Morphofunctional Reorganization of the Myocardium of Mouse-Like Rodents in Regions with Various Anthropogenic Contamination Levels

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Morphofunctional analysis (morphological and stereological) revealed stereotypic morphofunctional reactions (hemodynamic disturbances accompanied by considerable cardiomyocyte degeneration) in the myocardium of common field mice and common voles from various Altai regions. Changes in the capillary surface-volume ratio and the stroma/parenchyma volume ratio are typical of tissue reorganization of the myocardium. The nature of these changes depends on the level and type of contamination.

Key Words: *anthropogenic pollution; myocardium; mouse-like rodents; morphology; stereology*

During the late period after the exposure to radiation and chemical effects, long-term low-dose irradiation determines the intensity of functioning of many biocenoses and morbidity rate of humans living in the polluted territory [6,8]. The myocardium is one of the most radiation-resistant organs free of stochastic effects. However, analysis of delayed consequences of high-dose irradiation revealed pathological changes indicating the presence of nonstochastic effects of this treatment [2,3]. Cardiomyocyte (CM) lifetime is comparable with that of the organism. Thus, studies of the nature and direction of structural and functional changes in the myocardium allow us to elaborate prognostic criteria of morphofunctional states of various systems during long-term combined influences of technogenic factors.

Here, we studied specificity of adaptive reorganization in the myocardium (including analysis of cellular and subcellular mechanisms) of mouse-like rodents from Altai regions with various levels of anthropogenic pollution.

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MATERIALS AND METHODS

A morphofunctional analysis of 234 myocardial specimens from 2 mouse-like rodent species, common forest mice (*Apodemus sylvaticus*) and common voles (*Microtus arvalis*), was performed. The animals were taken from 3 Altai regions differed in technogenic pollution levels. The first territory was Uglovskii district (environs of Topol'noe and Laptev Log villages). The content of ^{137}Cs in the soil surpassed 158 mCi/km^2. The second territory was Loktevskii district (environs of Novenskoe village). The content of ^{137}Cs in the soil surpassed 105 mCi/km^2. The soil contained considerable amounts of heavy metals and their salts. The concentrations of lead, zinc, and copper in the surface soil layers reached 1000 mg/kg, 100-300 mg/kg, and 150 mg/kg, respectively. The third territory studied was Tyumentsevskii district (environs of Klyuchi), where the level of technogenic contamination was minimum. Therefore, this region served as control.

The heart was fixed in 10% neutral formalin, embedded in paraffin, and sections for histological examination were prepared. These sections were stained with hematoxylin and eosin using the Perls' staining procedure. Van Gieson's and Weigert's resorcin-fuchsin staining methods were used to visualize elastic fibers. The PAS staining was also conducted.

To obtain semithin sections, heart fragments (no more than 1 mm^3) were fixed in 4% paraformaldehyde, postfixed in 1% OsO_4, dehydrated routinely, and embedded into Epon-Araldite mixture. Semithin sections were stained with azure II. The PAS reaction was performed, and azure II was used to complete the staining. These sections were then used for tissue stereological analysis using a multipurpose test system [5]. Volume and surface densities of the main tissue structures were evaluated, and secondary stereologic parameters (surface/volume and volume/volume ratios of structures and stroma/parenchyma volume ratio) were calculated from primary stereologic values. The results were analyzed using Student's t test.

RESULTS

Weights of the body and heart and relative weights of the heart of adult common forest mice were nearly similar in all studied regions. By contrast, in common voles, weights of the body and heart were higher in

