ORIGINAL RESEARCH

Submitted: 17.7.2018; Accepted: 10.8.2018; Published online: 21.11.2018

The state of hydrolytic processes in the myocardium and its draining lymphocytes in rats under the effect of Doxorubicin under protective Trimetazidine premedication

Elena M. Frantsiyants[1], Ekaterina I. Surikova[1*], Irina V. Kaplieva[1], Lidia K. Trepitaki[1], Irina V. Neskubina[1], Elena V. Shalashnaya[1], Valeria A. Bandovkina[1], Larisa S. Kozlova[1], Yulia A. Pogorelova[1], Lyudmila A. Nemashkalova[1], Natalia D. Cheryarina[1], Irina S. Tavaryan[1], Olga V. Kozyuk[1], Lyudmila Y. Rozenko[1], Pavel G. Sakun[1], Vladimir S. Trifanov[1]

[1] Rostov Research Institute of Oncology, Ministry of Healthcare of the Russian Federation
Russia, 344037, Rostov-on-Don, 14th Line st. 63

[*] Corresponding author:
phone: +7 (904) 501-83-62, e-mail: super.gormon@yandex.ru

Aims

The aim hereof is to study the activity of cathepsin D (CatD), alkaline phosphatase (ALP) and acid phosphatase (AP), the antitryptic activity (ATA) of acid-stable inhibitors (ASI) in rats in heart tissue and its draining lymphocytes under introduction of Doxorubicin and protection with Trimetazidine.

Materials and methods

The study has been carried out in outbred male rats (50 animals) weighing 150-160 g: the reference group consists of 10 animals; group 1 (20 animals) has experienced injections of Doxorubicin in physiological saline at a dose of 2 mg/kg, 5 times; group 2 (20 animals) has undergone the above mentioned Doxorubicin medication after preliminary protection with Trimetazidine for 5 days at a dose of 35 mg/kg. In the myocardium and its draining lymphocytes determined has been the following: CatD, ATA, ASI, ALP, AP, ratios CatD/ATA, CatD/ASI and AP/ALP. The statistical significance has been evaluated using the Mann-Whitney criterion.

Results

In the group 1 animals in their myocardia and lymphocytes observed has been a statistically significant increase in the activity of CatD by 42.4 and 64.6%, respectively, AP and ALP by 2.2-2.3 times on the average, ASI and ATA by 1.8-1,9 times if to compare with the level in the reference animals. Ratios CatD/ATA, CatD/ASI in the myocardium have been considerably decreased by 25.4% and 24.2%, and in the lymphocytes all the ratios remained at the level of values in the reference animals. In group 2 in the myocardia and lymphocytes a significant decrease in enzyme activity by 23-31%, on the average, as compared with reference animals, has been observed, and, at the same time, all the ratios have been at the level of the reference animals.

Conclusions

The dynamics of the studied indicators recorded for the hearts in the group 1 rats reflect an intensification of catabolic processes and the activation of inflammation in myocardial tissue as a manifestation of the toxic effect of Doxorubicin. The dynamics of indicators in the group 2 rats demonstrates the lack of activation in the processes of autophagy/apoptosis and the absence of a pronounced local inflammation that is caused by the cardioprotective action of Trimetazidine.

Keywords

Cardiotoxicity, Doxorubicin, Trimetazidine, Cathepsin D, Acid phosphatase, Alkaline phosphatase

Imprint

Elena M. Frantsiyants, Ekaterina I. Surikova, Irina V. Kaplieva, Lidia K. Trepitaki, Irina V. Neskubina, Elena V. Shalashnaya, Valeria A. Bandovkina, Larisa S. Kozlova, Yulia A. Pogorelova, Lyudmila A. Nemashkalova, Natalia D. Cheryarina, Irina S. Tavaryan, Olga V. Kozyuk, Lyudmila Y. Rozenko, Pavel G. Sakun, Vladimir S. Trifanov. The state of hydrolytic processes in the myocardium and its draining lymphocytes in rats under the effect of Doxorubicin under protective Trimetazidine premedication. Cardiometry; Issue 13; November 2018; p.66-74 ; DOI: 10.12710/cardiometry.2018.13.6674; Available from: http://www.cardiometry.net/issues/no13-november-2018/hydrolytic-processes-in-the-myocardium

Introduction

The use of more intensive chemotherapeutic schedules in modern oncology not only leads to an increase in the duration of disease-free survival and the number of patients cured of cancer, but also to an increased risk of side effects, primarily produced the cardiovascular system. The development of cardiac complications against the background of antitumor chemotherapy results in a decrease in its effectiveness

(in case of reduction of chemotherapy courses provided to patients), a deterioration in the quality of life and its duration in those patients, who may be potentially cured, and especially those who have already had cardiovascular pathology [1-3].

