STEREOLOGIC ANALYSIS OF ULTRASTRUCTURAL CHANGES IN CARDIOMYOCYTES IN ADRIBLASTINE-INDUCED CARDIOMYOPATHY

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Antibiotics of the anthracycline series have antitumor properties but also a marked cardiotoxic action [8, 11]. The mechanism of action of these antibiotics is linked with inhibition of protein synthesis in human and animal tissues [4]. However, the pathogenesis of the cardiotoxic effect of antibiotics of the anthracycline series has not been adequately studied. Elucidation of this problem is of great importance not only to place the pathogenetic prevention of adriamycin-induced cardiotoxicity on a firm basis, but also to shed light on the general rules governing the development of toxic lesions of the myocardium under the influence of many chemical factors. The only data to be found in publications describing ultrastructural studies of cardiomyocytes under the influence of anthracyclines are descriptive in character. Yet the study of quantitative parameters would help to give an objective assessment of the structural and functional state of the cell.

This paper describes a study of quantitative ultrastructural changes in cardiomyocytes of rats treated with a toxic dose of adriblastine (ADB), an antibiotic of the anthracycline series.

EXPERIMENTAL METHOD

Ultrastructural changes in the myocardium were studied in 15 male albino rats weighing 180-200 g, receiving ADB intraperitoneally in a single dose of 20 mg/kg (experiments of series I) and in fractional doses of 5 mg/kg once a week for 4 weeks (series II). Control animals received an equal volume of physiological saline by the same scheme. The animals were decapitated under chloroform anesthesia on the 5th day after the experiment. The papillary muscle of the left ventricle was used as the test material, and it was removed and fixed by the perfusion method [5]. Sections were stained with uranyl acetate and lead [7]. The test material was studied in the IEM-7A electron microscope. The quantitative characteristics of the structural components of the cardiomyocytes were obtained in a stereologic investigation of ultrathin sections [2, 6], followed by the use of a set of calculations by which absolute integral values for the organ as a whole could be determined [6].

EXPERIMENTAL RESULTS

By the time of decapitation the body weight of all the animals was reduced: in series I by 21.2%, in series II by 17%. The weight of the ventricles of the heart was 74.6 and 74.9% of the control, respectively. At autopsy signs of heart failure were found, similar to those described *in vivo* under the influence of other antibiotics of the anthracycline series [4].

Electron-microscopic investigations of the ultrastructural organization of the cardiomyocytes showed that in some heart muscle cells segregation, collapse, and fragmentation of the nucleoli had taken place and the granular components of the nucleoli could not be detected. These findings are evidence of a profound disturbance of protein synthesis in the cardiomyocytes. The pictures of focal degradation of cytoplasmic structures with the formation of lysosomes and autophagosomes were combined with lytic changes in the myofibrils. Lysis of myofibrils in individual cardiomyocytes in the experiments of series I was considerable and exposed the cytoplasmic matrix; the T system remained intact under these circumstances. Besides

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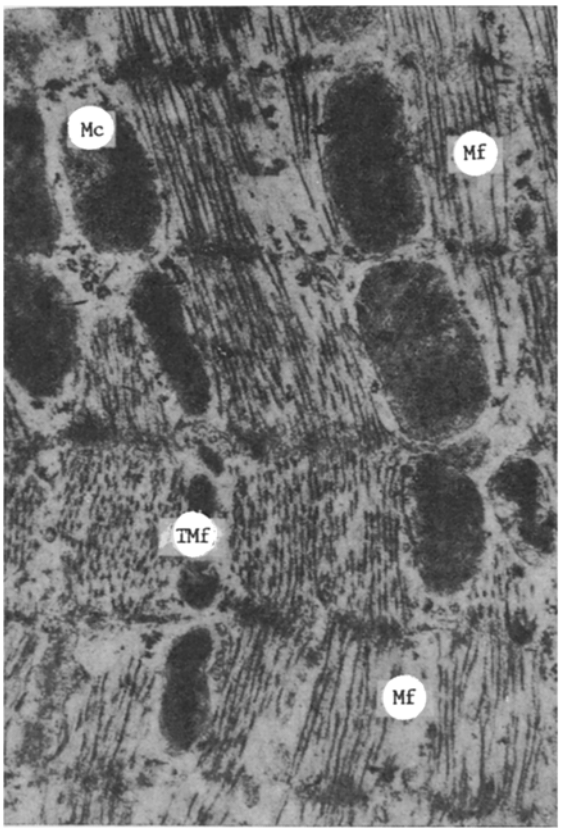

