DOI 10.1007/s10517-016-3363-9


# MORPHOLOGY AND PATHOMORPHOLOGY

# Ultrastructure of Cardiomyocytes and Blood Capillary Endotheliocytes in the Myocardium under Conditions of Experimental Mechanical Injury to the Heart

**V. P. Novoselov[1], S. V. Savchenko[1], A. N. Porvin[1], D. A. Koshlyak[1], A. P. Nadev[2], T. A. Ageeva[2], Yu. V. Chikinev[3], and A. S. Polyakevich[3]**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 161, No. 1, pp. 153-156, January, 2016
Original article submitted May 2, 2015

We studied ultrastructural changes in cardiomyocytes and blood capillary endotheliocytes in the ventricular myocardium in response to mechanical injury of the heart of varying severity in Wistar rats. Acute alterative changes in cardiomyocyte and endotheliocyte ultrastructure indicate impairment of the energy-producing, contractile, and protein-synthesizing functions of the cells after mechanical injury. These disorders play the key role in the development of acute contractile insufficiency of the myocardium in mechanical injury to the heart.

**Key Words:** *heart contusion; experiment; ultrastructure*

Most studies on heart pathomorphology are focused on myocardial injury in ischemia as a result of cardiovascular disease and toxic damage caused by chronic alcohol and narcotic intoxication [2-6,8,9,15]. Reports presenting morphological evaluation of thoracic injuries associated with myocardial damage are still rare and primarily performed at the light microscopy level [7,10,11,13,14]. It seems essential to carry out a morphological analysis of ultrastructural changes in cardiomyocytes and blood capillary endotheliocytes in the myocardium after mechanical injury (contusion) of the heart, because these changes play an important role in the development of acute contractile failure of the myocardium.

We evaluate ultrastructural changes in the mitochondria, ribosomes, sarcoplasmic reticulum, lysosomes, and glycogen of ventricular cardiomyocytes and in the mitochondria, ribosomes, luminal, free (cytoplasmic) and basal micropinocytotic vesicles of blood capillary endotheliocytes in ventricular myocardium after experimental mechanical injury of the heart of varying severity.

## MATERIALS AND METHODS

The study was carried out on Wistar rats ($n$=48; 180-200 g) bred under standard vivarium conditions on standard ration. The experiment was carried out under general anesthesia with diethyl ether. Immediately before the experiment, the animals under light ether narcosis were fixed on a special table in the supine position. ECG was recorded in standard leads using needle electrodes (LabLink complex, model V75-25A; Coulbourn Instruments), the data were processed by LabVIEW 5.1 software.

Mechanical injury to the heart was inflicted by dropping a load of 50 g from the height of 30 cm (free drop acceleration; contusion surface area 2 cm$^2$) onto the anterior chest surface at the site of maximum heart pulsation. Then, the animals were distributed

[1]Department of Forensic Medicine, [2]Department of Pathology, [3]Department of Hospital and Pediatric Surgery, Novosibirsk State Medical University, Novosibirsk, Russia. ***Address for correspondence:*** dr.serg62@yandex.ru. S. V. Savchenko

into two groups by the severity of mechanical injury to the heart according to ECG data. Medium-severe injury group consisted of 20 rats. The ECG manifestations of mechanical injury in these animals consisted of single and grouped ventricular extrasystoles and sinus bradycardia with gradual recovery of the normal rhythm. The animals were decapitated after heart rhythm recovery. Severe mechanical injury group consisted of 20 animals in which the above disorders in heart rhythm was followed (after 15-20 min) by ventricular fibrillation eventuating in asystole, after which the animals were decapitated. Control group consisted of 8 rats decapitated under deep anesthesia. The chest was opened directly after decapitation. The heart was isolated and fragments of the right and left ventricular myocardium were collected for studies.

Semithin sections (2-3 μ) were stained with toluidine blue. Myocardial sections were examined under a microscope in order to determine area for further ultramicroscopic studies. The myocardium was examined at ×100 and then at ×400 (Fig. 1).

After selection of appropriate sites in myocardial semithin sections, ultrathin sections (35-45 nm) were sliced on an LKB-880 ultratome and contrasted with water solution of uranyl acetate.

Transmission electron microscopy was carried out under a JEM 1010 electron microscope according to recommendations [12]. Ultrastructural organization of cardiomyocytes and blood capillary endotheliocytes of the right and left ventricular myocardium was studied.

Volume densities (Vv) of the cardiomyocyte mitochondria, ribosomes, sarcoplasmic reticulum, lysosomes, and glycogen and of blood capillary endotheliocyte mitochondria, ribosomes, luminal, free (cytoplasmic), and basal micropinocytotic vesicles of ventricular myocardium were characterized in electronograms (×8000) by the point method. The results were statistically evaluated with reference to previous data [1] by the ANOVA common linear model.