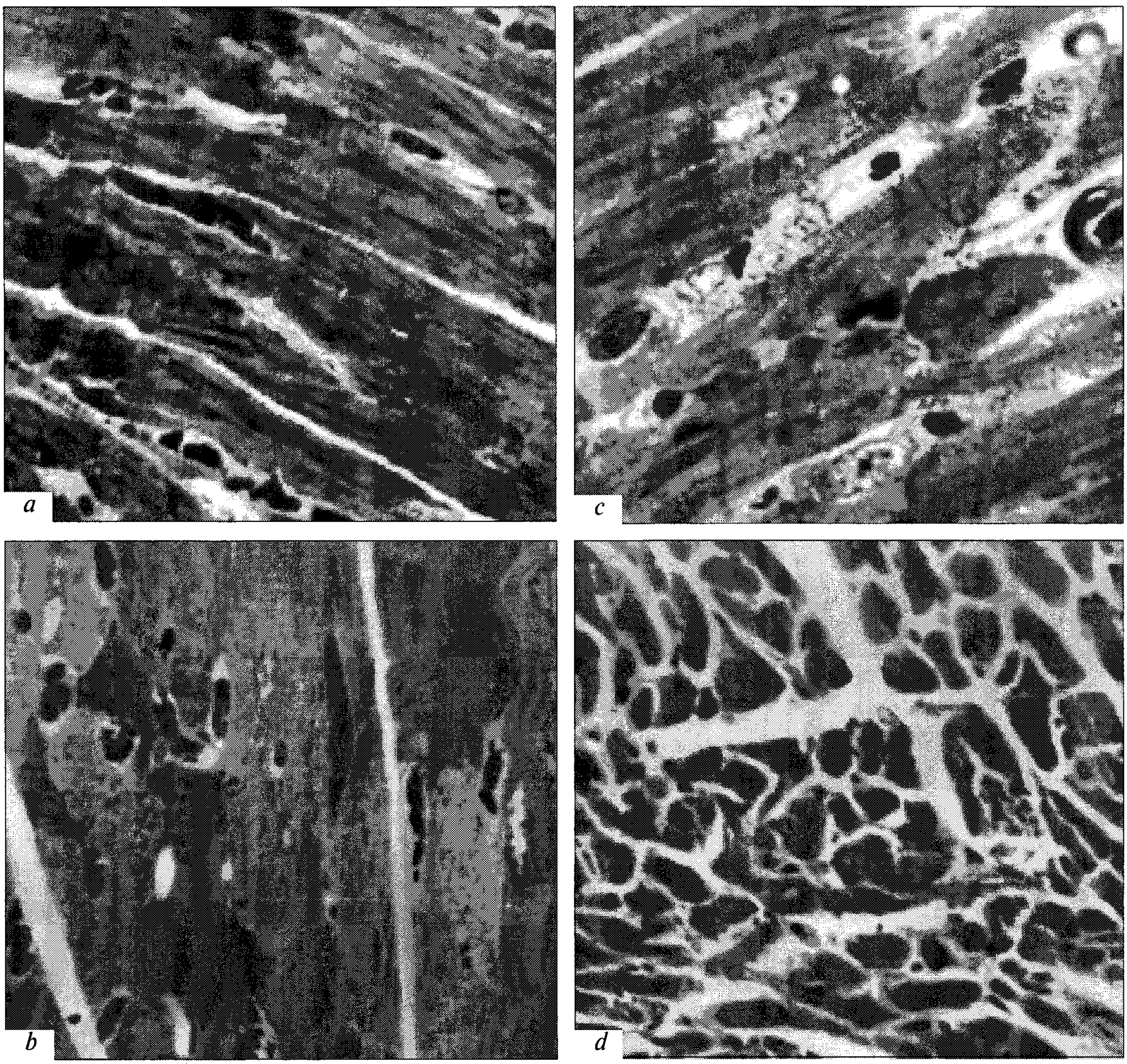

Fig. 1. Morphological changes in the myocardium of mouse-like rodents living in various Altai regions: *a*) myocardium of common forest mice living in Tyumentsevskii district, ×800; *b*) contracture-induced changes in cardiomyocytes of common forest mice living in Loktevskii district, ×1000; *c*) perinuclear and subsarcolemmal depletion in cardiomyocytes of common voles living in Uglovskii district, ×1000; and *d*) considerable intermuscular edema in the myocardium of common forest mice living in Loktevskii district, ×400. Semithin sections stained with azure II (*a-c*) and hematoxylin and eosin (*d*).

TABLE 1. Tissue Stereologic Parameters of the Myocardium of Common Forest Mice from Different Altai Regions with Various Ecological Conditions (*M*±*m*)

