Morphological Manifestations of Heart Remodeling in Anthracycline-Induced Dilated Cardiomyopathy

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The morphogenesis of anthracycline-induced dilated cardiomyopathy was studied after single sublethal dose of doxorubicin. Cardiomyocyte depopulation (up to 27%) and decrease in their regeneratory plastic reactions were the main mechanisms of cardiac failure development after anthracycline (doxorubicin) treatment, determining the type of heart remodeling by the dilatation variant. Cardiomyocyte elimination and atrophy during the development of anthracycline-induced regeneratory plastic cardiac insufficiency were paralleled by hypertrophy of remaining cardiomyocytes and diffuse and small focal sclerosis of the myocardium, which could be regarded as a correlated compensatory reaction of the connective tissue to the decrease in the number of muscle fibers.

Key Words: *dilated cardiomyopathy; heart remodeling; doxorubicin; cardiomyocyte number*

The prevalence and clinical significance of cardiomyopathies necessitate studies of their pathogenesis and main stages of morphogenesis, as well as of the molecular and cellular mechanisms of cardiomyocyte dysfunction. Dilated cardiomyopathy (a myocardial disease characterized by dilatation of cardiac ventricles and sometimes of the atria and contractile dysfunction) is the most prevalent pathology [2,6].

Dilated cardiomyopathy as the integral final phenotype can be caused by many etiological agents and genetic abnormalities [1-3]. The detected genetic defects (congenital and developing under the effects of mutagenic factors) include mutations in genes encoding sarcomer and cytoskeletal proteins, and enzymes providing energy and calcium homeostasis of cardiomyocytes [3,10]. These disorders leads to reduction of the strength of contractions and death of cardiomyocytes, the leading pathophysiological component in the development of heart failure determining heart remodeling by the dilatation variant [2,6,8].

Similar changes in the cardiomyocyte metabolic reaction develop during treatment with anthracycline antibiotics characterized by selective cardiotoxicity. Doxorubicin, the most effective drugs of this group, belongs to pleiotropic drugs by the spectrum of its effects on cardiomyocyte intracellular structures and molecules [9,11]. Simulation of chronic anthracycline cardiomyopathy helps us to evaluate the total spectrum of structural and functional injuries in cardiomyocytes and other cell populations of the myocardium, the type and scale of their death, and the main variants of heart remodeling.

We studied tissue reorganization in the myocardium, types of cardiomyocyte injuries and death causing dilatation remodeling of the heart during the development of anthracycline-induced cardiomyopathy.

MATERIALS AND METHODS

Doxorubicin hydrochloride (Ferrein) was injected intraperitoneally in a single dose of 10 mg/kg to 25 rats. The animals were decapitated 1, 3, 5, 7, 14, 21, and

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30 days postinjection. Control group consisted of 10 rats intraperitoneally injected with normal saline in a volume corresponding to their body weight simultaneously with experimental animals.

The heart was separated from the adjacent tissues and rapidly weighed. The samples were fixed in 10% neutral formalin. Paraffin sections were stained with hematoxylin and eosin with Perles' reaction, by the method of Van-Gieson with poststaining of elastic fibers by Weigert's resorcin-fuchsine; PAS reaction was carried out. Semithin sections were stained with 1% Azur II by the droplet method.

The total population of cardiomyocytes in cardiac ventricles was quantitatively evaluated by alkaline dissociation of fixed tissues [4,5]. Statistical processing of the results included estimation of the means for the parameters, estimation of dispersion and errors the means. The significance of differences was evaluated using Student's test.

RESULTS

Injection of a sublethal dose of doxorubicin to rats resulted in the development of chronic cardiac insufficiency over 30 days. Animal mortality attained 20%. Signs of congestive cardiac insufficiency were observed in all animals: hydrothorax (24%), hemorrhagic ascites (60%), congestive liver (50%), and anasarca (60%).

Development of chronic anthracycline cardiomyopathy in rats was associated with phase-wise changes in body and heart weights (Table 1). Up to day 5 body and heart weights decreased (by 15 and 26%, respectively), but then gradually increased (by 20 and 14% maximally by day 21). Body weight increased because of transudate accumulation in the abdominal and thoracic cavities; heart weight increased as a result of myocardial hypertrophy. Dilatation of heart ventricles was observed.