## RESULTS

Focal condensation of myofibrils (contracture strips) and slight fragmentation (dissociation) of individual myofibrils or their groups were observed in cardiomyocytes in medium-severe mechanical injury to the heart. In severe injuries, the myofibril fragmentation was pronounced: ruptures of myofibril groups. The mitochondria varied in sizes, were enlarged or shrank. However, quantitative analysis showed a trend to an increase of their total volume density. In medium-severe cardiac injury, the gaps between the cristae were dilated, the cristae were often deformed, sometimes with solitary ruptures (Fig. 2). In severe injury, clear foci because of edema sometimes occupied almost the total mitochondrial area. In addition, the number of crist ruptures was higher, they were sometimes partially and completely homogenous.

The number of ribosomes in cardiomyocytes was significantly lower in mechanical cardiac injury in

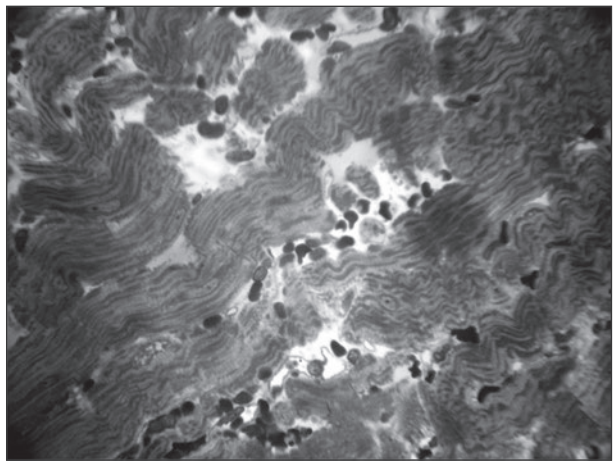

**Fig. 1.** Unevenly stained changed myofibrils (wave-like pattern) in severe heart contraction. Toluidine blue staining, ×400.

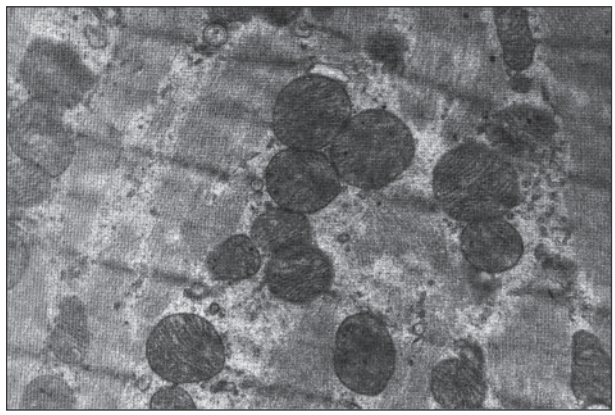

**Fig. 2.** Variability of mitochondrial size, crist deformation and ruptures in medium severe contusion of the heart. Electronogram, ×8000.

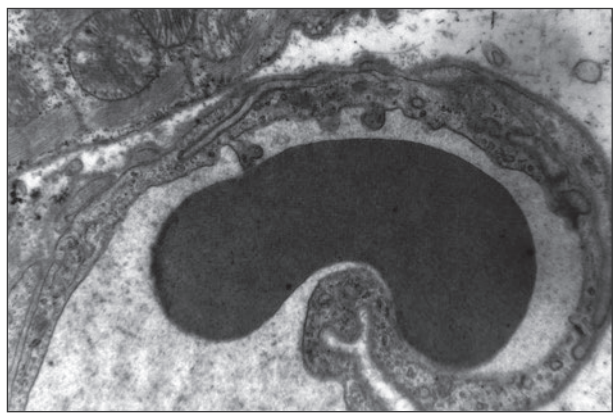

**Fig. 3.** High plication of endotheliocyte luminal surface with formation of microvilli and clasmatosis, sharp dilatation of pericapillary space in severe contusion of the heart. Electronogram, ×8000.

comparison with the control. The volume density of the endoplasmic reticulum increased, vesicles were dilated, and large vacuoles emerged in the cardiomyocyte hyaloplasm, making it look perforated. Lysosomes, virtually not found in cardiomyocytes of control animals, were significantly more numerous. The number of glycogen granules sharply decreased and in some areas they disappeared completely in comparison with glycogen level in control.

