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The role of endothelin-1 in the doxorubicin cardiotoxicity

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Abstract

Background: The cardiotoxicity of doxorubicin (Dx), an antineoplastic drug, is imposed by the development of cardiomyopathy and heart failure. The expression of endothelin-1 (ET-1) in myocardium under the action of Dx, directly correlates with the degree of cardiac dysfunction, mediated by endothelin A (ETA) receptor.
Material and methods: For prospective randomized study 2 groups of white rats (experimental group n=9, control group n=9) were used. During 2 weeks in the control group was administrated Dx (i/p, 4mg/kg in one dose, twice/week), cumulative dose – 16 mg/kg. The ET-1 effects were estimated at its peak action in concentration 10^{-7} M (mol), reproduced after 30 sec of endothelin stimulation.
Results: The functional parameters of isolated heart perfused in physiologic regime and in condition of volume and resistance overload under the ET-1 action in the group with Dx compared with the control one, were reduced considerably, namely: cardiac output (CO); left ventricle systolic pressure (LVSP); left ventricle end-diastolic pressure (LVEDP).
Conclusions: Under the ET-1 action on the isolated heart perfused in physiologic regime in the group with Dx – the LVSP and CO were reduced determining negative inotropic effect. At the volume overload test, under the ET-1 action, the diastolic impairment was more evident in the group with Dx, due to increased LVEDP. At the resistance overload test under the ET-1 action, the CO was reduced indicating the depreciation of myocardial contraction capacity.
Key words: doxorubicin cardiomyopathy, endothelin-1, coronary flow, heart reactivity.

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Introduction

Doxorubicin cardiotoxicity (doxorubicin, the antineoplastic drug efficient in treatment of leukaemia, lymphomas and sarcomas) is characterized by rapid evolution of cardiac failure imminent to doxorubicin (Dx) cardiomyopathy, inclusively at a young person, that is imposed as a cause of death or cessation of drug administration to oncologic patients. So, over several decades the researching of pathogenetical mechanisms of doxorubicin cardiomyopathy represents the main goal of cardiology and of course of oncology. Although many studies have been realized, till our days the pathophysiological prerogatives in conceptual plan have not been well established, the attempts to elaborate pathogenic therapy have at the base experimental values [1-3].

In this context, it is important to mention some successful mechanisms:

1. Development of myocardial energy deficiency is caused by endangerment of mitochondrial respiration and of oxidative phosphorylation [4]. The depletion of ATP reserves directly correlates with the severity of contractile dysfunction of myocardium and inability of it to realize the heart adaptation to hemodynamic and neuroendocrine efforts. A. Murabito et al. (2020) consider that doxorubicin (Dx) is accumulated in cardiomyocyte and binds to one of the phospholipids of mitochondrial membrane, cardiolipin, that in turn affects the electron transport and increases the membrane permeability for cytochrome C, responsible for activation of caspase 9 and initiation of apoptosis process [5]. Moreover, Dx reduces the ratio Bcl2/Bax (cardiac anti- and proapoptotic factors).

2. The activation of oxidative stress is due to exaggerated production of reactive oxygen species and depreciation of antioxidant system. It has been established that accumulation of Dx into the mitochondria in concentration more than 100 μM, represents the threshold level for the onset of lipid and protein peroxidation in cardiomyocytes. The administration of antioxidants, such as vitamin C and coenzyme Q10, in the experimental model of Dx cardiomyopathy in rats, has shown the improvement of left ventricle function at the effort and resistance [6, 7].

3. The impairment ratio between collagen type I and type III, caused by extracellular matrix metalloproteinase activation, determines one of the causes of pathologic myocardial remodeling with the eccentric pattern and dilation of left ventricle (LV), responsible for the disturbance of lusitropy function of the heart [8]. The evaluation of circulating level of different types of metalloproteinases (e.g. MMP-2

and MMP-9) has the predictive value of the risk of extracellular matrix remodeling of myocardium and exacerbation of heart failure.

