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Prevention of Diastolic Dysfunction Caused by Doxorubicin by Mitochondrial Antioxidant Plastomitin

Aim	An attempt to prevent the development of diastolic dysfunction (DD) with the mitochondrial antioxidant plastomitin on a model of doxorubicin-induced cardiomyopathy. DD is a type of chronic heart failure. Due to the increasing number of patients with this condition and the absence of effective therapy, development of means for DD correction is a relevant objective.
Material and methods	Cardiomyopathy was modeled in 17 rats by two subcutaneous injections of doxorubicin 2 mg/kg/week. The other group (n=17), also administered with doxorubicin, received plastomicin 0.32 mg/kg daily subcutaneously. Left ventricular function was evaluated with echocardiography (EchoCG) and cardiac catheterization with simultaneous pressure and volume monitoring.
Results	According to EchoCG data the ejection fraction remained unchanged in the experimental groups. Cardiac catheterization showed disorders of both myocardial contractility and relaxability only in the doxorubicin group.
Conclusion	A course of plastomitin in combination with the doxorubicin treatment can maintain normal heart contractility and thereby, prevent the known doxorubicin cardiotoxicity.

Keywords	Doxorubicin; heart failure; plastomitin; diastolic dysfunction; left ventricle; volume; pressure
For citation	Lakomkin V. L., Abramov A. A., Lukoshkova E. V., Prosvirnin A. V., Kapelko V. I. Prevention of Diastolic Dysfunction Caused by Doxorubicin by Mitochondrial Antioxidant Plastomitin. Kardiologiia. 2020;60(7):98–102. [Russian: Лакомкин В. Л., Абрамов А. А., Лукошкова Е. В., Просвирнин А. В., Капелько В. И. Предотвращение диастолической дисфункции, вызываемой доксорубицином, митохондриальным антиоксидантом пластомитином. Кардиология. 2020;60(7):98–102]

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Diastolic dysfunction (DD) is a form of chronic heart failure (CHF). It is less severe than systolic dysfunction with reduced ejection fraction, yet the prognosis is nearly the same, and its share among patients with CHF is beginning to predominate [1]. The lack of effective therapy exacerbates this situation. Thus, it is advantageous to prevent the development of CHF and DD, particularly in the early stage of cardiomyopathy.

The use of doxorubicin as an antitumor agent is often accompanied by cardiomyopathy. Oxidative stress is an essential component of the pathogenesis of this type of cardiomyopathy [2, 3]. It is known that doxorubicin-induced cardiomyopathy is characterized by impairment of mitochondrial function. This impairment is manifested, among other things, by decreased levels of the most critical link in the electron transport chain, i.e., coenzyme Q_{10}, in the myocardium of patients with systolic dysfunction. Treatment with this coenzyme improves the symptoms of CHF [4]. We used a combination of plastoquinone and triphenylphosphonium (SkQ_1, Plastomitin), which has been shown in several papers to be superior to coenzyme Q_{10} [5].

We have demonstrated recently that plastomitin successfully prevented systolic cardiac dysfunction occurring 8 wks after the initiation of the 4-wk course of doxorubicin [6]. The objective of the current study was to determine if plastomitin would prevent cardiac DD, which occurs in most experiments after 2-wk of doxorubicin administration.

Material and Methods

Wistar male rats with a bodyweight of 320–380 g were used in this study, which was carried out according to the principles of the 2000 Declaration of Helsinki and the 1985 International Guiding Principles for Biomedical Research Involving Animals (1985). In one group of animals (n=17), doxorubicin, 2 mg/kg, was administered subcutaneously twice a week, and in the other group (n=17), doxorubicin was administered concomitantly with subcutaneous plastomitin (SkQ_1 in 50% water/propylene glycol solution, 0.32 mg/kg daily), kindly provided by the Research Institute of Mitoengineering, Lomonosov Moscow State University. The control group (n=15) received an injection of isotonic NaCl solution. All animals were subjected to transthoracic echocardiography at baseline and two weeks later. About 50% of rats were used for cardiac studies, and the others were used for biochemical studies, the results of which will be presented in a separate paper.

Left ventricular (LV) catheterization was performed with a pressure-volume (PV) conductance catheter (Transonic Systems Inc., USA) by the method detailed earlier [6]. The

ISSN 0022-9040. Kardiologiia. 2020;60(7). DOI: 10.18087/cardio.2020.7.n1157

ADV500 Pressure-Volume Measurement System (Transonic Systems Inc., USA) was used for preliminary processing of signals from PV-catheter, and the LabChart Pro System with ADC PowerLab 4/35 (AD Instruments, Australia) provided data acquisition, recording and all necessary processing. The baseline measurements were made using multiple (10–40 times) records of parameters, which could be used later for calculation the mean values of the parameters that characterize cardiac function.