Tissue reorganization in the myocardium during the initial period of anthracycline-induced cardiomyopathy was mainly characterized by lythic changes in cardiomyocytes, their progressive atrophy, development of hemodynamic disorders, and diffuse sclerosis.

On days 1-5 after doxorubicin injection mosaic staining of cardiomyocyte sarcoplasm with acid dyes was observed (Fig. 1, *a*); cardiomyocytes with eosinophilic sarcoplasm were observed, indicating contracture injuries of myofibrils. On the other hand, cardiomyocytes with signs of sarcoplasm lysis were present in all layers of the myocardium (~50% of the total cardiomyocyte count). There were also cardiac myocytes with normal structure and unchanged tinctorial characteristics. Pronounced polymorphism of cardiomyocyte nuclei was observed (the nuclei were enlarged, their shape and tinctorial characteristics were altered), their location in the cells being often changed (the nuclei were shifted into the subsarcolemma zone).

Hemodynamic disorders manifesting in sharp venous plethora, acute focal hemorrhages, lymphostasis, were the most pronounced in the subepicardial and medial layers of the myocardium of both ventricles. Edema of the stroma led to dissociation and disorganization of muscle fibers (Fig. 1, *b*). Intramural arteries were mainly in a state of secondary paresis, capillaries in the intermuscular interlayers were dilated, filled with plasma and blood cells. Plasmorrhagias were seen in some sites of the myocardium; diffuse infiltration of the stroma with mononuclear cells was observed.

Cardiomyocyte atrophy was observed during this period (Fig. 1, *b*). Mononuclear cells accumulated around thinned muscle fibers, the number of small

TABLE 1. Body Weight and Quantitative Characteristics of Wistar Rat Hearts after Single Injection of Doxorubicin Hydrochloride (*M*±*m*)

Group	Body weight, g	Heart weight, g	Cardiomyocyte concentration per mg tissue, $\times 10^3$	Absolute count of cardiomyocytes in the heart, $\times 10^3$
Control	179.8±2.3	667.3±39.8	6.5±0.6	4318.0±233.3
Day postinjection				
1	182.9±3.8	576.0±21.0	5.6±0.3	3259.0±316.6*
3	166.3±4.7	540.3±33.2	6.1±0.5	3304.3±429.7
5	152.7±5.6*	494.7±37.8*	6.7±0.4	3285.0±242.9*
7	162.4±6.3	556.7±10.1	6.1±0.4	3390.3±207.0*
14	204.9±10.1	702.7±19.0	6.8±0.7	4752.3±364.1
21	216.3±11.5*	759.3±43.5	4.9±0.3	3748.0±269.2
30	203.3±17.2	675.5±18.5	4.7±0.2*	3144.0±187.0*

Note. *$p<0.05$ compared to the control.