Parameter	District		
	Tyumentsevskii	Loktevskii	Uglovskii
Morphometric assays			
Body weight, g	19.7±5.3	17.4±1.4	25.9±4.5
Heart weight, g	0.137±0.038	0.125±0.010	0.160±0.023
Relative heart weight, mg/g	7.1±0.5	7.2±0.2	6.4±0.4
Stereologic assays			
Volume density, mm^3/cm^3			
CM	869.9±4.5	876.4±6.0	875.4±5.9
CM nuclei	12.6±0.3	13.8±2.1	10.5±2.6
capillaries	105.8±4.0	97.2±7.6	101.3±2.4
endothelial cells	3.9±1.1	5.3±0.7	4.3±1.3
connective tissue cells	7.6±1.1	7.1±1.2	8.3±1.6
base substance and connective tissue fibers	0.3±0.01	0.2±0.01	0.3±0.01
Surface density, m^2/cm^3			
CM	0.110±0.005	0.118±0.007	0.125±0.005*
CM nuclei	0.009±0.001	0.010±0.001	0.008±0.001
capillaries	0.050±0.003	0.045±0.003	0.053±0.001
connective tissue cells	0.005±0.001	0.004±0.001	0.004±0.001
Surface-volume ratio, m^2/cm^3			
CM	0.124±0.005	0.131±0.009	0.141±0.005*
CM nuclei	0.701±0.052	0.761±0.088	0.749±0.048
capillaries	0.483±0.044	0.472±0.016	0.526±0.010^{++}
capillary/CM	0.057±0.003	0.051±0.003	0.061±0.001^{++}
Volume ratio			
stroma/parenchyma	0.134±0.006	0.124±0.010	0.129±0.005
capillary/CM	0.121±0.005	0.110±0.009	0.115±0.003
nucleus/CM cytoplasm	0.015±0.004	0.016±0.002	0.012±0.003

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared with the corresponding values in rodents from Tyumentsevskii district; $^{+}p<0.05$ and $^{++}p<0.01$ compared with corresponding values in rodents from Loktevskii district.

Uglovskii district (by 31% and 17%, respectively) and lower in Loktevskii district (by 12% and 9%, respectively) (Tables 1 and 2).

Microscopically, myocardium samples of common forest mice and common voles taken from Tyumentsevskii district had normal structures. CM were evenly stained, and their cross-striated pattern was well defined (Fig. 1, *a*). In some animals, few eosinophilic muscular segments with contracture-induced damages to myofibrils and cells with loosened light sarcoplasms were found. Necrobiotic changes in CM surrounded by mononuclear cell aggregate were observed in common forest mice. Hyperemia and moderate edema of the interfibrillar connective tissue were found in some animals.

The majority of rodents from regions with high levels of anthropogenic pollution (Loktevskii and Uglovskii districts) displayed a considerable heterogeneity of CM due to the presence of contracture-induced changes in cells, cells with loosened sarcoplasms (Fig. 1, *b*), and necrotic foci. In some animals, examination of semithin sections revealed perinuclear sarcoplasm depletion (Fig. 1, *c*). CM with more acidophilic sarcoplasm had hyperchromic and pyknotic nuclei. We observed a marked polymorphism of CM nuclei in common forest mice from Uglovskii district. In common forest mice from Loktevskii district, we observed loosening of muscular fibers due to considerable muscular and perivascular edema (Fig. 1, *d*). Uneven plethora and secondary paresis of intramural ar-

TABLE 2. Tissue Stereologic Analysis of the Myocardium of Common Voles from Different Altai Regions with Various Ecological Conditions (*M*±*m*)

Parameter	District		
	Tyumentsevskii	Loktevskii	Uglovskii
Morphometric assays			
Body weight, g	19.7±5.3	17.4±1.4	25.9±4.5
Heart weight, g	0.137±0.038	0.125±0.010	0.160±0.023
Relative heart weight, mg/g	7.1±0.5	7.2±0.2	6.4±0.4
Stereologic assays			
Volume density, mm^3/cm^3			
CM	875.5±5.5	852.5±4.4*	892.1±5.4*+
CM nuclei	14.1±3.8	12.1±2.3	16.0±1.2+
capillaries	100.7±8.2	123.2±2.5*	78.6±5.1*++
endothelial cells	2.3±0.4	5.0±0.9*	5.0±0.9*
connective tissue cells	7.2±1.7	7.1±1.6	9.8±0.5*+
base substance and connective tissue fibers	0.3±0.01	0.1±0.01	0.2±0.01
Surface density, m^2/cm^3			
CM	0.121±0.006	0.121±0.004	0.111±0.008
CM nuclei	0.008±0.002	0.009±0.001	0.011±0.001
capillaries	0.052±0.002	0.055±0.002	0.054±0.005
connective tissue cells	0.004±0.001	0.004±0.001	0.006±0.001
Surface-volume ratio, m^2/cm^3			
CM	0.136±0.006	0.140±0.006	0.123±0.010
CM nuclei	0.631±0.030	0.770±0.072	0.692±0.080
capillaries	0.520±0.037	0.451±0.024*	0.694±0.038*++
capillary/CM	0.059±0.003	0.065±0.002*	0.059±0.006
Volume ratio			
stroma/parenchyma	0.125±0.008	0.158±0.005**	0.102±0.006*++
capillary/CM	0.114±0.010	0.143±0.004**	0.087±0.006*++
nucleus/CM cytoplasm	0.016±0.004	0.015±0.003	0.018±0.002

teries were noted. Hemorrhages in intermuscular areas were observed. Atrophy and necrobiosis of some CM were accompanied by the formation of mononuclear infiltrates containing monocytes and fibroblasts. In 20% cases, diffuse infiltration and small regions of substitutive sclerosis were revealed.

