REGENERATIVE PROCESSES IN THE MYOCARDIUM DURING EXPERIMENTAL THERAPY OF HEART WOUNDS IN RABBITS

(UDC 616.12-001.4-085-092.9-07 : 616.127-003.9-07)

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(Presented by Active Member of the Academy of Medical Sciences of the USSR A. N. Bakulev)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 5, pp. 27-29, May, 1966
Original article submitted August 8, 1964

During recent years methods of operative treatment of heart disease have been used on a wide scale. This gives urgent importance to the development of therapeutic measures promoting the rapid healing of penetrating wounds of the heart and, in particular, of surgical wounds of the myocardium.

S. V. Andreev and co-workers previously suggested that a group of therapeutic measures including administration of vitamins of the B group, nucleic acids, methionine, and ATP be used to stimulate repair processes in the myocardium [1-4, 8]. Yu. V. Bukin has shown [5-7] that in experimental myocardial infarction the use of this therapeutic complex increases the rate of protein biosynthesis in the zone of injury and stimulates the formation de novo of the apoenzyme aspartate-glutamate transaminase (AGT).

In the present investigation a dynamic study was made of the effect of this therapeutic complex on the processes of regeneration of the myocardium in rabbits with penetrating wounds of the heart.

EXPERIMENTAL METHOD

Experiments were conducted on 95 chinchilla rabbits of both sexes, weighing from 2.0 to 3.5 kg. The operation was performed in aseptic conditions under ether anesthesia. The thorax was opened and the sternum split into two bony laminae. An accurately measured penetrating puncture or incised wound, 5 x 0.5 mm in extent, was created in the region of the upper third of the anterior wall of the left ventricle by means of a specially adapted ophthalmic knife. The therapeutic complex, consisting of methionine (50 mg), ATP (10 mg), vitamin B_6 (1-10 mg), vitamin B_{12} (30 μg), RNA (30 mg), and DNA (10 mg), was given daily to the rabbits, starting on the day of the operation, in aqueous solution by subcutaneous injection. The remaining rabbits acted as controls. The animals were sacrificed on the 1st, 5th, 10th, and 30th days after the operation.

For the histological and cytochemical investigations the material was fixed by intra-arterial injection of Shabadash's neutral fixing agent into the rabbits, to enter the contracted heart [9]. Sections 7 μ thick were stained with hematoxylin-eosin and picrofuchsin. Histological preparations were studied in transmitted and polarized light and in the phase-contrast microscope. Nucleic acids were detected by Brachet's method [10] and glycogen was demonstrated by Shabadash's histochemical method [9]. To identify the nucleic acids and glycogen, some sections were preliminarily treated with ribonuclease and diastase.

The AGT activity was determined by the method of Umbreit and co-workers [12]. The conditions of incubation of the myocardial tissue with the substrate mixture, containing 30 μg of phosphopyridoxal hydrochloride, were described previously by the authors [6]. The protein content of the tissue was determined by Lowry's method [11]. The results of control experiments showed that the protein content and the AGT activity varied only very slightly in different parts of the anterior wall of the left ventricle in normal conditions, within limits close to the limits of accuracy of the analytical methods used. The thoracotomy itself had no effect on the AGT activity in the heart dur-

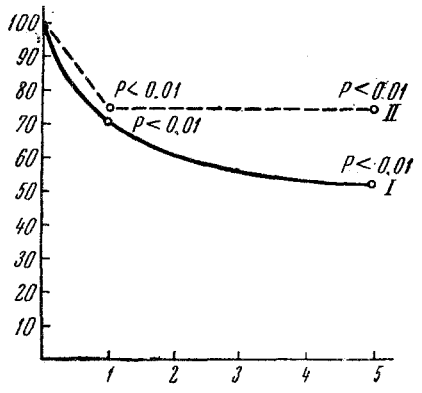

Fig. 1. Fall in AGT activity in traumatized area of the rabbits' heart after infliction of a puncture or incised wound. Along the axis of abscissas) days after operation; along the axis of ordinates) AGT activity as percentage of activity of enzyme in apex of left ventricle of the same heart. I) absolute activity of AGT [calculated per mg weight of moist tissue]; II) specific activity of AGT [calculated per mg protein].

ing the first 24 h after the operation, but the creation of the puncture or incised wound in the upper third of the anterior wall of the left ventricle led to a fall in the AGT activity after 24 h in the lower third of the anterior wall of this ventricle, at a distance of not less than 1.5 cm from the zone of injury, on the average by 13% ($P < 0.05$). Using this tissue as a control for comparing the activity of the enzyme in the zone of the wound, in this way only the minimal actual decrease in the activity of the enzyme in the necrotic focus was determined.

