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RESEARCH ARTICLE

SOME HISTOLOGICAL ASPECTS OF DOXORUBICIN CARDIOMYOPATHY AND ITS EXPERIMENTAL TREATMENT

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ABSTRACT

Chemotherapy in oncology patients is always followed by side effects of therapeutic agents. One of the most common effect is cardiotoxicity. Mainly it caused by anthracycline therapy. Doxorubicin (the most common anthracycline) cardiotoxicity usually difficult to estimate due to its pathogenesis. Doxorubicin – induced heart failure can manifest even in several years due to compensatory mechanisms. Clinical detection of this effect is difficult before heart contractile function decrease. In this article, we are going to estimateeffects of correction scheme, which include testosterone and experimental myocardium extract on doxorubicin-induced cardiotoxicity in Wistar rats model. In experiment, we used 4 groups of female rats. We histologically studied normal conditions myocardium in intact rats and then compared it with experimental animal's tissue.

INTRODUCTION

It is known, doxorubicin has dose-depended cardiotoxicity but it is one of the most effective component of chemotherapy in veterinary oncology. The side effect usually leads to irreversible dilated cardiomyopathy, associated with myocardial damage and metabolic disturbance. However, this process usually stay unmarked for a long time, until it manifested with biventricular heart failure and death[1,2,3]. Unfortunately, noninvasive methods can't show the real conditions of myocardium, so the "gold standard" method of early stage doxorubicin-induced cardiomyopathy is sub endocardial biopsy. It shows proper changes in cardiomyocytes: cellular vacuolization and lysis, interstitial edema, fibrosis, leucocytes infiltration[1,5,6].

In addition, oxidative stress and DNA damage, associated with doxorubicin action, leads to cellular energy supplement malfunction. Non-effective energy metabolism is connected with pathological dystrophy of myocardium, systolic\diastolic dysfunction, RAAS activation and hemodynamic malfunction[2,4,5].

In this kind of cardiomyopathy traditional heart failure treatment often is not effective. There are several drugs used as preventive, but there are a few evidences of its positive influence in animals and such information usually extrapolated from human medicine[2,3].

In our work, we decided to find out effects of testosterone and experimental myocardial extract on chronical doxorubicin-induced cardiomyopathy in Wistar rats model.

MATERIALS AND METHODS

Location and duration of study

This study was conducted in the vivarium of the Department of Therapy, St. Petersburg State Academy of Veterinary medicine, St. Petersburg, Russia. The experiment lasted for 2 month including animal acclimatization, inducing doxorubicin cardiomyopathy and experimental treatment.

Experimental design and grouping

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

Procedures for animal sacrifice and tissue harvest

The animals were euthanized on 35-37 day of treatment trial. Heart tissue was harvested and fixed in 10% formol saline for histological and histochemical analysis.

Histological methods

Sections from the ventricular free wall were prepared by standard methodology and stained with hematoxylin and eosin, Mallory, Van Gieson, metal hematoxylinewith picrofuchsin and with McManus periodic acid –Shiff stain.

RESULTS AND DISCUSSION

The cardiomyocytes of intact rats stained with hemotoxylin had normal cell morphology, eccentric nucleus location, cytoplasm striation. Examples stained by Van Gieson, Mallory showed non-significant amounts of fibers, located mostly near vessels (Fig.1). Cardiomyocytes stained with metal hematoxylin had many myofilaments in cytoplasm and very low spaces of non-specific cytoplasm near nucleus. Shiff-reaction showed non-significant amounts of glycogen in cytoplasm and brightly stained neutral polysaccharides of interstitial tissue (Fig.2).

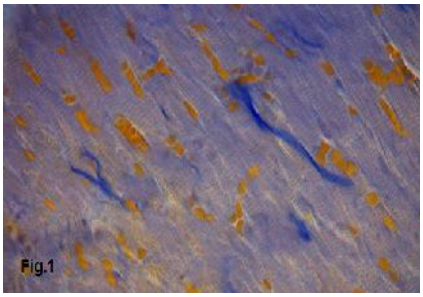

Figure 1 Heart; intact rat. Solitary collagen fibers (blue). Mallory stain, x400.

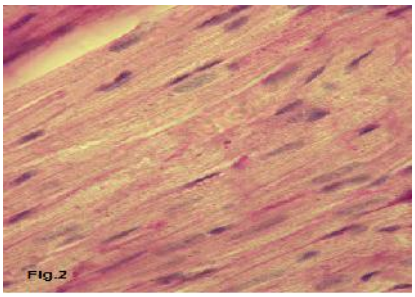

Figure 2 Heart; intact rat. Solitary glycogen grains (purple dots). Shiff-reaction, x400

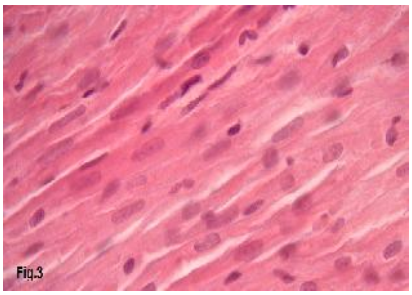

Figure 3 Heart; doxorubicin-control group rat. Thinned cardiomyocytes, increased interstitial space. hematoxylin and eosin, x400

In doxorubicin control group, we found loss of specific tissue pattern and striation, thinned cardiomyocytes (its diameter was almost equal nucleus), myocardial fibers lost their connection showed with enlarged interstitial spaces (Fig 3), and there were lots of vessel filled with erythrocytes. In examples stained by Van Gieson and Mallory there were many collagen fibers non-orderly located in interstitial spaces (Fig.4). Cardiomycytes stained with metal hematoxiylin and picrofuchsin showed lack of contractile material in their cytoplasm, significant spaces were free of myofibrils (Fig.5). Schiff-reaction was negative in cytoplasm, but significant in interstitial spaces; also, we found massive leucocytes migration in one of the probes (Fig.6).

