Changes in Myocardial Metabolism and Ultrastructure of Myocardial Microvessels during Pharmacological and Cold Cardioplegia under Hypothermic Conditions without Perfusion

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A combination of pharmacological and cold cardioplegia in with hypothermia without perfusion in open-heart surgery guarantee the reversible character of shifts in energy and free radical balance in the myocardium. However, this procedure can impair coronary micricirculation due to structural and functional changes in microvessel endothelium. Our results demonstrate that new cytoprotective approaches are extremely needed for cardiac protection during surgery.

Key Words: *myocardium*; *cardioplegy*; *metabolism*; *ultrastructure*; *microvessels*

Abundant experience is available in the field of pharmacological and cold cardioplegia (PCC) during open-heart surgery [10]. A large number of cardioplegic solutions were designed [9,12]. However, the problem of prevention of myocardial functional impairments is solved completely. Some difficulties consist in that distinct biochemical correlates of effectiveness of myocardial protection are not established yet. In this respect, a most promising approach is a complex morphobiochemical examination, which combines determination of indices of myocardial oxidative metabolism and ultrastructural analysis of coronary vessels, which cardioplegic solution enters first. The study of myocardial state during hypothermia without perfusion can give additional information, because this method of anti-ischemic protection differs principally from artificial circulation.

Our aim was to compare the dynamics of structural modifications developed in coronary microvessels (MV) and biochemical clinical indices of myocardial state in patients, which underwent open-heart surgery during hypothermia without perfusion and PCC.

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MATERIALS AND METHODS

The study included 22 patients aged 9.8±0.7 years with congenital ventricular septal defect undergoing synthetic patch plasty for the cardiac defect under conditions of hypothermia without perfusion. In all patients, PCC was produced with hyperosmolar normokaliemic solution cooled to 2-4°C before manipulation of the heart. Anesthetic management of deep hypothermia without perfusion was carried out [3]. The maximum depth of cooling (26.8-24.5°C) was achieved at a rate of 1°C per 6.7±0.7 min. The mean times of occlusion and reperfusion were 28.3±0.3 and 2.2±0.3 min, respectively.

Biochemical tests included measuring of glucose, lactate, and malonic dialdehyde (lipid peroxidation product) contents, and catalase activity by standard methods [2] in arterial and venous (coronary sinus) blood. Blood samples were taken before cooling, be-

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TABLE 1. Perfucion Capacity of Myocardial Microvessels and Population Composition of Endothelial Cells (EC) in Patients with Congenital Ventricular Septal Defect at Various Stages of Cardiac Defect Plasty under Conditions of Hypothermia without Perfusion and Normokaliemic Pharmacological and Cold Cardioplegia (*M*±*m*; % of Total Population in Section)

Parameter	At the end of hypothermia before occlusion (t_M=26.1°C; *n*=8)	At the end of occlusion (t_M=18.8°C; *n*=7)	During reperfusion (t_M=18.8°C; *n*=8)
Microvessels, %			
open	41.5±7.1	16.5±3.6*	27.8±6.7
closed	58.5±7.1	83.5±3.6*	72.3±6.7
EC basic type (with preserved ultrastructure)	47.5±1.5	34.6±2.7*	32.1±2.1*
with signs of necrosis			
colliquative (edematous)	2.4±0.8	1.9±0.7	9.9±1.5+
coagulation (hyperosmic)	1.6±0.9	9.5±1.6*	4.1±1.8+

Note. *p<0.05 compared to preocclusion period, +compared to occlusion period. t_M: myocardial temperature; *n*: number of patients.

fore termination of hypothermia just before cardiac defect correction, and 30 min after resumption of cardiac function.

Biopsy samples for electron microscopy were cut from the right atrial myocardium at the peak of hypothermia before the start of cardioplasty, at the end of circulatory arrest, and 30 min after circulation recovery. The samples were routinely fixed and processed for electron microscopy. Ultrastructural analysis of MV included evaluation of MV lumens and estimation of percentage of various morphological types of endothelial cells (EC) using methods described previously [1,14].

The data were statistically processed using Student's *t* test.

RESULTS

Clinical investigation revealed good recovery of cardiac function after surgical correction of congenital heart disease under conditions combined cardioplegia. Spontaneous sinus rhythm was observed in 6 patients (27.3%), most patients (54.5%) needed only one defibrillator discharge, and only 4 patients (18.2%) required multiple defibrillation. However, in the postoperative period, symptoms of acute heart failure (6 patients) and unstable hemodynamics as a consequence of low cardiac output syndrome (9 patients, 40.9%) were observed, both complications required intensive cardiotonic therapy.

