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Testosterone and myocardial extract as a correction scheme in case of experimental doxorubicin cardiomyopathy

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ABSTRACT

Cardiotoxicity is one of the most common side effects in anthracycline therapy. The clinic manifestation is often masked by myocardial compensatory mechanisms. But its ability soon decreases and the decompensation develops. The appeared changes in myocardium are developing due to anthracycline influence on genetic apparatus, metabolism intermediates and mitochondrial function. While these effects occur, hemodynamic changes are adding. If these processes couldn't be prevented we should medicate them. The most interesting component of treatment for a therapist is influence on energy metabolism. There are many proposed drugs but they are not always effective in doxorubicin cardiomyopathy. In this article we are going to estimate effects of the correction scheme which includes testosterone and experimental myocardium extract. As metabolism markers, we measured activity of total creatine kinase, lactate dehydrogenase, concentration of lactate, pyruvate and macroergic phosphates in tissue homogenates.

1. Introduction

Cardiomyopathy is the disease of myocardium which is connected with structure and functional changes leading to heart failure. Nowadays they are divided into primary and secondary ones. Primary ones affect only the heart (dilated, hypertrophic, restrictive cardiomyopathy and arrhythmogenic dysplasia of right ventricle); secondary ones are the result of damaging the other parts of the body (endocrine, toxic, metabolic, inflammatory ones).

Recently there has been a great breakthrough in understanding of pathophysiology, genetic predilections and molecular mechanisms of these pathologies due to modernization of scientific and clinic methods. This data gives the great opportunity to medicate this disease.

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One of the most common causes of toxic cardiomyopathy for animals is antitumor therapy. Due to repeated infusions these medications are accumulated until reaching the toxic concentration.

The most prevalent antitumor drug in veterinary medicine is doxorubicin and its combinations with vincristine and cyclophosphamide. It has acceptable effectiveness in antitumor therapy, but it has the negative side effect – cardiotoxicity. Doxorubicin cardiomyopathy is hardly cured and prevented. Its pathological influence could be hidden for a long time before its clinical manifestation. Despite medication it often leads to biventricular failure and death. Hence, the correction therapy should be developed.

2. Analysis of recent research

Doxorubicin cardiotoxicity can be developed in two ways: acute and chronic ones. The acute manifestation is connected with myocardial edema and can be reversed (Billingham et al. 1978). Chronic course is rare. It can be developed during several years and it requires a cumulate dose of more than 160 mg\ м2 for dogs (Mauldin, 1992).

Doxorubicin toxic effects can be described in several theories: oxidative stress; sympathoadrenal and cytokine systems' excessive activation; mitochondrial damage and disturbance in oxidative phosphorylation; DNA damage; Ca and Fe- associated toxicity. In metabolic aspect the lipid peroxidation is predominating (Takemura, 2007). Accumulation of free radicals damages lipid layer of cardiomyocytes and its mitochondria (Gille, 1997). As for DNA, doxorubicin breaks regeneration, reparation and intercalation of nucleotids' molecules. Also it leads to intensifying the emergence of free radicals, disturbance in compartment system of the cell and apoptosis (Schimmel, 2004).

It is known that the damaged myocardium needs extra energy to compensate the risen load. The doxorubicin-induced energy starvation decreases the work effectiveness of cardiomyocytes. Soon it is manifesting with biventricular failure (Swain, 2003). There are some researches which show that changes in energy metabolism will help to avoid energy starvation switching it from fatty acids oxidation to glycolysis breakdown (Chiu et al., 2001). In addition, there is some good experience of using hormonal medications (insulin, testosterone) (Tsang, 2008).

The tissue mediatory systems of myocardium have not been properly studied. We have assumed that myocardial extract of a healthy animal contains some of these mediators which will help myocardium to reorganize its energy metabolism for normal contractile function. In addition, we added testosterone due to its influence on gene expression of some ionic pumps and myosin's heavy chains synthesis (Golden, 2003).

The aim of this study is to estimate effectiveness of suggested correction scheme on some metabolic aspects of myocardial metabolism.

3. Statement of research objectives

- determine parameters which reflect energy metabolism in myocardium
- study the parameters of energy metabolism in normal rats and ones with doxorubicin-induced cardiomyopathy
- compare parameters changes caused by testosterone and correction scheme therapy.

4. Material and methods.

Animals. Thirty two female adult Wistar rats were taken. Their weight was 150-180 g. The rats were kept in standard vivarium conditions having free access to drinking water and food. After adaptation they were divided into 4 groups (n=8). There is a group of intact rats; a doxorubicin group consists of three subgroups: the control one (only doxorubicin cardiomyopathy induction – 8mg\kg + physiological solution 0,3 ml\animal), the dox-testosterone one (induced cardiomyopathy 8mg\kg + testosterone propionate 16mg\kg treatment), the dox-teststerone-extract one (induced cardiomyopathy 8mg\kg + testosterone propionate 16mg\kg + pig myocardial extract 0,2 ml\animal). The treatment lasted for 35 days.

The myocardium samples were taken at once after euthanasia, then they were rapidly homogenized in cold 0,05 mmol tris-buffer and 2,5% trichloracetic acid solution. Then samples were delivered to the laboratory where they were instantly examined.

Drugs. Doxorubicin – Andriamicin (Pfizer), Testosterone propionate 5% (Pharmak).

