Cardiopathy Modeling in Rats

T. P. Novgorodtseva, L. M. Isachkova, and O. G. Vostrikova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 7, pp. 114-117, July, 2002
Original article submitted April 17, 2002

Stages of reproduction of a polyetiological model of coronary failure in rats are described. The model is based on the development of electrolyte steroid cardiopathy. Morphological studies confirmed the presence of degenerative changes in rat myocardium and coronary vessels. The model is intended for studies of the development and remission of cardiac diseases.

Key Words: *cardiovascular diseases; cardiopathy; rats; experimental models*

There are various methods of modeling human disease in animals. Among well-known models of cardiovascular diseases are experimental atherosclerosis, dyslipidemias, and myocardial infarction. Rats as a biological species are little sensitive to spontaneous and experimental atherosclerosis and they are more often used for modeling dietary dyslipidemias. For scientific and practical purposes animal models of coronary failure accompanied by pathological changes in coronary vessels and characterized by minimum mortality (i.e. allowing long-term observation) are needed. The aim of our study was to create a model for studies of metabolic processes in cells, subcellular structures, organs, and tissues and regularities of their inheritance.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats (180.5±10.6 g) from Stolbovaya Breeding Center (Russian Academy of Medical Sciences). The rats were kept on a 14-day quarantine before the experiment. The experiments were carried out in accordance with Regulations for Studies Making Use of Experimental Animals (Supplement to the Order of Ministry of Health of the USSR No. 755, 12.09.77).

According to reports published in the 1950-70s, changes in the myocardium can be induced by pathogenic (hyperlipidemic, sclerotic, Mg-deficient) diets, injections of insulin, adrenocorticoid hormones after unilateral nephrectomy, sodium phosphate load against the background of therapy with highly active steroids or stress, *etc*. The pathogenic factors promoting the formation of cardiac necrosis in various biological species are described by J. Shosh *et al*. [5]. However, we found no recommendations on modeling disturbances in rat myocardium with these factors or their combinations. We hypothesized that the most pronounced injuries can be induced by a combination of these pathogenic factors.

Mg^{2+},K^+ deficiency produces a negative effect on the myocardium [5]. Diets with high Na^+ content aggravate myocardial damage induced by K^+ deficit, because sodium is toxic against the background of K^+ deficiency. K^+ loss can be caused by adrenal dysfunction. Aldosterone primarily regulates intracellular Mg^{2+} concentration, while the concentration of K^+ is its secondary target. Animal experiments revealed a relationship between dietary Mg^{2+} deficiency and atherosclerotic changes in vessels and functional disturbances of the heart rhythm [6-9]. Mg^{2+} deficiency is always associated with hyperlipidemia [4,7]. These data suggest that mineral balance (K^+/Na^+ and Mg^{2+}/Ca^{2+} ratios) is essential for damaged myocardium. These data led the authors to creation of a new experimental polyetiological model of cardiopathy based on electrolyte-steroid imbalance (ESC).

Sensitization of rat myocardium was attained via surgical removal of the kidney, replacement of drinking water with 1% NaCl solution, and special semisynthetic cardiovasopathogenic electrolyte-enriched diet. Intramuscular injections of hydrocortisone ace-

Institute of Medical Climatology and Rehabilitative Treatment, Vladivostok Affiliated Branch of Far-Eastern Research Center of Siberian Division of Russian Academy of Medical Sciences. ***Address for correspondence:*** curdeal@mail.ru. Novgorodtseva T. P.

0007-4888/02/1341-0100$27.00

tate and cold exposure served as the conditioning and resolving factor, respectively. The duration of exposure to pathogenic factors and pattern of disease development were determined by morphological changes in the hearts. Semisynthetic diets and salt mixtures are presented in Tables 1 and 2. ESC was modeled as follows: on day 3 after unilateral nephrectomy under ether narcosis the animals were switched to Na^+ and Ca^{2+}-enriched diet against the background of K^+ and Mg^{2+} deficiency. During this period drinking water was replaced with 1% NaCl solution and the animals received daily intramuscular injections of 1.5 mg/100 g hydrocortisone acetate (corresponds to the pharmacological dose). On day 13 of the experiment, the animals were exposed to cold stress (4 h at 4°C). The optimal temperature and duration of cold exposure were selected experimentally. On day 17 and the animals were returned to standard vivarium conditions.