4. The activation of inflammatory response, according to the results of several fundamental researchers, is one of the important mechanisms leading to dysfunction of heart inclusively doxorubicin cardiotoxicity [9-11]. In this context X. Xinyong et al. (2020) have demonstrated the role of transmembrane receptors, Toll-like, in the onset of inflammatory response of cardiomyocyte and interstitial macrophage to the action of signal molecules derived from damaged cardiac cells – Damage Associated Molecular Pattern (DAMP), resulting in increased production of cytokines [12]. The expression of Tumor Necrosis Factor-α (TNF-α) in murine myocardium subjected to action of Dx is increased in direct ratio with the cumulative dose of anthracycline. In one of previous fundamental researches, we have demonstrated that administration of monoclonal antibody of TNF-α attenuates the cardiotoxicity of doxorubicin, manifested by the improvement of inotropy of the heart [13].

5. The activation of neuro-endocrine system is characterized by initial activation of sympathetic adrenal system followed by excessive synthesis of endothelin-1 (ET-1). The expression of ET-1 in myocardium subjected to action of doxorubicin (Dx) correlates directly with the severity of structural disturbances, remodeling and degree of cardiac dysfunction, effects of this oligopeptide is mediated by ETA receptor [14, 15]. The functional activity of ET-1 is characterized by increasing concentration of calcium into the cardiomyocyte and smooth vascular muscle, which denotes the increased cardiac contraction and constriction of coronary arterioles. Evidently, that inotropy stimulation of the myocardium requires also additional energy expenditure, which can compromise the heart adaptation to the action of stressful and effort factors, especially those, compared with catecholamine and angiotensin II (Ang II), the time of ET-1 metabolisation is much longer. Furthermore, in the myocardial interstitium there are granules that can store ET-1 formed by endothelial coronary and cardiac cells and which are realized in huge amount at the paracrine and endocrine actions, triggered by hypoxia, ischemia, energy depletion, oxidative stress, etc. Thus, ET-1 imposes notable pathogenetic contribution in the onset and exacerbation of cardiac failure, but the particularities of its action on the heart functionality in doxorubicin affection are still poorly explained.

Material and methods

The doxorubicin cardiomyopathy of the heart has been reproduced on the white rats by administration of Dx intraperitoneal (cumulative dose 16 mg/kg during 2 weeks, 2 injections/per week in one dose 4.0 mg/kg). Rats have been sacrificed by euthanasia (thiopental sodium, 0.4 mg/kg) after 10 days from the last injection of anthracycline, because doxorubicin is a drug that due to reduced clearancei s accumulated in the body.

The isolated heart has been perfused in working regime according to the Neely-Rovetto method, functional indices of left ventricle (LV) were estimated by the technical registration device of the parameters in real time "Bio-Shell" (Australia) or by recorder Linearcorder Mark WR3101 (Germany) connected to mechanical sensor.

The effort reactivity of the heart has been studied by the increasing filling pressure of the left atrium to the value of 25cm H_2O (volume overload) or of the pressure in aorta to the value of 120 cm H_2O (volume overload). The effects of endothelin-1 (ET-1) were estimated at the peak of its action in concentration of 10^{-7} M (mol) or, when the action of ET-1 during 30 sec on the isolated heart preceded the manoeuvres of volume and resistance overload. At the same time, the premedication of isolated isovolumic heart with ET-1 in concentration of $5x10^{-7}$ M during 30 sec, gave the opportunity to appreciate the impact of ET-1 on the myocardium tolerance to ischemia-reperfusion stress (30 min ischemia and 45 min reperfusion).

The obtained data exposed by value of M±m (mean and standard error) were compared and statistically analysed according to t-Student criteria in relation to the records of the control group (intact rats) or in relation with indices estimated before the effort test. Margin of error less than 5 % was considered admissible, but deviation from the reference value – statistically significant ($p<0.05$).

Results

The estimation of functional indices of isolated heart in condition of physiologic effort perfusion (pressure in the left atrium and aorta is 15 and respectively, 80 cm H_2O) already has shown serious dysfunction of left ventricle (LF) in the group with doxorubicin (tab. 1).