RESULTS

The dynamics of the fall in AGT activity in the necrotic focus of the rabbits' myocardium after wounding is shown in Fig. 1. The absolute activity of the enzyme in the zone of injury fell on the average by 30% during the 24 h after the operation. The protein content in the zone of necrosis fell by a mean value of only 6% during the 24 h after the operation, and as a rule, moreover, there was no increase in the water content of the injured tissue. The specific AGT activity fell on the average by 25% during the first 24 h after the operation. This particular enzyme is one of the soluble cytoplasmic proteins; in the conditions of myocardial ischemia and of injury to the cell membranes, this feature is evidently responsible for the rapid movment of AGT from the muscle fibers into the intracellular space and the blood stream. This is in good agreement with the results of the histological investigations. The microscopic study of the tissue sections showed that in the region of the wound canal the muscle fibers next to the defect became necrotic and developed pathological changes during the 24 h after wounding. Outside the layer of necrotic muscle, around the wound canal, lay a zone of dedifferentiated muscle fibers showing reactive changes, whose sarcoplasm was homogenized and slightly basophilic. In this zone the muscle fibers, as a rule, did not contain glycogen, thus demonstrating the hypoxic state of the tissue lying next to the necrotic focus.

In the later stages, on the 5th day after the operation, the biochemical changes in the myocardium under the influence of trauma became more intensive. The protein concentration in the myocardium in the zone of injury fell on the average by 27% ($P < 0.01$). The absolute activity of AGT fell on the average by 50% (see Fig. 1).

Histological investigation of the injured zone revealed considerable destructive changes at this period in the myocardial tissue. The wound canal was filled with necrotic masses, granulation tissue, and fibrin clots. At the edges of the wound canal the muscle fibers showed degenerative changes and were surrounded by developing connective tissue. The regenerative processes were ill defined; both in the cavity of the canal and near its edges only isolated mitoses were observed among the connective-tissue cells.

In the rabbits receiving the therapeutic complex, on the 5th day after the operation signs of regeneration were clearly developed in the traumatized myocardial tissue. The AGT activity in the zone of injury of the myocardium was lower – on the average by 40% – in the treated animals than in the controls. As is clear from Fig. 2, in the control rabbits the protein concentration in the focus of injury was lowered by 20-40% (mean 27%), while in the rabbits receiving the therapeutic complex for 5 days the tissue protein level was lowered by 5-30% (mean 15%); the difference was statistically significant ($P < 0.01$).

Morphologically, at the margin of the wound canal in the treated animals muscle buds with nuclei at their ends dividing by amitosis were found. The wound canal was largely cleared of necrotic masses; the granulation tissue contained many myoblasts with a high concentration of nucleic acids in their cytoplasm and also in their nucleoli. Mitoses were seen among the myoblasts and some myoblasts were united to form symplasts.

The biochemical and histochemical data thus revealed a higher level of protein synthesis in the traumatized zone of the myocardium of the treated animals. At later periods after the operation (on the 10th and 30th days) the wound canal in the treated animals was much smaller than in the controls. Whereas in the untreated rabbits healing of the wounds took place by connective tissue development, in the treated animals not only connective tissue but

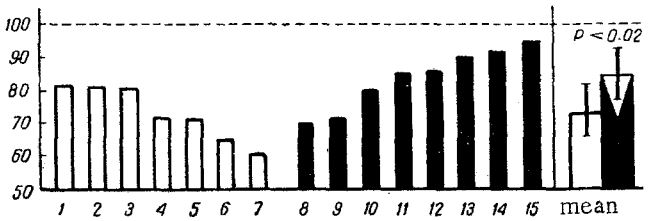

Fig. 2. Protein content in injured area of rabbits' myocardium on 5th day after creation of a puncture or incised wound and subsequent stimulant therapy. Along the axis of abscissas) No. of animal [Nos. 1-7) control rabbits; Nos. 8-15) treated rabbits]; along the axis of ordinates) protein concentration in zone of injury as a percentage of the protein concentration in the healthy tissue of the apex of the left ventricle of the same heart.

also new muscle fibers were formed. In individual cases, in the treated rabbits the edges of the wound canal were closed together by the newly formed muscle tissue.

SUMMARY

The object of study was the influence of biologically active substances on the regenerative processes in the myocardium of rabbits with penetrating wounds of the heart. It was found that a combined administration of methionine, ATP, vitamins B_6 and B_{12}, RNA, and DNA made it possible to intensify appreciably the regenerative processes in the lesion focus in the heart. In rabbits subjected to therapy the protein level in the lesion area on the 5th day after the operation showed an average decrease of only 15% ($p < 0.01$), and in control animals 27%. The activity of aspartate-glutamatetransaminase in the heart in the lesion area was somewhat higher in the treated animals as compared to the control ones. The healing of the wound in rabbits given a therapeutic complex was accompanied by the regeneration of muscle fibers. In some cases, the lips of the wound closed due to regenerated tissue.

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