EFFECT OF THE ANTHRACYCLINE ANTIBIOTICS RUBOMYCIN AND RUBOXYL ON MORPHOLOGY AND ELECTRICAL ACTIVITY OF THE RAT MYOCARDIUM

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Difficulties with the use of anthracycline antibiotics in the chemotherapy of malignant neoplasms are associated with their toxic side effects on heart muscle, leading to disturbance of centricular conduction and the development of cardiomyopathy and heart failure [5-7, 9]. The experimental study of new derivatives of the anthracyclines has shown that one of the most effective preparations of this series is ruboxyl, a nitroxyl analog of rubomycin; during its use, moreover, no significant ECG changes characterizing the cardiotoxic action of the anthracyclines were found [3].

The aim of this investigation was to compare morphological changes in the working tissue of the left ventricular myocardium of rats during chronic administration of rubomycin and ruboxyl and to compare the results with changes in contractile and electrical activity of the isolated papillary muscles in order to estimate the cardiotoxic action of these antibiotics.

EXPERIMENTAL METHOD

The rat is a convenient model with which to study the cardiotoxic action of anthracycline antibiotics [7, 8]. Both after a single dose and after chronic administration of Adriamycin and other anthracyclines in different doses, considerable changes are observed in working myocardial tissues, with histological characteristics similar to those found in man [8]. The toxic action of these preparations was studied during chronic administration to noninbred rats weighing 180-200 g. Rubomycin was injected intraperitoneally in a dose of 0.7 mg/kg daily for 30 days. Ruboxyl was given by the same scheme but in a dose of 3 mg/kg. Rats not treated with antibiotics served as the control. The rats were killed 8-11 weeks after the last injection and a papillary muscle was removed from the left ventricle in order to study contractile and electrical activity. For histological investigations the hearts were fixed at the same time in Bouin's fluid or Shaffer's mixture. Subsequent treatment of the tissue followed the standard method [2]. The force of isometric contractions of the papillary muscles, the transmembrane potential, and its derivative were measured by the method described previously [1]. The rubomycin used in the experiments were obtained from a "Mosmedpreparaty" and the ruboxyl was synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR.

TABLE 1. Effect of Rubomycin and Ruboxyl on Percentage of Pycnotic Nuclei and Parameters of Heart Muscle Function

Experimental conditions	Pycnosis of nuclei, %	Force of isometric contractions, mg	Maximal velocity of leading edge, (V_{max}), V/sec	Duration of AP at 2/3 of repolarization level, msec	Resting potential, mV
Control	0,40±0,11 (n=5)	231,0±17,5 (n=11)	111,2±3,9 (n=11)	31,4±2,4 (n=11)	−80,2±2,1 (n=11)
Rubomycin	31,20±0,85** (n=3)	72,5±8,1*; (n=9) 202,±12,5 (n=2)	68,8±8,2* (n=4)	42,5±7,9 (n=4)	−77,2±2,1 (n=4)
Ruboxyl	1,60±0,90 (n=6)	64,5±8,6*; (n=7) 204,3±17,9 (n=13)	112,3±6,9 (n=8)	37,1±6,9 (n=8)	−80,1±2,8 (n=8)

Legend. *$p < 0.05$, **$p < 0.01$ compared with control.

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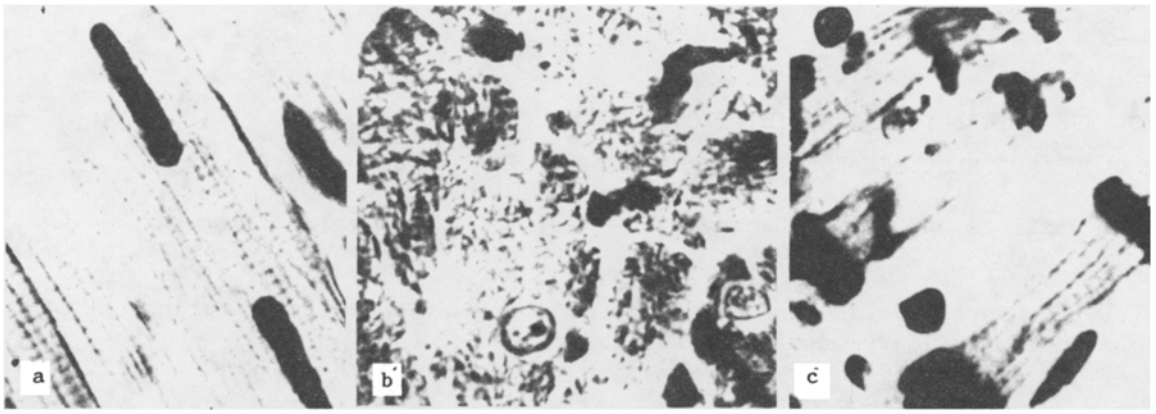