Table 1

The values of functional indices of isolated heart perfused in physiologic regime

Functional indices	Groups		
	Control (n=9)	Dx (n=9)	p
Aortic jet velocity (AJ), ml/min	21.5±1.4	12.8±0.8 -40.47%	<0.01
Cardiac Output (CO), ml/min	37.4±1.9	23.6±1.3 -36.90%	<0.01
Left ventricular systolic pressure (LVSP), mm Hg	143.5±8.2	106.2±6.3 -25.99%	<0.05
Heart rate (HR), 1/min	289±13	245±11 -15.22%	<0.05
Left ventricular enddiastolic pressure (LVEDP), mm Hg	4.7±0.26	12.6±0.78 +168%	<0,01
Diastolic Stiffness of LV, mm Hg/ml	29.8±1.7	62.5±4.4 +109.73%	<0.01
+dP/dTmax, mm Hg/sec	8565±208	6320±165 -26.21%	<0.05
-dP/dTmax, mm Hg/sec	6710±174	5045±120 -24.81%	<0.05

Note: p – value of significance vs control; ± – relative deviation from the control group.

So, the main indices of pumping function of left ventricle (AJ and CO) are by 40.47% reduced from the control group, but systolic pressure generated by LV reaches the averages of 75% from control values of parameter. Also, is remarked the evident decline of diastolic relaxation of LV, being given increased LVEDP and diastolic stiffness by 168% and, respectively 109.73%.

Also, it is attested the disturbance of contraction and isovolumetric relaxation of the heart, and main parameters that characterized these important phases of cardiac cycle (e.g. +dP/dTmax and -dP/dTmax) are decreased significantly by 26.21% and respectively 24.81% compared with the control group.

There are important evidences that reflect the inotropy response of isolated heart perfused in physiologic regime under the action of ET-1 (tab. 2).

Table 2

The values of functional indices of isolated heart perfused in physiologic regime under the action of ET-1

Indices/lot	Action ET-1 (10^{-7} M)	
LVSP (mm Hg)	**Initial**	**Stimulation**
Control	141.7±8.2	177.3±8.6 +25.1%vs initial
Dx	104.9±6.4*	95.4±6.6* -9.1%vs initial
CO (ml/min)	Initial	Stimulation
Control	36.9±1.9	42.7±2.2 +15.7%vs initial
Dx	23.6±1.3*	21.5±1.5* -9%vs initial
HR (1/min)	Initial	Stimulation
Control	284±11	297±15 +4.6%vs initial
Dx	243±10*	250±16* +2.9% vs initial
LVEDP (mm Hg)	Initial	Stimulation
Control	4.9±0.29	6.1±0.55 +24.5%vs initial
Dx	12.5±0.87*	17.7±1.26* +41.6% vs initial

Note: * - p<0.05 vs control

The obtained results describe 3 important features:

First of all, inotropic response of the heart in the doxorubicin disorder is compromised. Unlike the control heart, it is manifested by the positive inotropic effect, being given by the increased value of left ventricular systolic pressure (LVSP) at the peak of stimulation by 25.1%, but in the Dx group it has been reduced by 9.1% that denotes the negative inotropic effect. As a result, cardiac output increased in case of positive inotropic effect with the ratio of 15.7%, but decreased cardiac output (CO) compared with initial value – by 9% that is characteristic for negative inotropic effect.

Secondly, ET-1 has increased the diastolic rigidity in both groups, fact that explains the imminent effect of this oligopeptide for increasing the concentration of calcium in cardiomyocyte, but unlike catecholamines it doesn't stimulate analogical lusitropy function of the myocardium. However, in the group of Dx the value of left ventricular enddiastolic pressure (LVEDP) has increased more at the peak of stimulation by about 70% compared with the control one: 41.6% vs 24.5%.

Thirdly, for the action of ET-1 it is not characteristic the notable chronotropic effect, but heart rate (HR) increased up to 4.6% from the initial values, both in the control and the Dx groups.

The detrimental effects of ET-1 have been manifested especially in the context of adaptive processes of the heart in the condition of volume and resistance effort, because both tests require from the heart the engaging of intrinsic mechanisms of contraction and relaxation capacity.