Fig. 1

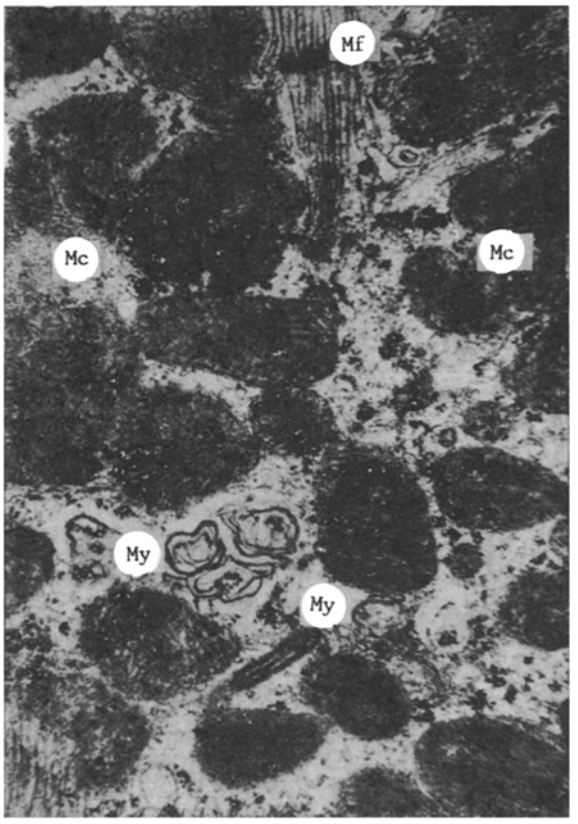

Fig. 2

Fig. 1. Changes in myofibrils in cardiomyocytes under the influence of ADB. Lysis of myofibrils (Mf), transversely oriented myofibrils (TMf). Mc) Mitochondria. 25,000 ×.

Fig. 2. Ultrastructural changes in cardiomyocytes under the influence of ADB. Concentration of myelin bodies (My), thinning and destruction of myofibrils (Mf). Mc) Mitochondria. 24,000 ×.

lytic changes in the myofibrils, examination of longitudinal sections revealed myofibrils with a transverse orientation (Fig. 1). Cardiomyocytes with swollen mitochondria, a translucent matrix, and fragmented cristae were frequently seen. Mitochondrial membranes were indistinct and showed partial lysis. In many sections collections of myelin bodies of different shape and diffuse myolysis were found (Fig. 2). The ultrastructural changes described in the cardiomyocytes of the rats in experiments of series I and II were identical, and in some cases differences were only quantitative in character.

The study of the stereologic parameters of structural components of the cardiomyocytes in rats in the experiments of series I and II compared with the control showed parallel changes in all absolute values characterizing the volume and the surface density of organelles (Table 1). The volume of the mitochondria was reduced compared with the control by 54.5 and 43.4%, respectively, in the experiments of series I and II. The surface area of these organelles in the experiments of series I was reduced by 45.5% and in series II by 40.7%. The ratio of the surface area of the mitochondria to their bulk density in the experiments of series I fell by 17.3%, evidence of an increase in size of the mitochondria. The volume of the myofibrils was the most demonstrative parameter, for it was considerably reduced compared with the control, indicating marked lysis and disturbance of regeneration of these structures. Accordingly the ratio of the volume of the mitochondria to the volume of the myofibrils was increased in experiments with a single injection of ADB (Table 1). The equivalent nature of the changes in volume of the cardiomyocytes reflects an adequate response of the muscle cells to an equal dose of the antibiotic, whether given all at once or fractionally.

Stereologic investigations show intensive structural changes in the cardiomyocytes and degradation of their ultrastructural components, consisting not of a simple reduction in size of the structures, but of a fundamental qualitative change. The mechanism of action of ADB,

TABLE 1. Results of Stereologic Analysis of Ultrastructures of Cardiomyocytes after Administration of ADB to Rats

Parameter	Control	Series I	Series II
Absolute total volume, mm^2			
of cardiomyocytes	474,4±6,96	286,1±8,16[a]	286±3,33[a]
of mitochondrion	133,5±3,26	87,4±1,02[a]	75,6±2,21[a, б]
of myofibril	280,8±3,86	151,7±6,62[a]	166±3,16[a]
Other structures of sarcoplasm	60,1±3,83	47,4±1,53[a]	43,9±2,13[a]
Absolute total surface density of mitochondria, $mm^2 \times 10^3$	645,6±20,03	355,2±22,0[a]	382,7±17,08[a]
Ratio of surface density of mitochondria to their bulk density, $mm^{-1} \times 10^2$	48,5±2,15	40,6±2,4[a]	50,5±1,33[б]
Ratio of bulk density of mitochondria to bulk density of myofibrils	0,48±0,015	0,58±0,023[a]	0,45±0,011

Legend. a) $P \leq 0.01$ compared with control, b) $P \leq 0.05$ compared with experiments of series I.

incidentally, is based on inhibition of protein synthesis in cardiomyocytes. However, the pathogenesis of the cardiotoxicity of the antibiotic evidently cannot be explained by this fact alone. According to data in the literature the toxic action of antibiotics of the anthracycline series may be linked with activation of lipid peroxidation (LPO) [10, 13]. Adriamycin, on entering the cell, functions as a pro-oxidant [10]. It has been shown that this antibiotic can undergo single-electron reduction *in vivo* with the formation of a semiquinone [9]. Diffuse myolysis of cardiomyocytes is known to be due to liberation of hydrolytic enzymes. Liberation of the latter is associated with LPO activation [3]. The formation of myelin-like structures also is connected with free-radical oxidation of membrane lipids [1].

On the basis of the data in the literature cited above and of our discovery of the character of ultrastructural changes in cardiomyocytes, it can be postulated that the pictures of concentration of myelin bodies, diffuse myolysis, and labilization of mitochondrial membranes can evidently be taken as morphological features of LPO activation.

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