TIME COURSE OF CHANGES IN ARYL SULFATASE ACTIVITY IN THE INITIAL PERIOD OF ACUTE EXPERIMENTAL FOCAL MYOCARDIAL ISCHEMIA

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UDC 612.172.014.46.014.014.49+615.9.015.44:616.127-092.19

KEY WORDS: myocardial ischemia; rabbits; aryl sulfatase; lysosomes.

Current opinion [5-7] is that an increase in activity of the lysosomal system plays an important role in cardiomyocyte destruction in acute focal myocardial ischemia. However, there have been virtually no direct investigations the results of which would enable this process to be characterized quantitatively and the time course of changes in the lysosomal system described during the development of cardiac ischemia. Attempts to assess the state of cardiomyocyte lysosomes by the use of a histochemical reaction for acid phosphatase activity [2] suffer from the shortcoming that this enzyme can play the role of lysosomal enzyme only when biochemical tests are carried out in a pure fraction of these organelles or when methods of electron histochemistry are used, when activity of the enzyme may be visually linked with concrete organelles, for acid phosphatase is not confined to lysosomes [1]. It may therefore be considered important to study the behavior of the cardiomyocyte lysosomal apparatus by other histochemical reactions which could characterize only lysosomes, and not other subcellular structures.

The aim of this investigation was to assess the state of the cardiomyocyte lysosomal system in acute focal myocardial ischemia by means of a histochemical reaction for aryl sulfatase, which is regarded [4] as a specific marker of lysosomes.

EXPERIMENTAL METHOD

Experiments were carried out on male Chinchilla rabbits (21) weighing 2.5-3.5 kg, divided into seven groups. The control group consisted of three animals, and a model of acute focal myocardial ischemia was produced on the other rabbits under acute experimental conditions and superficial hexobarbital anesthesia, with controlled respiration, by ligation of the descending branch of the left coronary artery at the junction between its upper and middle thirds. The rabbits were killed by extirpation of the heart 1, 2, 5, 10, 20, and 40 min after ligation of the coronary vessel (three animals in each group). Aryl sulfatase activity was revealed histochemically [3] in frozen sections through the left ventricle (the section was cut so as to include ischemic, juxtanecrotic, and "intact" zones of the myocardium). Activity of the enzyme was estimated as the number of dark brown granules in 30 fields of vision (for the heart of each rabbit) under immersion magnification. The data were subjected to statistical analysis on a "Commodore-64" personal computer. Correlation between the parameters was assessed as strong if the coefficient of correlation (r) $\geq |0.7|$, as of average strength when $r = |0.69\text{-}0.3|$, and as weak when $r = |0.29|$. The significance of correlation was estimated by the usual statistical methods, on the basis of our own program for the personal computer.

EXPERIMENTAL RESULTS

Aryl sulfatase activity in the normal myocardium is very low, evidence of weak activity of the lysosomes in intact cardiomyocytes. A completely different picture was observed in the zone of ischemia, where there was a progressive increase in the number of enzyme granules with lengthening of the duration of the pathological process (Fig. 1; Table 1).

Department of Pathological Physiology, Patrice Lumumba Peoples' Friendship University, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 105, No. 3, pp. 274-276, March, 1988. Original article submitted July 17, 1987.

TABLE 1. Results of Quantitative Analysis of Aryl Sulfatase Activity in Ischemic Zone of Left Ventricle of Rabbit at Different Periods of Ischemia (M ± m)

Experimental conditions	Average number of fields of vision with granules per rabbit	Average number of granules per field of vision	Average number of granules per field of vision containing granules
Normal	9,3±0,8	1,6±0,3	5,2±0,3
Ischemia			
1 min	10,0±0,03	2,3±0,4	6,9±0,3
2 min	14,0±0,9	3,4±0,4	7,2±0,3
5 min	17,3±0,3	6,4±0,7	11,8±1,0
10 min	13,3±0,3	6,5±0,8	14,4±0,7
20 min	13,7±0,8	9,7±1,2	21,3±2,0
40 min	30 (all fields of vision	32,0±1,2	

Legend. After 40 min of ischemia granules of enzyme were found in all fields of vision in all animals.

