H₂O₂ – hydrogen peroxide, NAD(PH)⁺ – nicotinamide adenine dinucleotide (phosphate), O₂^{•-} – superoxide anion, OH – hydroxyl radical, SOD – superoxide dismutase.

The second application point of Mexicor in this model of pathology is glutathione. The concentration of disulfide forms of glutathione GSSG in the body is normally found in the tissue and blood of mammals is maintained at levels many times lower than for GSH. Oxidative stress can lead to a significant accumulation of GSSG in the liver and release it into the blood. The increase in the concentration of GSSG in the blood plasma, in turn, can cause oxidation of thiol groups of proteins basolateral membranes of tissue cells and its inactivation. The main mechanisms causing the depletion of the functionality of the glutathione system in doxorubicin model of pathology, are the inhibition of activity of glutathione reductase enzyme from the oxidized form (glucose-6-phosphate dehydrogenase), that leads to critical decline reduced glutathione, and then to the decrease in the activity of glutathione-dependent enzymes of antiradical protection, activation of free radical processes and death of cardiomyocytes [37-41]. As directed pharmacological protection of cardiomyocytes there is pathogenetically explained the administration of drugs, whose action is directed at correcting the glutathione system. One of such directions can be the partial compensation of the antioxidant load attributable to the glutathione system. As such drug 3-hydroxypyridine derivatives, which is Mexicor can be used [31].

Conclusion

Based on the results of study it can be concluded that the maximum increase of stability of the contractile apparatus of cardiac muscle was reached due to administration of the Mexicor at the dose of 85.7 mg/kg/day, which convincingly demonstrate a pronounced cardioprotective effect in in model of doxorubicin-induced cardiomyopathy.

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