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MORPHOLOGICAL MANIFESTATIONS OF ANTHRACYCLINE CARDIOMYOPATHY IN THE VENTRICULAR MYOCARDIUM OF RATS

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KEY WORDS: anthracycline cardiomyopathy; plastic insufficiency of the heart; right and left ventricular myocardium; cardiomyocyte ultrastructure; morphometry.

Anthracycline cardiomyopathy of laboratory animals is a convenient and promising model with which to study the general rules of development of plastic cardiac insufficiency [2, 5, 11]. During investigation of the left ventricular myocardium by light and electron microscopy, combined with stereologic analysis and the method of cell isolation by alkaline dissociation of the fixed myocardium, the writers discovered the fundamental rules governing involutional reorganization of cardiomyocyte ultrastructure and quantitative reduction of the population of these cells in the absence of any necrotic changes in the myocardium [9, 11]. However, contractile cardiac insufficiency has not been investigated when structural metabolism in the muscle tissue of the right ventricle is disturbed.

The working myocardium of the right ventricle, despite similarity in the ultrastructural organization of its cardiomyocytes with those of the left ventricular myocardium, presented considerable autonomy, manifested as greater resistance to metabolic damage, high sensitivity to hemodynamic overloads [6], and differences in the dynamics and rates of growth and of aging of the myocardium during postnatal development [1, 10]. The degree of involvement of the cardiomyocytes in the elimination process during anthracycline cardiomyopathy of the right ventricle is therefore an interesting problem.

The aim of this investigation was to discover whether any differences exist in the sensitivity of the right and left ventricular myocardium to the action of the anthracycline antibiotic rubomycin, which depresses DNA-dependent RNA synthesis in the cardiomyocytes, by concentrating on the study of "disappearance" of some cells from the population of ventricular cardiomyocytes [9].

EXPERIMENTAL METHOD

Experiments were carried out on 15 male Wistar rats weighing 180 ± 20 g, divided into two groups. The experimental animals (n = 10) received a single intraperitoneal injection

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TABLE 1. Results of Quantitative Morphological Analysis of Cardiomyocyte Populations of Right and Left Ventricular Myocardium of Rats with Anthracycline Cardiomyopathy ($\bar{x} \pm m$)

Parameter	Right ventricle		Left ventricle	
	control	experiment	control	experiment
Number of animals	5	5	5	5
Weight of myocardium, mg	143.4±17,5	129,0±10,0	519,0±41,0	347,6±25,0**
Concentration of muscle nuclei, $\cdot 10^3$	40,07±3,31	34,36±3,86	28,98±1,13	27,28±1,77
Absolute number of muscle nuclei, $\cdot 10^6$	5,56±0,37	4,31±0,34*	15,16±1,57	9,29±0,24**
Absolute number of cardiomyocytes, $\cdot 10^6$	3,01±0,21	2,32±0,18*	7,69±0,82	4,73±0,13**
Number of nuclei in 1000 cardiomyocytes	1849±9	1859±12	1972±7	1966±8

Legend. *$p < 0.05$. **$p < 0.01$.