TABLE 2. Results of Quantitative Analysis of Aryl Sulfatase Activity in "Intact" Zone of Left Ventricle of Rabbit at Different Times of Acute Focal Ischemia (M ± m)

Experimental conditions	Average number of granules per field of vision	Average number of granules per field of vision containing granules
Normal	1,6±0,3	5,2±0,3
Ischemia		
1 min	1,5±0,8	5,0±0,7
2 min	1,9±0,8	4,75±0,9
5 min	2,4±1,1	6,0±0,9
10 min	2,4±1,04	6,0±0,5
20 min	3,0±1,3	7,5±1,1
40 min	4,3±1,6	8,6±1,0

The results of quantitative analysis of myocardial preparations in two different versions are given in Table 1: on average for 90 fields of vision (30 fields of vision each for the preparation obtained from each rabbit), and on average for the number of fields of vision in which granules of the test enzyme were observed at each time.

It will be clear from Table 1 that after the 1st minute of ischemia aryl sulfatase activity was increased in the zone of ischemia and rose progressively with lengthening of the period of the process. Correlation analysis showed strong significant positive correlation to be present between the duration of ischemia and the number of enzyme granules (both on average for all fields of vision and on average for fields of vision in which enzyme activity was detected) ($r = +0.96$ and $r = +0.99$, respectively). Although the localization of enzyme activity at first glance does not correlate to this degree with the duration of ischemia (after ischemia for 10-20 min a significant decrease was found in the number of fields of vision in which granules were present), nevertheless strong, significant positive correlation was found between this parameter and the period of ischemia, although admittedly the coefficient of correlation was lower than in the previous groups ($r = +0.86$).

The shift of the distribution of values characterizing the number of enzyme granules in individual fields of vision was to the left at all times of ischemia; on the whole this is evidence of a uniform increase in the number of these granules in the myocardium (large local concentrations of granules are rare).

Data on the change in aryl sulfatase activity in the "intact" zone (peripheral regions of the preparation, as far as possible from the zone of ischemia) are given in Table 2.

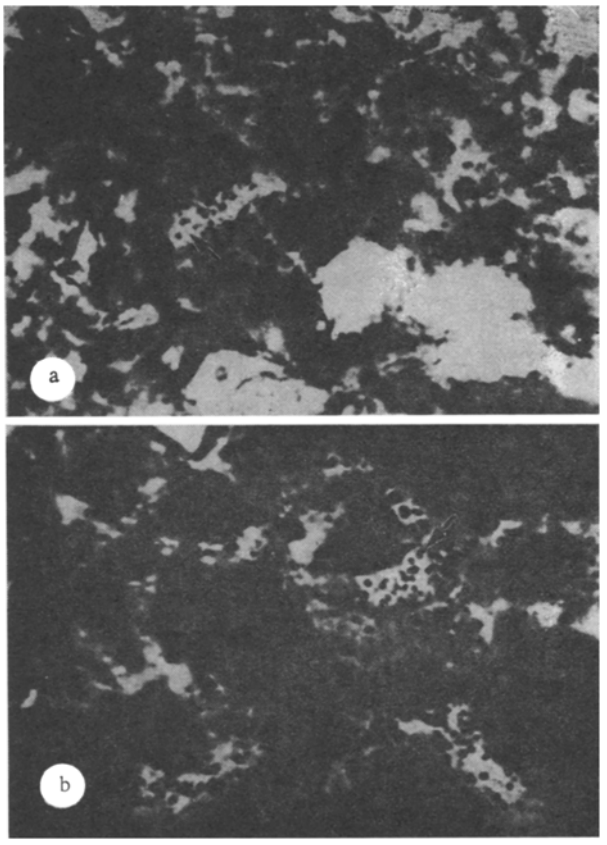

Fig. 1. Left ventricular myocardium of a rabbit showing zone of ischemia.

