Anthracycline-Induced Cardiomyopathy is Manifested in Decreased Protein Synthesis, Impaired Intracellular Regeneration, and Non-Necrotic Death of Cardiomyocytes

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The cytostatic anthracycline antibiotic daunomycin hydrochloride led to the development of plastic myocardial insufficiency characterized by impaired intracellular regeneration of cardiomyocytes and progressive involution of cytoplasmic structures. Morphological signs of plastic myocardial insufficiency included fragmentation, annulation, or collapse of nucleoli in cardiomyocyte nuclei, lysis of myofilaments, sarcomeres, or myofibrils, focal degradation of the cytoplasm, and intensive autophagy. Fatal anthracycline-induced cardiac insufficiency was associated with massive cardiomyocyte loss due to their non-necrotic death and elimination. Our findings indicate that anthracycline-induced cardiomyopathy in laboratory animals is a convenient model for studying general mechanisms underlying the pathogenesis of regenerative and plastic cardiac insufficiency in humans.

Key Words: *regenerative and plastic insufficiency; anthracycline-induced cardiomyopathy; cardiomyocytes; apoptosis; biological modeling*

Cardiomyopathies (CMP) are myocardial diseases of unknown etiology leading to the development of acute or chronic cardiac insufficiency [4]. High incidence and clinical importance of CMP determine the necessity of studying their etiopathogenesis, main stages of morphogenesis, and molecular and cellular mechanisms underlying impairment of cardiomyocyte (CM) functions. The search for new adequate models and objects is of considerable importance in this respect [6].

Previous studies showed that disturbances in regeneration of structural and, particularly, contractile proteins associated with suppressed transcription or translation underlie the pathogenesis of CMP [2]. Thus, CMP are accompanied by impaired intracellular regeneration and development of regenerative and plastic insufficiency of parenchymal cell. Some substances and compounds, including anthracycline antibiotics used as antitumor drugs, directly suppress matrix properties of DNA and inhibit key enzymes of protein synthesis. Anthracycline antibiotics doxorubicin (adriamycin) and daunomycin (rubomycin or rubomycin hydrochloride, RH) are most widely used in medical practice. Apart from general cytotoxicity, these compounds possess selective cardiotoxicity and can cause severe or lethal cardiac insufficiency. These peculiarities limit their use in clinical practice. Studies of anthracycline-induced CMP would allow us to understand general mechanisms of structural reorganization accompanying regenerative and plastic insufficiency of the myocardium, elaborate diagnostic pathomorphological criteria, and prevent progressive involution and apoptosis in CM.

Here we studied peculiarities of anthracycline-induced CMP during various regimens of RH administration.

MATERIALS AND METHODS

Anthracycline-induced CMP was induced in 100 animals. Series I was performed on 40 male Wistar rats

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weighing 160-220 g. We determined the maximum toxic dose of RH that induced cardiac insufficiency and circulatory disturbances in 100% animals. Intraperitoneal injection of RH in single doses of 10 and 15 mg/kg did not cause animal death. Two rats died 12 days after administration of 20 mg/kg RH. RH in a dose of 30 mg/kg induced death of 100% animals within 4-7 days postinjection. RH in a cardiotoxic dose was injected to 60 rats. The animals were decapitated 1-24 h and 1-5 days after RH administration. Before decapitation (4-5 days after treatment) all rats were in grave (terminal) conditions.

In series II, RH was injected repeatedly. Group 1 rats received 3 intraperitoneal injections of 10 mg/kg daunomycin hydrochloride with 7-day intervals and decapitated 5 days after the last injection. In group 2 the rats first received 10 mg/kg RH (intraperitoneally) and then weekly 5 mg/kg RH for 5 weeks. The animals were decapitated 5 days after the last injection. Group 3 rats were weekly administered with 5 mg/kg RH for 6 weeks and decapitated 3-6 days after the last injection.

Control animals received intraperitoneal injections of physiological saline in an equivalent volume.

