

КЛИНИЧЕСКИЕ ДИСЦИПЛИНЫ

THE STRUCTURAL-FUNCTIONAL CHARACTERISTICS OF MYOCARDIUM IN THE EXPERIMENTAL CRUSH SYNDROME

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The investigation of the pathogenesis mechanisms and the morphological manifestations of crush syndrome are still actual. There is still a persistent likelihood of occurrence of the situations that can possibly lead to the given pathology: the lesions as a rule gain an incremental character, and what is more, the developing crush syndrome in such cases occurs in its heaviest form.

The exploited model of the experiment has enabled to mitigate CS; the lighter form of the syndrome has made the development of a more sustainable model of CS possible, which is proved by the morphological changes of myocardium.

Key words: crush syndrome, myocardium

The syndrome of long-term compression (SLC), crush syndrome are not the only the terms used to name the heavy pathology occurring in different hazards and casualties; such as earthquakes, wars, mine collapses, explosions, timber-cutting, terrorist attacks [6].

The likelihood of natural hazards and catastrophes is still high, hence the issue of investigating CS has not only retained its topicality, but also has been ranked as a top priority in the face of the Earth's escalating seismic activity and the strengthening effect of anthropogenic factors [4].

The ruinous Spitak's earthquake of 1988 incurring numerous human casualties has made the problem more than actual for the Republic of Armenia. Furthermore, the investigation of mechanisms and ultrastructural properties of damaged inner organs in case of CS has become one of the most important goals of both Theoretical and Practical Medicine of Armenia.

Nowadays works on the experimental modelling of CS with a subsequent investigation of the dynamics of microscopic and ultrastructural changes of various organs are not numerous.

Because of the complexity of the given disease's clinical picture, unfortunately often resulting in lethal outcome, the establishment of a meticulous profile of CS manifestations in different organs and tissues has gained utmost importance.

Materials and Methods of Investigation. The experiment was carried out on out-breed viripotent male rats of 130—150 body mass. Their CS was triggered with the compression of a pelvic limb with a special setting [1]. The surface of the compression of the inner pelvic limb comprised 2,14 sm, the strength of the compression — 150 kPa. The exposition took 1 hour. The material for the investigation was taken on the 7th and the 30th days after decompression, i.e. in the mid- and post-periods of the CS. Evidently the severity level of CS was assumed as light, as we did not witness lethal outcomes even in a month after the decompression.

4 groups with 6 animals in each were experimented in the given in research.

Material extraction was carried out *in vivo*. The animals were dosed with ether anaesthesia to the point when they lost their corneal reflex.

The material was fixed in 10% formalin for the photooptical investigation and was then moulded in paraffin. The patterns were stained with the usage of haematoxylin and eosin method. The examination of the histostructure of organs was carried out with photomicroscope.

The fragments of organs of 1mm were fixed in 2% p-D Glutaraldehyde on 0,2 M cacodylate buffer (pH 7,4) in the temperature of 40 °C within 2 hours for electric microscopic examination. The dehydration of the material was done in acetone of increased concentration. The moulding was performed with Epon Araldite in accordance with Mollenhauer (1964). Semi-thin and ultra-thin fragments were prepared on ultratom LKB (III type, Sweden). The semi-thin fragments were stained with Pyronin and Toluidine Blue and were examined with photomicroscope. The ultra-thin slices were contrasted in lead quotes according to Reynolds and were studied with electric microscopes JEM-IOOB and JEM-100CX.

Investigation 1. Cardiotoxicity. The investigation was carried out on tissue culture of contracting myocardial chick embryo explants (the number is given in tables). They were dissected in 1—2 mm slices from 6—8 days-old chick embryo hearts and were cultivated in Plexiglas in a shape of a washer of 3,1 cm³ on a coverslip in “Needle” sphere in the temperature of 37 °C. An hour later the experiment was in the stage, when the testing serums and tissue extracts of animals with CS were placed in the cameras. The registration of the contraction parameters of explants was done with electrocardiograph in accordance with photoelectric principle of registration. 30minutes after the beginning of the cultivation the explants started to contract rhythmically in the frequency of 90—120 beats per minute. The testing materials (with the volume of 0,1 ml) were placed in cameras and were examined in terms of amplitude, frequency and rhythmic contractions within 10 m.