The premedication of the isolated heart with ET-1 has reduced the increasing rate of CO in the condition of increasing left atrial pressure up to 25 cm H_2O (tab. 3).

Table 3

ET-1 effect on the adaptive capacity of the isolated heart in the condition of effort with volume overload

Indices	Effort with volume overload without ET-1 (n=9)		Effort with volume overload preceded by ET-1 (n=9)	
	Control	**Dx**	**Control**	**Dx**
CO, ml/min	54.4±3.6 +47.43%	32.5±2.9 +37.71% p<0.01	51.3±4.7 +39.02%	28.6±2.6 +21.19% p<0.001
LVEDP, mm Hg	6.8±0.52 +38.78%	19.3±1.83 +54.4% p<0.01	7.5±0.72 +53.06%	24.8±2.44 +98.4% p<0.001

Note: +% – relative increment vs initial index; p – significance vs control

At effort test with volume overload of LV, the cardiac output and LVEDP are the main important parameters that estimate functional feasibility of the heart for adaptive processes. According to the physiologic entity of the test with volume overload, the values of these indices are increased in both groups. So, CO has increased in the control group by 47.43%, but in the group of Dx the rate of rise was lower – 37.71%. On the other hand, elevation of control LVEDP came to 38.78%, but in doxorubicin affection it was considerably more – 54.4%, that indicates the depreciated diastolic relaxation capacity.

In case of premedication with ET-1 the heart response was limited, especially in the group with Dx. The increasing rate of CO has been reduced by 43.8% (from 37.71 up to 21.19%), while in the control group it decreased only by 17.73% (from 47.43% up to 39.02%). This phenomenon is determined by the more pronounced alteration of diastole by ET-1 in the Dx group, because LVEDP increased by 98.4%, while in the control group the increasing of this index was 53.06%. As a result, the absolute mean value of LVEDP in doxorubicin disorder has become above 3.3 times greater than that from the control group (24.8 vs 7.5 mm Hg).

Premedication of the heart with ET-1 has compromised essentially the adaptive ability in the condition of increasing peripheral resistance by elevation pressure in aorta up to 120 mm Hg (tab. 4).

Table 4

Effect of ET-1 on the isolated heart adaptation in the condition of effort with resistance

Indices	Effort with resistance without ET-1		Effort with resistance preceded by ET-1	
	Control (n=9)	Dx (n=9)	Control (n=9)	Dx (n=9)
LVSP, mm Hg	176.4±11.3 +22.9%	126.3±11.5 +20.4% p<0.05	172.8±14.7 +20.42%	111.5±10.6 +6.29% p<0.001
CO, ml/min	29.2±2.4 -21.92%	16.3±1.2 -30.93% p<0.01	25.4±2.23 -32.08%	11.4±1.22 -51.7% p<0.001

Note: +% – relative increment vs initial index; p – significance vs control

The increasing rate of LVSP in the condition of effort with resistance without ET-1 premedication didn't differ considerably in both groups (from 20.4% in Dx up to 22.9% in the control group), although the recoil of absolute index compared with the control one was considerable, 28.4% (126.3±11.5 vs 176.4±11.3 mm Hg).

When the test with effort and resistance was reproduced under the action of ET-1, the contraction capacity of the myocardium in the Dx group reduced more considerably. So, the increasing rate of LVSP in the control group depreciated from 22.9% to 20.4%, but in the Dx group – from 20.42% to 6.29%. In this context it is mentioned that the difference of absolute value of LVSP increased from 28.4% up to 35.47%.

The disturbance of heart contractility under the action of ET-1 in the Dx group manifested by decreasing much more significantly CO at elevation of pressure in aorta. So, depreciation of CO in the control group was 52.08%, but in doxorubicin disorder – 51.7%. As a result, the cardiac output in the Dx group was depreciated by 55.12% compared with control index.

Therefore, the ET-1 action has manifested the negative inotropic effect in doxorubicin disorder of the heart and has been imposed by the exhaustion of adaptive capacity of the myocardium in the condition of effort with volume and resistance.

