EFFECT OF AN ASSISTED CIRCULATION AFTER COMBINED COLD CARDIOPLEGIA ON MYOCARDIAL PHOSPHOLIPID COMPOSITION AND ULTRASTRUCTURE

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The assisted circulation (AC) has proved its worth in complicated forms of myocardial infarction, in heart failure after operations with an artificial circulation, and in unstable angina [1, 3, 5, 8]. However, there are no data in the literature on the use of AC after combined cold cardioplegia.

The object of this investigation was to study relations between the phospholipid (PL) level in the myocardium and the state of the membranous ultrastructures when an AC was used after hypothermic perfusion and cold cardioplegia in experimental animals.

EXPERIMENTAL METHOD

Healthy mongrel dogs were used. Under balanced anesthesia hypothermic perfusion was carried out with the AIK-5M apparatus. In the course of 60-90 min the animal was cooled to 26°C. The aorta was then clamped and 250-300 ml of 5% glucose solution, cooled to 4°C, was injected intra-arterially, causing cardiac arrest. Cardioplegia was maintained by external irrigation of the heart with the same solution. The aorta was occluded for 50-60 min. An AC with the

TABLE 1. Composition of PL in Myocardium (in mg/g wet weight of tissue) in Course of Experiment (M ± m)

Parameter	Control (n = 8)	Stages of experiment			
		I (n = 22)	II (n = 20)	III (n = 6)	IV (n = 6)
Unidentified fraction	0,65±0,02	0,70±0,05	0,67±0,03	0,81±0,09	0,72±0,07
P		>0,05	>0,05	>0,05	>0,05
Lysophosphatidylcholines	0,84±0,04	0,89±0,05	1,13±0,09	1,12±0,08	1,00±0,06
P		>0,05	<0,01	<0,02	<0,05
Sphingomyelins	1,34±0,06	1,38±0,09	1,42±0,06	1,34±0,05	1,32±0,07
P		>0,05	>0,05	>0,05	>0,05
Phosphatidylcholines	7,51±0,12	6,45±0,45	4,61±0,61	2,35±0,61	3,07±0,56
P		>0,05	<0,001	<0,001	<0,001
Phosphatidylinositol with phosphatidylserine	1 39±0,07	1,69±0,18	1,65±0,08	2,22±0,23	2,05±0,21
P		<0,05	<0,05	<0,01	<0,02
Phosphatidylethanolamines	3,61±0,41	2,86±0,40	2,46±0,36	1,26±0,19	1,49±0,12
P		<0,05	<0,05	<0,001	<0,001
Polyglycerophosphatides	3,26±0,22	3,09±0,26	3,15±0,21	4,84±0,29	3,60±0,26
P		>0,05	>0,05	<0,001	>0,05
Total NPL	13,21±0,54	11,44±0,49	9,12±0,86	6,73±0,81	6,88±0,88
P		<0,05	<0,001	<0,001	<0,001
Total APL	4,65±0,22	4,71±0,24	4,72±0,61	7,06±0,99	5,65±0,72
P		>0,05	>0,05	<0,05	>0,05
NPL/APL ratio	2,84±0,21	2,45±0,29	1,93±0,16	0,97±0,12	1,24±0,20
P		>0,05	<0,01	<0,001	<0,001
Grand total of PL	18,61±0,63	17,06±1,10	15,09±0,90	13,94±0,36	13,25±0,40
P		>0,05	<0,01	<0,001	<0,001

Legend. P relative to control.

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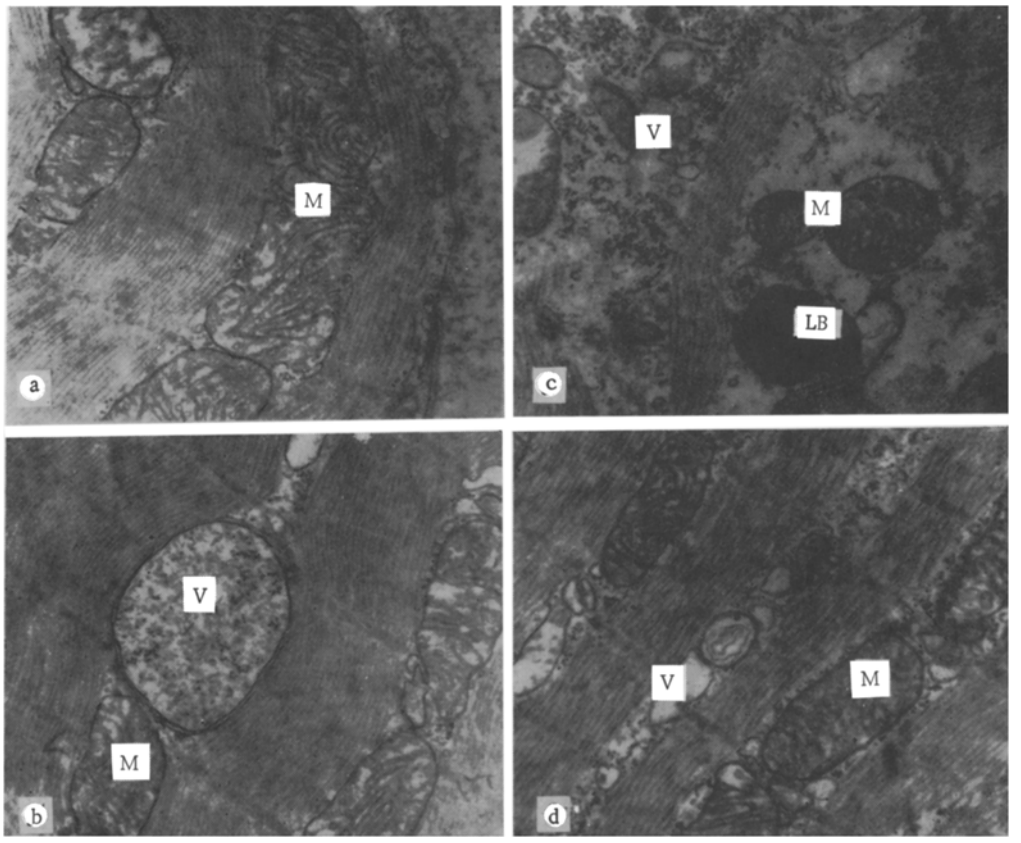