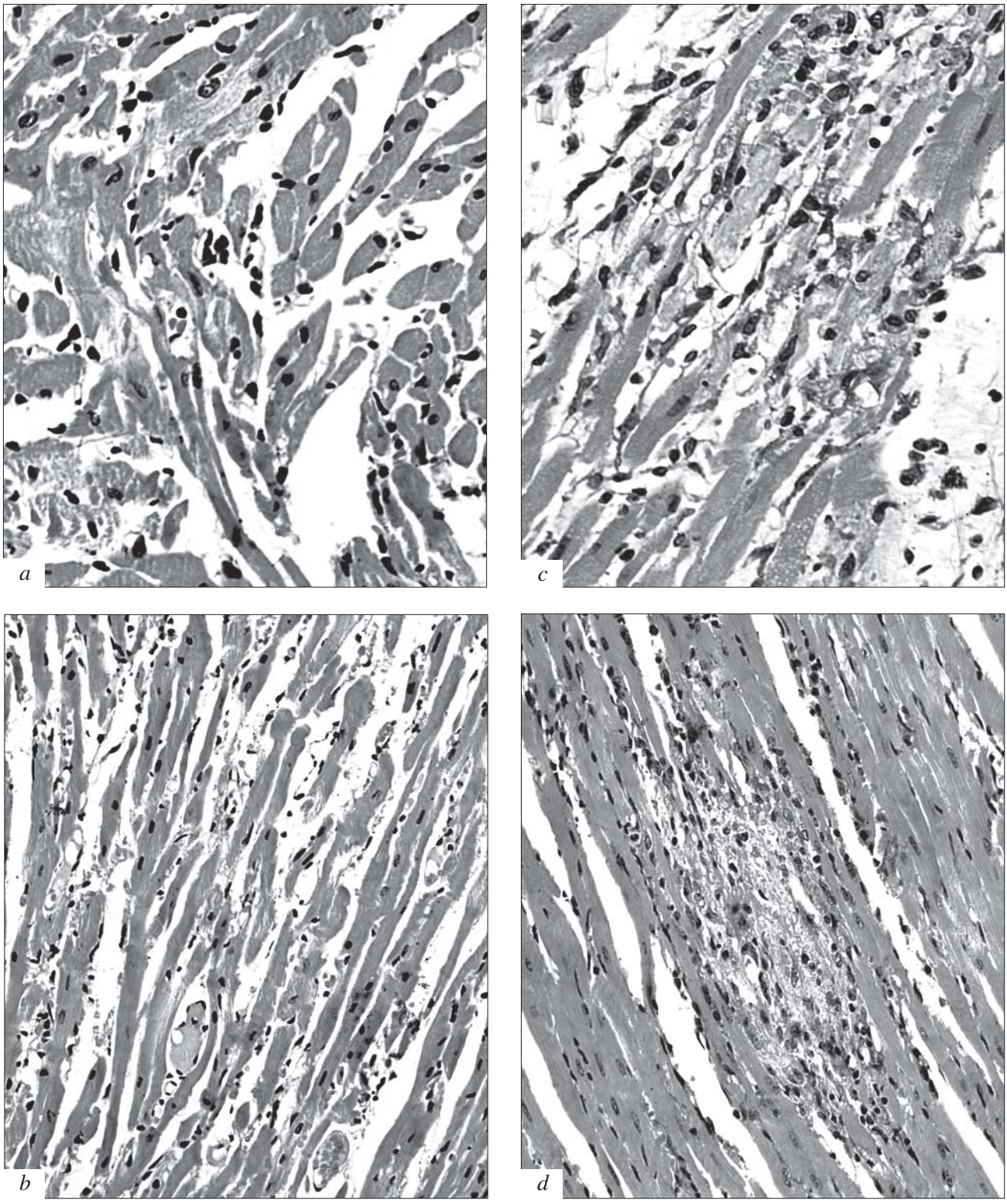

Fig. 1. Morphological changes in the myocardium of Wistar rats during development of anthracycline-induced dilated cardiomyopathy. Hematoxylin and eosin staining. *a*) mosaic lesions of cardiomyocytes (contractures and lythic injuries) 3 days after injection of doxorubicin, ×450; *b*) pronounced edema of interstitial connective tissue after 5 days of experiment, ×240; *c*) disintegration of muscle fibers, mononuclear infiltration in the focus of cardiomyocyte death 7 days postinjection, ×300; *d*) focal and diffuse cardiosclerosis after 21 days of experiment, ×240.

focal infiltrations increased. Myocardial stroma was abundantly infiltrated.

After 7 days of the experiment the number of cardiomyocytes with lythic injuries, which could be considered as morphological manifestations of their regenerative plastic insufficiency, appreciably increased; involution processes manifested (Fig. 2, *a*). Pronounced necrobiotic changes of cardiomyocytes were seen. In these cells the sarcoplasm was appreciably condensed and the nuclei were hyperchromatic; pronounced lipid infiltration of individual cardiomyocytes was seen (Fig. 2, *b*). Diffuse infiltration of the interstitium by mononuclears increased in foci of pronounced necrobiotic changes; there were small bundles of collagen fibers and accumulations of glycosaminoglycans. The absence of inflammatory cellular infiltration suggests that these changes in cardiomyocytes could be regarded as morphological manifestations of apoptotic death characteristic of doxorubicin (anthracycline) treatment.

Cardiomyocyte apoptosis caused disintegration of muscle fibers (Fig. 1, *c*); elimination of cardiomyocytes along muscle fibers was observed. The myocardium in these sites had honeycomb, but not fibrous structure, which impaired its contractility.

These changes in the parenchymatous compartment were observed in the presence of pronounced hemodynamic disorders (venous and capillary plethora, spasm of intramural arteries, lymphostasis). Pronounced interstitial and perivascular edema was observed in all layers of the myocardium, which led to destruction of fibers in muscle bundles. Small foci of cardiosclerosis with muscular segments between these foci formed in some animals.

