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Cumulative cardiotoxic effect of bleomycin in experiment

A. Gozhenko[1], O. Bestanchuk[1], O. Kaschenko[2], T. Narbutova[2]

[1]Ukrainian Research Institute of Transport Medicine of the Ministry of Health of Ukraine, Odessa, Ukraine
[2]Odessa National Medical University, Odessa, Ukraine

Abstract

The aim of the study was to evaluate the cumulative toxic effect of bleomycin under experimental conditions

Material and Methods. The study was conducted in the Research Institute of Transport Medicine during 2016-2021. The experimental model of the cardiotoxic effect of the bleomycin was performed using the medication "Bleocin" manufactured by Nippon Kayaku Co., Ltd. (Japan). According to the task, the study was performed on 10 mature rats of both sexes of the Wistar line with a body weight of 237 ± 20 g.

Rats were housed in standard vivarium conditions of Odessa National Medical University. Animals were divided into 2 groups: experimental group (n = 5) and control (n = 5). Bleomycin animals of the experimental group were obtained intraperitoneally at a dose of 0.5 IU / kg on 1st and 8th days. Withdrawal of animals from the experiment was performed on the 5th, 14th and 28th day of the experiment, followed by morphological and morphometric examination.

The material after weighing and morphometry was fixed with a neutral 10% formalin solution and poured into paraffin. Histological sections were stained with hematoxylin-eosin, MSB, Van Gizon. Performed light microscopy.

After two weeks there was a decrease in myocardial weight by 7-10% from baseline, there were pronounced dystrophic changes in the myocardium. Repeated administration of bleomycin has a cumulative cardiotoxic effect leading to irreversible changes in the myocardium and endothelial dysfunction clinically manifested by heart attack, vascular pathology at significant cumulative doses of bleomycin. Morphofunctional changes of the right ventricle are prominent and considered to be pathognomic for bleomycin toxicity.

Conclusions: 1. Bleomycin has a cumulative toxic effect on the myocardium of mammals

2. The severity of the cardiotoxic effect of bleomycin is proportional to the tissue concentration of the drug, which is proportional to the duration of exposure

Keywords: **bleomycin; cardiotoxic effect; cancer patients**

Modern advances in medicine have increased the survival of cancer patients, but at the same time increased morbidity and mortality caused by side effects of treatment [1-3], with their most common manifestations are cardiovascular disease [1, 2]. Treatment of many malignancies requires the use of chemotherapeutic agents and radiation therapy. These methods significantly improve the survival of cancer, but often cause cardiotoxic effects in the early or long term after treatment, manifested by arrhythmias, valvular heart disease, pericarditis, myocardial necrosis, heart failure and others. [1].

To define this complication in the treatment of cancer patients, the term "cardiotoxicity" is proposed, which includes various adverse events that lead to dysfunction of the cardiovascular system on the background of drug and radiation therapy [4]. Cardiotoxicity is most often seen with the use of multicomponent treatment regimens, a combination of chemotherapy and radiation therapy, especially with the use of high-dose chemotherapy. Cardiotoxicity is the cause of the direct effect of treatment on cardiac function and structure or may be the cause of the accelerated development of cardiovascular disease, especially in the presence of known risk factors. To date, there are a large number of studies and published a lot of data on some specific manifestations of pharmacological and / or radiation-induced cardiotoxicity.

There are three forms of cardiotoxic effects of antitumor drugs [4, 5]: 1. Acute cardiotoxicity is rare and usually not accompanied by clinical signs. It develops at the time of chemotherapy or immediately after it (a few hours). Manifested by arrhythmias, nonspecific changes in the terminal part of the ventricular complex, asymptomatic decrease in ejection fraction, transient heart failure, acute myocarditis or myopericarditis, very rarely - sudden

myocardial infarction or sudden death. The changes are reversible and regress within 1 month. Rarely, acute cardiotoxicity is manifested by life-threatening complications and precedes the development of heart failure.

2. Early (subacute) cardiotoxicity. Most often, manifestations of heart failure develop 3 months after treatment. The main manifestations are similar to those of acute cardiotoxicity [5].

3. Late (chronic) cardiotoxicity can develop in the period from one to ten years and more after chemotherapy and / or radiation therapy [5, 6]. The main manifestations of cumulative cardiotoxicity are myocardial contractility disorders, development of degenerative cardiomyopathy with subsequent decrease in left ventricular contractile function (LV), possible development of dilated cardiomyopathy (DCMP) [6]

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Assessment of the cardiotoxic effect of bleomycin was performed by observation of experimental animals, standard morphological examination (evaluation of macrodrugs, morphological examination - light microscopy of histological sections stained with hematoxylin-eosin, MSB, Van Gizon) [7].

