INJURY TO VENTRICULAR CARDIOMYOCYTES IN EXPERIMENTAL MASSIVE PULMONARY EMBOLISM

M. S. Tverskaya, V. V. Karpova, A. O. Virganskii, I. Zh. Satylganov, I. G. Saganelidze, and M. A. Chumakova

UDC 616.127-091+616.131-008.331-07

KEY WORDS: experimental massive pulmonary embolism; heart; injury to cardiomyocytes

In massive pulmonary arterial embolism (MPAE) the most serious hemodynamic disturbance is acute right ventricular failure. In order to understand. The mechanisms of its development it is essential to study injuries to ventricular cardiomyocytes, which are closely connected with structural and metabolic changes in the myocardium. The most sensitive methods of detecting metabolic injuries to the myocardium at the present time are polarization and electron microscopy [8].

The aim of this investigation was to study the state of the ventricular cardiomyocytes in acute experimental MPAE on the basis of parallel light-optical, polarization-microscopic, and electron-microscopic investigations.

EXPERIMENTAL METHOD

The investigation was carried out on 26 mongrel dogs weighing 15-20 kg under closed chest conditions and with natural breathing. Premedication consisted of intramuscular injection of trimeperidine (10 mg/kg); general anesthesia during the experiment was given by fractional intravenous injection of thiopental sodium (20 mg/kg). A description of the method of creating the model of acute MPAE, the plan of catherization, and methods of recording the hemodynamic parameters were all described previously [1]. Animals in which a model of MPAE was created were divided into two groups. Experimental group 1 consisted of 10 dogs in which no signs of circulatory failure were observed in the course of 6 h (compensated MPAE). Experimental group 2 consisted of 10 dogs in which MPAE was accompanied by the development of irreversible decompensation. The control group consisted of 6 dogs. Animals of the control and first experimental groups were sacrificed by intravenous injection of a lethal dose of thiopental sodium 6 h after the experiment began. Material for morphologic study was taken from the right ventricle (RV), left ventricle (LV), left anterior papillary muscle (APM), and fixed in 10% neutral formalin, buffered by Lillie's method, and embedded in paraffin wax. Sections 5-7 μ thick were stained with hematoxylin-eosin, by Van Gieson's method, with PAS reagent and amylase, and with Regaud's hematoxylin. The relative volume of the interstitial tissues and of injured and intact cardiomyocytes was determined in sections stained by Regaud's method with the aid of a television image analyzer (STZ-2M), using a special program of photometric analysis of histologic preparations [2]. To determine the character and degree of injury to the cardiomyocytes, polarization (MBI-15 instrument) and electron microscopy was used. Material for electron microscopy was taken from the subendocardial parts of RV and LV and fixed for 1.5 h in 2.5% glutaraldehyde, made up in phosphate buffer (pH 7.4). As well as by standard procedures, preparations of the specimens included incubation in 1% tannic acid solution after treatment in 1% OsO_4 solution, followed by dehydration of the tissue and embedding in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in the HU-12A transmission electron microscope ("Hitachi," Japan). The relative

N. I. Pirogov Russian National Medical University. (Presented by Academician of the Russian Academy of Medical Sciences V. S. Savel'ev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 9, pp. 319-322, September, 1992. Original article submitted February 26, 1992.

