ULTRASTRUCTURAL CHANGES OF CARDIOMYOCYTES IN POSTINFARCTION VENTRICULAR REMODELING

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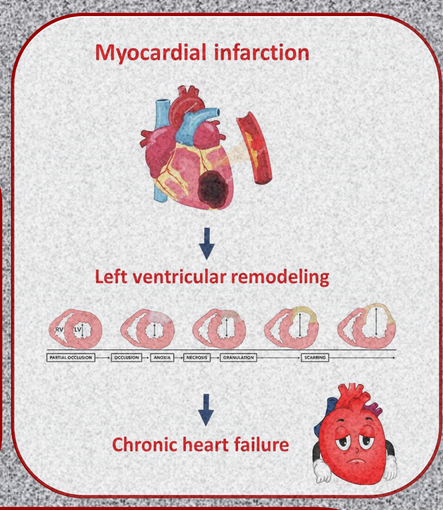
ABSTRACT. Ischemic lesions of the heart, including myocardial infarction, are the most common pathologies of the human cardiovascular system. After the acute stage of disease necrotic cells are replaced by scar tissue, rearrangement of heart structure and function occurs and the disease becomes chronic. Post-infarction left ventricular remodeling occurs at different levels of system organization and allows a certain degree of cardiac function compensation. This process involves cellular and molecular mechanisms beginning days after myocardial infarction and persisting for weeks and months after the initial insult both at the site of infarction as well as in the surviving unaffected areas. Currently, no effective therapies exist for this ischemic complication, and the mechanisms driving left ventricular dilatation during chronic post-infarction remodeling remain poorly understood. Therefore, in our study we explored ultrastructural changes of cardiomyocytes in the intact and border zones of post-infarction myocardium at chronic stages of the disease on rat model.



METHODS

Experiments were carried out on adult Wistar rats (4 months old, 250–300 g). Animals were divided in two groups*: control rats* and *rats with myocardial infarction (MI)*. Myocardial infarction was caused by permanent ligation of the left coronary artery. In order to evaluate the effectiveness of ligature imposition, standard 3-lead electrocardiogram was carried out *(Fig.1)*. Probes were collected 2 and 26 weeks after the surgery. Left ventricular ultrathin sections of control and from the *border (b)* and *intact zone (int)* of infarcted myocardium *(Fig.2)* for electron-microscopic studies were obtained with ultratome-LKB III (Sweden), and viewed with electronic microscope LIBRA 120 Carl Zeiss (Germany). Morphometric analysis of obtained pictures was made using ImageJ program *(Fig.3)*. Myocardial infarction severity was evaluated after animal autopsy visually and on histological sections of the heart *(Fig. 4, 5)*.

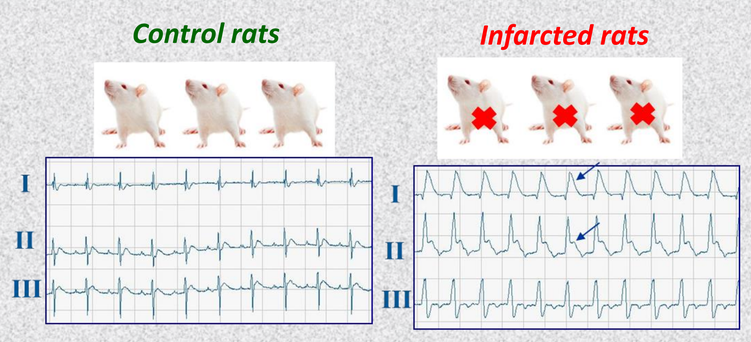


Fig. 1. *ECG of the rat before coronary occlusion (left) and 10 min after (right). I, II, III – standard leads; ↓ – ST-segment elevation. ST-segment elevation after coronary occlusion indicates the occurrence of ischemic changes in the myocardium.*

