MORPHOLOGICAL STUDY OF MYOCARDIAL REVASCULARIZATION BY LASER

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Ischemic heart disease (IHD) occupies a leading place among diseases leading to reduced working capacity and to death. Several methods of surgical correction of the circulation in the myocardium have been suggested for its treatment, including restoration of the blood flow in the affected coronary arteries by their anastomosis with the internal mammary artery [4, 10] or by autografting a segment of vein between the aorta and the affected arteries [3, 5]. However, operations on the coronary arteries in IHD are practicable on by no means all patients because of the extent of the lesion, the patient's age, and the principal or concomitant pathology.

Experiments carried out by a number of investigators to study revascularization of the myocardium by the creation of multiple channels in the myocardium, communicating with the chamber of the left ventricle, with a fine needle [6, 9] did not prove successful because of rapid obliteration and sclerosis of the artificial channels [8].

With the development of powerful carbon dioxide lasers operating under pulsed conditions, experiments have been conducted to study laser revascularization of the myocardium (LRM) [7]; these have shown that thin channels formed by laser beams in the myocardium preserve their lumen for a long time after the operation. In the control group after ligation of the anterior descending branch (ADB) of the left coronary artery all the animals died from myocardial infarction. The survival rate in the group of animals after laser revascularization, performed immediately after ligation, was 83%, and if performed before ligation it was 100%.

EXPERIMENTAL METHOD

A carbon dioxide laser with emission energy of up to 20 J and pulse duration 8-100 msec was constructed for the experiments by members of the staff of the P. N. Lebedev Physical Institute, Academy of Sciences of the USSR (Dr. Phys.-Math. Sci. R. V. Ambartsumyan, Cand. Phys.-Math. Sci. E. P. Markin, and E. L. Koshelev), under the scientific direction of Academician N. G. Basov. Experiments were carried out on 30 mongrel dogs weighing 18-24 kg. After premedication with morphine (0.1 mg/kg) the animals were anesthetized by intraperitoneal injection of 25 mg/kg pentobarbital; the trachea was intubated and the lungs were artificially ventilated during the operation.

Left-sided thoracotomy (in the fourth-fifth intercostal space) and pericardiotomy were performed. From 20 to 30 microperforations were produced on the anterior surface of the left ventricle by pulsed laser (Fig. 1). Bleeding from the outer orifices of the channels was stopped by gentle pressure with a swab containing hemostatic sponge for 2-4 min. The pericardium was closed by interrupted cat gut sutures and the chest wall was closed in layers with removal of air from the pleural cavity. To prevent septic complications, broad-spectrum antibiotics were administered to the animals during the operation and for the first 3 days thereafter. There were three series of experiments. In series I (four dogs) only ADB of the left coronary artery was ligated. In series II (10 dogs) LRM was carried out initially, after which ADB of the left coronary artery was ligated. In series III (16 dogs) only LRM was carried out. In the final stage of the experiments, after 1, 3, 9-12, 20, 30, and 80 days and also after 6 and 12 months, thoracotomy and pericardiotomy were repeated and ADB of the left coronary artery was ligated. During the experiments the ECG of all the animals

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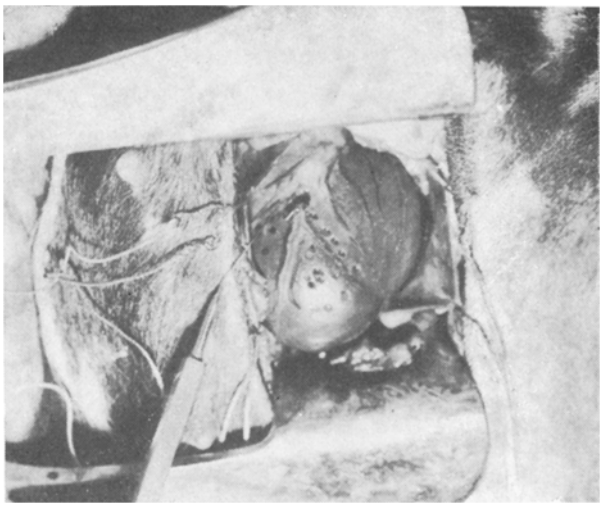

Fig. 1. Surface of left ventricle after treatment with pulsed laser.