The hearts were examined under light and polarization microscopes. For light microscopy, the samples were fixed in 10% neutral formalin. Deparaffinized sections were stained with hematoxylin and eosin, colloidal iron-PAS reaction, and by the method of van Gieson and then examined under a Docuval light microscope. For electron microscopy, myocardial samples from survived rats were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, and then processed routinely [8]. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined under Tesla BS500, JEM 100B, and JEM 1010 electron microscopes (acceleration voltage 80 kV).

The volume density of muscle fibers, vessels, and connective tissue elements was measured stereologically. The absolute weights of these structural elements were estimated. The absolute number of CM and their nuclei in ventricular myocardium was evaluated after alkaline dissociation of fixed tissues [8].

RESULTS

During CMP caused by a single injection of RH in a cardiotoxic dose experimental animals progressively lost body weight (more than 20% of the initial body weight 4-5 days postinjection). Body weight loss was due to the cytotoxic effect of this antibiotic on the epithelium of the gastrointestinal tract [11]. However, fractional administration of RH caused more than 30% body weight loss against the background of less pronounced dyspepsia [11,15], which probably attested to cardiac cachexia accompanying anthracycline-induced CMP.

Subcutaneous edema, transudation into body cavities, venous plethora of parenchymal organs, and progressive dyspnea and acrocyanosis were found in experimental animals. Intraperitoneal injection of RH led to the formation of focal necroses in omental fat, aseptic inflammation, and adhesive peritonitis without intestinal obstruction. These changes not accompanied by exudation into the peritoneal cavity indicated that the drug was injected properly.

Fractional administration of 15-20 mg/kg RH produced toxic effects: decreased motor activity, anorexia, and dyspepsia. RH in a dose of 25 mg/kg caused pronounced dyspepsia, adynamia, and dyspnea. Autopsy revealed hydropericarditis, ascites, and enlargement and plethora of the liver. Immediately before decapitation, heart rate in most animals decreased to 64-68 bpm. In 87% rats the left ventricle was enlarged, and the myocardium was lighter colored than in the control.

We evaluated the contribution of alteration and plastic changes in CM into impairment of contractile functions of the myocardium. In our experiments all models of anthracycline-induced CMP were not associated with the development of necrotic changes in CM at all stages of cardiac insufficiency, including the terminal period. We observed no intracellular myocytolysis characterized by the loss of contractile properties of CM due to myofibril lysis [1,9].

The selective cardiotoxicity of RH was manifested in impaired or suppressed protein synthesis in CM, which contributed to the development of plastic insufficiency and diffuse collagenation of the stroma [8]. The initial stages of plastic myocardial insufficiency were characterized by fragmentation, annulation, or collapse of nucleoli in CM nuclei, lysis of myofilaments, total lysis of sarcomeres and myofibrils, focal degradation of the cytoplasm and mitochondria, and intensive autophagy. Involution of cytoplasmic structures was accompanied by the release of residual bodies, myelin-like structures, and autophagosomes from the cytoplasm of CM through the sarcolemma and intercalated discs into the intercellular space followed by their absorption by macrophages. Total swelling of mitochondria related to destabilization of their membranes was the late ultrastructural sign of plastic myocardial insufficiency.

Despite the existence of various ultrastructural signs of plastic myocardial insufficiency (Fig. 1), their contribution to the impairment of contractile function should be evaluated stereologically by changes in the total weight of working myocardium and total number of CM (Fig. 2). Single injection of RH in a cardiotoxic dose caused apoptosis and non-necrotic death of 36% CM without structural changes in the myocardium (Table 1). After fractional administration of RH, the