Recording for defining the LV diastolic stiffness was performed in the next step. For this purpose, the right ventricle inflow was restricted by partial occlusion of inferior vena cava using a ligature preliminary arranged under this vena between the diaphragm and the liver. This allowed obtaining a series of heart cycles with gradual decrease in LV filling. Occlusions were repeated in every animal by 3–5 times with intervals of 10–15 min, and the most successful was used for further processing. In this step the LabChart software defined, in accordance with the main international recommendations [7], the beat-by-beat values of the end-diastolic pressure and volumes and fitted to resulting curve the relationship $P=C * e^{\beta V}$, from which the calculated value of β (with dimension of ml^{-1}) is used as an exponential constant (coefficient) of LV diastolic stiffness.

At the end of experiment, the animals were killed with an overdose of urethane.

The findings were statistically analyzed with computational algorithms available in GraphPad Prism (version 8.4.0). The normality of data distributions was confirmed with the Kolmogorov-Smirnov and D'Agostino-Pearson tests. Multiple comparisons were evaluated by an analysis of variance (ANOVA) with F-test and the Brown-Forsythe test. The significance of differences in mean values was determined after taking into account the multiplicity of comparisons with the Dunnett T3 test. The results are expressed as mean and standard error of the mean (M±SEM).

Results

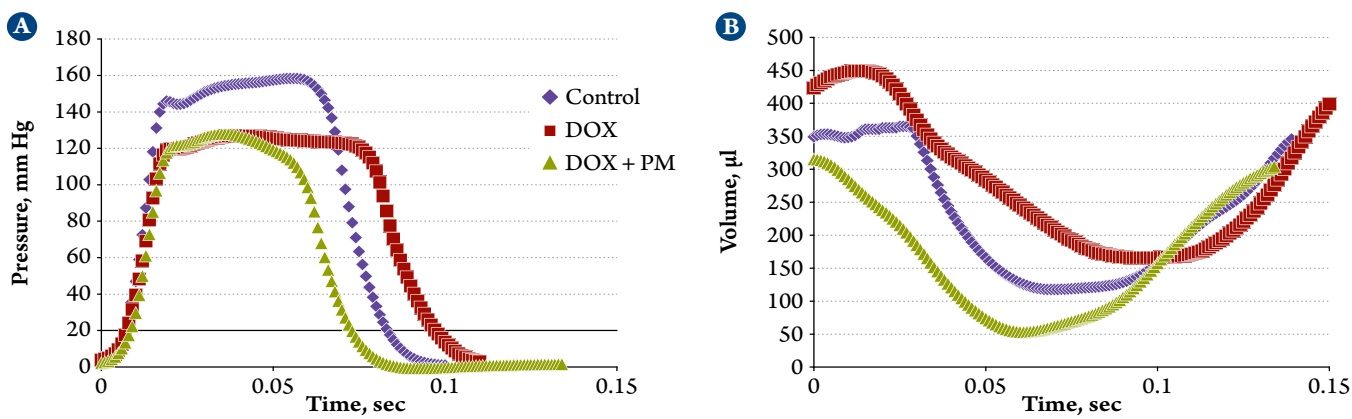
After 2 wks of treatment, the echocardiographic findings were comparable in all groups (Table 1). There was a significant decrease in the LV end-diastolic dimension in the doxorubicin group and a significant increase in E/A, reflecting LV early filling velocity, in the doxorubicin + plastomitin group.

Table 1. Echocardiographic indicators of cardiac function in rats 2 wks after initiation of treatment with doxorubicin or doxorubicin + plastomitin

Variable	Control	Doxorubicin	Doxorubicin + Plastomitin
Number of rats	15	17	17
Weight of rats, g	380±12	364±14	374±8
Heart rate, bpm	371±3	366±2	371±2
LV end-diastolic dimension, mm	7.1±0.1	6.5±0.2*	6.9±0.1
LV fractional shortening, %	48±2	48±2	45±1
LV end-diastolic volume, ml	0.41±0.02	0.37±0.01	0.38±0.01
LV ejection fraction, %	74±2	73±2	70±1
E/A	2.16±0.08	2.14±0.09	2.76±0.17*#

* p<0.05 versus control group; # p<0.05 versus doxorubicin group; LV, left ventricle.
E/A, ratio between peak early (E) and atrial-induced (A) mitral flow velocities.

