Dynamics of Reparative and Adaptive Processes in the Myocardium after Irradiation with High-Intensity Laser

V. L. Kovalenko, A. I. Kozel', S. V. Evdokimov, and L. V. Abramovskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 469-472, October, 1997
Original article submitted March 20, 1997

Reparative and adaptive processes in the myocardium after irradiation with a high-intensity Nd:YAG laser are accompanied by an increase in the number of blood vessels in the damage-adjacent and remote areas. The results obtained indicate that laser revascularization of the myocardium can be employed in the treatment of some forms of ischemic heart disease.

Key Words: *reparation; adaptive processes; myocardium; high-intensity laser radiation*

Surgical methods of correcting blood supply to the myocardium have been widely applied during the last two decades. However, operation on coronary arteries can be performed in no more than 50% of patients: they are impossible in case of diffuse and distal atherosclerotic lesions in coronary arteries. Therefore, the development of new methods of revascularization of ischemized myocardium is an urgent problem in modern cardiovascular surgery [10,11]. Transmyocardial laser revascularization is prospective in the treatment of certain forms of ischemic heart disease [8,9]. As we are aware, there is no systematized information on the morphogenesis of reparative processes and structural and functional reorganization of the myocardium after irradiation with a high-intensity laser and on the effectiveness of transmyocardial laser revascularization.

MATERIALS AND METHODS

Chronic experiments were carried out on 34 mongrel dogs. An Nd:YAG-laser (Raduga-1) served as a source of laser radiation (wavelength 1.06 μ, 20-30 W in continuous mode, exposure time 1-2 sec). A quartz monochannel light guide was used, which allowed us to work effectively on any surface of contracting heart. The procedure was optimized on 5 dogs.

The choice of an adequate damage stimulating reparative and adaptive processes in the myocardium was based on coherence, monochromaticity, high energy density, and precise orientation of the laser. The fact that laser radiation produces a wide variety of effects upon interaction with biological tissues was taken into consideration.

The procedure was performed under endothracheal anesthesia combined with intravenous administration of kalipsol. After thoracotomy, II sutures were applied on the anterior surface of the left ventricle in the vessel-free zone, and 4 transmyocardial channels 1 mm in diameter were made in the center of the suture.

General condition of the dogs during postoperative period was satisfactory, without complications. Adequate spontaneous breathing and stable hemodynamics were restored after extubation. There were no arrhythmias and other cardiac disturbances. In most dogs, behavioral reactions were restored within several hours after operation.

The animals were sacrificed several hours after operation and on days 1, 5, 10, 20, 30, 60 and 90 by intrapulmonary injection of 1.0-1.5 g sodium thiopental.