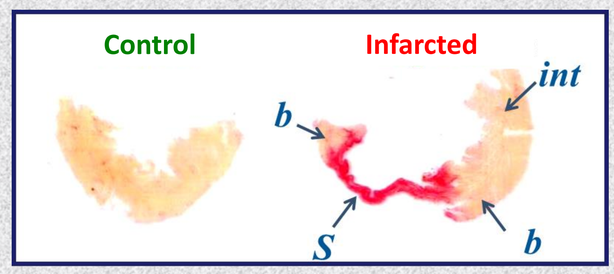


Fig. 2. *Left ventricular transverse histological sections of control and infarcted rats 26 weeks after surgery. Picrosirius red staining. Scar is red stained, and cardiac muscle tissue is yellow. S – scar, b - border zone, int - intact zone.*

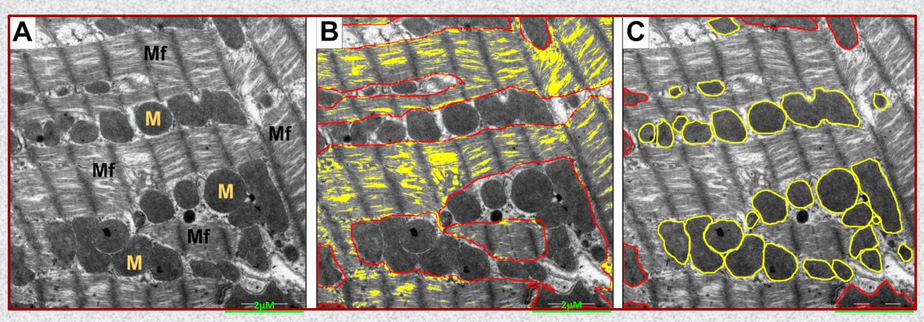


Fig. 3. *Examples of compartment selection, TEM. A. Representative section of CMC showing myofibrils (Mf) and intermyofibrillar mitochondria (M). B. Image from section A with red outlines of the myofibril regions and yellow shading of intermyofibrillar empty space subtracted from myofibril regions to calculate myofibrillar density. C. Mitochondrial area (yellow) and mitochondrial volume fraction determination (yellow +red). Scale bar – 2 μM.*

RESULTS

Morphometric analysis of myofibrils, mitochondria, and excitation-contraction coupling structures of cardiomyocytes (CMC) revealed similar remodeling processes in both zones at 2 weeks post-infarction, characterized by decreased myofibril density, reduced mitochondrial area and contacts between T-tubules and sarcoplasmic reticulum. However, at 26 weeks post-infarction, we observed increased mitochondrial area, increased volume fraction of intermyofibrillar mitochondria, and altered morphology of mitochondrial cristae without a reduction in T-tubule and sarcoplasmic reticulum contacts. This data highlight factors contributing to ventricular dilatation and may inform the development of new therapeutic strategies to delay heart failure progression.

Structural and histological changes of left ventricular after coronary occlusion.

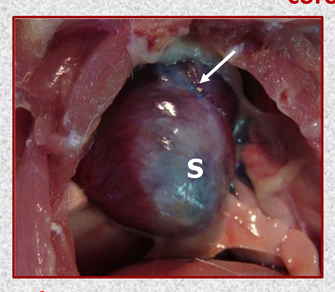


Fig. 4. *Rat heart 26 weeks after coronary occlusion. S - scar, ⇩ - place of ligature.*

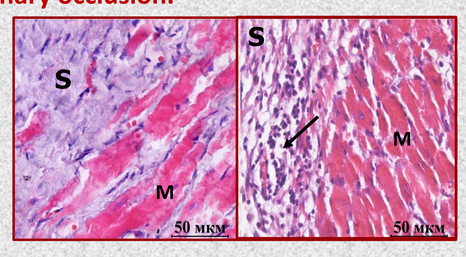
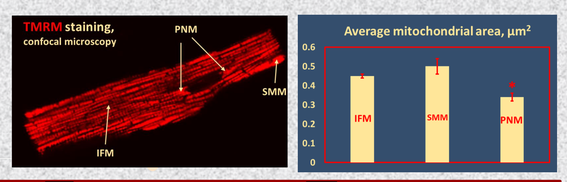