Fourteen days postinjection diffuse foci of cardiomyocyte necrobiosis infiltrated with mononuclear cells were enlarged, "interwoven" with bundles of collagen fibers. Intracellular regeneration of cardiomyocytes was enhanced in the myocardium of survivors, which resulted in hypertrophy of these cells and gradual increase of heart weight (Fig. 2, *c*). Disintegration of muscle fibers at the intercalated disks was observed during the same period (Fig. 2, *c*). These changes reflecting disorders in the biosynthesis of protein components of desmosomes could be indicative of the initial stages of cardiomyocyte apoptosis. Dissociated cardiomyocytes can form temporary compounds with extracellular matrix [12], but then they are destroyed and absorbed by macrophages.

Pronounced hypertrophy of the greater part of cardiomyocytes was observed at later periods of the experiment (days 21-30). On the other hand, in some cells we observed appreciable vacuolation of the sarcoplasm resultant from dilatation of cisterns of the Golgi complex (Fig. 2, *d*), which indicated deep disorders in cardiomyocyte structure. These changes in the Golgi complex can be caused by impaired permeability of intracellular membranes resultant from modification of their physicochemical characteristics and by destruction of the cytoskeleton proteins creating the framework for intracellular structures and determining their orientation.

Abundant diffuse mononuclear infiltration of myocardial stroma was observed; cardiosclerotic foci formed at sites of cardiomyocyte death (Fig. 1, *d*). The stroma was still edematous with persisting venous and capillary plethora and spasm of intramural arteries. Perivascular bundles of collagen fibers grew larger.

Quantitative evaluation of the absolute number of cardiomyocytes in rat heart ventricles after single injection of 10 mg/kg doxorubicin showed phase-wise changes in this parameter. Cardiomyocyte concentration per 1 mg heart tissue virtually did not change until day 21 (Table 1). Heart weight decreased over 7 days, especially after 5 days of the experiment (by 26%, $p<0.05$). Decreased heart weight was responsible for 21-25% decrease in the total count of cardiomyocytes in the heart during the first 7 days of the experiment ($p<0.05$). By day 14 the absolute count of cardiomyocytes was restored and reached the control level. By days 21-30, with development of anthracycline cardiomyopathy, heart weight increased, which was paralleled by dilatation of the left and right ventricles. Cardiomyocyte concentration per 1 mg heart tissue notably decreased during this period (by 25 and 28%, respectively), which caused a decrease in the total count of cardiomyocytes in the organ by 13 and 27%, respectively. Cardiac hypertrophy during this period was caused solely by cardiomyocyte hypertrophy.

Quantitative data on the absolute count of cardiomyocytes in the heart during the development of anthracycline-induced dilated cardiomyopathy suggest that cardiomyocyte population not only decreases under the effect of the cardiotoxic agents, but can be restored as well. However, despite recovery of the total cardiomyocyte count by day 14 after a single dose of cardiotoxic compound doxorubicin this parameter notably decreased by day 30.

Cardiomyocyte apoptosis can play an important role in the development of dilated cardiomyopathy. Analysis of experimental and clinical studies indicates that low level of apoptotic death of cardiomyocytes can be more hazardous for cardiac ventricle hemodynamics than equivalent focal necrosis of cardiomyocytes [8]. For example, heart failure after coronary artery occlusion develops as a result of focal death of 40-50% left-ventricular cardiomyocytes [7], and similar result was observed in diffuse loss of 10-20% cardiomyocytes [13]. Development of dilated cardiomyo-