State Medical Academy; State Institute of Laser Surgery, Chelyabinsk

0007-4888/97/0010-1034$18.00

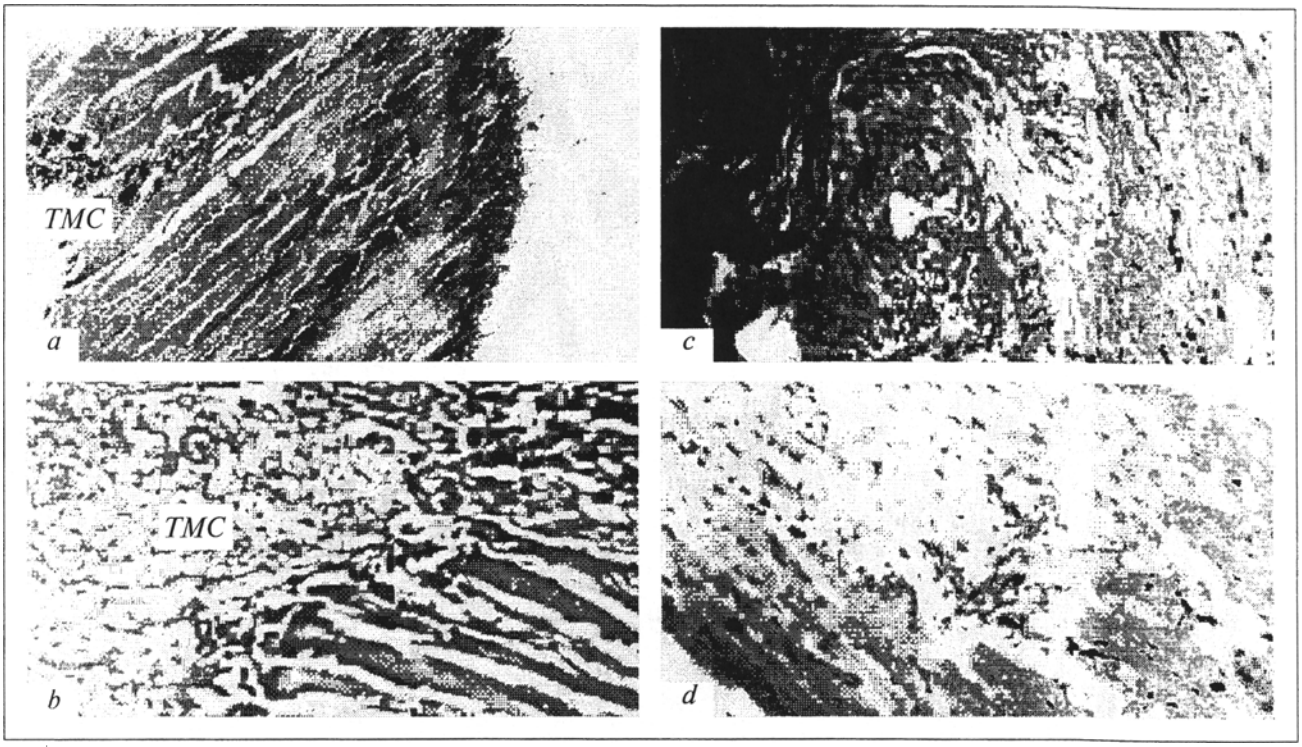


Fig. 1. Changes occurring in the transmyocardial channel (*TMC*) zone and in remote myocardium. *a*) relaxation and disintegration of cardiomyocytic fibrils is the laser channel wall, 1 day after irradiation, polarization microscopy; *b*) fibrin thrombus in the laser channel, focal necrosis of cardiomyocytes with inflammatory-reparative reaction, day 5; *c*) blood vessels of different calibers in the area of thrombus organization and necrotic foci in the laser damage zone, day 30; *d*) newly formed blood vessels in a remote area of myocardium, day 60. *b-d*) staining with hematoxylin and eosin. ×100.

The heart was opened through the right ventricle and septum so that to preserve the irradiated myocardial zone. Pieces of the myocardium perpendicular to the channel were dissected in the subepicardial, intramural, and subendocardial zones, and a specimen from the upper posterior area of the ventricle were collected for histological studies.

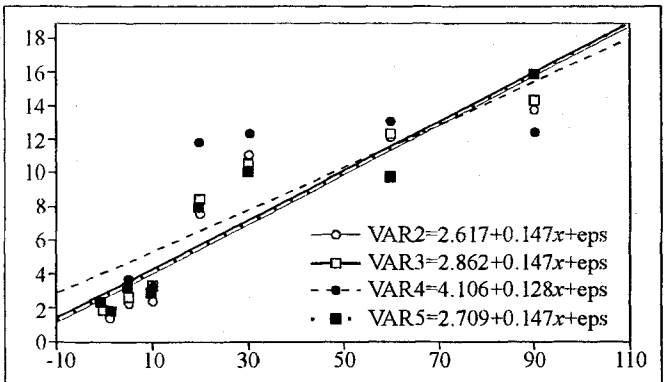
The pieces were fixed in 10% neutral formaldehyde, dehydrated in alcohols, and embedded in paraffin. Sections were stained with hematoxylin and eosin, picrofuchsin by the method of Van Gieson, orcein by the method of Unna—Taenzer, and silver-impregnated by the method of Gomori; fibrin was visualized by the method of Shueninov. Some sections were used for PAS reaction with saliva amylase as a control. Cryostat sections were stained for fats with Sudan III. The state of myocardial contractile fibers was assessed in polarized light using the criteria described elsewhere [6].

Morphometric studies were performed using a Quantimet 500 image analyzer and the grid and ruler for microscopical stereometric measurements. The total area of damage, the number of blood vessels in arbitrary unit of area, the relative numbers of blood vessels of different calibers, and the stroma—parenchyma ratio were determined. Data were statistically analyzed using special software. The significance of differences was evaluated by the Pierson test.

RESULTS

Transmural laser channels (diameter up to 1 mm) with a narrow scab were observed several hours after the procedure. The sings of thermal "vaporization" and microcirculatory disturbances were developed in the adjacent area. Polarization microscopy of this area revealed a blood clot in the channel and cardiomyocytes with relaxed and disintegrated myofibrils (isotropic zone); cardiomyocytes with contractures were seen at the periphery. The total area of the damage was 8.5 mm^2.