Figure 1. Trends in left ventricular pressure (A) and volume (B) in control rats and in rats treated for 2 wks with doxorubicin (DOX) or with doxorubicin + plastomitin (DOX+PM)



The group symbols are identical in parts A and B.

Table 2. Cardiac pump function in rats after two injections of doxorubicin or doxorubicin + plastomitin

Variable	Control	Doxorubicin	Doxorubicin + Plastomitin
Number of experiments	7	7	7
Cardiac minute volume, ml/min.	118±9	105±10	102±8
Heart rate, bpm	430±9	412±12	395±15
LV end-diastolic volume, ml	0.41±0.03	0.47±0.02	0.42±0.03
Cardiac performance, mm Hg x ml	37.3±2.6	30.4±2.9	32.8±2.7
Peak ejection rate, ml/sec	15.5±2.7	9.3±1.4	12.2±2.1
Ejection fraction, %	63±2	57±4	64±4
Peak left ventricular filling velocity, ml/sec	10.5±2.8	10.2±1.0	11.4±1.7
Arterial elasticity (Ea), mm Hg/μl	0.54±0.04	0.48±0.04	0.51±0.03

Table 3. Cardiac contractile function in rats after two injections of doxorubicin or doxorubicin with plastomitin

Variable	Control	Doxorubicin	Doxorubicin + plastomitin
Number of experiments	7	7	7
LV systolic pressure, mm Hg	142±7	120±4*	134±5
Maximum LV pressure rate in mm Hg/sec	15190±1353	9690±755**	11900±1144
Contractility index, sec^{-1}	147±8	113±5**	131±8
Maximum rate of LV pressure decrease, mm Hg/sec	12240±870	6950±541***	10480±1005#
Relaxation index, sec^{-1}	84±6	56±5**	77±5#
Relaxation rate constant, sec^{-1}	125±5	89±3***	114±5##
LV diastolic pressure, mm Hg	1.1±0.3	3.2±1.1	0.3±0.4
LV end-diastolic pressure, mm Hg	4.9±0.7	7.2±2.0	3.1±0.7

*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ versus the control group; #, $p < 0.05$, ##, $p < 0.01$ versus the doxorubicin group; LV, left ventricle.

Changes in the LV pressure in rats treated with doxorubicin + plastomitin differed from those in the doxorubicin group. The highest LV systolic pressure was detected in the control group, and it was lower in the other two groups (Figure 1, A). However, there was a significant difference between the two experimental groups in the duration of systole. Compared to the control group, the duration of systole was much longer in the doxorubicin group and shorter in the doxorubicin + plastomitin group. Changes in LV volume in the same experiments are shown in Figure 1, B. The doxorubicin group apparently differed from the control group by having a reduced ejection rate, although the filling rate was roughly the same.

Doxorubicin and doxorubicin + plastomitin did not lead to significant disturbances in cardiac pump function (Table 2). However, there was a trend of increasing diastolic volume and decreasing LV ejection fraction, cardiac performance, and ejection rate in the doxorubicin group. These changes were much less in the doxorubicin + plastomitin group. At the same time, the measurements of the LV contractility function made it possible to detect apparent changes in the myocardial contractility and relaxation in the doxorubicin group (Table 3). The maximum rate of LV pressure development and contractibility index decreased by 36 and 23%, respectively. The rates of myocardial relaxation decreased by 39–43%. There was a clear trend of increasing LV diastolic pressure. Thus, the signs of DD were observed in the doxorubicin group. In the doxorubicin + plastomitin group, myocardial contractility and relaxation were significantly superior to that of the doxorubicin group and did not differ from that of the control group. These changes were accompanied by reduced LV diastolic pressure. Thus, addition of plastomitin completely eliminated the signs of DD observed in the doxorubicin group.

Calculation of the exponential constant of LV diastolic stiffness (see Material and Methods for details) showed the values of 14.3±0.9 ml^{-1} in the control group, 8.1±1.9 ml^{-1} ($p<0.05$) in the doxorubicin group, and 9.4±1.5 ml^{-1} in the doxorubicin + plastomitin group. Thus, a decrease in LV diastolic stiffness was observed in both doxorubicin groups, though the multiple comparison of the doxorubicin + plastomitin group values with the values of other two groups did not reveal statistically significant difference for any of them.