The volume density of vesicles increased in myocardial blood capillary endotheliocytes, depending on the severity of heart injury. The volume density of organelles in myocardial capillary endotheliocytes was higher in the left *vs.* right ventricle in cardiac injury. In control animals the volume density of micropinocytotic vesicles in myocardial capillary endotheliocytes was greater by the basal surfaces of cells. The differences in the volume densities of micropinocytotic vesicles concentrated by the basal and luminal surfaces of endotheliocytes in ventricular myocardial capillaries tended to be less manifest in medium severe injuries. In severe injuries the volume densities of micropinocytotic vesicles, concentrated by the luminal surfaces of myocardial capillary endotheliocytes were higher than the volume densities of micropinocytotic vesicles located at the basal surfaces of endotheliocytes.

Mechanical cardiac injury was associated with high plication of the endotheliocyte luminal surface, up to formation of microvilli. Severe injury was associated with clasmatosis – rupture of endotheliocyte microvilli from the body of the cells (Fig. 3). Quantitative analysis showed an increase of the mitochondrial volume density in mechanical injury to the heart. The structural changes in endotheliocyte mitochondria were similar, with changes in the cardiomyocyte mitochondria. In addition, the numbers of ribosomes and glycogen granules in endotheliocytes decreased significantly in comparison with control.

The findings indicated that mechanical injury (contusion) of the heart was associated with an increase in the volume densities of mitochondria, sarcoplasmic reticulum, and lysosomes and a decrease in the volume densities of ribosomes and glycogen in ventricular cardiomyocytes. In ventricular myocardial blood capillary endotheliocytes the volume density of mitochondria and micropinocytotic vesicles increased and the volume density of ribosomes decreased. The degree of these disorders depended on the severity of mechanical injury to the myocardium. Acute alterative changes in cardiomyocyte and endotheliocyte ultrastructure indicated a reduction of the energy-producing, contractile, and protein-producing functions of the cell in response to mechanical injury. It seems that these disorders played the key role in the development of acute contractile insufficiency of the myocardium in mechanical injury to the heart.

## REFERENCES

1. G. G. Avtandikov, *The Basics of Quantitative Pathology* [in Russian], Moscow (2002).
2. L. V. Kaktursky, *Sudden Cardiac Death (Clinical Morphology)* [in Russian], Moscow (2000).
3. L. V. Kaktursky, Clinical morphology of alcoholic cardiomyopathy, *Arkhiv Patol*., **71**, No. 5, 21-22 (2009).
4. L. V. Kaktursky, Primary (idiopathic) cardiomyopathies, *Arkhiv Patol*., **73**, No. 3, 18-20 (2011).
5. L. B. Mitrofanova and Kh. K. Amineva, Macroscopic and Organometric Analysis of Heart in Pathology: Guidelines for Physicians, Ed. G. B. Koval'sky [in Russian], St. Petersburg (1998).
6. L. M. Nepomnyaschikh, Morphogenesis of Principal General Pathological Processes in the Heart [in Russian], Novosibirsk (1991).
7. V. P. Novoselov, S. V. Savchenko, E. A. Romanova, and V. G. Tsimmerman, *Pathomorphology of the Myocardium in Heart Contusion* [in Russian], Novosibirsk (2002).
8. V. S. Paukov and Yu. A. Erokhin, Pathologic anatomy of hard drinking and alcoholism. *Arkhiv Patol*., **66**, No. 4, 3-9 (2004).
9. V. S. Paukov and L. V. Kaktursky, *Pathology*, Eds. M. A. Pal'tsev, V. S. Paukov, and E. G. Ulumbekova [in Russian], Moscow (2002) pp. 202-254.
10. S. V. Savchenko, Current issues of the expert opinion about heart morphology. *Vestn. Sudeb. Med*., **1**, No. 3, 5-8 (2012).
11. Yu. I. Sosedko, Forensic diagnosis of cardiac injuries in blunt trauma, *Sud.-Med. Ekspertiza*, No. 6, 13-17 (2001).
12. B. S. Weakley, *A Beginner's Handbook in Biological Electron Microscopy* [Russian translation], Moscow (1975).
13. V. V. Yurasov, A. I. Filatov, D. A. Koshlyak, and M. V. Rassadovsky, Case of undiagnosed bruising of the heart in blunt closed trauma of chest. *Vestn. Sudeb. Med*., **1**, No. 2, 44-46 (2012).
14. M. Elie, Blunt cardiac injury. *Mt Sinai J. Med*., **73**, No. 2, 542-552 (2006).
15. A. J. Ruttenber, H. D. Kalter, and P. Santinga, The role of ethanol abuse in the etiology of heroin-related death. *J. Forensic Sci*., **35**, No. 4, 891-900 (1990).