Premedication with ET-1 depreciated more notably the heart tolerance in the Dx group compared to the control one, at the action of ischemia (30 min), as so reperfusion (45 min), attested by the evaluation of enddiastolic pressure of LV (tab. 5).

The LVEDP value at 30 min action of ischemia has increased by 21.2% in the Dx group, in case the premedication of isovolumic heart with ET-1. It is important to mention that in the control group the elevation of LVEDP in similar condition constituted only 11.8%. So, ET-1 increased the

Table 5

Value LVEDP of isovolumic heart in ischemia and reperfusion

Group	Ischemia (min)		Reperfusion (min)	
	30	Deviation ET-1 (%)	45	Deviation ET-1 (%)
Control (n=9)	41.7±2.3		14.9±1.1	
ET-1 + control (n=9)	46.6±3.6 p1<0.05	+11.8%	20.2±1.8 p1<0.05	+35.6%
Dx (n=9)	63.7±4.4 p2<0.05		22.8±1.4 p2<0.05	
ET-1 + Dx (n=9)	77.2±6.9 p1<0.05 p2<0.01	+21.2%	35.7±2.7 p1<0.05 p2<0.01	+56.6%

Note: p1 – significant vs index before ET-1 action; p2 – significant vs control

difference of LVEDP between the Dx and the control groups from 52.76% up to 65.67% that was appreciated at 30 min of ischemia action. ET-1 affects the functional recovery of myocardium after reperfusion in both groups, being given elevation of LVEDP. However, the rising of this index was greater in the Dx group: 56.6 vs 35.6%. As a result, the value LVEDP estimated at 45 min of reperfusion increased in the Dx group compared with the control one by 76.73% (initial increment constituted 53.02%).

Discussion

The repeated action of doxorubicin in cumulative dose of 14 mg/kg during 14 days leads, in our study, to the myocardial contractility disturbance, especially in effort test with resistance, manifested by increasing decline of cardiac output and systolic pressure of LV compared with the control group. Impairment of pumping function of LV explains the phenomena of doxorubicin cardiotoxicity.

The recent reported data have shown that Dx can induce sarcopenia caused by disturbed synthesis of sarcomere contractile proteins, and concomitantly with detrimental effect on ATP synthesis, it can be a serious cause of functional incompetence of heart for adaptation to hemodynamic and neuroendocrine efforts [16, 17]. The action of ET-1 on the isolated heart perfused in physiologic regime has manifested in the group with Dx by decreasing of systolic pressure of left ventricle and of cardiac output at the peak of stimulation, that from pathophysiologic point of view is described as negative inotropic effect.

Endothelin-1 (ET-1), as angiotensin II (Ang II) or adrenergic agonists, is one of the important natural stimulators of myocardial inotropy, which in the control group has been imposed by elevation of LVSP, followed by increasing of aortic jet velocity and cardiac output. The negative inotropic effect specific for ET-1 can be recorded as notable pathogenetic mechanism of onset and exacerbation of

heart failure in patients with doxorubicin cardiomyopathy, because hemodynamic effort or homeostasis disturbances (e.g. hypoxia, ischemia, oxidative stress, augmentation of immune-inflammatory response, hyperglycemia, acidosis, etc.) lead to excessive realizing of ET-1.

A. Luu et al. (2018) consider that limitation of endothelial dysfunction is a conclusive benefit in doxorubicin disorder of the myocardium, due to limitation of the factors that stimulate realizing of ET-1, or by the factors that decrease detrimental effect of it, such as nitric oxide, prostacyclin, antioxidant enzymes, etc [18]. In pathophysiology of doxorubicin cardiomyopathy, endothelin 1 is viewed as neuroendocrine factor with mitogenic properties, such as growth factor, prooxidant, proinflammatory, etc.

Through the mechanisms of negative inotropy of ET-1in Dx affection can be underlined the coronaroconstriction action superior to reactivity of the intact heart, demonstrated in recent researches [19]. The diminished coronary functional reserve increases energy depletion and cardiomyocyte overloading with calcium that is detrimental to diastolic relaxation and realizing of Starling law. The effect of cardiomyocyte overload with calcium, specific for doxorubicin cardiotoxicity has been demonstrated by us through decreasing of myocardial tolerance to the ischemia-reperfusion impact [13].