 0007-4888/92/0009-1364$12.50

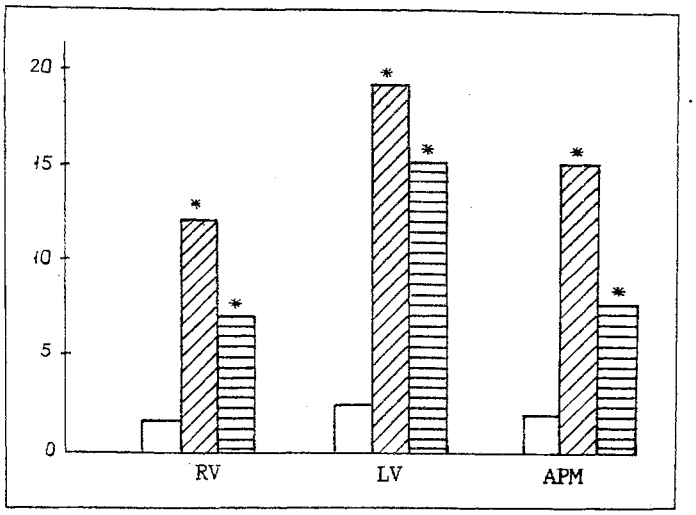

Fig. 1. Injuries to cardiomyocytes of ventricles in MPAE, revealed by staining by Regaud's method. Ordinate, relative volume of injured cardiomyocytes (in percent). Unshaded columns – control group; obliquely shaded – experimental group 1 (compensated MPAE); horizontally shaded – experimental group 2 (decompensated MPAE). *$p < 0.001$, differences compared with control group are significant.

volume of the myofibrils and of their injured areas was estimated stereologically on electron micrographs. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The control group was characterized by solitary foci of injuries, detectable on staining by Regaud's method, and occupying a small volume of cardiomyocytes of RV and LV (Fig. 1). Under these circumstances polarization microscopy revealed no pathological changes in the cardiomyocytes of the ventricles. According to the results of electron microscopy, the relative volume of the myofibrils was 58.1 ± 3.1% in RV and 48.3 ± 3.7% in LV, whereas in their injured areas the corresponding values were 1.0 ± 0.6% and 0.1 ± 0.1%.

In MPAE the relative volume of the injured cardiomyocytes, estimated histosterometrically, was much increased in both ventricles (Fig. 1). Examination of sections in polarized light and electron-microscopic study revealed significant differences in the character and degree of damage to the cardiomyocytes between the two experimental groups (Fig. 2).

In compensated MPAE injuries to the cardiomyocytes in both ventricles consisted (Table 1; Fig. 2c) of contractural changes of the I degree (increased anisotropy of the A-disks without shortening of the I-disks) and of the II degree (increased anisotropy and approximation of the A-disks, shortening of the I-disks), which are generally interpreted as reversible disturbances [8]. The reversible character of these injuries was confirmed by the absence of plasma seepage into the cardiomyocytes, revealed by the PAS reaction with amylase control. According to the results of the ultrastructural study, normal packing of unchanged myofibrils could be seen in the cardiomyocytes of RV and LV, and the relative volume of the myofibrils (51.0 ± 5.0% and 54.4 ± 1.8%) and of their injured areas (1.8 ± 0.6% and 1.1 ± 0.4%) did not differ significantly ($p > 0.1$) from the control value.

In cases when MPAE was accompanied by the development of decompensation, lesions of the ventricular cardiomyocytes consisted, not only of contractural changes of the I and II degree (Table 2; Fig. 2d), but also of contractural changes of the III degree (conglomerates typical of anisotropy were present), and also of cloudy swelling degeneratian of the myofibrils (disappearance of the cross striation, multiple masses of anisotropic substances). These injuries, which are interpreted as irreversible [8], were more marked in the myocardium of RV. Further evidence of