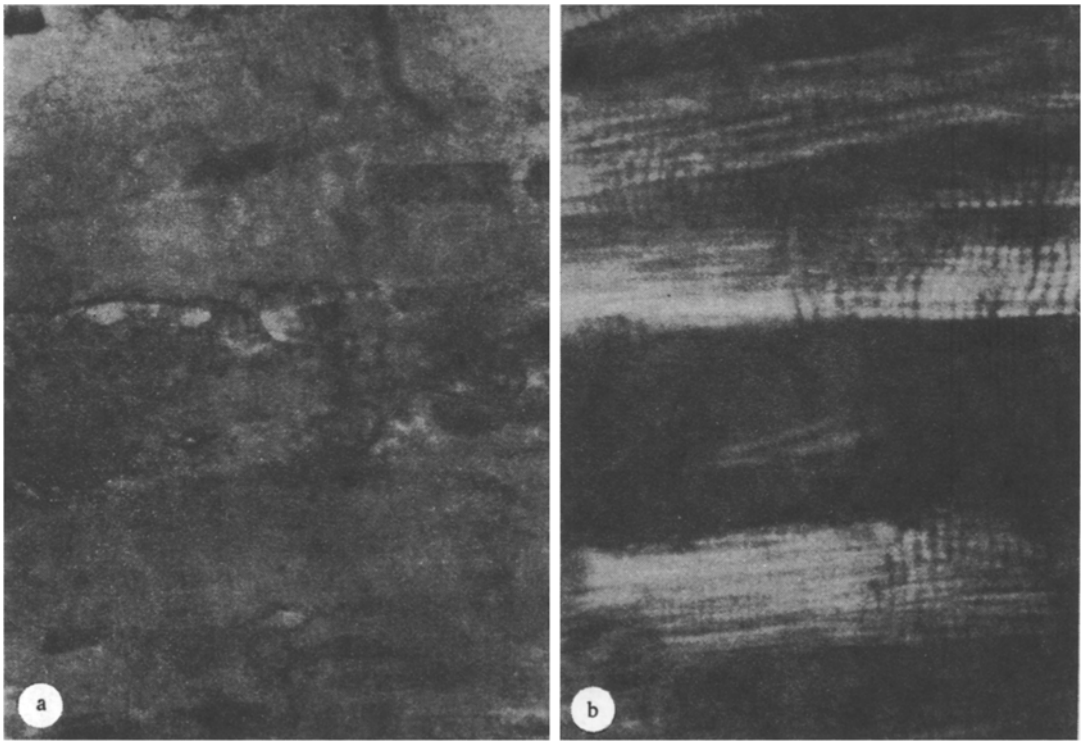

Fig. 1. Myocardium of rat 4 days after injection of cardiotoxic dose of rubomycin. a) Atrophied muscle fiber surrounded by accumulation of glycosaminoglycans. Colloidal iron—PAS—hematoxylin, 800 ×; b) the same, in polarized light.

of rubomycin hydrochloride in the form of a 0.2% aqueous solution, in a dose of 30 mg/kg. Those rats which survived (n = 5) 5 days after injection of rubomycin were decapitated under chlorofrom anesthesia. Rats of the control group (n = 5) received an intraperitoneal injection of physiological saline at the same time as the experimental animals, in a volume corresponding to body weight (15 ml/kg). The experimental and control animals were weighed at the beginning and end of the experiments. The hearts were removed, cardiac contractions were stopped by cold, and the atria were removed and fixed in cold 4% paraformaldehyde, made up in 0.1 M phosphate buffer, pH 8.0, for 10 days. After accurate dissection, the left ventricular myocardium with the ventricular septum and the right ventricular myocardium were weighed separately on torsion scales and the results obtained were used in the subsequent calculations. Samples weighing 20-25 mg were excised from the walls of both ventricles and subjected to alkaline dissociation followed by counting of the absolute number of muscle nuclei and cells [4]. The numerical data were subjected to statistical analysis and compared by Student's test.

Fig. 2. Ultrastructural changes in a cardiomyocyte of a rat 5 days after injection of cardiotoxic dose of rubomycin; close packing of residual organelles in atrophied muscle cell (23,900 ×).

Samples of tissue from the right and left ventricles of the recently killed animals, for electron microscopy, were postfixed with 1% OsO_4 solution in the same buffer, pH 7.2-7.4 and, after dehydration, embedded in a mixture of styrene and butyl methacrylate. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the Tesla BS 500 electron microscope under an accelerating voltage of 60 kV. Histological investigations were carried out on dying and killed animals. Tissue samples were postfixed in a 12% solution of neutral formalin. Parafin sections through the right and left ventricular myocardium were stained with hematoxylin and eosin, and a combination of colloidal iron and PAS-hematoxylin. The NU2 universal biological light microscope was used for general and polarization microscopy.

EXPERIMENTAL RESULTS

Half of the rats which received a cardiotoxic dose of rubomycin died on the 4th-5th day with evidence of heart failure and circulatory disturbances. In animals killed in the preagonal state these phenomena were also maximally exhibited. The body weight of the experimental rats by the end of the experiment had fallen by 20-23% whereas that of the controls had increased by 10-15%. The decrease in body weight of the experimental animals was due primarily to the temporary cytotoxic action of the anthracycline antibiotics on the epithelium of the gastrointestinal tract [15], but the development of cardiac cachexia likewise cannot be ruled out. Increased catabolism in the organs and tissues may perhaps be linked with hypoxia due to deficiency of the blood supply [12].

General light microscopy and polarization microscopy of the myocardium of the rats which died and were killed with anthracycline cardiomyopathy revealed no focal necrotic or necrobiotic changes, in agreement with results obtained previously [9, 11] and with data described by other workers [15]. Ultrastructural signs of disturbance of protein synthesis in the cardiomyocytes of the working right ventricular myocardium were similar to those described in the papillary muscles of the left ventricle [3, 8, 11]. They consisted of a combination of changes such as fragmentation and a circular arrangement of the nucleoli in the nuclei, absence of granular glycogen, ribosomes, and polysomes, focal degradation of the cytoplasm, a decrease in the number and thickness of the myofibrils, and instability of the mitochondrial membranes in individual cardiomyocytes. On careful examination of serial histological and ultrathin sections from samples of the myocardium of many animals,

freely lying atrophied cardiomyocytes could be seen in the intermuscular spaces (Figs. 1 and 2). These changes correspond to the terminal stage of irreversible plastic insufficiency [11].