It is remarkable that premedication of the isolated heart with ET-1 even 30 sec before the hemodynamic effort, limited the adaptive-compensatory capacity, in this way underlining the role of diastolic relaxation disturbance in heterometric regulation of heart in volume overload, as the role of compromised myocardial inotropy in homeometric regulation of heart in resistance overload.

The obtained data indicate the key mechanisms of diastolic and systolic disturbance in doxorubicin disorder, these being determined by significant decreasing the velocity of relaxation(-dP/dTmax) and isovolumetric contraction (+dP/dTmax) of the heart. The functional component of these phases is orchestrated mainly by the energetic potential, necessary for adequate turnover of the calcium cations, and it is dominant in the intrinsic system of the heart, such as: (1) increasing cardiac output in heart overload with volume by the formation of filling pressure for realizing Starling mechanism; (2) reaching the "mechanical stress", by the accumulated kinetic energy, sufficiently to overcome increased peripheral resistance in effort with resistance.

In estimation of functional severity and prognostic of heart failure, the values, such as isovolumic relaxation time and isovolumic contraction time of the heart are used as variables for echocardiographic appreciation of *Tei* index, considered as an index of precocious evaluation of cardiac dysfunction based on reduced ejection time of LV, due to increasing relaxation time and isovolumic contraction time, or decreasing values +dP/dTmax and -dP/dTmax [20].

W. Border et al. (2020) consider in this context that echocardiographic indices of isovolumic relaxation and contraction of the heart are important functional predictors of precocious heart disorders in oncologic children who take anthracycline [21]. Thus, the effort tests which underline the functional dysregulation of isovolumic relaxation and contraction of the heart, which are absent in rest condition, have a great diagnostic value in doxorubicin cardiotoxicity.

The drugs that modulate activity of ET-1 (nonspecific blockers of receptors ETA/ETB and inhibitory of ET-1 conversion enzyme) are used in the treatment of cardiac failure, and especially in pulmonary arterial hypertension, in which pathogenesis of the ET-1 has crucial role, promoting the von Euler reflex [22, 23]. So, it is a great benefit for modulation the activity of ET-1 in patients who administrate doxorubicin, especially due to the fact that the level of circulated ET-1 elevates with increasing cumulative dose of Dx and directly correlates with severity of injury and cardiac dysfunction [24].

Conceptually the ET-1 effect is important for reducing myocardial tolerance under the action of ischemia-reperfusion syndrome. The impact of this is imposed by cardiomyocyte overload with calcium and excessive realizing of oxygen free radicals. The premedication of the heart with ET-1 potentiated these mechanisms and in such a way limited the capacity of intrinsic system of the myocardium to diminish impact of calcium overload and oxidative stress. It is important to mention, in this context, that myocardial ischemia increases the level of ET-1 realized by interstitial granules and expression of vasoconstrictor receptors [25]. On the other hand, cardiotoxicity of Dx also is imposed by the increasing ET-1 expression and disturbance of coronary reactivity depending on the endothelium [13]. Another mechanism is determined by the decreasing expression of NO under the action of ET-1, that compromises myocardial resistance to ischemic reperfusion impact [26].

Conclusions

Cardiotoxicity of doxorubicin is characterized by significant decreasing of contraction and relaxation velocities of left ventricle, as well as by negative inotropy effect under the ET-1 action *in vitro*, which disturbs heterometric and homeometric cardiac regulation, and myocardial tolerance to ischemia and reperfusion.

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Author's contribution
LT reviewed the scientific literature, designed the study, interpreted the data, performed the analytical part of the laboratory work and interpreted the data.

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Ethics approval and consent to participate
The research project was approved by Ethics Committee of *Nicolae Testemitanu* State University of Medicine and Pharmacy (Protocol No 40, 05.12.2016).

Conflict of Interests
No competing interests were disclosed.