Many anti-tumor drugs show cardiotoxicity, among them is a large group of Anthracycline antibiotics, which so far are widely used in various chemotherapeutic schedules due to their high efficacy [4]. The cardiotoxicity developing during their use can be acute, chronic or delayed, manifesting years after the termination of therapy [5-8]. Cardiotoxic effects of anthracyclines are attributed to the type 1 cardiotoxicity, characterized by the development of apoptosis or myocardial necrosis as a result of the cumulative dose-dependent effect. Cardiotoxicity of this type is dangerous due to its limited reversibility, resistance to therapy, and it causes a deterioration in cardiac prognosis. Nowadays various methods of cardiac protection are being actively developed against the background of oncological patients therapy with Anthracycline-based drugs [9].

Histological signs characterizing the cardiotoxicity of Anthracyclines include, in particular, an abundant vacuolization of cardiomyocytes, disorganization of myocardial fibers that is related to the mechanism of the action of these chemotherapeutic agents on myocardial cells. The enhancement of free radical oxidation in cardiomyocytes offers an increased permeability in their internal membranes, mitochondrial dysfunction, exit of the Ca ions and lysosomal enzymes with their entry into the cytoplasm, and development of apoptosis or necrosis [9,10].It is known that Trimetazidine, used in cardiology, has a protective effect in case of ischemia and development of oxidative stress in cardiomyocytes due to modulation of their energy metabolism, restoration of the intracellular ATP level, reduction of the intracellular acidosis and calcium dysfunction intensity and activation of antioxidant protection [11].

In this connection, in our opinion, it would be intriguing to study the activity of hydrolytic processes occurring in the heart tissue after the Doxorubicin medication under the preliminary protection by Trimetazidine, as it is known that proteolytic enzymes, changing the functional properties of proteins and thus controlling various biochemical processes, play an important role in the physiology both of individual organs and the organism as a whole.

Thus, the aim of our study is to study the activity of a number of proteolytic enzymes and the activity of proteolysis inhibitors in the heart tissue and its draining lymphocytes in rats in case of the Doxorubicin use under the Trimetazidine preliminary protection.

Materials and methods

The study has been carried out on outbred male rats (50 animals) weighing 150-160 g. The animals have been kept under natural lighting conditions with free access to water and food. All the studies have been performed in accordance with applicable international norms and regulations (European Communities Council Directive 86/609/EEC) and the associated National Order Regulation No.267 dd. 19.06.03 "Approved Rules and Regulations for Laboratory Practices" issued June 19, 2003 by the Ministry of Healthcare of Russian Federation. Doxorubicin and Trimetazidine (Preductal MB) have been used in our experimental work. The animals have been divided into 3 groups as follows: the reference group (10 animals) has received injections of a sterile physiological saline; group 1 (20 animals) has been treated with intravenous injections of Doxorubicin in a physiological saline at a dose of 2 mg/kg, 5 times; group 2 (20 animals) has undergone the above-mentioned Doxorubicin therapy after their preliminary protection with Trimetazidine for 5 days at a dose of 35 mg/kg administered perorally through the feeding tube.

Seven days after the termination of the Doxorubicin administration, the rodents have been decapitated, their hearts have been rapidly removed and chilled; the hearts were homogenized in the physiological saline, and lymphocytes have been isolated from the heart tissue according to a density gradient of Fikkol/Verografin of 1.078 g/cm3 (3x106 cells/ml working concentration of the lymphocyte suspension); we have obtained the 10% cytosolic fractions prepared using a 0.1 M potassium phosphate buffer with a pH of 7.4 containing 0.1% Tween-20 and 1% BSA. In the heart tissue and its draining lymphocytes, the activity of cathepsin D (CatD) [12] and acid-stable inhibitors (ASI), general antitriptical activity (ATA) [13], and the activity of alkaline phosphatase (ALP) and acid phosphatase (AP) have been determined by the unified method with the use of Olvex Co. reagent kits. The obtained results have been recalculated per gram of the heart tissue or per mg of protein in lymphocytes. The protein concentration has been assessed by

Table 1. Indicators of the hydrolytic system in heart tissue and its draining lymphocytes, when using Doxorubicin and Trimethazidine in the experiment

	Reference group		Group 1 (introduction of Doxorubicin in physiological saline)		Group 2 (introduction of Doxorubicin after Trimetazidine protection)	
	Myocardium (/g tissue)	Lymphocytes (/mg protein)	Myocardium (/g tissue)	Lymphocytes (/mg protein)	Myocardium (/g tissue)	Lymphocytes (/mg protein)
CatD (nm)	528±51,6	83,0±8,0	752±64,3 p_1<0,05	136,6±12,3 p_1<0,05	400±39,2 p_1<0,05 p_2<0,05	57,1±4,2 p_1<0,05 p_2<0,05
ATA (nm)	1,1±0,2	5,3±0,4	2,1±0,3 p_1<0,05	9,8±0,8 p_1<0,05	0,8±0,2 p_2<0,05	3,7±0,3 p_1<0,05 p_2<0,05
ASI (nm)	2,5±0,4	8,1±0,5	4,7±0,6 p_1<0,05	15,1±1,6 p_1<0,05	1,8±0,4 p_2<0,05	5,7±0,5 p_1<0,05 p_2<0,05
AP (nm)	13200±121,6	10,9±1,0	27300±254,6 p_1<0,05	22,4±2,3 p_1<0,05	10100±96,4 p_1<0,05 p_2<0,05	7,7±0,8 p_1<0,05 p_2<0,05
ALP (nm)	1100±98,3	3,8±0,9	2400±22,7 p_1<0,05	8,8±0,9 p_1<0,05	840±76,4 p_1<0,05 p_2<0,05	2,9±0,3 p_1<0,05 p_2<0,05
CatD/ ATA	478,8±44,2	15,7±1,6	358,1±34,2 p_1<0,05	13,9±1,2	504,3±47,3 p_2<0,05	15,4±1,2
CatD/ ASI	221,5±19,3	10,2±1,0	160±14,2 p_1<0,05	9,0±0,8	212,2±20,1 p_2<0,05	10,0±0,9
AP/ALP	12±1,1	2,9±0,3	11,4±1,0	2,5±0,2	12,0±1,1	2,7±0,3