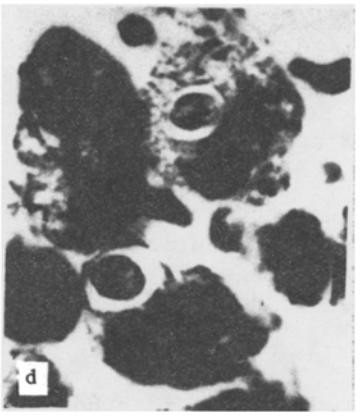

Fig. 1. Ultrastructure of rat myocardium after chronic administration of rubomycin. a, b) Intensification of chromatin coagulation in altered cardiomyocyte nuclei; c) contractural lesions and disintegration of myofibrils in myocardial muscle fibers; d) vacuolation of cardiomyocytes (transverse section). Here and in Figs. 2 and 3: hematoxylin and eosin, 900 ×.

EXPERIMENTAL RESULTS

The study of histological preparations of the heart from rats receiving rubomycin or ruboxyl for 30 days showed that the character of the morphological changes produced by these antibiotics differed, as also did their severity. Rubomycin had a distinct toxic action on the heart tissue. Zones of irreversible changes in the cardiomyocytes were found in the myocardium. The morphological signs of these changes were increased coagulation of chromatin, its concentration into coarse aggregates inside the nucleus, and widening of the zones of condensed chromatin (pycnosis; Table 1), possibly reflecting inhibition of nuclear synthetic processes. Changes were observed in the shape of the nucleus: in some areas of the myocardium the nuclei were greatly shortened (Fig. 1b), whereas in others they were lengthened and became rod-shaped (Fig. 1a). Like Adriamycin [4, 7, 8], rubomycin had a strongly destructive action on the contractile apparatus of the cardiomyocytes. Under the influence of rubomycin different zones could be distinguished within the cardiomyocyte: zones with strongly contracted or destroyed myofibrils and zones of homogeneous cytoplasm free from myofibrils (Fig. 1c). These changes were accompanied by vacuolation of the cardiomyocytes (Fig. 1d), the predominant feature of disturbances of myocardial tissue due to the toxic action of anthracycline antibiotics [7]. Besides the disturbances of cardiomyocyte morphology noted above, disturbance of the blood supply to the working myocardium, expressed as the formation of focal hemorrhages, must also be mentioned.

Unlike rubomycin, ruboxyl caused virtually no pathological changes in the morphology of the heart tissue. Just as in the heart muscle of the control animals (Fig. 2a, b) cardiomyocytes containing pale oval or slightly elongated nuclei were clearly visible in the rats receiving ruboxyl. The myofibrils were uniformly arranged throughout the sarcoplasm outside the nucleus (Fig. 3a, b).

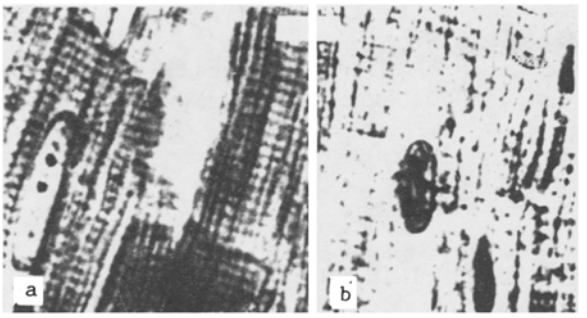

Fig. 2

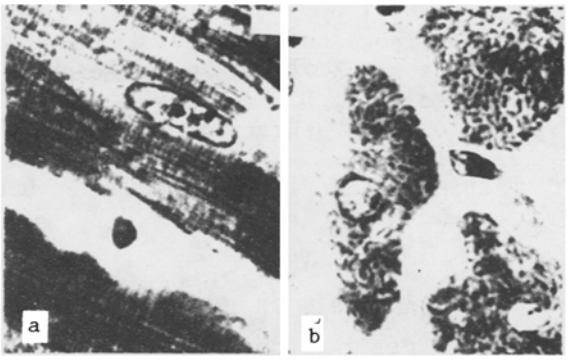

Fig. 3

Fig. 2. Ultrastructure of myocardium of control rats. Here and in Fig. 3: a) longitudinal section; b) transverse section.

Fig. 3. Ultrastructure of rat myocardium after chronic administration of ruboxyl.

The results of the morphological investigations of the myocardium correlated with changes in contractility and electrical activity of the isolated papillary muscles of the animals receiving rubomycin and ruboxyl (Table 1). In most of the muscles tested (9 of 11), isolated from the heart after chronic administration of rubomycin, the force of contraction was much weaker than in the control animals. Administration of ruboxyl caused less severe changes in contractility. Contractions close to the control values (204 ± 17 mg) were recorded in 13 experiments, and only in seven experiments was the amplitude of the contractions lower.