Table 1

Cardiotoxic activity of blood serum and extracts of animal organs with CS

Animal groups		Period of material extraction	Number of contractions per m	
			Blood Serum	Heart extract
CS	(n = 15)	7 days after decompression	14,2 ± 1,1	21,5 ± 2,1
CS	(n = 14)	30 days after decompression	33,6 ± 3,2	44,2 ± 3,5
Intact control	(n = 25)	—	90,5 ± 6,1	

Investigation 2. Histostructure of Myocardium. The whole digital material accumulated from the immunological investigation was statistically processed in accordance with Student's and Fisher's criteria.

Results and their Discussion. The peculiarities of histological and submicroscopic organisation of myocardium in month's time period right after the decompression.

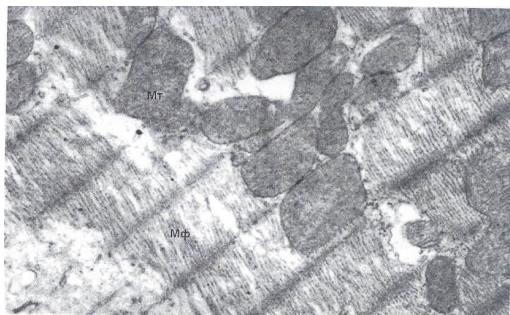
The interim period of the CS (on the 7th day after decompression).

The microscopic examination of myocardium reveals the mosaic character of the damage in retractive cardiomyocytes (Cm). Along with survived cells, Cms with no banding patterns and homogenising sarcoplasm are detected. Muscle fibres are damaged, in some areas — disconnected and positioned as separate necrotic fragments. The cardiac myocyte nucleuses manifest signs of deprivation with chromatin and look vesicular. The vessels of arterioles are full-blooded; staza of erythrocytes is visualised in the capillaries. A homogeneous mass of pale-pink colour is identified in the intercellular junctions, i.e. connective tissues around the blood vessels.

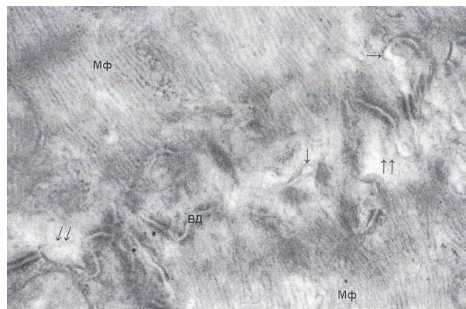
On the 6th day after the decompression the electromicroscopic examination exposes signs of intercellular Cm edema, particularly more vivid in the areas surrounding the nucleus. Nucleuses of most of the Cm possess scalloped outlines; the pANCA tank is sometimes expanded, the chromatin is mostly diffused. Granular component usually dominates in 2—3 nucleuses.

The organelles are characterized with homogeneous structure. Most of the myofibril in Cm are in parallel positions and have sarcomeren organisation. However many of them are characterized with an underpressure of myofilaments and are not quite multidirectional in the sarcomere and with disappeared clear A-, I-disks and M-lines. Besides local contractura of myofibril, destruction of hearth of sarcomeres, blurring Z-lines, damage and extinction of myofilaments are detected (pic. 1).

The characteristic features detected in the areas of crimped intercalated disks are reflected in the destruction of hearth of adjoining terminal plasmolemmas, local divergence between the membranes of intercalated disks, as well as the disconnection of myofibril, that is obviously the consequence of intracellular edema (pic. 2).



Picture 1. The electric microphoto of the fragment of cardiomyocyte of the left stomach on the 7th day after decompression. Destruction of sarcomeres of myofibrils (Mf) on the background of intracellular edema. Mt — mitochondria. Zoomed by 20200



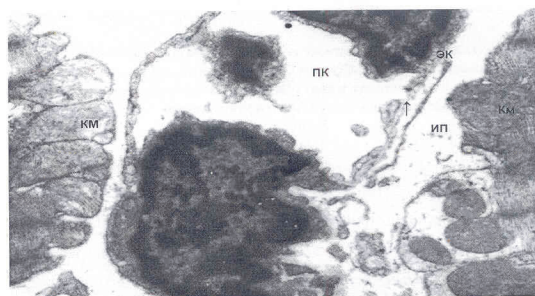
Picture 2. Electric microphoto of the fragment of myocardium of the left stomach on the 7th day after the decompression. Display of the intercalated disk (ID) between the two. Mf — myofibril. The local expansion between the membranes are indicated with arrows; disconnection of Mf from membranes are pointed with arrows. Zoomed by 24000

Moreover, the detected hypertrophied forms of contractile organelles reflect certain intracellular regenerating processes.