Note: The data are represented in the M±m form. Statistically significant variances are as follows: p_1 in relation to the values in the reference animals; p_2 in relation to the values in group 1.

Lowry method. The values of ratios CatD/ATA, CatD/ASI and AP/ALP have been calculated

The statistical processing of the results has been carried out with the original Statistica 10.0 software; in each group the mean value ± standard error of the mean (M ± m) has been determined. The significance of differences has been evaluated using the Mann-Whitney nonparametric criterion. Variance at a significance level $p < 0.05$ have been considered to be statistically significant.

Results

The results of the study are presented in Table 1 herein. In the group 1 rats, in their heart tissue we observed a statistically significant increase in all the studied parameters compared to the level in the reference group: cathepsin D activity has been 42.4% higher, ATA and ASI have been increased by 1.9 times, and the acid and alkaline phosphatase activity has been reported to be 2,1 and 2,2 times higher, respectively. At the same time, the CatD/ATA and CatD/ASI values, which characterize the state of the protease inhibitor system, have been statistically significantly reduced by 25.4% and 24.2%, respectively, and the AP/ALP ratio has not differed from that in the reference group. A significant increase in the following studied parameters has also been observed in the lymphocytes in the rodents of this group: CatD has been increased by 64.6%, ASI and by ATA 1.8 times, AP has been found 2.1 times and ALP 2.3 times greater, respectively. However, the values of ratios CatD/ATA, CatD/ASI and AP/ALP remained at the level of the reference unaffected animals.

In the group 2 animals we observed counter-tendency dynamics of the studied parameters, both in the heart tissue and lymphocytes. When introducing Doxorubicin after the Trimetazidine protection, a statistically significant decrease in CatD by 24.2%, AP and ALP on the average by 23.6% in the heart tissue compared to that in the reference animals, but no statistically significant changes in the ATA, ASI, CatD/ATA, CatD/ASI and AP/ALP values have been recorded. In lymphocytes we observed a statistically significant decrease in all the studied parameters by 23-31%, and, at the same time, no significant change in the ratios of CatD/ATA, CatD/ASI and AP/ALP in comparison with the corresponding level in the reference group has been identified. As a result, in animals, who

received Doxorubicin after protection with trimetazidine, in the heart tissue, statistically significantly lower activity of CatD (by 46.8%), AP and ALP (2.2 times on the average), ATA and ASI (by 61, 8% on the average) in comparison with the group 1 animals' indicators, have been detected. At the same time, the values of CatD/ATA and CatD/ASI have been found to be significantly higher: their increases were 38-39%. In the lymphocytes a statistically significantly lower level of all the studied parameters has been observed, namely, they decreased by 2.4-3.0 times in comparison with the level in group 1.

Analyzing the dynamics of the hydrolytic system activity indicators, we can note the same direction of the changes found in the heart tissue and its draining lymphocytes. In the group with the use of Doxorubicin introduced in physiological saline, a significant increase in the activity of all components of the studied system has been revealed in comparison with the level in the reference animals, and, as opposed to it, in the group of animals, who had received Trimetazidine protection, a decrease in the activity of the hydrolytic system components has been observed. As a result, in the group 1 animals, a small, but statistically significant, decrease in the CatD/ATA and CatD /ASI values in the heart tissue may indicate a predominance of proteolytic activity inhibition processes, while in the group 2 animals the absence of changes in these ratios (in comparison with the level in the reference animals) indicates that the protease inhibitor system is balanced, and the hydrolytic processes activity is generally maintained at the reference level. Attention should also be drawn to the absence of statistically significant changes in these values for lymphocytes in both groups of animals that may indicate a more balanced response by lymphocytes to the medication action if to compare with the reaction of the myocardium.

Discussion and Conclusions

Various mechanisms of cardiotoxicity of Anthracyclines have been described in the present-day literature, but the main triggers are the development of free-radicals stress, mitochondrial dysfunction (with disordered metabolism of high-energy phosphates) and dysregulation of the calcium homeostasis. The next step is a change in gene expression, activation of the ubiquitin-proteasome system, apoptosis and induction of immunogenic reactions. Thus, it is obvious that the Anthracycline cardiotoxicity is a multifactorial multi-stage process leading to apoptosis of cardiomyocytes. As a result of the myocardium damage, clinical signs of heart failure develop [14,15].