The results obtained by alkaline dissociation of the fixed myocardium are given in Table 1. The mean data show that the weight of the right ventricular myocardium in rats with anthracycline cardiomyopathy was reduced on average by 10%. The fact that the change in this parameter was not significant can be explained by the marked intermuscular edema of the myocardium, one sign of which was a reduction in the concentration of muscle nuclei per milligram of tissue [11]. The reduction in weight of the left ventricular myocardium considerably masked the difference in concentration of the muscle nuclei, and amounted to 33% of the control level. The cardiomyocyte population and the number of their nuclei in the right ventricular myocardium decreased by 23 and 22% respectively, and by 38 and 39% in the left ventricular myocardium. Differences between the data in the experiment and control with respect to those parameters are statistically significant and demonstrate the great sensitivity of the left-ventricular cardiomyocytes to the toxic action of rubomycin.

The cause of these differences must be sought in differences in the mechanism of interaction of anthracycline cytostatics with nuclear DNA. The results of a study of the pharmacokinetics point to selectively strong and prolonged binding of molecules of anthracycline antibiotics with the cells of rapidly growing hematopoietic tissues, tumors, and working myocardium [14]. Common to all these cells is a high level and rate of synthesis of structural proteins. In particular, the level of RNA synthesis in cardiomyocyte nuclei of the ventricles is higher than that in the striated somatic muscles and in connective-tissue cells [7, 13].

Anthracycline antibiotics are antimetabolites with intercalating action, which insinuate themselves between the base pairs of uncondensed double stranded DNA [16]. The more uncondensed and, consequently, the more synthetically active the DNA, the larger the number of antibiotic molecules that can insinuate themselves into it, blocking the mechanism of DNA-dependent RNA synthesis.

It can thus be reliably concluded that the myocardium of the right ventricle, in each separate time interval in the phase of intensive protein synthesis, contains a smaller proportion of the cardiomyocyte population than the left ventricular myocardium.

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MORPHOLOGICAL FEATURES OF NATRIURETIC FACTOR SECRETION BY ATRIAL CARDIOMYOCYTES IN SPONTANEOUSLY HYPERTENSIVE RATS

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KEY WORDS: natriuretic factor; atrial cardiomyocytes.

In 1956, Kisch [4], in an electron-microscopic study of guinea pig cardiomyocytes, found small granules which were present only in the atrial cardiomyocytes (ACM). In a more detailed study of the ACM and the system forming specific granules, Jamieson and Palade [3] expressed, for the first time, the view that ACM possess a secretory as well as a contractile function. De Bold et al. [1, 2] proved that the atrial granules contain a substance which has a powerful natriuretic and diuretic action, which they called atrial natriuretic factor (ANF). Subsequent investigations showed that ANF is a short polypeptide, which not only possesses natriuretic and diuretic activity, but also has a relaxing action on smooth-muscle cells and can inhibit the renin–angiotensin–aldosterone system [6]. It has been suggested that ANF plays an important role in the genesis of arterial hypertension.

The aim of this investigation was a morphological assessment of the system synthesizing and secreting ANF in the cardiomyocytes of the right atrium in rats with experimental hypertension. For this purpose, the number, size, and distribution of specific granules were studied by electron microscopy and the rough endoplasmic reticulum and Golgi lamellar complex in the ACM were studied in spontaneously hypertensive (SHR) and normotensive (WKY) rats of the control group.

EXPERIMENTAL METHOD

The experimental material consisted of the auricles of the right atrium of SHR (spontaneously hypertensive Kyoto-Wistar) and WKY (normotensive Kyoto-Wistar) rats of two age groups (Table 1). The animals were decapitated under ether anesthesia in the morning, material was immersed in a 2.5% solution of glutaraldehyde in phosphate buffer (pH 7.4), after which it was postfixed with osmium and embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB-III Ultrotome. The sections were stained with uranyl acetate and lead citrate [5, 7] and examined and photographed in the Hitachi IIE electron microscope (Japan) under standard magnification on the photographic plate of 6100; electron micrographs were printed under a standard magnification of twice (final magnification 12,200). In each case all cardiomyocytes having a nucleus regardless of in which plane the section was cut, and in no fewer than three ultrathin sections repaired from different areas of the auricle, were photographed. The panoramas of the cells were subjected to statistical analysis by means of the MOP-Videoplan computerized morphometric system, using standard statistical programs from Kontron (France).

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