Fig. 5. *Histology sections of rat left ventricular 26 weeks after coronary occlusion. S – scar, M - cardiac muscle tissue. ↓ - leukocyte infiltrate. Hematoxylin and Eosin staining*

Mitochondrial subpopulations



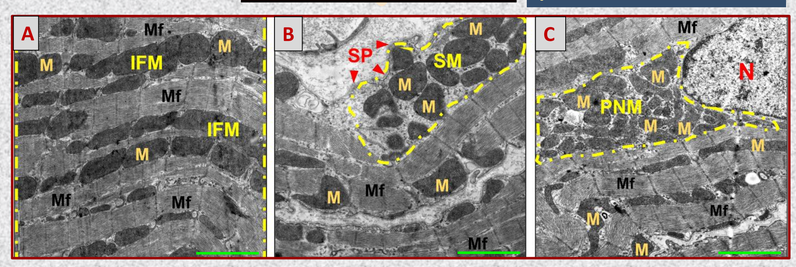


Fig. 8. *Longitudinal sections of CMC with regions of interfibrillar (IFM) - left, subsarcolemmal (SM) - middl, and perinuclear mitochondria (PNM) – right. N—nucleus, SL—sarcolemma, Mf—myofibrils; M—mitochondria. Scale bar – 2 μM.*

Remodeling of T-tubules and their contacts with Sarcoplasmic cisternae.

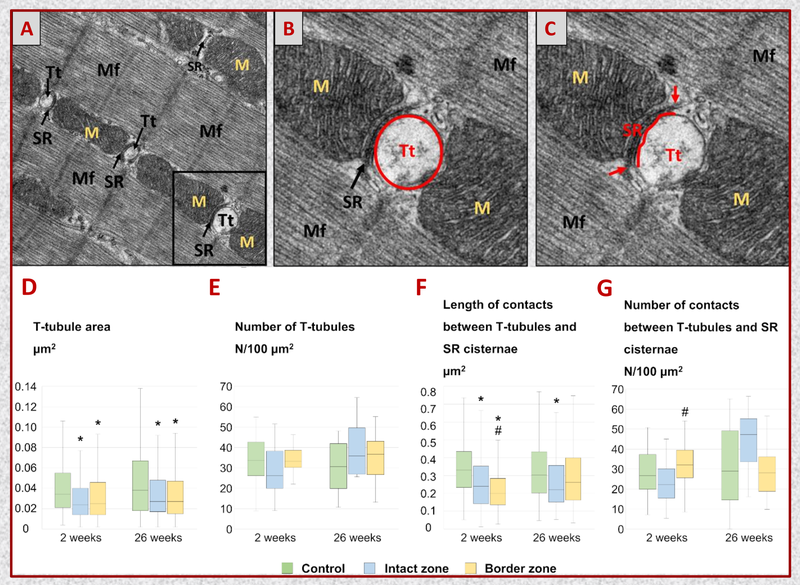


Fig. 11. *Parameters of excitation-contraction coupling in border and intact zones of myocardium at 2 and 26 weeks post-infarction. A — Section of a cardiomyocyte showing T-tubular lumens (Tt) and terminal cisternae of the sarcoplasmic reticulum (SR). (B-C) Magnified insets from A showing red outlines of T-tubule areas (Tt) and red lines measuring SR length (red arrows). Areas (D) and number (E) of T-tubules, length (F), and number (D) of T-tubule contacts with sarcoplasmic cisternae. Mf—myofibrils; M—mitochondria. Asterisks indicate p < 0.01 between control and infarct groups; # —indicate differences between border and intact zones in the infarct group.*

Ultrastructural changes of cardiomyocytes 26 weeks after myocardial infarction.