Despite the understanding of importance of nucleus- and mitochondria-related events in the cardiotoxicity induced by Doxorubicin, nowadays an idea of a decisive role of functioning of lysosomes and the associated autophagy dysregulation in cardiomyocytes is being extensively elaborated [16,17]. Autophagy is a universal cellular process, which is treated to be a protective mechanism, which maintains the cell viability due to the elimination by isozymal proteases (cathepsins A, B, D, H and L) of the damaged cells or undesired proteins and organelles. In pathological states, autophagy is initiated to protect cells from stress stimuli or, alternatively, to contribute to cell death. The obtained data demonstrate the key role of autophagic processes in cardiac physiology and pathology: an accumulation of damaged proteins and organelles in cardiomyopathies of various genesis has been discovered [18]. Conducted were studies, which attribute the Doxorubicin-induced cardiotoxicity to the dysregulation of macroautophagy, mitophagy, and chaperone-mediated autophagy through the disruption of functioning of the transcription factors, which are also responsible for the regulation of the lysosomal function [19,20]. In cardiomyocytes exposed to Doxorubicin, changes in proteolytic processes are detected, but the role they play in the Doxorubicin-provoked cardiotoxicity remains unclear. The degree of the dysregulation of autophagy in response to the influence produced by Doxorubicin depends on the type of cardiotoxicity model used, on the concentration of the active substance and the duration of the Doxorubicin action [20].

During the formation of defective mitochondria in cells, the process of mitophagy (the elimination of the improperly functioning mithochondria) in lysosomes is triggered, which, as revealed, is sensitive to the Doxorubicin exposure [21,22].This form of autophagy is of a great importance in cells with a high density of mitochondria, in particular in cardiomyocytes. The Doxorubicin-induced disorder of mitophagy through the dysregulation of various cytosolic and mitochondrial signaling pathways leads to a deterioration in the physiological elimination of damaged mitochondria and to the accumulation thereof, that increases the production of active oxygen species and aggravates the ATP deficiency [20].

An important point is that structural changes in the heart tissue appear much earlier than clinical manifestations of heart failure. This means that the compensatory mechanisms are capable of supporting the heart performance for a certain time against the growing myocardium damage.

In accordance with the above, it can be assumed that an enhancement in the activity of lysosomal enzymes such as cathepsin D, acid and alkaline phosphatases, observed in this study in the group 1 animals, bearing witness to the activation of proteolytic processes in the heart tissue, may be a manifestation of the acute toxic effect produced by Doxorubicin upon its introduction in a physiological saline. In the study by Leto G. et al. (1987) it is shown that changes in the cathepsin D activity in the cardiac muscle in mice have been detected after each introduction of Doxorubicin [23]. In the research work done by Hilfiker-Kleiner D. et al. (2007) it has been found that enhancing the expression of cathepsin D and its activity can mediate a progression of heart failure through prolactin cleavage [24]. In our case, an activation of the autophagy processes, in particular mitophagy, probably takes place that may be interpreted as a protective reaction of heart tissue in response to Doxorubicin damage of the most important cardiomyocyte organelles: mitochondria. In the review by Ghosh R, Pattison JS. (2018) it is shown that an exposure to Doxorubicin intensifies autophagic processes, and inhibition of these processes weakens the manifestations of cardiotoxicity induced by Doxorubicin. The authors suggest a possible protective role of the autophagy processes in the Doxorubicin-induced cardiotoxicity [18].

For a long time it has been believed that the main function of cathepsin D is to cleave damaged proteins in lysosomes and phagosomes as a result of activation of endocytosis and autophagy. However, it has now become known that, besides, cathepsin D undertakes many physiological functions associated with the activation of precursors of a number of enzymes, hormones, growth factors, thus participating in the maintenance of the tissue homeostasis, development of the apoptosis processes, remodeling of the extracellular matrix and generation of an immune response [25-27]. Analyzing the results obtained in our study, we can also assume that an increase in the activity of cathepsin D in the heart in the group 1 rats is an indication of the activation of the cardiomyocyte apoptosis processes as a result of the Doxorubicin action, since it is known from the literature that cathepsin D is one of the key mediators of the induced apoptosis (via caspase-dependent and caspase-independent mechanisms), and its pro-apoptotic effect can be produced both at the expense of the proteolytic activity and in a way independent thereon [26,28-31]. However, depending on the intracellular context and stress stimulus cathepsin D can manifest not only proapoptotic, but also anti-apoptotic effect. The study by Sagulenko V. et al. (2008) shows that an elevation of the expression of cathepsin D in neuroblastoma cells leads to a weakening of Doxorubicin-induced apoptosis due to activation of the Akt path, one of the main paths of the cell survival [32]. The investigation work by Oliveira C.S. et al. (2015) discovers an antiapoptotic effect of cathepsin D in colorectal cancer cells under the activation of the mitochondrial apoptotic cascade [33]. Du F. et al. (2017) demonstrate that increasing the expression of cathepsin D promotes the viability of renal tubule cells, preventing lysosomal apoptosis and stabilizing the membrane potential of mitochondria under toxic effects of glycosylation products [34]. In our study, however, it is impossible to unambiguously identify which sort of effects, pro- or anti-apoptotic, is produced by cathepsin D in this case.