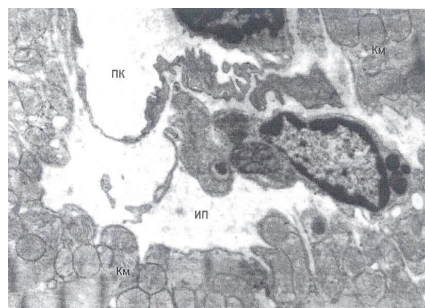
The interstice of myocardium usually looks edemic on the 7th day after the decompression, particularly in pericapillary areas. As for the areas in close distance to the capillaries in Cm, dystrophic changes are precisely manifested there and the changes are expressed in focal contractura of myofibril, destruction and lysis of myofilaments, swelling of mitochondria. These observations raise interest in relation to the well-known factor of blood serum toxicity in case of CS and can obviously testify for the prominence of the toxicity factor's role in myocardium damage.

The wall of blood capillaries is often thinned and with defects, particularly occurring in the gapping between endotheliocytes (pic. 3). The basal membrane of endothelium is sometimes discontinuous or does not exist at all.

The characteristic features for the given stage are intussusception of thinned areas of endotheliocytes in pericapillary areas (pic. 3). Deformed blood capillaries with folded endothelial are occasionally detected.



Picture 3. The electric microphoto of the fragment of cardiomyocyte of the left stomach on the 7th day after decompression. Gapping between endothelial cells (EC) — pointed out with arrows. Pellucidity of capillaries (PC); cardiomyocytes — Cm; interstitial space (IS). Zoomed by 15000



Picture 4. Electric microphoto of the fragment of myocardium of the left stomach on the 7th day after the decompression. Detection of the protrusion of the thinned area of endothelial cells (EC) to the pericapillary space. (PC) — protrusion of capillaries; cardiomyocytes — Cm; interstitial space (IS). Zoomed by 10500

The above-mentioned signs are undoubtedly testifying about the growth of permeability haematopoietic lineages in myocardium, which results in intercellular edema, which in its turn is a consequence of intracellular edema and dystrophy of Cm.

Thus, both on the 7th day of decompression in lab animal myocardium and in the earlier stages CS [3; 8] destructive signs are observed in almost all components of myocardium, which is compliance with the results of other investigators.

On the 7th day after decompression submicroscopic examination of myocardium has revealed various manifestations of pathology of cells on the level of ultrastructures, the fundamentals of which were defined by A.P. Avtsin and V.A. Shahlamov (1979); particularly the identified changes concerned both Cm and the elements of interstitia and first and foremost endothelial walls of blood capillaries [2].

The latest period of crush syndrome (on the 30th day of decompression). Even in a month's time after the decompression normalization of submicroscopic structure

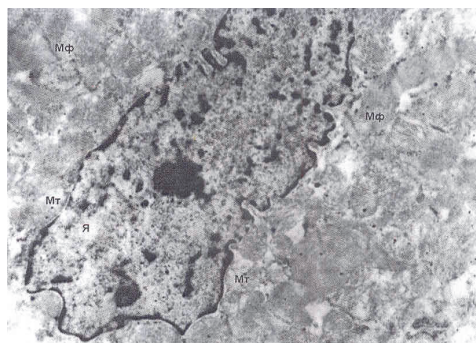
of myocardium does not occur. In most Cms intracellular edema is detected. Some of the observed Cms show signs of hydropic {vacuolar} degeneration (pic. 5).

The nucleus of such cells is prone to pycnosis and rhexis. The myofibrils lose their sarcomere organisation, Z-lines are not identified. Myofilaments for myofibrils are homogenized and form thick grassy turf masses.

The changes of contractile apparatus are varied — from contractual damages to disintegration of myofibril into separate sarcomeres with total destruction of myofilament hearths (pic. 6).