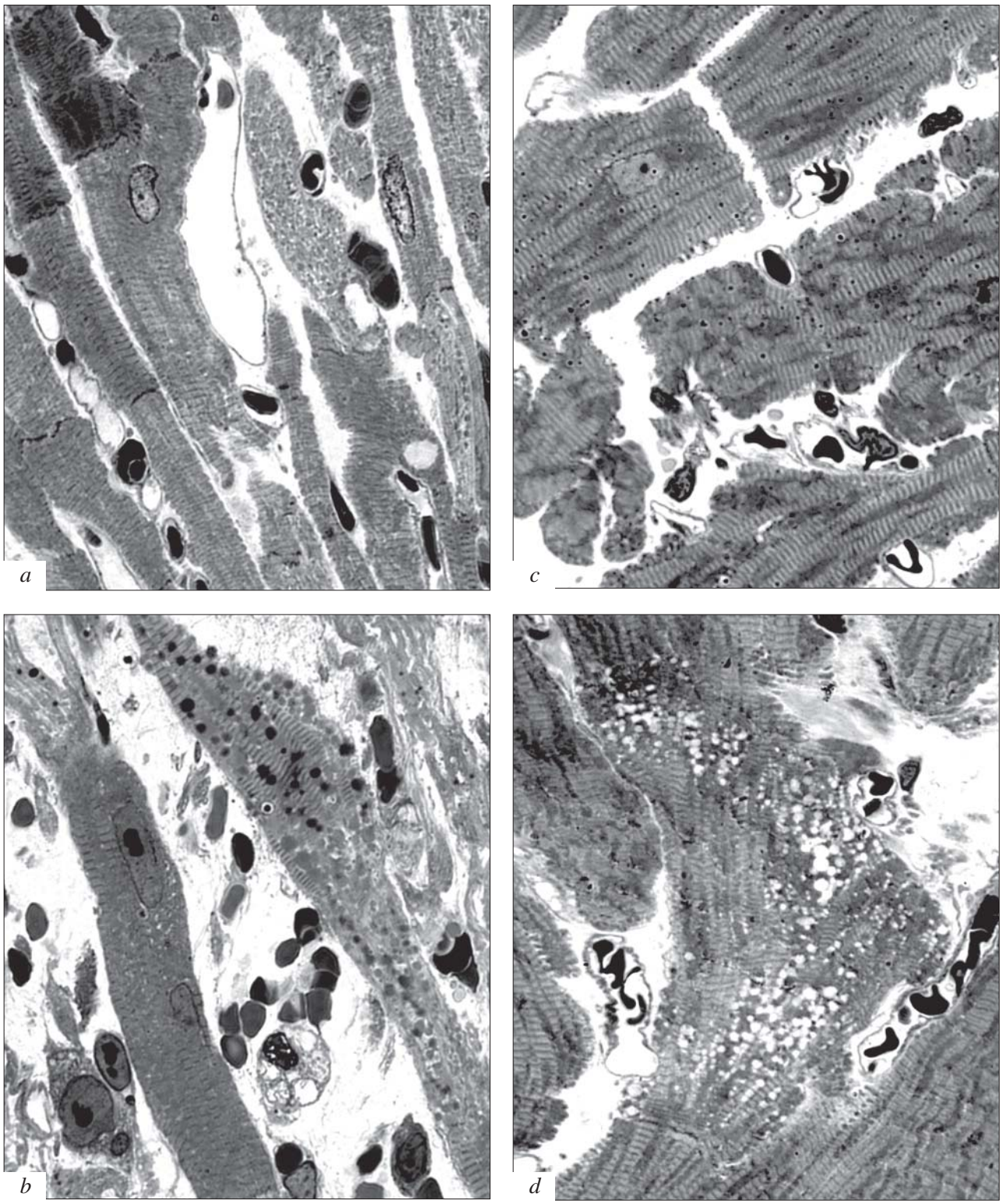

Fig. 2. Structural changes in cardiomyocytes of Wistar rats during development of anthracycline-induced dilated cardiomyopathy. Semithin sections. Azur II staining, ×1180. *a*) pronounced atrophy of cardiomyocytes 7 days after injection of doxorubicin; *b*) focus of cardiomyocyte necrobiosis and their resorption by macrophages after 7 days of experiment; *c*) disintegration of muscle fibers at the insertion disks, cardiomyocyte hypertrophy 14 days after injection of doxorubicin; *d*) pronounced hypertrophy of cardiomyocytes and pronounced vacuolization of sarcoplasm (dilatation of the Golgi complex) after 21 days of experiment.

pathy was detected in transgenic mice with cardiospecific activation of caspase-8 and a serin-threonin kinase Mst1, stimulating cardiomyocyte apoptosis [14, 15]. Diffuse elimination of ~27% cardiomyocytes from cardiac ventricles under the effect of doxorubicin observed in our experiments was also paralleled by heart remodeling by the dilatation variant.

The results of quantitative studies of cardiomyocyte population during the development of dilated cardiomyopathy suggest that the regeneratory plastic insufficiency of cardiomyocytes is the key factor in tissue reorganization, determining the type of remodeling and, eventually, diffuse apoptosis of these cells.

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