In doxorubicin-testosterone group, we defined mostly similar to intact rats tissue pattern, but there were sites with high amount of collagen fibers and sporadic cell autolysis. Shiff-reaction showed low concentrations of glycogen in myocytes cytoplasm, as it was in intact group.

In last doxorubicin-testosterone-extract group we found almost normal-sized cardiomyocytes, morphologically similar to intact probes. There were low amount of collagen fibers, connected with microcirculatory vessels (Fig.7). Metal hematoxylin – picrofuchsin stained probes showed non-altered myofibrils location and thin perinuclear spaces with unspecific cytoplasm. Shiff-reaction showed high amount of glycogen grains in cytosol and normal distribution of neutral polysaccharides connected with interstitial tissue (Fig.8).

In addition, we measured cardiomyocytes diameter, results are shown in Table 1.

Table 1 Cardiomyocytes diameter

Group	Intact	Control-doxorubicin	Doxorubicin-testosterone	Doxorubicin-testosterone-extract
M±m	14,31±0,45	5,83±0,444	10,97±0,6125	13,53±0,446

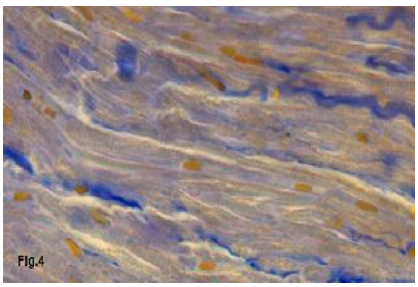

Figure 4 Heart; doxorubicin-control group rat. High amounts of separate collagen fibers (blue). Mallory stain, x400

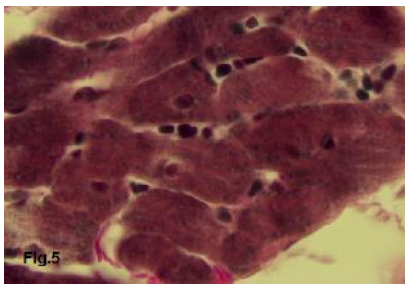

Figure 5 Heart; doxorubicin-control group rat. Myofilaments are located only in submembrane spaces of the cell, increased non-specific cytoplasm around nucleus. Metal hematoxiylin and picrofuchsin stain, x400

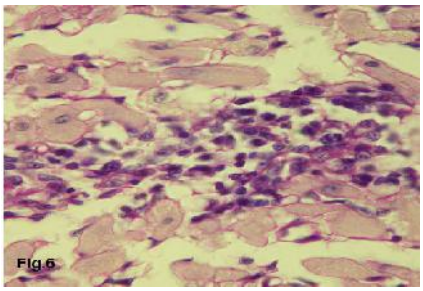

Figure 6 Heart; doxorubicin-control group rat. Enlarged interstitial spaces, fibroblastic proliferation, leucocytes infiltration and migration. Glycogen grains are not detected. Shiff-reaction, x400

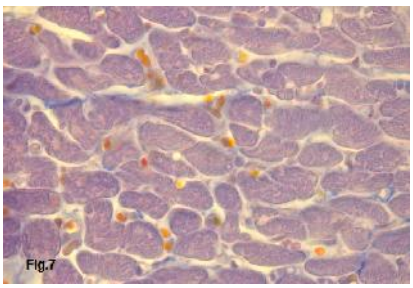

Figure 7 Heart, DTE group rat. Solitary collagen fibers (blue). Mallory stain, x400

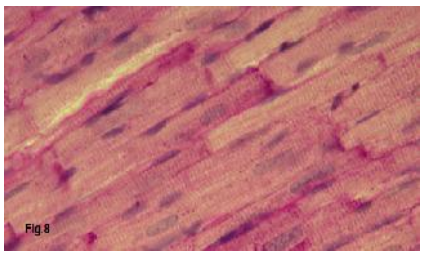

Figure 8 Heart, DTE group rat. High amounts of glycogen (purple dots). Shiff-reaction, x400

Measurements show that doxorubicin-induced cardiomyopathy is combined with cardiomyocyte dystrophy and atrophy. In groups treated with testosterone and its combination with myocardial extract we found a partial restoration of cell mass.

CONCLUSION

Histomorphological and histochemical changes show mechanisms involved in doxorubicin- induced cardiomyopathy.

Damage of cardiomyocytes is notable in all experimental groups, but in presence of correction components, we defined positive dynamic in cells rehabilitation. We noted reduction of fibrotic processes, recovery of myofilaments and glycogen grains in cytoplasm.

References

1. Billingham ME, Mason JW, Bristow MR, Daniels JR: Anthracycline cardiomyopathy monitored by morphologic changes. *Cancer Treat Rep* 1978; 62: 865–872.
2. Broder H, Gottlieb RA, Lepor NE: Chemotherapy and cardiotoxicity. *Rev Cardiovasc Med* 2008; 9: 75–83
3. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Swain SM, Whaley FS, Ewer MS Cancer. 2003 Jun 1; 97(11):2869-79
4. Mauldin GE, Fox PR, Patnaik AK, Bond BR, Mooney SC, Matus RE: Doxorubicin-induced cardiotoxicosis: clinical features in 32 dogs. *J Vet Intern Med* 6:82–88, 1992.
5. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L: Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* 2004; 56: 185–229.
6. Schaper J, Meiser E, Stammler G: Ultrastructural morphometric analysis of myocardium from dogs, rats, hamsters, mice and from human hearts. *Circ Res*