During the experimental stage, the research was guided by the provisions of the "European Convention for the protection of vertebrate animals used for experimental and

other purposes" (Strasbourg, 1985), as well as the Law of Ukraine № 3446-IV of 21.02.2006, Kyiv "On protection animals from cruel treatment "[8].

Statistical processing of the obtained data was performed by methods of variance, correlation and regression analysis using Statistica 14.0 software (TIBCO, USA) [9]. The null hypothesis was accepted at $p>0,05$.

Results

On the 14th day of the experiment (after two injections of bleomycin), the heart weight was 0.94 g ± 0.12 grams. Macroscopically on the back - the right surface at the base of the heart appeared small dense foci of yellow-gray color. Microscopically, the ICR and large vessels showed the same changes as after the first injection, however, draws attention to the more pronounced edema of all components of the walls and perivascular space, marked expansion of the perinuclear space with the formation of cisterns of smooth muscle cells and their margination of chromatin nuclei.

On the 14th and 28th days, the degree and prevalence of the described changes increases, necrosis of cardiomyocytes (CMC), sclerosis of the subendocardial areas of the myocardium develops.

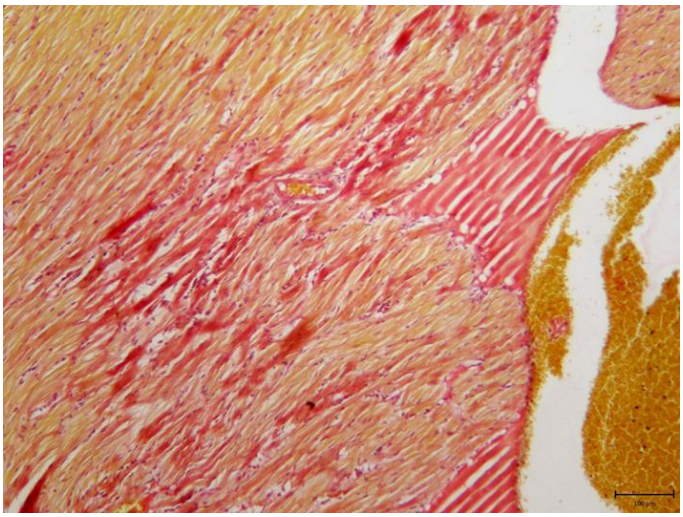

Fig. 1. A fragment of the rat myocardium after double administration of bleomycin. Large focus of contracture and postcontractural damage to cardiomyocytes of the left ventricular wall (left). Loss of fibrin on the surface of the endocardium on the right. Erythrocytes fill the lumen of the ventricle. Stained by MSB X100.

All the above changes in the myocardium (contractural degeneration, lateral myocytolysis, endocardial damage with the formation of foci of necrosis, fibrosis, most in the endocarditis of the right ventricle, basal interventricular septum), microvascular bed, heart vessels (venules, veins, arteries) toxic effect of the drug Bleomycin not only on the vascular

bed, which is manifested by the destruction of vascular walls, adversely affecting the myocardium, but also on the endocardium, more right heart and interventricular septum (Fig. 1, 2).

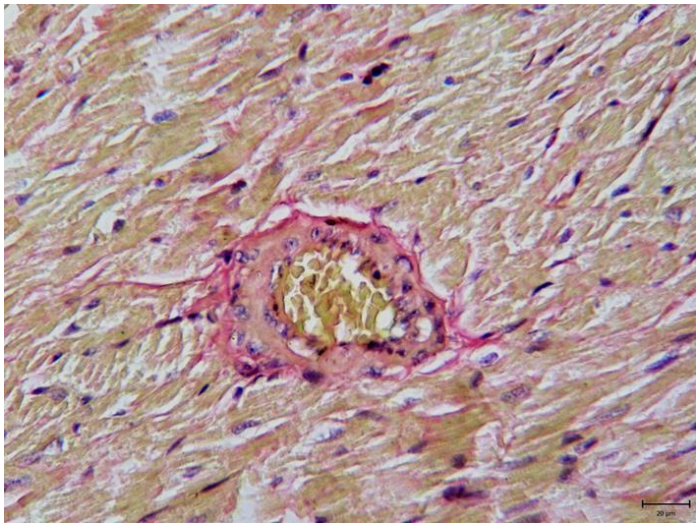

Fig. 2. A fragment of the rat myocardium after double administration of bleomycin. In the wall of the small artery the phenomenon of edema, the nucleus of smooth cells with deep or crescent-shaped chromatin. Dissociation and fragmentation of myofibrils are observed in cardiomyocytes. Stained by Van Gizon, X400.