Tissue stereologic analysis showed that volume densities of CM and CM nuclei were the same in common forest mice from contaminated regions (Table 1). This contributes to the constant nucleus/cytoplasm volume ratio. The surface density of CM in mice from Uglovskii district increased significantly (by 14%). Therefore, the surface-volume ratio of CM increased also by 14%. The surface and volume densities of capillaries in these animals tended to increase and decrease, respectively. Thus, their surface-volume ratio was higher by 9% and 11% than that in mice from Tyumentsevskii and Loktevskii districts, respectively. The capillary/CM surface-volume ratio in mice from Uglovskii district was 7% and 20% above the corresponding values in animals from Tyumentsevskii and Loktevskii districts, respectively.

In common voles from Uglovskii district, the volume density of CM was higher by 2% and 5% than that in animals from Tyumentsevskii and Loktevskii districts, respectively (Table 2). A significant decrease in this parameter (by 3%) was found in rodents from Loktevskii district. We found no considerable changes in the surface density and CM/nucleus surface-volume ratio in common voles living in these regions. The nucleus/cytoplasma ratio also did not change.

The volume density of capillaries in common voles from Uglovskii district decreased by 22% and 36% compared with that in animals from Tyumentsevskii and Loktevskii districts, respectively. This parameter in common voles from Loktevskii district increased by 22%. The retention of the surface density of capillaries at the level typical of common voles from Tyumentsevskii district contributed to a considerable increase in the surface-volume ratio of these structures in common voles from Uglovskii district compared with that in animals from Tyumentsevskii and Loktevskii (by 33% and 54%, respectively) and a decrease in this parameter in animals from Loktevskii district.

The opposite changes in CM and capillary volume density in common voles induced a considerable increase in the capillary/CM volume and surface-volume ratio in animals from Loktevskii district (by 25% and 10%, respectively). The capillary/CM volume ratio in common voles from Uglovsii district decreased compared with that in rodents from Tyumentsevskii and Loktevskii districts (by 24% and 39%, respectively). However, the capillary/CM surface-volume ratio in these animals was at the level typical of rodents from Tyumentsevskii district. The volume density of endothelial cells increased by 117% in animals from Loktevskii district. The volume density of connective tissue cells in common voles from Uglovsii district increased by 36% and 38% compared with that in rodents from Tyumentsevskii and Loktevskii districts. Thus, the stroma/parenchyma volume ratio decreased considerably in voles from Uglovsii district. However, this parameter increased by 26% in animals from Loktevskii district.

Thus, the following stereotypic nonspecific morphofunctional reactions were found in the myocardium of rodents living in various Altai regions with considerable anthropogenic pollution levels: degeneration, atrophy, and necrobiosis of CM with the formation of substitutive sclerotic foci; moderate intermuscular and perivascular edema; unequal vascular and capillary plethora; and hemorrhages in intermuscular areas. The most considerable changes in the myocardial architectonics were observed in common voles. Combined effects of physical and chemical pollutants increased the stroma/parenchyma volume ratio and decreased the capillary surface-volume ratio. Chronic effects of low-dose irradiation decreased the stroma/parenchyma volume ratio and increased the capillary surface-volume ratio.

The structural reorganization of the myocardium observed under impaired ecological conditions is determined by various factors. The trigger factor is the interaction of ionizing radiation and xenobiotics with various cell components (chromatin, membranes, and macromolecules) [1,7]. These interactions result in reorganization of cell functioning with the formation of a new phenotype and, thus, realization of a genetic program determined by the type and intensity of affecting factors. The realization of a new genetic program in mammalian cells is accompanied by the development of the general adaptive syndrome manifested in structural and functional rearrangement, especially at the tissue level [2,4,9,10]. Our findings indicate that these morphofunctional changes represent a new adaptive form resulting from anthropogenic modifications of the nature.

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