Picture 5. Electric microphoto fragment of cardiomyocyte of the left stomach on the 30th day after decompression. Vakuolige Dystrophie of cells are demonstrated. N — nucleus; Mt — mitochondria; Mf — myofibrils. Zoomed by 13500



Picture 6. Electric microphoto fragment of cardiomyocyte of the left stomach on the 30th day after decompression. Depravation of myofibrils is demonstrated (Mf), as well as destructive changes of mitochondria (Mt) in circumnuclear areas. N — nuclear. Zoomed by 13500

The inhomogeneity of the morphological picture is quite depictive. Thus, along with Cm, the latter being in state of dystrophy and necrobiosis, heart muscle cells with characteristic signs of compensatory intracellular regeneration are detected showing features of myofibrillar hypertrophy and mitochondria. A month after decompression the examined organ retains some features of interstitial edema, various deformations and dystrophic changes in the tissue elements of microvasculature.

The examination of capillary network reveals the phenomenon of psuedo-angiomatosis which is a consequence of the tortuous course of capillaries, and possibly neoplasms. The thin capillary wall in weak zooming is presented in structureless electron-dense layer as a result of endotheliocyte cytoplasm seal. Capillary wall breaks are not rare. In some exceptional cases sarcolemma disruptions occur that inflict organelle's penetration into interstitial space.

The mentioned changes testify for the retention and even deterioration increased capillary permeation, and that probably supports the detention of dystrophic changes in the muscle tissue and heart stroma possibly because of the penetration of toxic substances from plasma.

Thus, in a month's time after the decompression normalization of submicroscopic structure of myocardium does not occur. Cm nuclear changes, as well as transformations of energy system and contractile machinery are detected then; and even more some get deeper in their transformation. For example, Cm features nuclear pycnosis with a de-

veloping sarcoplasm necrotic change. It makes sense highlighting, that the identified long-term (within a month after the decompression) persistent and destructive changes of Cm in case of the experimental CS are in full compliance with the data accumulated by clinicians, that cover a considerable amount of time (three months and more) needed for recovery from the effects of CS, of course on the condition that there should be no lethal outcome in its early stages [8]. One of the prime factors resulting in greater damage of myocardium is undoubtedly toxemia [6]; the considerable role in the development of destructive processes because of biomembrane damage is assigned to lipid peroxidation, inevitably developing in case of oxygen suffocation of tissues [8].

Conclusions

1. The tested model of limb compression of lab animals (rats) can be recreated, and can trigger development of light form of crush syndrome, which is characterised with a manifestation of the main pathogenic bundle of the given disease, such as toxemia, multiple organ dysfunction syndrome, and immunodeficiency.

2. On the 7th day of decompression, i. e. in the midperiod of crush syndrome, the myocardium ultrastructure of the left stomach of the lab animals is characterised with destructively affecting changes in cardiomyocyte and vascular endothelium, as well as with morphological features of increased hemocapillary permeability.

3. The ultrastructure signs of damage of cellular and tissue structures are more prominent in contact areas of blood capillary walls, which proves the significance of toxemia factor in the development of cellular pathology in case of extreme crush syndrome.

4. On the 7th day after decompression submicroscopic organization of the examined organs identifies a low intensity level of intracellular regenerating processes.

5. The ultrastructural changes, supposing morphological subtract of multiple organ dysfunction syndrome (for example in the examination of myocardium), are more vivid in midperiod and do not vanish in later stages, persisting at least till the 30th day after decompression on the backstage of the morphological manifestations of intracellular reparative processes.

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СТРУКТУРНО-ФУНКЦИОНАЛЬНАЯ ХАРАКТЕРИСТИКА МИОКАРДА ПРИ ЭКСПЕРИМЕНТАЛЬНОМ КРАШ-СИНДРОМЕ

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Исследование патогенетических механизмов и морфологических проявлений краш-синдрома остается актуальным. Постоянно существует вероятность возникновения ситуаций, которые могут привести к данной патологии; само поражение носит, как правило, массовый характер, а развивающийся при этом краш-синдром представляет собой один из самых тяжелых видов приобретенных заболеваний.

Использованная в эксперименте модель позволила вызвать нетяжелую форму КС, которая дала возможность получения состоятельной модели КС, что подтверждено морфологическими изменениями миокарда.

Ключевые слова: синдром длительного сдавления, миокард

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