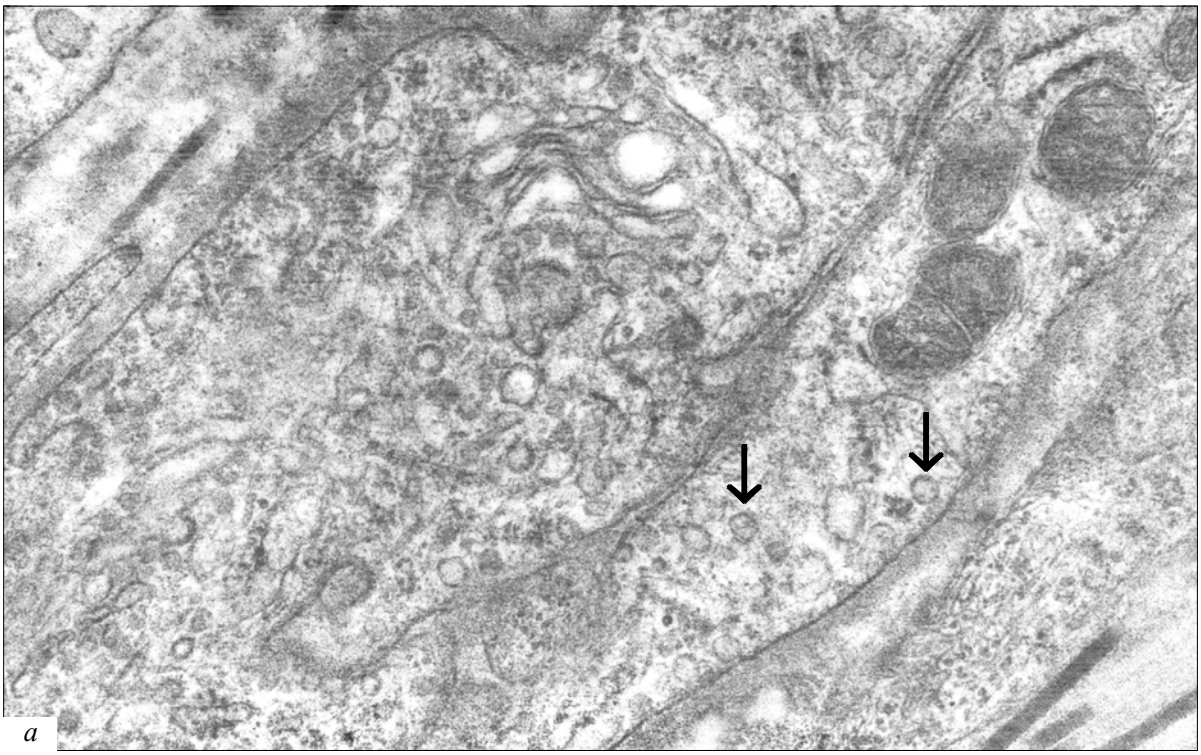
Analysis of laboratory data revealed a correlation between the postoperative functional disorders and myocardial structural and metabolic changes, which developed at various stages of operation including the period before aortic occlusion. Thus, a relatively high number of closed MV was observed in myocardial vascular bed even at the stage of cooling, suggesting the fall of coronary perfusion (Table 1). Most closed MV were collapsed, others were drastically narrowed due to EC nucleus protrusion into the lumen (physiological regulatory mechanisms of microcirculation activated by EC cytoskeleton rearrangements) [11].

Specific feature of myocardial metabolism during hypothermia was a pronounced lactacidemia (Table 2). In 36.4% patients it was also accompanied by appea-