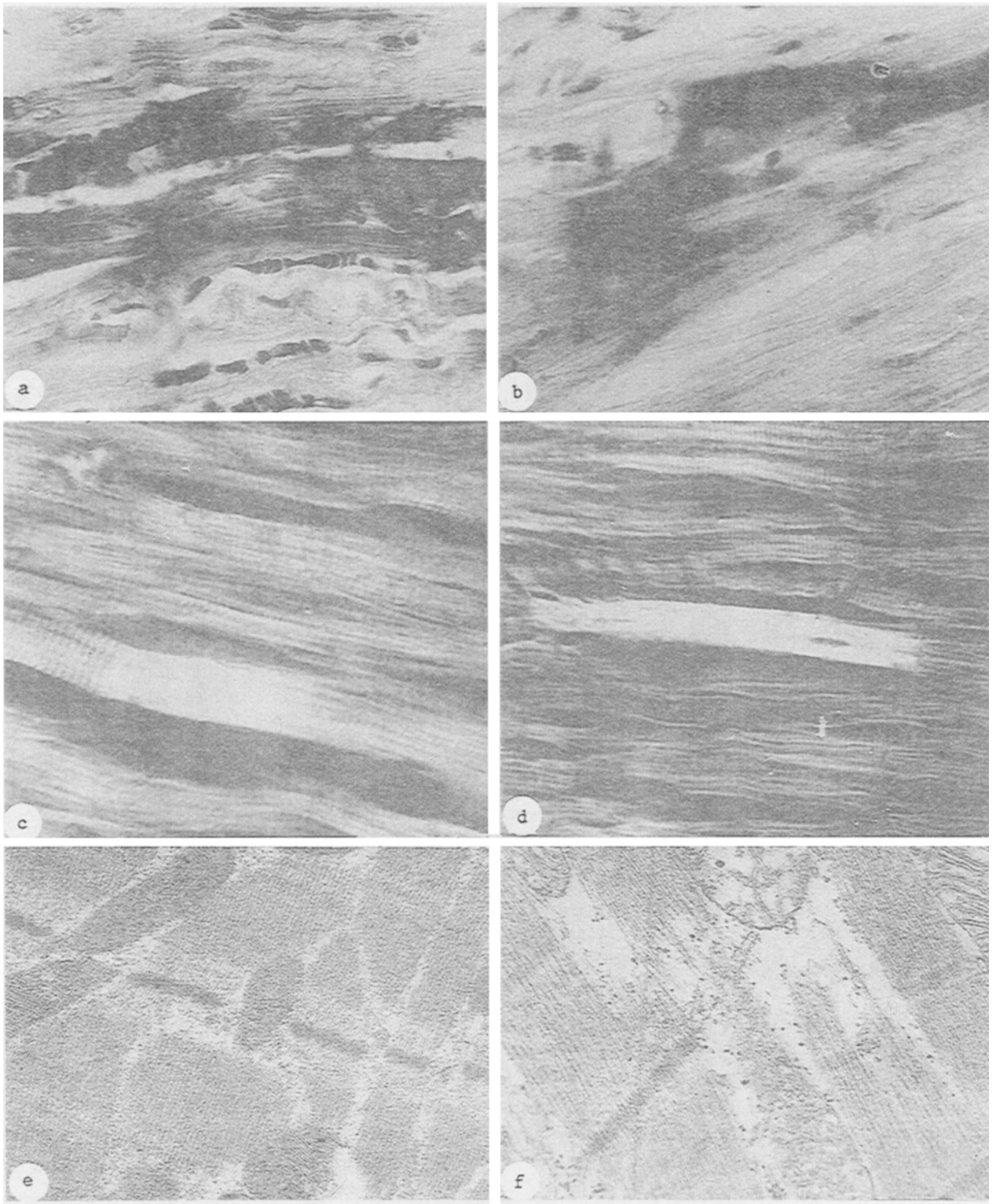

Fig. 2. Cardiomyocytes of wall of RV in compensated (a, c, d) and decompensated (b, e, f) MPAE. a, b) large foci of myocardial damage (stained with Regaud's iron-hematoxylin, 500×); c) contractural lesions of the I-II degree (stained with hematoxylin-eosin, polarization microscopy, 500×); d) contractural lesions of the III degree (stained with hematoxylineosin, polarization microscopy, 500×); e) normal packing of intact myofibrils and mitochondria (electron microscopy, 20,000×); f) partial destruction of myofibrils and cristae of mitochondria, intracellular edema (electron microscopy, 25,000×).

TABLE 1. Injuries to Ventricular Cardiomyocytes in MPAE Revealed by Polarization Microscopy

Injury	Severity of injuries					
	experimental group 1 (compensated MPAE)			experimental group 2 (decompensated MPAE)		
	ПЖ	ЛЖ	ПСМ	ПЖ	ЛЖ	ПСМ
Contractural changes of the I degree	++	++	++	+	++	+
Contractural changes of the II degree	+	+	+	+	++	+
Contractural changes of the III degree	—	—	—	++	+	+
Cloudy swelling degeneration of myofibrils	—	—	—	+	+	+
	—	—	—	—	—	—

Legend. −) lesions absent or mild; +) lesions moderately severe; ++) lesions severe.