Determination. Lactate dehydrogenase, Creatine kinase, lactic acid and pyruvate were estimated by commercial kits (Olvex Diagnosticum). Total concentration of macroergic phosphates were estimated by the method of determination of phosphate concentration before and after extracted samples were boiled.

5. Results and discussion

Experimental data is shown in table 1. Metabolites and myocardial enzymes activity level in the intact group showed the intensity and energy metabolism pathway in normal conditions and without external disturbance. However, the specific changes were seen in the three experimental groups.

Table 1. Some aspects of myocardial energy metabolism during the experiment.

Group	Creatine kinase, U\mg wet weight	Lactate mkmol\g wet weight	Pyruvate, mkmol\g wet weight	Lactate ehydrogenase, U\g т wet weight	Macroergic phosphates, mkmol\g wet weight	NAD\NADH ratio
Intact	8,67±0,53	22,32±4,08	0,27±0,06	445,5±14,8	4,77±0,77	131,7±31,1
Control-doxorubicin	2,56±0,63	9,46±0,96	0,6±0,18	269,96±55,5	2,56±0,63	609,2±182,3
Doxorubicin-testosterone (DT)	9,26±0,48	10,03±1,94	0,9±0,23	368,21±46,83	5,39±1,48	1688,18±756,7
Doxorubicin-testosterone –extract (DTE)	16,5±1,76	15,03±1,67	1,45±+0,17	412,13±37,96	7,28±1,38	905,8±100

The doxorubicin activity on oxidative phosphorylation in myocardium was indicated by lowering of ATP regeneration; it was shown in the control group where availability of macroergic molecules decreased almost twofold ($P<0.05$). This impaired synthesis in doxorubicin group is based on several mechanisms: inhibition of nucleoids synthesis and DNA intercalation; activation of lipid peroxidation and mitochondria damage; effect on Ca and Fe homeostasis. Hence, the myocardial energy metabolism decreased by inhibition of synthesis of glycolysis protein-clinging enzymes, fatty acids oxidation and tricarboxylic acids cycle as well as biological oxidation. Also in the control group the enzymatic activity of lactate dehydrogenase (by 1,65 times) and creatine kinase (by 3,4 times) decreased; and the decrease twice lower that the level of lactic acid. The high rates of pyruvate could be explained by impaired pyruvate dehydrogenase (PDH) action and slowed pyruvate oxidation. The high NAD\NADH ratio may be caused by decreased NAD- reduction ability of energy metabolism reactions. It leads to oxidative phosphorylation and ATP-regeneration failure. It is known that heart failure there can be switch of the myocardial metabolism from fatty acids oxidation to glycolysis, and its intensity depends on failure severity (Reccia et al., 1998).

Next we estimate influence of testosterone (DT group) and its combination with experimental myocardial extract (DTE group) on a rat's heart with doxorubicin-induced cardiomyopathy. Testosterone infusion

prevents depression of lactate dehydrogenase (LDH) and creatine kinase (CK) activity. Moreover, it intensified the synthesis of macroergic molecules (5,39±1,48 mkmol\g), twofold in comparison with the control group. Thus, the reactivation of oxidative phosphorylation in myocardium appeared and it became almost the equal to the intact rats. At the same time, the NAD\NADH ratio dramatically increased in comparison with the intact and control rats (both $P<0.05$). This situation showed the activation of all reserve myocardium mechanisms in order to provide cardiomyocytes with enough energy and but possibly it can turn into decompensation. It could be caused due to changes in energy metabolism pathways - inhibition of fatty acids oxidation and malate-aspartate shuttle hyper activation.

In DTE group, we should admit more significant intensification of metabolism in comparison with all doxorubicin-treated groups. The concentration of macroergic compounds reaches the highest value in all groups (7,28±1,38 mkmol\g). The effectiveness of oxidative phosphorylation is proved by high activity rates of CK- from 1,8-6,5 folds higher ($P<0.05$ for all groups). The LDH activity almost reached its ratio in the intact group. The concentration of lactate also raised, pyruvateis dramatically increased (1,45±+0,17 mkmol\g). The NAD\NADH ratio decreased by 46,4% in comparison with testosterone treated group, but it is still much higher than in the intact rats.

The pyruvate concentration may show high rates of glycolytic processes, which is possible due to compensation mechanisms. Pyruvate isn't used in oxidative metabolism and it concentrates in cytosol. Similarly, the high NAD\NADH ratio is caused by the increased oxidative phosphorylation activity and high demand in NADH for electron transport chain. It is proved by increased activity of CK and amount of macroergic molecules. However, the ways they are created are still debatable.

6. Conclusions

Nowadays, the data shows that in heart failure the energy is wasting on the cellular level (Saavedra et al., 2002). It leads to impaired contractile function and it is connected with oxygen and substrates' delivery. In the end, it causes the energy starvation. Thus, in untreated doxorubicin-induced cardiomyopathy group we see the decreased effectiveness of all energy-producing systems.

Here we admitted a preventive effect of testosterone and myocardial extract combined administration in the case of doxorubicin-induced cardiomyopathy in rats activating the energy-supplying mechanisms. The measured parameters have showed that this correction scheme has positive influence on pathogenesis and in less severe conditions it could greatly reduce doxorubicin effects.

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