The changes observed in contractile function under the influence of these antibiotics were evidently connected with destruction of the contractile apparatuses themselves and disintegration of myofibrils, which was particularly marked under the influence of rubomycin.

The action of the antibiotics on electrical activity of the myocardial cells differs most clearly. As Table 1 shows, chronic administration of rubomycin reduced the maximal rate of rise of the leading edge of the action potential (AP) considerably (V_{max} = 68.8 ± 8.2 V/sec) and increased the duration of AP, but the reduction of the resting potential was not significant (only 3 mV). Under the influence of ruboxyl the first derivative of AP remained at the control level, and the increase in the duration of AP was not statistically significant.

The decrease in the first derivatives of AP, unaccompanied by membrane depolarization, suggests that the cause of the persistent disturbance of ventricular conduction described in the literature and characterizing the toxic action of anthracyclines [9] is inhibition of the inward fast Na current, of which $\dot{V}_{max}$ is the measure. Incidentally, depression of Na conductance correlated with the appearance of pycnosis of the nuclei during rubomycin administration. With ruboxyl pycnosis of the nuclei was not found, nor were there any changes in $\dot{V}_{max}$. Condensation of chromatin in the nuclei under the influence of rubomycin indicates inhibition of nuclear synthesis. This is in agreement with data in the literature on the action of anthracyclines as intercalating agents [4] and free radical inducers. In mitotically stable "adult" myocardial cells free-radical damage and intercalation of the antibiotic with DNA affect RNA-polymerase activity, and this leads to a disturbance of synthesis and to loss of structural proteins in the myocyte. Disturbance of integral Na-conductance, appearing only when a long time (1-2 months) has elapsed after the end of rubomycin administration, may therefore be due to inhibition of synthesis of channel-forming proteins and a decrease in the number of channels in the sarcolemma.

Correlation between the morphological and functional changes is evidence of a reduction of damage to the myocardium as a result of modification of the structure of the rubomycin molecule by introduction of a nitroxyl group.

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MORPHOMETRIC ANALYSIS OF THE TOXIC ACTION OF ETHANOL OF INTRAPHASE NUCLEAR CHROMATIN OF RAT HEPATOCYTES

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Alcohol-induced liver damage occupies an important place in the organic pathology of patients with alcoholism, but many biological aspects of this urgent medical problem have received little study [11, 13]. This applies in particular to the mechanism of action of ethanol at levels of structural and functional organization of the hepatocytes whose study has become possible only in recent years thanks to the appearance of computer-based high-resolution scanning photometric systems [2]. Among objects at this level, the most interesting in our opinion is the supramolecular organization of the interphase nuclear chromatin (INC) of the hepatocytes, structural modification of which is regarded as an evolutionarily consolidated method of genome regulation [12], damage to which is linked with the negative effect of pathogenic factors in the normal course of the life cycle of the cell [10]. However, instruments used for this purpose permit the accurate recording only of a set of quantitative parameters of structure of INC, whereas the degree to which the latter reflect relationships that exist objectively, and in fact determine the behavior of this complex and little-studied system, although hidden from direct observation, under conditions of exposure to ethanol is still unknown.

TABLE 1. Effect of Ethanol on DNA and RNA Content and Structural Parameters of Interphase Nuclear Chromatin of Rat Hepatocytes (in % of control, taken as 100%; M ± m, n = 10)

Morphometric parameter	Dose of ethanol, g/kg			
	4	6	8	10
DNA (nucleus)	110±1,6	81±1,8}	66±4,4	52±8,4
RNA (cytoplasm)	109±1,4	87±3,7	73±2,1	63±2,1
Diameter of chromatin granules (Ag)	118±6,8	116±5,4	118±5,5	124±6,9
Steepness of optical profile of granule (∇)	92±2,8	131±3,4	162±3,6	192±5,1
Integral optical density (mass) of granule (DgAg)	124±8,3	85±6,2	100±7,1*	118±6,9
Optical density of granule (Dg)	102±3,6*	71±2,4	83±3,4	100±3,2*
Height of granule (Hg)	120±7,2	86±5,4	89±6,3*	78±7,3
Distance between granules (M)	124±6,8	115±6,2*	134±7,1	121±7,0
Contrast of nucleus (K)	107±3,1*	116±4,0	95±2,3*	62±4,2
Optical density of pale regions of nucleus (D min)	93±2,1	63±3,7	82±3,3	105±2,3*

Legend. Asterisk indicates that differences are not significant; in all other cases $p \leq 0.05$ compared with control.

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