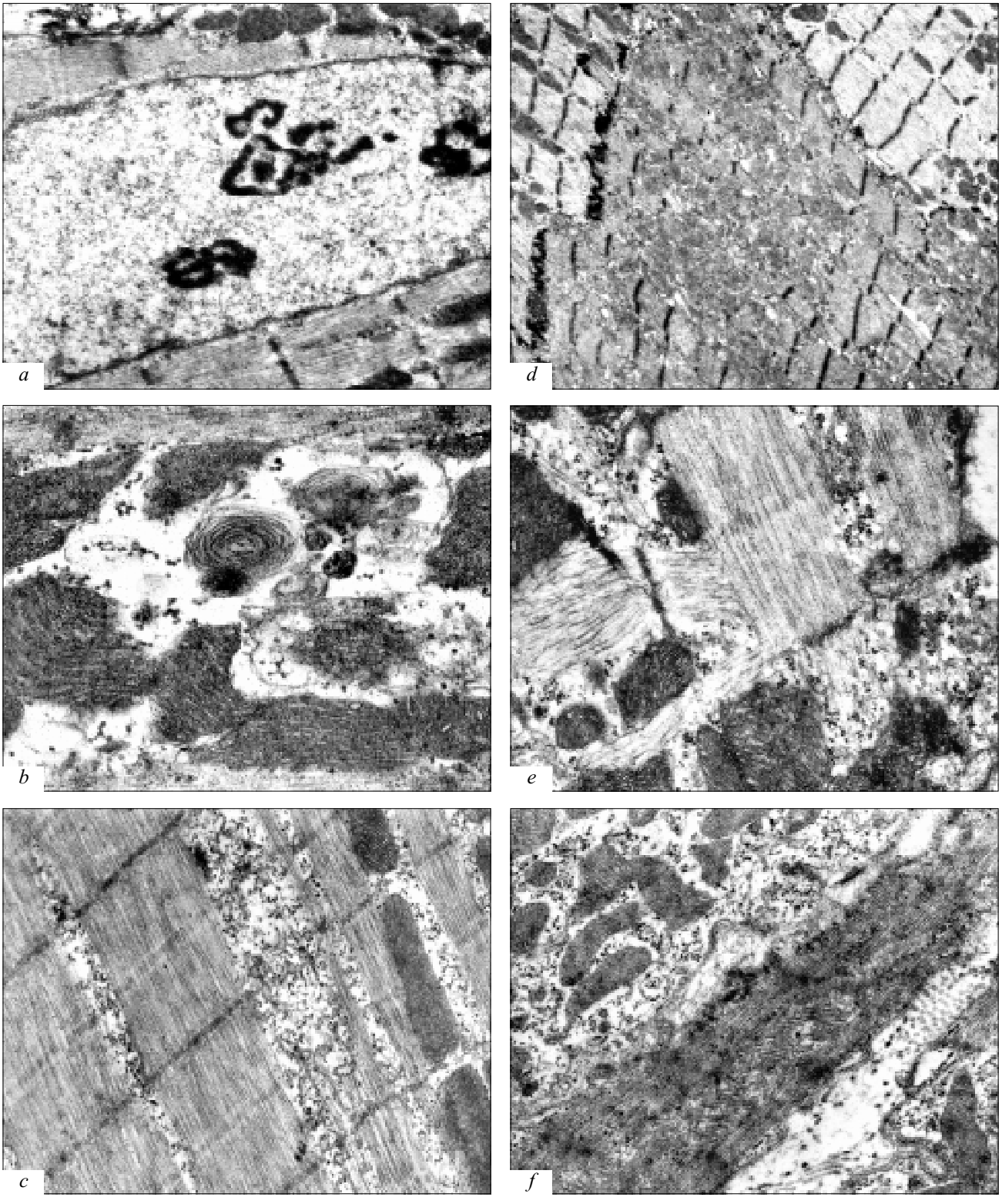

Fig. 1. Ultrastructural changes in rat cardiomyocytes during anthracycline-induced cardiomyopathy: fragmentation of nucleolar nucleolemma in the nucleus (*a*, ×16,100), numerous autophagolysosomes containing granular glycogen and myelin figures (*b*, ×51,500), myofilament lysis and thinning of myofibrils (*c*, ×25,800), destruction and swelling of mitochondria (*d*, ×9700), formation of new myofilaments on ribosomes and disorientation of newly formed myofibrils (*e*, ×41,000), atrophy of muscle cell with dense position of preserved organelles (*f*, ×23,900).