At double administration of bleomycin thrombosis of small arteries, a sludge of erythrocytes met more often. At the level of light microscopy, especially in areas of physiological disorientation of the cardiomyocyte chain, mainly in the posterior basal parts of the heart (posterior interventricular sulcus), microfoci of their swelling with destruction of the sarcolemma and homogenization of myofibrils, accompanied by leukocytosis. (Fig. 3).

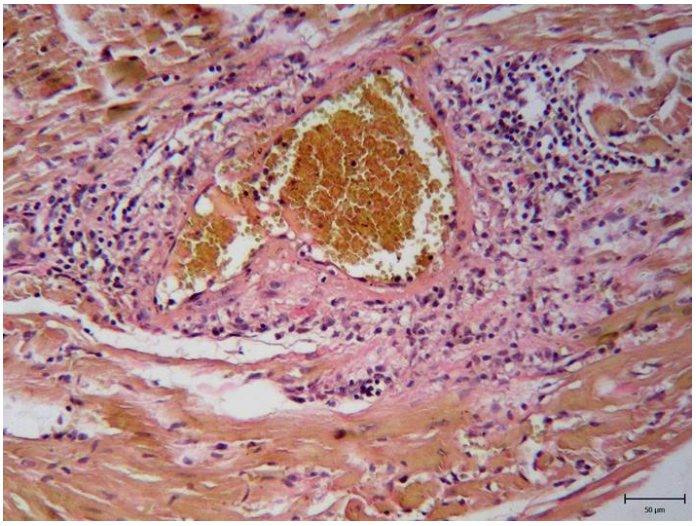

Fig. 3. Fragment of the rat myocardium after double administration of bleomycin. Necrotizing arteritis with massive periarterial leukocyte infiltration. Stained by Van Gizon, X400.

Similar to the described changes were found in the wall of the right ventricle (PN) and the interventricular septum (IBS), on the border of the wall of the right ventricle with the interventricular septum. Thus, a single injection of bleomycin into the body of rats causes severe morphological changes in the structures of the heart. Changes, first of all, develop in vessels of heart (veins, arteries, vessels of MCR) with formation of sludges, stasis, microthrombi. This demonstrates the presence of bleomycin, in addition to cardio- and pulmotoxic also endotheliotoxic effects. With increasing frequency of administration of the drug increases the severity and prevalence of the described changes, necrosis of the CMC (Fig. 4). Such pathological changes may indicate a pronounced cardiotoxic effect of the drug.

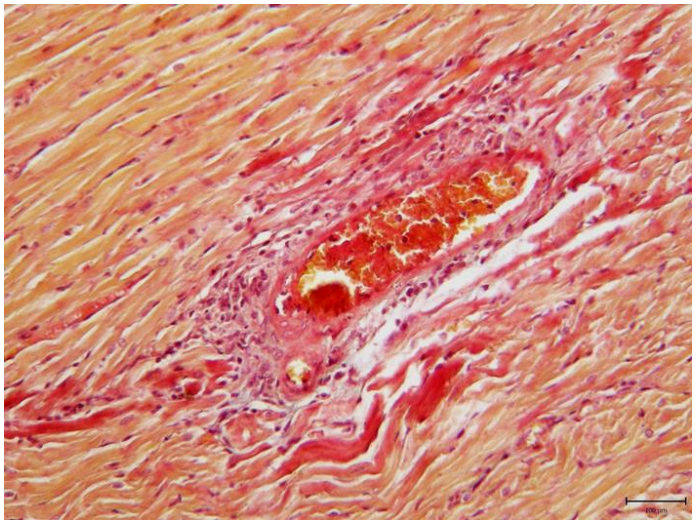

Fig. 4. Necrobiosis and necrosis of the elements of the artery wall, accompanied by a significant leukocyte response and contractural degeneration of the CMC. Staining with hematoxylin and eosin X200.

Summing up the results of the pathomorphological study, it should be noted that the model used was adequate to reflect the cardiotoxic effects of therapeutic / subtoxic doses of bleomycin. The predominant lesion of the pancreas and reproductive system can be considered as evidence of the need for use in patients receiving bleomycin as part of complex chemiotherapy or as monotherapy, fundamentally different approaches to clinical monitoring. It is possible to predict that in such patients for a long time there will be no recommendation of systolic function of left ventricle. More likely is the presence of diastolic dysfunction, or minimal changes in myocardial perfusion, which can be detected using high-resolution methods.

After the second administration of the drug there were contractural degenerations of cardiomyocytes and marginal lysis of individual cardiomyocytes, damage to the microcirculatory tract of all heart vessels with increasing necrosis of cardiomyocytes both due

to ischemia and due to apoptosis. Thus, there was both direct and indirect cardiotoxic effect, and its severity increased in proportion to the duration of exposure.

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