Discussion

The results of this study are consistent with our previous findings that plastomitin reduced the severity of CHF after 4-wk administration of doxorubicin [6]. The current prevention of DD was due to a significant improvement of myocardial relaxation and contractility. Thus, we suggest that plastomitin has a direct effect on Ca^{2+} transport in cardiomyocytes, which is also consistent with our previous

ISSN 0022-9040. Kardiologiia. 2020;60(7). DOI: 10.18087/cardio.2020.7.n1157

experiments that showed a significant decrease in the rate and intensity of arrhythmias caused by hydrogen peroxide or adrenaline [5]. Moreover, long-term (3-wk) administration of SkQ_1 in a moderate dose of 0.2–2.0 nM/kg of body weight increased the relaxation index of isolated hearts of normal rats and decreased arterial vessel tone [8].

As a mechanism of the possible effect of plastomitin on Ca^{2+} transport, we can put forward the following hypothesis. It is known that the Ca^{2+} concentration in myoplasm is determined by the amount of Ca^{2+} released from the sarcoplasmic reticulum through the ryanodine receptor channel protein RyR2 when triggered by excitation-contraction coupling. The amount of Ca^{2+} released depends on several factors, including the degree of oxidation of cysteine thiol groups making up the channel. Moderate oxidation of these groups increases the protein sensitivity to the levels of Ca^{2+} [9, 10] and thus contributes to the opening of the channel and the release of Ca^{2+}.

Mitochondria use this same mechanism to regulate the amount of Ca^{2+} activating myofibrils. This link is carried out by changing the amount of superoxide produced when oxygen molecules pass through the electron transport chain. Increased oxygen consumption, such as during physical activity, is accompanied by a moderate increase in the formation of superoxide and a corresponding increase in Ca^{2+} and the strength of contraction. The increased delivery of Ca^{2+} to mitochondria is accompanied by the increased rate of oxidative phosphorylation and their synthesis of ATP [11, 12], i.e., so that the increased function has sufficient energy supply.

However, when doxorubicin damages or inhibits the electron transport chain, which occurs when the amount of superoxide or hydrogen peroxide formed during the dismutation reaction increases many times, a large number of cysteines are oxidized, and the output of Ca^{2+} from the channel is reduced. The superoxide action on RyR2 is an essential relationship between the function and its power supply, and the RyR2 channels are under the constant tonic influence of redox regulators [13]. This mechanism is an example of the effective use of cellular energy reserve, for if the strength of the contractions is not limited, the ATP reserve will be exhausted quickly. Plastomitin molecules may become embedded in the electron transport chain, and replace damaged coenzyme Q_{10} molecules [5], and thus maintain the proper level of ATP synthesis and the normal levels of myofibril-activating Ca^{2+}.

Increased myocardial compliance is an important factor that compensates for reduced myocardial contractility, thus increasing the area of possible actomyosin interaction. In our experiments, reduced diastolic elasticity or increased myocardial compliance were observed in both doxorubicin groups. Myocardial compliance, within the physiological range of sarcomere lengths, is determined by the state of titin [14], the largest protein in the myocardium and which connects the ends of the myosin filaments to the Z-line sarcomere boundary. When contraction develops, myosin filaments bond with actin filaments, shifting the Z-line, and compress the titin spring. When Ca^{2+} is eliminated from myofibrils, the spring-like titin structure ensures an elastic relaxation of the shorted myofibrils even in the absence of a stretching force, as been observed in isolated cardiomyocytes. Unlike skeletal muscle, myocardial titin contains extensible isoform N2BA, as well as the elastic isoform N2B. The former allows the ventricular myocardium to stretch when blood enters from the left atrium.

Analysis of available data on changes in the N2BA/N2B ratio in different types of cardiomyopathy shows a wide variability in different disease models. An increased N2BA/2B ratio is typical of dilated cardiomyopathy or ischemic cardiomyopathy with reduced ejection fraction [15], and a decreased ratio is typical of concentric cardiomyopathy or hypertension [16]. Similar changes were observed in CHF with normal ejection fraction [17]. Increased levels of the N2BA isoform are mainly typical of situations involving increased diastolic volume, and decreased levels are typical for situations when it is necessary to overcome increased resistance. Our findings on increased myocardial compliance suggest a shift in the N2BA/N2B ratio to the predominance of N2BA.

Conclusion

Treatment with plastomitin in combination with doxorubicin is capable of maintaining the normal contractile function of the heart and prevents the known toxic effects of doxorubicin on the myocardium.

Funding

The study was supported by the Russian Foundation for Basic Research (grants # 1801500271 and # 2001500027).

No conflict of interest is reported.

The article was received on 20/04/20

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ISSN 0022-9040. Kardiologiia. 2020;60(7). DOI: 10.18087/cardio.2020.7.n1157