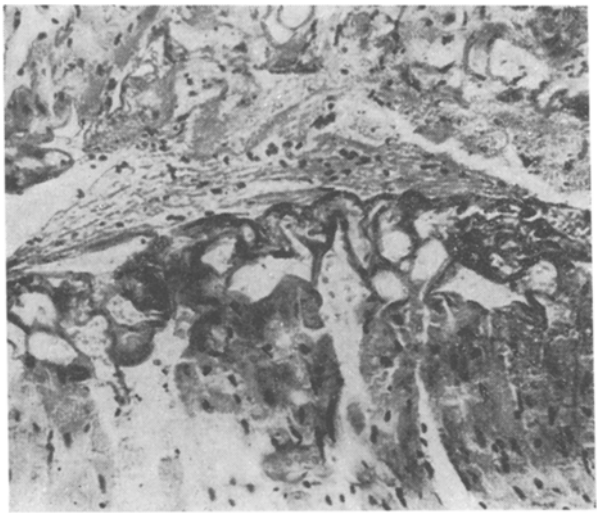

Fig. 2. Thermal coagulation necrosis and vacuolation of cytoplasm of cardiomyocytes 24 h after treatment with laser. 120 ×.

and parameters of the hemodynamics and blood gas composition were recorded. Animals of series II and III were given an injection of a lethal dose of anesthetic to terminate the experiment. Material for histological investigations was fixed in 10% neutral formalin solution and in Bouin's fixative and embedded in paraffin wax. Preparations were stained with hematoxylin and eosin and picrofuchsine, the PAS reaction was carried out according to McManus, and sections were impregnated with silver nitrate by Gordon's method.

EXPERIMENTAL RESULTS

In series I all the animals died from ventricular fibrillation associated with established myocardial infarction, indicating inability to tolerate ligation of branches of the coronary arteries. Of the 26 animals in series II and III one dog died from ventricular fibrillation resulting from heating of the conducting systems of the heart by laser radiation, because several perforations were formed in the region of the apex.

Histological investigations showed that channels 200-400 μ in diameter were formed in the myocardium by laser radiation and, during the first 10-40 min after the operation they were filled with blood cells (Fig. 2). The mechanism of formation of the perforations in the myocardium by laser consists of instantaneous evaporation of the tissue along its trajectory, and the residual energy of the radiation is absorbed by blood in the left ventricle.

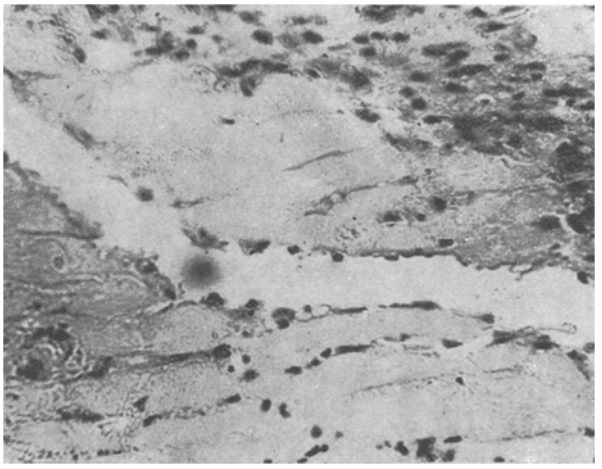

Fig. 3. Endothelized laser channel 30 days after application. Granulation tissue transformed into fibrous tissue in areas of myocardium surrounding channel. Hematoxylin-eosin, 120 ×.

After 24 h the formation of an occluding thrombus began in the epithelial part of the channel. In the rest of its course the channel had the appearance of a slit 200-380 μ in diameter filled with blood cells. The channel walls consisted of myocardial tissue undergoing thermal coagulation necrosis to a depth of 37.6 ± 2.4 μ thick and an adjacent two or three layers of cardiomyocytes with vesiculated cytoplasm (Fig. 2). This can be attributed to the thermal effect of the laser, and the extremely rapid and intensive evaporation of the inter- and intracellular fluid followed by edema of the cells. Outside the zones described above, the cardiomyocytes preserved their normal histological structure.

After 3-9 days the diameter of the channels was reduced to 120-250 μ, and it could be traced throughout the thickness of the wall of the left ventricle in the form of a narrow slit. Granulation tissue continued to develop around the channels, with numerous newly formed capillaries and proliferating macrophages and fibroblasts, without neutrophilic infiltration at the boundary between intact tissues and those undergoing coagulation necrosis. This is evidence of the aseptic productive character of the inflammatory reaction, characteristic of healing of laser wounds [1, 2].

By the 10th-14th day the inner surface of the laser channels acquired an endothelial lining, and 1 month after the experiment began they had the appearance of capillaries of sinusoidal type, and resembled the veins of Thebesius in structure (Fig. 3). After 6-12 months the diameter of the channels was reduced to 10-14 μ. One result of transformation of the granulation tissue was formation of fibrous tissue with numerous microvessels around the laser channels.