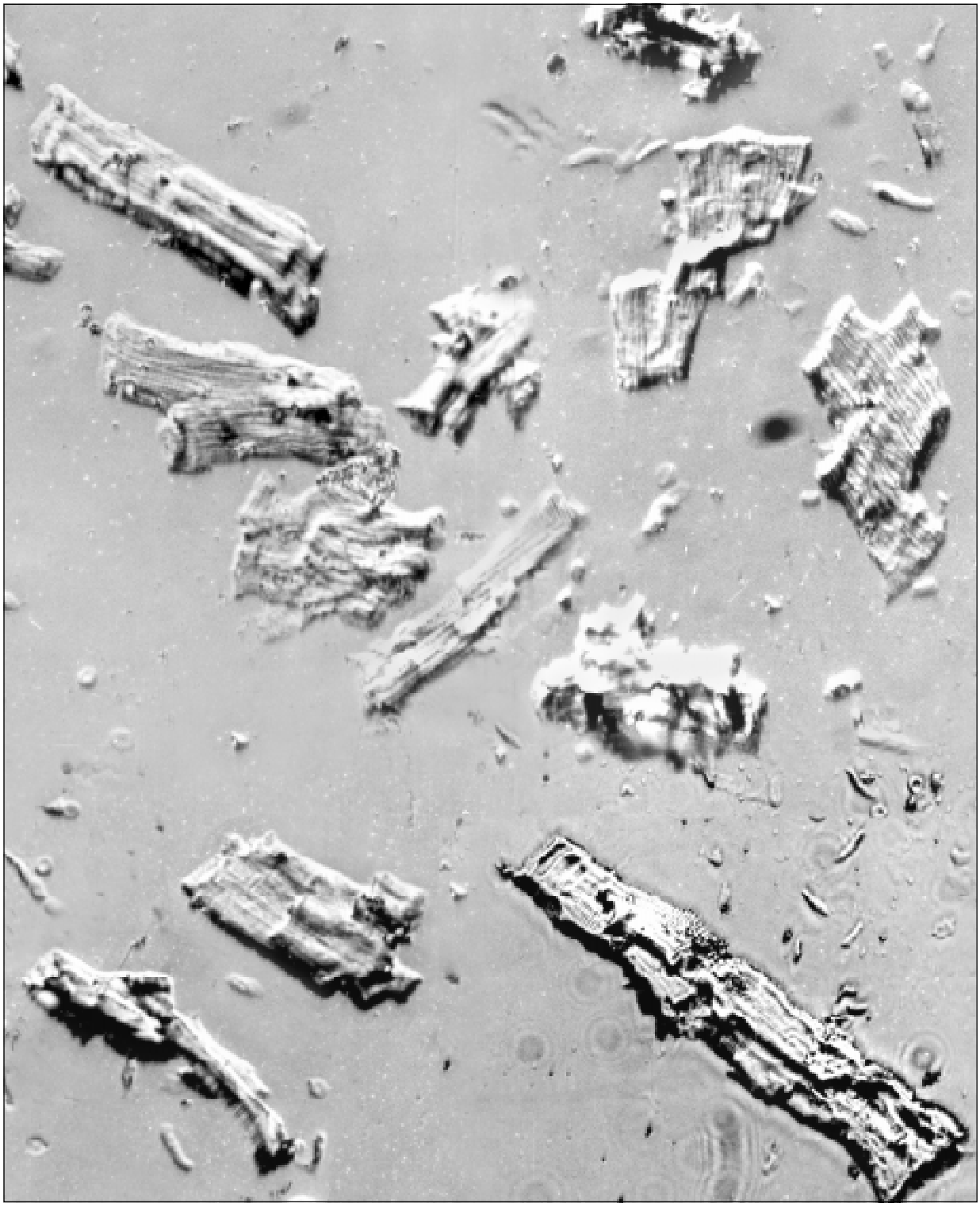

Fig. 2. Cardiomyocyte suspension after alkaline dissociation of the myocardium. Cardiomyocytes with various numbers of nuclei. Orsein and light green staining. Polarized light, ×1250.

number of CM decreased to a lesser extent (by 18% compared to the control, Table 1). The volume density of CM decreased in all experimental groups. The content of interstitial fluid and connective tissue elements increased, while the volume density of vessels and capillaries remained unchanged (Table 2). Irreversible contractile cardiac insufficiency developed after the loss of not less than one third of the total weight of CM.