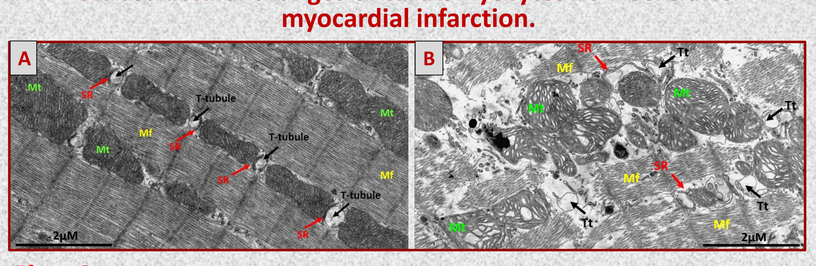


Fig. 6. *Electronograms of CMC in control (A) and infarct groups (B) 26 weeks after coronary occlusion. Mf — myofibrils; Mt — mitochondria, SR — sarcoplasmic reticulum, Tt – T-tubule. Scale bar – 2 μM.*

Mitochondria parameters in post-infarction period.

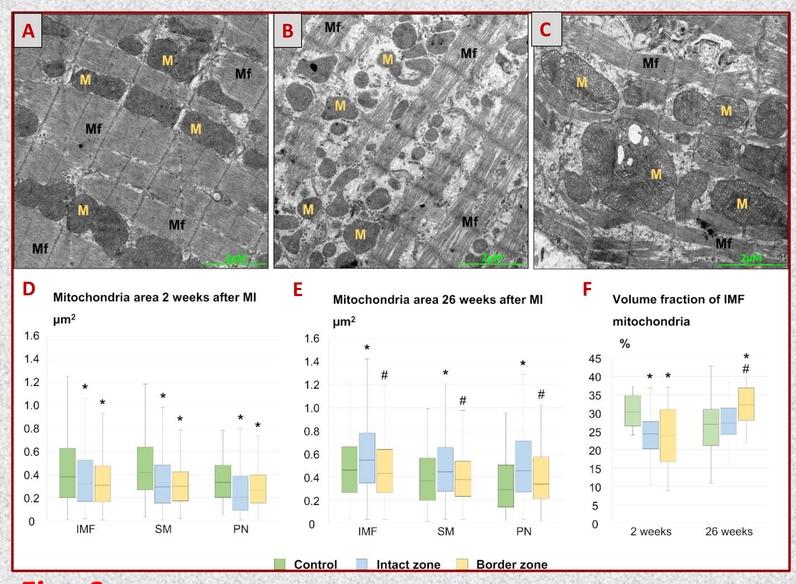


Fig. 9. *Electronograms of CMC in control (A) and infarct groups (B—2 weeks, increased number of smaller and irregularly arranged mitochondria; C — 26 weeks, enlarged mitochondria), border zones. Areas of intermyofibrillar (IMF), subsarcolemmal (SM), and perinuclear (PN) mitochondria in intact and border zones of myocardium at 2 (D) and 26 (E) weeks. (F)—volume fractions of IMF mitochondria. Asterisks indicate p < 0.01 between control and infarct groups; # —indicate differences between border and intact zones in the infarct group.*

Myofibril parameters and cardiomyocyte size in border and intact zones of myocardium at 2 and 26 weeks post-infarction.