In our opinion, an enhancement in the activity of phosphatases in the heart tissue in response to the Doxorubicin effects also deserves attention. Although these enzymes have been studied for a long time, but their biological functions in the body are still not finally determined. At present, it is known that acid phosphatase is defined as a multifunctional enzyme that catalyzes not only hydrolytic dephosphorylation reactions, but also plays an important role in the ATP-dependent regulation of the calcium homeostasis and, as supposed, can participate in the reactions of generation of reactive oxygen species (due to the presence of iron atoms in the active center) [35-37]. For some isoforms of acid phosphatase of plant origin, the connection with the intracellular production of reactive oxygen species (mainly in the process of cell autolysis) and the manifestation of the peroxidase activity under stress conditions are shown [38,39]. Despite the fact that alkaline phosphatase is available in great numbers in many tissues in the organism and there is a rich variety of its isozyme forms, its exact physiological function remains to a great extent still unexplored. It has been found that this enzyme is of great significance in phosphoric and calcium metab-

olism; some studies show the participation of alkaline phosphatase in the regulation of protein phosphorylation, apoptosis and development of cardiovascular diseases [40,41].

The activation of cathepsin D, acid and alkaline phosphatases, detected in this study, could be considered in general as a marker of intensification of catabolic processes in rat myocardial tissue resulting from Doxorubicin toxicity. The simultaneous considerable activation of ASI and ATA leads to a change in the proteinase inhibitor system towards weakening of intracellular proteolysis, as evidenced by a decrease in the CatD/ATA and CatD/ASI values. An increase in the antitryptic activity and the tissue-related acid-stable inhibitors' activity can be associated with the functioning of the inflammation regulation mechanisms in the heart tissue, and the resulted proteinase-inhibitory balance probably reflects the severity of the inflammation. The triggering of inflammation in the myocardial tissue can also be indicated by the activation of proteolytic enzymes in the myocardium-draining lymphocytes. To date, it is known that various cathepsins, including cathepsin D, are key modulators of congenital and adaptive immune reactions, and they are involved in the activation/inhibition of cytokines, which play a crucial role in the development of inflammatory reactions, and an imbalance in the functioning of cathepsins in the immune system cells can lead to chronic inflammation, autoimmune disorders and, as a result, cause pathological damages to cells and tissues [42]. Thus, the observed dynamics of the hydrolytic system performance in the heart tissue in rats upon introduction of Doxorubicin in a physiological saline represents a complex picture of the manifestation of the acute chemotherapy-drug induced toxicity and the protective response of the organism to myocardial damage.

The possibility of cardioprotection in cancer patients treated with the use Anthracycline-based schedules has been actively explored in recent decades, resulting in defining several approaches that differ in their protection mechanism. One of them is the use of cardioprotective agents (for example, Decrazoxane, a free radical acceptor), and any molecule, which is capable of directly reducing myocardial damage, slowing down the sequential remodeling of the myocardium or contributing to the restoration of its physiological metabolic activity, can have a cardioprotective effect [9,43,44]. The use of Trimetazidine (Preductal MB), which precedes the introduction of Doxorubicin, in the group 2 animals in the context of our study, falls into the above approach pattern.

Trimetazidine is a medical drug of metabolic action with a large evidence base in cardiology, which has demonstrated its high efficacy and safety in chronic heart failure both of ischemic and nonischemic genesis. The molecular mechanism of the action of this drug is associated with its capability to slow down the oxidation of fatty acids and enhance the oxidation of glucose and, in doing so, contribute to the preservation of energy metabolism in cardiomyocytes. As a result of the activation of glycolysis, which supplies ATP, the transmembrane Ca2+ ion flux is normalized. The use of Trimetazidine diminishes intensity of intracellular acidosis. Improving the metabolism of membrane phospholipids, Trimetazidine reduces the permeability of membranes and increases their resistance to damage, weakens leukocyte infiltration of reperfused and ischemic tissues of the heart, contributing to a decrease in the size of the myocardial damage focus. Under intensive physical loads, the use of Trimetazidine retards the development of tissue hypoxia and enhances the contractility of the myocardium [45]. In case of the experimental myocardial infarction, the preventive introduction of Trimetazidine promotes a decrease in the necrosis zone [46]. The experimental study by Salouege I. et al (2014) shows that the Doxorubicin therapy accompanied by Trimetazidine medication during 3 days results in a decrease in the surface of apoptotic and necrotic areas, as well as of areas with lymphoid infiltration and inflammatory reaction in the histological sections of the rat heart, that has demonstrated the cardioprotective action of Trimetazidine in the model of the acute Doxorubicin-induced cardiotoxicity [47].