According to the ECG data and results of electron-microscopic and histochemical investigations, no evidence of the formation of a myocardial infarct was obtained in the animals of series II and III, indicating that laser revascularization of the heart muscle is effective. To obtain information on the time course of the circulation in the myocardium after laser revascularization, when the animals of series III were withdrawn from the experiment, ^{131}I was injected into the left ventricle 6-12 months after the beginning of the experiment, after preliminary ligation of all afferent and efferent vessels of the heart. The aorta was occluded above the orifices of the coronary arteries. Tests carried out on the "Hemoliter" and "Volumetron" apparatuses showed that sample of blood from the right ventricle contained radioactive label. This is evidence of the passage of blood from the left ventricle into the laser channels and the capillary and venous network of the myocardium, and thereafter into the common collector opening into the right atrium, namely the sinus venosus.

The results are thus evidence of the promising nature of LRM as a method for clinical use. The operation of LRM is simpler, more effective, and less dangerous than operations on the coronary arteries. It can be performed under normothermic conditions without the use of an artificial circulation, not only for the treatment of IHD, but also as a method of prevention of myocardial infarction due to occlusive lesions of the coronary arteries.

LITERATURE CITED

1. V. N. Galankin and K. V. Botsmanov, Byull. Éksp. Biol. Med., No. 10, 463 (1979).
2. V. I. Eliseenko, O. K. Skobelkin, and E. I. Brekhov, Byull. Éksp. Biol. Med., No. 1, 106 (1982).
3. M. D. Knyazev and R. A. Stegailov, Vest. Khir., No. 12, 18 (1979).
4. V. I. Kolesov, Vestn. Khir., No. 8, 9 (1978).
5. R. G. Favaloro, J. Thorac. Cardiovasc. Surg., 58, 178 (1969).
6. A. Goldman, S. M. Greenstone, F. S. Preuse, et al., J. Thorac. Cardiovasc. Surg., 31, 364 (1956).
7. M. Mirhoseini and M. M. Caiton, J. Microsurg., 2, 253 (1981).
8. R. Pifarre, Ann. Surg., 168, 871 (1968).
9. P. K. Sen, T. E. Udwadia, S. G. Kinare, et al., J. Thorac. Cardiovasc. Surg., 50, 181 (1965).
10. A. M. Vineberg, J. Int. College Surg., 22, 503 (1954).

QUANTITATIVE CHARACTERISTICS OF HEMATOPOIETIC MICROENVIRONMENT TRANSFER

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During heterotopic bone marrow transplantation a new bone marrow organ with bony capsule and medullary cavity is formed and is colonized by hematopoietic cells. In the heterotopic bone marrow organ (HBMO) the donor's microenvironment acts [8] although the hematopoietic cells in it are recipient's cells [1]. Heterotopic transplantation is essentially transfer of the hematopoietic microenvironment [2, 4]. The transfer is effected by clonogenic bone-marrow mechanocytes (CBMM), which preserve their donor origin in HBMO [1, 3]. The size of the HBMO is a characteristic which can be used to judge the factors regulating morphogenesis of the bone marrow stroma. It has been shown that the size of HBMO depends on the quantity of transplanted bone marrow tissue, but this relationship is multifactorial [9, 10]. In the present investigation parameters of transplants determining the size of HBMO are analyzed.

EXPERIMENTAL METHOD

(CBA × C57BL)F_1 mice weighing 20-22 g were used. Fragments of bone marrow flushed out of the medullary cavity of the femur were transplanted beneath the renal capsule of syngeneic recipients by the method in [1]. There were three series of experiments: I) marrow from one femur of each of three donors was transplanted into three separate recipients, and the marrow of the remaining three femora was transplanted together into the 4th recipient (six experiments), II) marrow from one femur was transplanted in its entirety into one recipient, marrow from the 2nd femur was divided into four parts: 1/2, 1/4, and 2 parts each of 1/8 were grafted each into a separate recipient (nine experiments). III) 10 Min before removal of the marrow the donors were irradiated in doses of 1.5-7.0 Gy on a cobalt source with dose rate of 28.2 rads/min; fragments of 1/4 or 1/8 of the marrow of the irradiated femora were transplanted, and in the control, fragments of the same size of unirradiated bone marrow were grafted (five experiments, 111 irradiated and 21 control grafts). After 2.5 months each HBMO was removed, a suspension was prepared from the cells of its medullary cavity, and the number of cells was counted in a Goryaev's chamber. The results were subjected to statistical analysis.

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