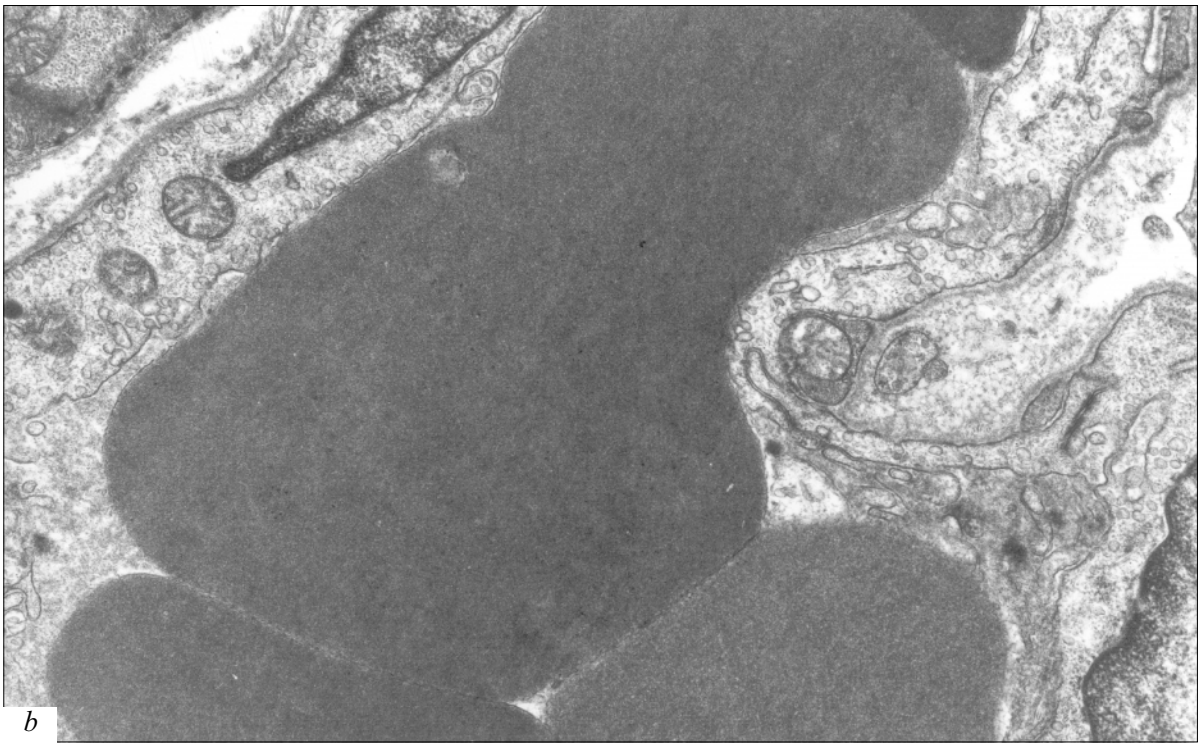
TABLE 2. Changes in Oxidative Metabolism in Myocardium and Lipid Peroxidation in Arterial Blood/Coronary Sinus Blood During Surgical Correction of Congenital Heart Defect under Conditions of Hypothermia without Perfusion and Pharmacological and Cold Cardioplegia (*M*±*m*; *n*=22)

Parameter	Before occlusion		Reperfusion
	normothermia	at the end of hyperthermia	
Glucose, mM	5.48±0.56/—	7.24±0.63**/7.34±0.68	15.5±1.2*/16.9±1.4*
Lactate, mM	0.81±0.11/—	3.12±0.34*/3.01±0.30	5.19±0.41*/5.02±0.35*
Free fatty acids, mM	0.30±0.03/—	0.53±0.05*/0.50±0.04	0.40±0.02**/0.36±0.04**
Malonic malonic dialdehyde, μM	5.30±0.21/—	5.66±0.28/5.97±0.35	9.05±0.43*/9.74±0.47*
Catalase, mcat/l	61.6±7.29/—	69.6±4.5/73.1±5.6	118.4±8.7*/112.8±7.3*

Note. *p<0.01, **p<0.05 compared to previous stage.



a



b

Fig. 1. Ultrastructure of right atrial microvessel in a patient with congenital ventricular septal defect at the stage of cooling before surgical correction of cardiac defect under conditions of hypothermia without perfusion combined with pharmacological and cold cardioplegia (*a*) and at the stage of reperfusion after correction of the defect (*b*). Fragment of cytoplasm of basic type endothelial cell (*a*): active Golgi complex with long cisterns, mitochondria with homogeneous matrix and clearly seen crista, numerous transport vesicles (indicated by arrows), ×43,500; erythrocytes in a vessel, ×11,250.

rance of lactate in coronary sinus blood. Thus, the disturbances in myocardial metabolism associated with hypoxia [16] developed before aortic occlusion. In addition, the increase in blood levels of high-energy molecules was accompanied by their reduced utilization (Table 2). In most cases, lactate and fatty acid metabolism was more preferable compared to glucose (33.9%) metabolism. Arteriovenous differences in lactate and fatty acid levels was positive in 63.6 and 64.7% patients, respectively.