The animals were sacrificed by decapitation under ether narcosis, the heart was removed and fixed in 10% neutral formalin. Tissue fragments were dehydrated in ascending ethanol concentrations and embedded in paraffin. Deparaffinated sections were stained with hematoxylin and eosin. Histological preparations were examined under an Amplival optic microscope. Microphotographs were made using Svema FN films (64 U).

TABLE 1. Composition of Semisynthetic Diet

Ingredients	Quantity, g/kg body weight
Casein	300
Codliver oil	10
Margarine	45
Fat	45
Dry yeast	20
Dry bile	10
Salt mixture	50
Starch	510
Vitamin D_2, mg	15

TABLE 2. Salt Mixture

Ingredients	Quantity, g/kg body weight
$Na_2HPO_4 \times 12H_2O$	170
$NaH_2PO_4 \times 2H_2O$	170
NaCl	270
$NaClO_4 \times H_2O$	80
Ca lactate	150
$CaCO_3$	150
$FeCl_3 \times 7H_2O$	6.04
$MnCl_2 \times 4H_2O$	1.60
$ZnCl_2 \times H_2O$	1.36
$CuSO_4 \times 5H_2O$	0.16
KI	0.80

RESULTS

The development of experimental cardiopathy was paralleled by changes in coronary vessels, which were confirmed by morphological studies. The thickness of arteriolal and venular walls varied, the lumen was narrowed, the wall was deformed and disordered, and the epithelium was sometimes destroyed (Fig. 1, *a*). Adherence of erythrocytes to the damaged endothelium attested to initiation of clotting. Corrosion of endothelium and pyknosis of endotheliocyte nuclei were seen in foci of lesions. Mucoid and fibrinoid degeneration of the vascular wall was seen. Thrombosis, homogenization, and vacuolation of muscular layer of vascular wall with angiosclerosis were seen in some venules. Cardiomyocyte degeneration and cytolysis with the loss of transverse striation were observed around these vessels (Fig. 1, *b*). Diapedesis of erythrocytes and small hemorrhages were detected in the myocardium. Plethoric vessels were seen under the epicardium. In some vessels mucoid degeneration was associated with perivascular infiltration with lymphocytes and histiocytes.

Histomorphological analysis showed that experimental ESC reflects pathological changes in the heart typical of cardiac diseases. The development of experimental cardiopathy is associated with minimum mortality of laboratory animals. Animal mortality during ESC modeling was 2% (rats died 24 h after cold stress exposure). Postmortem examination revealed extensive irregular whitish spots with clear-cut borders on the anterior wall of the left and right ventricles. Rats with experimental ESC were used in the next stage of the experiment, which lasted for 12 months; no unexpected deaths occurred.

Hence, ESC is a convenient model associated with necrosis and characterized by minimum mortality of laboratory animals, which allows the use of this model in dynamic studies for investigation of the pathogenetic and sanogenetic mechanisms of cardiovascular diseases.

REFERENCES

1. L. M. Belkina, N. B. Korchazhkina, Yu. G. Kamskova, and N. A. Fomin, *Pat. Fiziol.*, No. 2, 18-21 (1997).
2. D. S. Sarkisov and P. I. Remizov, *Experimental Simulation of Human Diseases*, Ed. A. A. Vishnevskii [in Russian], Moscow (1960).
3. P. A. Torkunov, N. S. Sapronov, N. Yu. Novoselova, and N. N. Nalivaeva, *Pat. Fiziol.*, No. 2, 21-23 (1997).
4. A. M. Shilov, I. S. Svyatov, M. I. Chubarov, and I. D. Sanodze, *Klin. Med.*, No. 4, 35-37 (1998).