By the end of the first day, black clumps of the scab were seen in the channel wall and myocytolysis was progressing (Fig. 1, *a*). Pyknosis and lysis of the nuclei, homogenization and increased eosinophilia of the sarcoplasm as well as intense PAS reaction were observed in damaged muscle fibers. PAS reactions did not disappear after incubation of the sections with the saliva amylase. In the area of contractures, the sarcoplasm was intensely stained by eosin, and contained numerous clumps and deformed nuclei. On the second day after operation, the total area of



	VAR2	VAR3	VAR4	VAR5
$P_\alpha <$	0.4159	0.4427	0.4502	0.3753
$P_\gamma >$	0.5841	0.5573	0.5498	0.6247

Fig. 2. Changes in the number of blood vessels in the myocardium after laser irradiation. VAR2) subendocardial zone; VAR3) intramural zone; VAR4) subepicardial zone; VAR5) remote myocardial area. Abscissa: time after irradiation, days; ordinate: number of blood vessels per arbitrary unit area. Probability of mistake (P_α) and confidential probability (P_γ) are given in the table.

the damage focus decreased to 4.9 mm^2. This phenomenon can be explained by regression of the consequences of "vaporization" and microcirculatory disorders. The borderline between damaged and intact myocardium was clearly seen.

By the end of the 5th day, transmyocardial channels were filled with fibrin thrombi; karyocytolysis was observed in blood cells. Adjacent cardiomyocytes were subjected to coagulative necrosis or myocytolysis (Fig. 1, *b*). Exudative-proliferative reactions in the channel were characterized by focal and diffuse mononuclear infiltration with individual granulocytes. The narrow peripheral zone of the damage differed from intact myocardium by fatty dystrophy and higher intensity of PAS reaction in the sarcoplasm. In all zones of the myocardium, the area of the damage tended to decrease.

By the 10th day, macroscopically the channels looked like interrupted bundles. Some segments of the channels with thrombi and part of necrotic area were replaced by young granulation tissue containing fibroblasts, individual macrophages, and considerable amount of capillaries. A dense network of argyro- and fuchsinophilic fibers was observed.

On day 20, the channels were completely obliterated. We did not reveal any signs of their functioning. However, other researchers observed endothelialization of transmyocardial laser channels and their anastomizing with the left ventricle capillaries [5]. On the other hand, it was reported that transmyocardial channels cannot function due to hydraulic processes preventing blood flow through them during isometric contraction of the heart [12]. The damage was represented by connective tissue with predominating fibrous structures with a dense capillary network; some capillaries were transformed into arterioles. In intact myocardium of the left ventricle, the number of *de novo* blood vessels increased considerably, while the stroma—parenchyma ratio remained unchanged. A tendency toward an increase in the number of small arteries was observed, which agrees with the findings of others [7,11].

By the end of the 1st month, stellate scars with an area of 2 mm^2 replaced the channels. The scars were rapidly vascularized, with a sharp increase in the number of arterioles and small muscular arteries (Fig. 1, *c*); veins were also seen. Metabolic processes in the adjacent myocardium normalized, as evidenced by disappearance of fatty dystrophy and PAS staining. Proliferation of endothelial cells and *de novo* formation of capillaries, arterioles, and small muscular arteries were observed in adjacent and remote myocardium. Elastic fibers and the elastic membrane were seen in the muscular arteries type. The stroma-parenchyma ratio tended to increase as a result of greater area occupied by newly formed blood vessels and stable content of the nuclei in cardiomyocytes, stable volume of the nuclei, and stable content of fibers.

After 2 months, the fibroblastic processes stabilized, while the newly formed vessels continued differentiating. The scar became loose at the periphery, and the number of arterioles and small muscular arteries with the elastic membrane in it increased 2-fold. A tendency toward endothelial cell proliferation and formation of capillaries, arterioles, and small arteries was preserved in intact myocardium (Fig. 1, *d*) was kept.

At the end of the observation period, the scar area was not greater than 0.5 mm^2. Its vascularization increased considerably compared with that at the early periods of observation. A tendency toward an increase in the number of blood vessels and in the stroma—parenchyma ratio was observed in the entire myocardium of the left ventricle (Fig. 2).

Our results indicate that the inflammatory-reparative reaction and adaptive regeneration occurring in the myocardium after laser damage [4] have specific features, for example, a weak leukocytic infiltration. This may be due to bacteriostatic effect of laser radiation.

The dynamics of myocardial necrosis caused by laser radiation differs considerably from that of infarction [1]. Bearing in mind the principles of material maintenance of adaptive processes [3], it can be suggested that necrotic foci with persistent elements of so-called laser scab act as an inductor of regeneration [2] and adaptive processes in the myocardium,

which are characterized by pronounced angiogenesis on the entire damage.

Thus, causing a comparatively small damage to the myocardium, high-intensity radiation induces transformation of the distal vascular bed in the entire left ventricle myocardium.

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