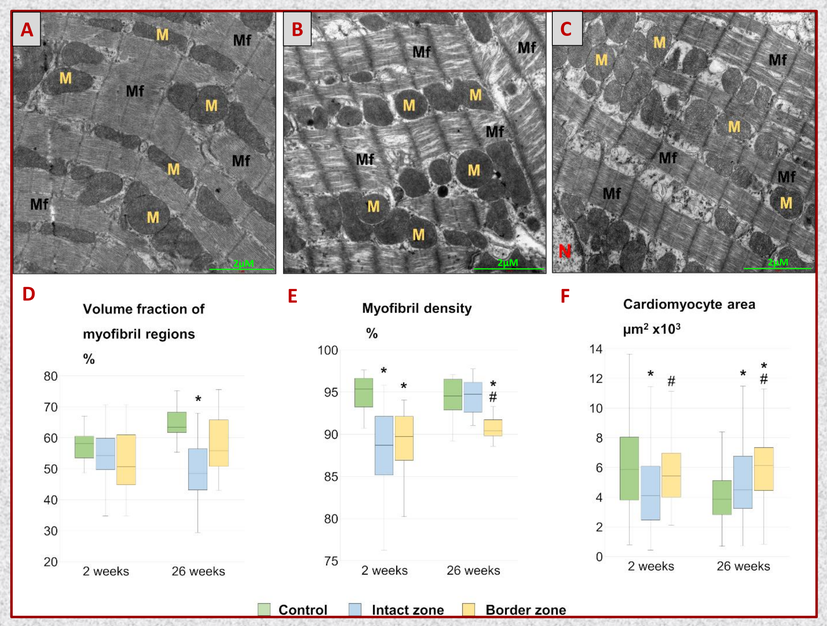


Fig. 7. *Representative electronograms of myocardium sections in control (A—dense myofibrils) and intact zone of infarct groups in 2 (B —loose myofibrils) and 26 (C —decreased myofibril regions) weeks after MI. Mf—myofibrils; M—intermyofibrillar mitochondria. Scale bar – 2 μM. D. Volume fractions of myofibrillar regions. E. Density of myofibrils. F. Area of cardiomyocyte. Asterisks indicate p < 0.01 between control and infarct groups; # —indicate differences between border and intact zones in the infarct group.*

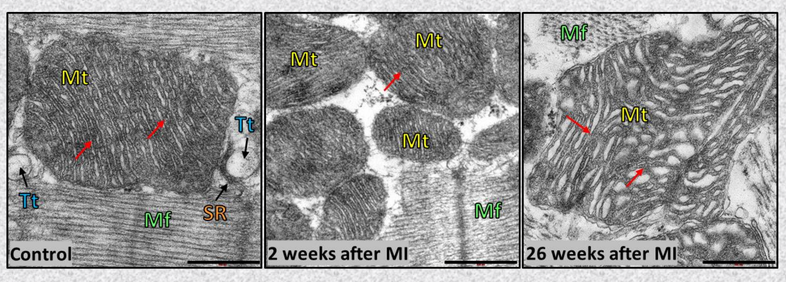


Fig. 10. *CMC mitochondria of control (left) and experimental rats in 2 (central) and 26 weeks (right) after myocardial infarction. Mt– mitochondria, Tt – T-tubule, SR – sarcoplasmic reticulum. The red arrows indicate the mitochondrial cristae. Scale bar – 500nm.*

Summarizing

Zone	Energy consumption			Energy supply		Load adjustment	
	Myofibril density	Cardio-myocyte area	Volume fraction of myofibril regions	Mito-chondria area	Volume fraction of mito-chondria	T-tubule area	Length of TS-contacts
2 weeks							
Intact	↓		---				
Border	↓	---	---	↓	↓	↓	↓
26 weeks							
Intact	---	↑	↓	↑	---	↓	↓
Border	↓	↑	---	---	↑	↓	---

Fig. 12. *Adaptation strategies post-infarction depending on myocardial zones' proximity to the scar. Arrows indicate changes in the infarct group relative to control; dashed arrows indicate no changes. Red arrows — decreased values; green arrows — increased values. Gray cells — similar or no changes in intact and border zones; orange cells — different changes in zones.*

CONCLUSION

Studying post-infarction remodeling mechanisms is crucial for predicting disease progression and selecting therapy. Our study highlights the specificity of late-stage remodeling in the border zone, where constant inflammation limits adaptation. Specific regional myocardial therapy could help restore myofibrillar and mitochondrial apparatus in this zone, maintaining functional activity.

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