The experimental study by Moustafa A.M. et al. (2006) shows the preventive administration of Trimetazidine performed 5 days prior to initiation of the Doxorubicin therapy changes the dynamics of the creatine phosphokinase-MB level that indicates a considerable reduction in heart damage. At the ultrastructural level, the use of Trimetazidine leads to a lack of mononuclear cell infiltration of the myocardium, a decrease in markers of oxidative stress, and an increase in the antioxidant activity that is evidence for the normal functioning of the mitochondria [48].

Analyzing the results obtained in the group 2 animals, we did not observe the activation of the

cathepsin D hydrolytic enzymes, acid and alkaline phosphatases both in the heart tissue and its draining lymphocytes that can be considered as a sign of the absence of the activation of autophagy and apoptosis processes (or their minor expression). On the contrary, we observed a slight decrease in the activity of those enzymes in comparison with those in the reference animals, as well as a slight decrease in the inhibitor activity of heart-draining lymphocytes. However, the absence of significant changes in the CatD/ATA, CatD/ASI and AP/ALP values as compared to the reference group indicates the proteinase/inhibitor system balance is available both in heart tissue and its draining lymphocytes and that a pronounced local inflammatory process with the use of the above Doxirubicin schedule of doxorubicin introduction is not present. In our opinion, such results may be determined by the Trimetazidine premedication, which normalizes the cardiomyocytes energy metabolism and the intracellular medium acidity, reduces the leukocyte infiltration in the heart tissue and prevents the development of acute the Doxorubicin-induced cardiotoxicity.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

All the authors read the ICMJE criteria for authorship and approved the final manuscript.

References

1. Chavez-MacGregor M, Zhang N, Buchholz TA, Zhang Y, Niu J, Elting L et al. Trastuzumab-related cardiotoxicity among older patients with breast cancer. Journal of Clinical Oncology. 2013;31(33):4222-8. doi: 10.1200/JCO.2013.48.7884.

2. Accordino MK, Neugut AI, Hershman DL. Cardiac effects of anticancer therapy in the elderly. Journal of Clinical Oncology. 2014;32(24):2654-61. doi: 10.1200/JCO.2013.55.0459.

3. Han X, Zhou Y, Liu W. Precision cardio-oncology: understanding the cardiotoxicity of cancer therapy. NPJ Precision Oncology. 2017; 1(1):31. doi:10.1038/s41698-017-0034-x.

4. Perevodchikova NI. Guide to chemotherapy for neoplastic diseases. 4-th ed. Moscow: Prakticheskaya medicina. 2018. [in Russian]

5. Gendlin GE, Emelina EI, NikitinIG, VasyukYuA. Modern view on cardiotoxicity of chemotherapeutics in oncology including anthracyclines. Russian Journal of Cardiology. 2017; 143(3):145-54. http://dx.doi.org/10.15829/1560-4071-2017-3-145-154. [in Russian]

6. Yandieva RA, Saribekyan EK, Mamedov MN. Cardiotoxicity of cancer therapy. The International Journal of Heart and Vascular Diseases. 2018; 17(6):3-11. [in Russian] https://cyberleninka.ru/article/n/kardiotoksichnost-pri-lechenii-onkologicheskih-zabolevaniy.

7. Swain S, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer. 2003;97(11):2869-79. doi: 10.1002/cncr.11407.

8. Mercuro G, Cadeddu C, Piras A, Dessì M, Madeddu C, Deidda M, et al. Early epirubicin-induced myocardial dysfunction revealed by serial tissue dopplerechocar-diography: correlation with inflammatory and oxidative stress markers. Oncologist. 2007;12(9):1124-33. doi: 10.1634/theoncologist.12-9-1124.

9. Kerkhove D, Paciolla I, Arpino G. Classification by Mechanisms of Cardiotoxicity. Anti-Cancer Treatments and Cardiotoxicity. Mechanisms, Diagnostic and Therapeutic Interventions. 2017. https://doi.org/10.1016/B978-0-12-802509-3.00003-0.

10. Maurea N, Coppola C, Rienzo A. Changes of Myocardial Structure and Function. Anti-Cancer Treatments and Cardiotoxicity Mechanisms, Diagnostic and Therapeutic Interventions. 2017. https://doi.org/10.1016/B978-0-12-802509-3.00008-X.

11. Dézsi CA. Trimetazidine in Practice: Review of the Clinical and Experimental Evidence. American Journal of Therapeutics. 2016;23(3):e871-879. doi: 10.1097/MJT.0000000000000180.