the irreversible character of the injuries to the cardiomyocytes also is given by the positive PAS reaction with amylase control. The presence of plasma seepage into the cardiomyocytes also indicates a disturbance of permeability of their sarcolemma [8]. This confirms our previous suggestion [3] that the relatively low level of LVH in the ventricular myocardium in MPAE can be explained by escape of cytoplasmic enzymes from the cell due to increased permeability of its membranes. Electron-microscopic investigation revealed partial destruction of the myofibrils and widening of the interfibrillar spaces (Fig. 2f) in the cardiomyocytes of both ventricles. The results of stereologic analysis showed a decrease ($p < 0.001$) in the relative volume of the myofibrils (30.2 ± 3.1% and 33.5 ± 1.8%) and an increase ($p < 0.001$) in the volume of the injured myofibrils (8.4 ± 1.4% and 13.3 ± 1.4%) in RV and LV respectively. Hence, compared with compensated MPAE, in this particular case there were severe pathomorphologic changes in the ventricular cardiomyocytes indicating irreversible damage and a decrease in the number of contractile elements, which could be responsible for the development of heart failure in the animals of this experimental group.

The most common stage in the development of intracellular dystrophies of varied genesis is known to be a disturbance of energy metabolism [4], leading rapidly to a change in the state of the myofibrils, and consequently, this factor is considered to be a sensitive criterion of the early stages of metabolic injury to the myocardium [8]. The writers showed previously [6] that in MPAE accompanied by the development of heart failure, there is a marked decrease in activity of enzymes involved in catabolic reactions in the ventricular myocardium, and also a decrease in the number of mitochondria and an increase in the fraction of injured mitochondria. Thus the results of the previous investigation are in agreement with those now obtained, which demonstrates the more profound injuries of a metabolic character to the ventricular cardiomyocytes in cases of MPAE when irreversible decompensation develops.

A comparative study of pathomorphological changes in the myocardium of RV and LV reveals injuries to the cardiomyocytes that are on the whole similar in character and severity in these parts of the heart in MPAE accompanied by decompensation (Table 1). However, the relative importance of the possible damaging factors in the genesis of these changes is different for RV and LV. The relatively low activity of catabolic enzymes, including enzymes of cellular respiration, in the cardiomyocytes of RV, in the presence of a sharp increase in its hemodynamic load [1, 6] points directly to insufficiency of the energy supplying processes, and is evidently the leading cause of the development of metabolic injuries in the myocardium of this part of the heart. Pathomorphological changes in the myocardium of LV, in our opinion, are due mainly to the action of systemic pathological influences, the most important of which in MPAE are hypoxia and activation of the sympathicoadrenal system [5, 9]. The role of these factors in the genesis of the pathomorphological changes in the myocardium of LV is all the more important because according to data in the literature [7], left-ventricular cardiomyocytes are more sensitive to their damaging action than those of the right ventricle.

Thus in MPAE accompanied by the development of decompensation, marked irreversible injuries of a metabolic character, affecting the contractile system of the cardiomyocytes, take place in both ventricles. The presence of these injuries in the myocardium of RV, which works in MPAE under conditions of a greatly increased hemodynamic load, may be the cause of development of heart failure of right-ventricular type.

Material for the electron-microscopic investigation was provided by E. I. Tikhvinskaya.

REFERENCES

1. A. O. Virganskii, M. S. Tverskaya, and R. V. Rogulenko, Byull. Éksp. Biol. Med., **110**, No. 12, 577 (1990).
2. A. V. Zhukotskii, V. V. Kilinovskii, and L. V. Nemirovskii, Trudy II Mosk. Med. Inst., **86**, 123 (1978).
3. V. V. Karpova, M. S. Tverskaya, A. O. Virganskii, et al., Byull. Éksp. Biol. Med., **111**, No. 2, 130 (1991).
4. G. N. Kryzhanovskii, Arkh. Patol., **36**, No. 5, 3 (1974).
5. L. D. Makarova, N. A. Sergeeva, L. D. Chirkova, et al., Grudnaya Khir., No. 5, 30 (1989).
6. M. S. Tverskaya, V. V. Karpova, A. O. Virganskii, et al., Byull. Éksp. Biol. Med., **1**, No. 3, 327 (1992).
7. A. Hecht, Introduction to Experimental Bases of Modern Pathology of Heart Muscle [Russian translation], Moscow (1975).
8. Yu. G. Tsellarius, L. A. Semenova, and L. M. Nepomnyashchikh, Focal Injuries and Myocardial Infarction: Light, Polarization, and Electron Microscopy [in Russian], Novosibirsk (1980).
9. A. Ansari, Clin. Cardiol., **9**, 449 (1986).