According to modern concepts, these metabolic changes can result from impaired function of membrane-bound enzymes playing an important role in the transport high-energy substrates into parenchymal cells [5]. Low perfusion capacity of coronary microcirculation also contributes to these changes. The observed preferential utilization of fatty acids and decreased glucose consumption during hypothermia without perfusion can be an adaptive response to intraoperative stress [7]. At the cellular level, these adaptive shifts towards predominant utilization of fatty acids improve EC integrity maintenance, confirmed by MV ultrastructural analysis. Despite the effect of hypothermia on cell homeostasis, the organelles with synthetic and transport functions were harmonically developed in most EC (Fig. 1, *a*). These EC (basic type, Table 1) constitute the structure-functional basis of the endothelium, which is responsible for cell tolerance to ischemia associated with cardiac arrest [14].

The number of closed MV in the myocardium significantly increased during ischemia (Table 1). Open MV often contained flocculated or osmiophilic substance, which resulted from of blood segmentation during circulatory arrest and coagulation of coarse-dispersed components [6] enhanced due to hemodilution with cardioplegic solutions [15]. When fatty acid consumption prevails over their oxidation, it can result in detergent-like effects on plasma membrane [13] aggravating the degree of ischemic injury to the myocardium.

Ultrastructural analysis of MV manifested in a sharp decrease in basic type EC population and pronounced growth of hyperosmic cell population (Table 1), dying by mechanism of coagulation necrosis due to impairment of protein and carbohydrate metabolism [4]. Changes in membrane permeability due to enhanced lipid peroxidation can also contribute to EC hyperosmolarity. The fact that in 50% patients the arteriovenous difference for malonic dialdehyde observed before occlusion was negative (Table 2) confirmed this assumption. These processes also resulted in the appearance of a large number of free EC fragments and round-shaped membrane structures containing transparent matter in MV.

During reperfusion, no significant increase was observed in MV patency compared to the occlusion period (Table 1). Among other factors responsible for MV narrowing, the primary role was played by endothelial protrusion and sludge phenomena (Fig 1, *b*). Electron microscopy also revealed enhanced interstitial edema accompanied by the appearance of myelin figures, vesicles, and degenerating cardiomyocyte organelles in interstitial tissue. MV endothelium characterized by considerable increase in proportion of edematous cells subjected to colliquative necrosis, while population of basic type EC remained unchanged (Table 1). However, increased number of patients with positive arteriovenous difference for glucose, lactate, and fatty acids during reperfusion (50, 70, and 71%, respectively, Table 2) indicated the recovery of myocardial metabolism to its normal state.

During reperfusion, ultrastructure of coronary MV was characterized by a lesser number of EC with signs of coagulation necrosis (Table 1). At the same time, a noticeable increase in both arterial and venous blood level of malonic aldehyde (Table 2), and a greater percent of patients (from 50 to 68.2%) with negative arteriovenous difference for malonic aldehyde indicated production of peroxidation metabolites in the myocardium at this stage. Simultaneous activation of catalase (Table 2) did not compensate accumulation of secondary lipid peroxidation products. This, in turn, could result in membrane permeability changes and, as a consequence, a higher number of edematous EC, while the proportion of basic type EC remained below the normal (Table 1). It is most likely that activation of lipid peroxidation is responsible for poor coronary microcirculation at this stage of operation.

Weak endothelium-dependent vasodilation responses to specific agents [8] due to inhibition of endothelium-dependent dilating factor by peroxidation products is a possible reason responsible for MV did not open during reperfusion. Another cause could be endothelial micro- and macroclasmatosis in MV induced by accumulation of toxic peroxidation metabolites during reoxygenation and rewarming of ischemic tissue.

Thus, we revealed some trigger mechanisms of functional disorders in the postoperative heart. Despite some shifts in myocyte metabolism associated with inhibition of membrane-dependent enzymes, the use of PCC in combination with hypothermia without perfusion in most patients provided rapid recovery of cardiac function after resumption of circulation. Changes in perfusion capacity of coronary microvessel and structural and functional modification of EC membranes involved in the regulation of transport of high-energy substrates through the endothelium could result in some postoperative complications, which require intensive therapy.

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