It should be emphasized that plastic insufficiency of CM is reversible in case of early recovery of pro-

TABLE 1. Quantitative Analysis of Ventricular CM in Wistar Rats after Single and Fractional Administration of RH in Cardiotoxic Dose (*M*±*m*)

Parameter	Control	RH administration	
		single	fractional
Weight of cardiac ventricles, mg	631.5±25.4	526.9±20.1**	533.1±21.2**
Concentration of CM nuclei, 10^3/mg tissue	29.32±0.74	23.15±0.84*	28.68±0.22
Number of CM nuclei, 10^6	18.87±0.85	12.02±0.39*	15.46±1.03*
Number of CM, 10^6	9.64±0.43	6.16±0.19*	7.89±0.52**

Note. Here and in Table 2: *p<0.001, **p<0.01, and ***p<0.05 compared to the control.

TABLE 2. Stereological Analysis of the Myocardium in Wistar Rats after Single and Fractional Administration of RH in Cardiotoxic Dose (*M*±*m*)

Parameter	Control	RH administration	
		single	fractional
Body weight, g	211.0±5.0	164.5±7.3**	146.8±7.9*
Weight of cardiac ventricles, mg	598.0±32.2	526.0±29.9**	524.7±21.5**
Volume density			
muscle fibers	0.672±0.007	0.471±0.013*	0.520±0.014*
vessels	0.161±0.005	0.183±0.012	0.140±0.013
connective tissue	0.106±0.005	0.171±0.007*	0.170±0.007*
interstitial fluid	0.061±0.005	0.175±0.007**	0.170±0.010*
Absolute total weight, mg			
muscle fibers	402.6±24.8	248.0±17.5*	270.7±6.3*
vessels	95.9±4.3	95.6±6.3	74.5±8.5*
connective tissue	62.9±2.8	90.0±7.1***	89.2±5.3*
interstitial fluid	36.6±2.2	92.4±6.7*	90.3±7.9*
Connective tissue/muscle nuclei ratio	2.04±0.05	3.06±0.02*	2.91±0.03**

tein synthesis. Morphological signs of intracellular regeneration, including formation of new myofilaments and normalization of the structure and number of myofibrils, are revealed after the appearance of ribosomes in CM cytoplasm. Disorientation of newly formed myofibrils observed in individual CM at the early stage of intracellular regeneration [3] is not specific for anthracycline-induced damages.

The cytostatic effects of anthracycline antibiotics on various cells were extensively studied. However, there is no agreement regarding the type of anthracycline-induced damages to CM. It was reported that these drugs produce a cytostatic effect by intercalating between base pairs in the double helix of DNA [16]. The cytotoxic effect of anthracycline antibiotics is realized through generation of free radicals and direct inhibition of genes encoding structural proteins and key enzymes involved in the synthesis of macroergic compounds [12,13]. These changes attenuate anabolic processes, which leads to destruction, lysis and reduction of main cytoplasmic organelles in CM, progressive atrophy of CM, and apoptosis [5,7]. Therefore, primary culture of CM treated with doxorubicin for several hours is now more often used to study the molecular and cellular mechanisms of apoptosis in CM [10,14,17]. This approach is more convenient for evaluating intracellular reactions, but does not allow us to study all pathogenetic stages of anthracycline-induced CMP.

These results indicate that RH-induced CMP is an appropriate model for studying structural reorganization during regenerative and plastic insufficiency of the myocardium and evaluating the mechanisms underlying the cardiotoxic effect of anthracycline antibiotics.

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