12. Ogloblina OG. Acid-resistant proteins are inhibitors of mammalian proteinases. Structure, properties, biological role. Biochemistry. 1982; 47(10):1587-1599. [in Russian]

13. Veremeenko KN, Goloborodko AI, Kizim AI. Proteolysis in norm and in pathology. Kiev. 1988. [in Russian]

14. Matyash MG, Kravchuk TL, Vysotskaya VV, Chernov VI, Goldberg VE. Anthracycline-induced cardiotoxicity: mechanisms of development and clinical manifestations. Siberian Journal of Oncology. 2008;30(6):66-75. [in Russian]

15. Octavia Y, Tocchetti CG, Gabrielson KL, Janssens S, Crijns HJ, Moens AL. Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. Journal of Molecular and Cellular Cardiology. 2012; 52(6):1213-1225. doi: 10.1016/j.yjmcc.2012.03.006.
16. Dirks-Naylor AJ. The role of autophagy in doxorubicin-induced cardiotox. Life Sci. 2013; 93(24):913-6.
17. Koleini N, Kardami E. Autophagy and mitophagy in the context of doxorubicin-induced cardiotoxicity. Oncotarget. 2017;8(28): 46663-80. doi:10.18632/oncotarget.16944.
18. Ghosh R, Pattison JS. Macroautophagy and Chaperone-Mediated Autophagy in Heart Failure: The Known and the Unknown. Oxidative Medicine and Cellular Longevity. 2018; doi: 10.1155/2018/8602041.
19. Li DL, Wang ZV, Ding G, Tan W, Luo X, Criollo A, et al. Doxorubicin blocks cardiomyocyte autophagic flux by inhibiting lysosome acidification. Circulation. 2016; 133(17):1668-87. doi: 10.1161/CIRCULATIONAHA.115.017443.
20. Bartlett JJ, Trivedi PC, Pulinilkunnil T. Autophagicdysregulation in doxorubicin cardiomyopathy. Journal of Molecular and Cellular Cardiology. 2017; 104:1-8. doi: 10.1016/j.yjmcc.2017.01.007.
21. Gharanei M, Hussain A, Janneh O, Maddock H. Attenuation of doxorubicin-induced cardiotoxicity by mdivi-1: a mitochondrial division/mitophagy inhibitor. PLoS One. 2013; 8(10):e77713. doi: 10.1371/journal.pone.0077713.
22. Dirks-Naylor AJ, Kouzi SA, Bero JD, Phan DT, Taylor HN, Whitt SD, et al. Doxorubicin alters the mitochondrial dynamics machinery and mitophagy in the liver of treated animals. Fundamental & Clinical Pharmacology. 2014; 28(6):633-642. doi: 10.1111/fcp.12073.
23. Leto G, Tumminello FM, Gebbia N, Rausa L. Cathepsin D: A possible biochemical marker for anthracycline cardiomyopathy. Medical science research. 1987; 15(23):1471-2.
24. Hilfiker-Kleiner D, Kaminski K, Podewski E, Bonda T, Schaefer A, Sliwa K. et al. A cathepsin D-cleaved 16 kDa form of prolactin mediates postpartum cardiomyopathy. Cell. 2007; 128(3):589-600. doi: 10.1016/j.cell.2006.12.036.
25. Benes P, Vetvicka V, Fusek M. Cathepsin D--many functions of one aspartic protease. Critical reviews in oncology/hematology. 2008; 68(1):12-28. doi: 10.1016/j.critrevonc.2008.02.008.
26. Masson O, Bach AS, Derocq D, Prébois C, Laurent-Matha V, Pattingre S, Liaudet-Coopman E. Pathophysiological functions of cathepsin D: Targeting its catalytic activity versus its protein binding activity? Biochemistry. 2010; 92(11):1635-43. doi: 10.1016/j.biochi.2010.05.009.
27. Pereira H, Oliveira CS, Castro L, Preto A, Chaves SR, Côrte-Real M. Yeast as a tool to explore cathepsin D function. Microbial Cell. 2015; 2(7):225-34. doi: 10.15698/mic2015.07.212.
28. Ollinger K. Inhibition of cathepsin D prevents free-radical-induced apoptosis in rat cardiomyocytes. Archives of Biochemistry and Biophysics. 2000; (373):346-51.
29. Kagedal K., Johansson U., Ollinger K. The lysosomal protease cathepsin D mediates apoptosis induced by oxidative stress. FASEB Journal. 2001;15:1592-4.
30. Emert-Sedlak L., Shangary S., Rabinovitz A., Miranda M.B., Delach S.M., Johnson D.E. Involvement of cathepsin D in chemotherapy-induced cytochrome c release, caspase activation, and cell death. Molecular Cancer Therapeutics. 2005;4:733-42.DOI: 10.1158/1535-7163.MCT-04-0301.
31. Beaujouin M, Baghdiguian S, Glondu-Lassis M, Berchem G, Liaudet-Coopman E. Overexpression of both catalytically active and -inactive cathepsin D by cancer cells enhances apoptosis-dependent chemo-sensitivity. Oncogene. 2006; 25:1967-73.doi: 10.1038/sj.onc.1209221.
32. Sagulenko V, Muth D, Sagulenko E, Paffhausen T, Schwab M, Westermann F. Cathepsin D protects human neuroblastoma cells from doxorubicin-induced cell death. Carcinogenesis. 2008; 29(10):1869-77. doi: 10.1093/carcin/bgn147.
33. Oliveira CS, Pereira H, Alves S, Castro L, Baltazar F, Chaves SR. et al. Cathepsin D protects colorectal cancer cells from acetate-induced apoptosis through autophagy-independent degradation of damaged mitochondria. Cell Death and Disease. 2015; (6):e1788. doi: 10.1038/cddis.2015.157.
34. Du F, Wang T, Li S, Meng X, Zhang HY, Li DT. et al. Cathepsin D protects renal tubular cells from damage induced by high glucose independent of its enzymatic activity. American Journal of Translational Research. 2017; 9(12):5528-37.
35. Kaija H, Alatalo SL, Halleen JM, Lindqvist Y, Schneider G, Väänänen HK, Vihko P. Phosphatase and oxygen radical-generating activities of mammalian purple acid phosphatase are functionally in-