It will be clear from Table 2 that not until the 40th minute did the number of enzyme granules per field of vision containing granules significantly exceed the normal value. However, correlation analysis, taking account of the tendency for the number of granules to change depending on the period of ischemia, showed strong significant positive correlation between these parameters (for the total number of fields of vision $r = +0.98$ and for fields of vision in which granules were present, $r = +0.96$). The number of fields of vision in which granules were found in the "intact" zone throughout the period of investigation did not differ significantly from normal.

Thus in an experimental model of acute focal myocardial ischemia, aryl sulfatase activity increases progressively in the ischemic zone with an increase in the duration of the process; this increase is manifested as an increase in the number of enzyme granules in the myocardium and also in the number of fields of vision in which these granules are detected. Meanwhile in the "intact" zone activity of this enzyme rises only a little during ischemia. These results are evidence that an increase in permeability of the lysosomal membranes takes place during sudden anoxia of heart muscle, whereas cardiomyocyte hyperfunction is a much less important labilizer of these structures. This hyperfunction must evidently reach a certain degree in order to affect lysosomal membrane permeability. The results confirm the view that immobilization of enzymes contained in the lysosomes may be an important pathogenetic factor in cardiomyocyte damage associated with myocardial infarction.

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EVALUATION OF THE RESULTS OF ARTERIAL RECONSTRUCTIONS ACCOMPANIED BY ARTERIOVENOUS ANASTOMOSIS

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UDC 616.134+616.137]-007.271-089.844-089.86-089.168

KEY WORDS: arteriovenous anastomosis; electrodiagnostic methods.

Reports have recently been published on a new type of operation intended for use in the treatment of patients with obliterative diseases of the limb arteries. These operations consist of arterial reconstructions accompanied by the formation of an arteriovenous anastomosis (AVA), used as the last opportunity to prevent amputation of the limb in cases when, because of insufficient capacity of the drainage vessels, traditional reconstructive operations on arteries had little prospect of success. Formation of an AVA provided a new drainage channel through the veins, and thus increased the velocity of blood flow in the prosthesis, preventing its thrombosis [1, 3-5]. However, these reports have been few in number and opinions regarding the effectiveness of the suggested method conflict. The main objection is that when an AVA is formed most of the blood flow is "dumped" from the prosthesis into the vein, and for that reason the volume of the blood flow entering the distal arterial bed may be insufficient to supply the limb with blood after the operation [6].

Because of the absence of unanimity on this question it was decided to carry out an experimental study. An electromagnetic flowmeter was used for the hemodynamic measurements, and blood supply and the intensity of ischemia were estimated by means of an electrodiagnostic method, namely a study of the electrical excitability of the limb muscles after the operation.

EXPERIMENTAL METHOD

Experiments were carried out on 25 adult mongrel dogs of both sexes weighing from 14 to 22 kg. The animals were divided into five experimental groups with five dogs in each group. Dogs of group 1 (control) underwent the operation of ileofemoral replacement by a graft of human umbilical vein (diameter 5 mm) and a model of incompetence of the drainage channels was created. This was done by ligating and dividing all collaterals of the femoral artery between its anastomosis with the graft and the site of origin of the ramus saphenus (Fig. 1). The femoral artery also was itself ligated just below the origin of the r. saphenus. The prosthesis developed thrombosis in all the animals of this group during the 1st day after the operation.

In addition to arterial replacement and creation of a model of incompetence of the drainage channels, in the other 20 dogs an AVA was formed between the femoral artery and femoral vein. In all cases the AVA was situated 2-3 cm below the prosthesis, and only the dimensions of the AVA differed: in dogs of group 2 the diameter of the anastomosis was 1-2 mm, i.e., 20-40% of the diameter of the graft; in dogs of groups 3, 4, and 5 its diameter was 2-3 mm (40-60%), 3-4 mm (60-80%), and 4-5 mm (80-100%), respectively.

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0007-4888/88/0003-0321$12.50