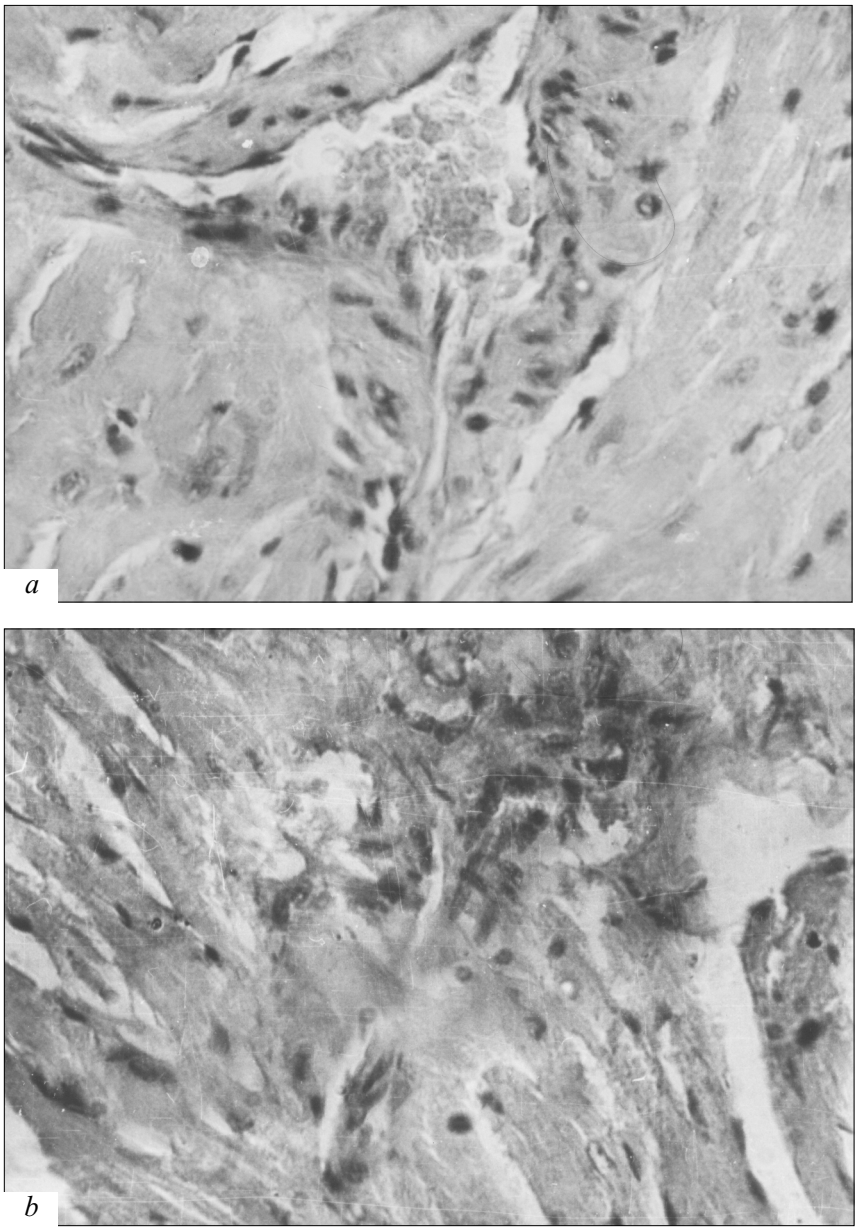

Fig. 1. Experimental rat (male). Hematoxylin and eosin staining. *a*) venule with unevenly thickened wall and lumen deformation, erythrocyte adhesion at the site of endothelial injury, ×82; *b*) fragment of cardiomyocyte with fibroblastic reaction, ×150.

5. J. Shosh, T. Gati, L. Chalai, and I. Deshi, *Pathogenesis of Diseases of Civilization* [in Russian], Budapest (1976).
6. S. Bloom, *Magnesium*, **4**, 82-95 (1985).
7. J. Britton, I. Pavord, K. Richards, *et al.*, *Lancet*, **344**, 357-362 (1994).
8. Y. Raissiguier, *Magnesium*, **5**, 182-190 (1986).
9. M. S. Seelig, *Magnesium Deficiency in the Pathogenesis of Diseases: Early Roots of Cardiovascular, Skeletal, and Renal Abnormalities*, New York (1980).