dependent. Biochemical and Biophysical Research Communications. 2002; 292(1):128-32. doi: 10.1006/bbrc.2002.6615.
36. Mitić N, Valizadeh M, Leung EW, de Jersey J, Hamilton S, Hume DA. et al. Human tartrate-resistant acid phosphatase becomes an effective ATPase upon proteolytic activation. Archives of Biochemistry and Biophysics. 2005; 439(2):154-64. doi: 10.1016/j.abb.2005.05.013.
37. Räisänen SR, Alatalo SL, Ylipahkala H, Halleen JM, Cassady AI, Hume DA, Väänänen HK. Macrophages overexpressing tartrate-resistant acid phosphatase show altered profile of free radical production and enhanced capacity of bacterial killing. Biochemical and Biophysical Research Communications. 2005; 331(1):120-6. DOI: 10.1016/j.bbrc.2005.03.133.
38. Bozzo GG, Raghothama KG, Plaxton WC. Structural and kinetic properties of a novel purple acid phosphatase from phosphate-starved tomato (Lycopersiconesculentum) cell cultures. Biochemical Journal. 2004; 377(2):419-28. doi: 10.1042/BJ20030947.
39. Veljanovski V, Vanderbeld B, Knowles VL, Snedden WA, Plaxton WC. Biochemical and molecular characterization of AtPAP26, a vacuolar purple acid phosphatase up-regulated in phosphate-deprived Arabidopsis suspension cells and seedlings. Plant Physiology. 2006; 142(3):1282-93. doi: 10.1104/pp.106.087171.
40. Sharma U, Pal D, Prasad R. Alkaline Phosphatase: An Overview. Indian Journal of Clinical Biochemistry. 2014; 29(3):269-78. doi:10.1007/s12291-013-0408-y.
41. Haarhaus M, Brandenburg V, Kalantar-Zadeh K, Stenvinkel P, Magnusson P. Alkaline phosphatase: a novel treatment target for cardiovascular disease in CKD. Nature Reviews. Nephrology. 2017; 13(7):429-42. doi: 10.1038/nrneph.2017.60.
42. Conus S, Simon HU. Cathepsins and their involvement in immune responses. Swiss Medical Weekly. 2010; 140:w13042. doi: 10.4414/smw.2010.13042.
43. Shujkova KV, Emelina EI, Gendlin GE, Storozhakov GI. Cardiotox. of modern chemotherapeutic drugs. Atmosfera. Novostikardiologii. 2012; 3:9-19. [in Russian]
44. Vasyuk YuA, Shkolnik EL, Nesvetov VV, Shkolnik LD, Varlan GV, Pilshchikov AV. Disorders of myocardial metabolism on the background of chemotherapeutic treatment, as well as the possibility of their correction. Consiliummedicum. Kardiosomatika. 2013; 4(2):11-5. [in Russian]
45. Shchetinin PP. The role of metabolic cardioprotection in the pharmacotherapy of ischemic heart disease.Aktualnye problem gumanitarnyh I estestvennyhnauk. 2015; (8-2):125-9. [in Russian]
46. Dehina L, Vaillant F, Tabib A, Bui-Xuan B, Chevalier P, Dizerens N, et al. Trimetazidine demonstrated cardioprotective effects through mitochondrial pathway in a model of acute coronary ischemia. Naunyn-Schmiedeberg's archives of pharmacology. 2013; 386(3):205–15.doi: 10.1007/s00210-012-0826-z.
47. Salouege I, Ben Ali R, Ben Saïd D, Elkadri N, Kourda N, Lakhal M, Klouz A. Means of evaluation and protection from doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. Journal of Cancer Research and Therapeutics. 2014; 10(2):274-8. doi: 10.4103/0973-1482.136557.
48. Moustafa AM, Shalahy AA. Impact of trimetazidine on doxorubicin-induced acute cardiotoxicity in mice: a biochemical and electron microscopic study. Egyptian Journal of Histology. 2006; 29(1):125–36.

List of abbreviations

ATA - antitryptic activity
ATP - adenosine triphosphate
BSA- bovine serum albumin
CatD - cathepsin D
ASI - acid-stable inhibitors
AP - acidic phosphatase
ALP - alkaline phosphatase