Fig. 1. Ultrastructure of myocardium at different stages of cold cardioplegia and after AC: a-d) stages I, II, III, and IV respectively. M) Mitochondria, V) vacuoles, LB) lipofuscin bodies. 35,000 ×.

AVK-4 apparatus was used when it was impossible to disconnect the artificial circulation apparatus because of severe cardiac failure.

The artificial ventricle was connected between vena cava and aorta and the blood was oxygenated. The volume of the ventricle was 100-150 ml, the stroke ejection 50-60 ml, duration of systole 210-230 msec, and it was synchronized relative to the P wave on the ECG.

Myocardial tissue for morphological and biochemical investigations was taken in the surgical stage of anesthesia (I), before removal of the clamp from the aorta (II), in the parallel circulation stage (III), and after the effective use of the artificial ventricle (IV). Material for electron microscopy was fixed with glutaraldehyde and osmium and embedded in Epon-Araldite.

Phospholipid fractions were determined by thin-layer chromatography [4].

EXPERIMENTAL RESULTS

In the surgical stage of balanced anesthesia the total PL level was unchanged, but this proportion was observed in the composition of individual PL. In particular, the content of phosphatidylinositol and phosphatidylserine was increased whereas that of phosphatidylethanolamine was reduced (Table 1). The membranous ultrastructures of the myocardium likewise showed no marked changes, although reduction of the cristae of the mitochondria with translucency of the matrix was observed in individual myocytes (Fig. 1a).

In the second stage of the experiment before removal of the clamp from the aorta (cardioplegia) a decrease in the total PL level was observed mainly on account of phosphatidylcholine. An increase in the number of vacuoles surrounded by a single membrane (Fig. 1b), single lipid granules, and myelin figures were found in the myocytes. However, in some parts of the myocardium there were no significant changes in the membranous structures.

During the development of cardiac failure and in the stage of the parallel artificial circulation (III) considerable fluctuations were observed in the phospholipid composition of the cardiac myocytes and a fall in the total PL level. The ratio of neutral PL (NPL) to acid PL (APL) (NPL/APL) fell by almost two-thirds. The phosphatidycholine and phosphatidylethanolamine concentrations decreased by 38.6 and 31.9% of the control level respectively, but the concentrations of phosphatidylserine and phosphatidylinositol increased, possibly due not only to mutual conversions, but also to disturbances of the initial stages of biosynthesis [2].

The rise in the lysophosphatidylcholine level (by 33.3%) accompanied by a parallel fall in the phosphatidylcholine concentration can also be explained by activation of the corresponding phospholipases.

The increase in the concentration of polyglycerophosphatides which, as we know, play an important role in regulation of enzyme activity of the respiratory chain [6], was probably compensatory-adaptive in character, caused by inhibition of activity of these enzyme systems under the experimental conditions used.

Reduction of the cristae was observed in most mitochondria; the mitochondria were swollen and in some of them the outer membrane was ruptured, with vacuole formation. The layers of the nuclear membrane showed significant changes characterized by dissection of their contours. Swellings were found in some places, in the form of vacuoles and vesicles. Numerous vacuoles, surrounded by a single-layered membrane, were found between the myofibrils and myocytes, especially in the zones of edema and beneath the plasmalemma. The number of free myelin structures was increased, and they were distributed both in the myocytes and in the interstitial tissue (Fig. 1c). Individual myelin structures were found in the lumen of the microvessels.

The use of an AC for 30-60 min, restoring the normal cardiac ejection from the left and right ventricles and the intracardiac hemodynamics, did not restore the normal phospholipid composition of the myocardium. However, a decrease in vacuolation and restoration of the membranous structures of the mitochondria and nuclear membranes were observed in the electron microscope (Fig. 1d).

Cold cardioplegia, carried out by the chosen method, thus leads to marked disturbances of PL metabolism and of the membranous structures of the myocytes, and these are evidently responsible for the disturbances of myocardial contractility.

The use of an AC restores the intracardiac hemodynamics and blood supply to the myocardium, has a beneficial effect on the ultrastructure of the myocyte membranes, but does not restore the normal phospholipid composition of the myocardium.

LITERATURE CITED

1. V. I. Burakovskii and V. G. Barvyn', in: Current Problems in Therapy [in Russian], Kiev (1976), p. 98.
2. G. A. Grivanov, Usp. Sovrem. Biol., 80, No. 3, 382 (1975).
3. A. Kh. Kasymov et al., Kardiologiya, No. 11, 90 (1979).
4. I. V. Ovchinnikov et al., Med. Zh. Uzbekistana, No. 11, 38 (1978).
5. B. V. Petrovskii, in: The Assisted Circulation [in Russian], Tashkent (1980), p. 3.
6. V. P. Skulachev, Usp. Sovrem. Biol., 77, No. 2, 125 (1974).
7. I. A. Shabanova, Biokhimiya, No. 6, 1155 (1967).
8. B. V. Petrovskii and V. I. Shumakov (editors), Problems with the Artificial Heart and Assisted Circulation [in Russian], Moscow (1970).