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Ultrastructure of Myocardial Capillary Endothelium in Children with Congenital Heart Disease

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Cardiosurgical stress initiates endothelial injury of the colliquation necrosis type (without activating coagulation necrosis) in coronary capillaries of infants aged under 1 year. The dark cells exhibited high tolerance to operation stress in the presence of labile ultrastructural response of endothelial cells of the main and light types. The percentage of dark cells does not change during surgical intervention, which is a sign predicting a favorable course of the postoperative period.

Key Words: *Fallot's tetralogy; congenital ventricular septal defect; myocardium; endothelial cells; ultrastructure*

Due to introduction of innovation technologies of surgical treatment of cardiovascular diseases, correction of congenital heart diseases during the first years or even months of life became one of the main trends of modern cardiosurgery. However, the problem of operation stress factors tolerance infant patients is still far from being solved. The myocardium of newborn mammals is functionally more resistant to ischemic stress than the heart of an adult individual. However, laboratory models differ from the heart of a child with a developmental defect, because these infants suffer from chronic hypoxia which significantly reduces myocardial resistance to long intraoperative ischemia of the organ [1].

Disagreement between the results of clinical and experimental studies are determined by many circumstances essential for the pathogenesis, for example, age-associated characteristics of myocardial structure, biochemistry, and function [11]. On the other hand, age-specific structural organization of various compartments of the heart remain insufficiently studied, for example, the ultrastructure of the myocardial capillary endothelial cells (EC) in infants aged under 1 year, including the cell status under conditions of cardiosurgical stress.

We carried out ultrastructural analysis of the time course of morphological changes in the coronary capillary EC in infants aged under 1 year at various stages of surgical correction of congenital heart disease.

MATERIALS AND METHODS

Electron microscopic analysis was carried out on diagnostic biopsy specimens of the right atria of 10 patients (mean age 8.9±0.6 months), operated on for Fallot's tetralogy and congenital ventricular septal defect. The main stage of the operation was carried out under conditions of artificial circulation and pharmacological and cold cardioplegia. Biopsy

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specimens were collected before the defect correction after induction of artificial circulation directly before occlusion of the aorta at body temperature of 33.6±0.5°C; at the end of aorta occlusion period, which lasted for 42.1±5.7 min; after coronary bloodflow resumption at 36.70±0.13°C after 26.00± 3.06 min of reperfusion.

Biopsy specimens for electron microscopy were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde and postfixed in 1% OsO_4, after which were treated by standard methods.

Intraoperative changes in EC forming the inner lining of the right atrial capillaries, were evaluated as described previously [6]. The profiles of EC lining all exchange capillaries in the section, were estimated at three stages of the operation at the initial magnification ×10,000. Five morphological variants of EC were distinguished in the total cell population, based on differences in electron density of the cytoplasm and degree of the microvesicular system development: cells of the main type, clear, dark, edematous, and hyper-osmium cells. The percentage of each variant in the total number of EC in a section was determined.

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

RESULTS

Before occlusion of the aorta, the main type and dark EC predominated in the right atrial capillary endothelium (Table 1). The number of clear EC was much lower in the total population, the percentage of edematous and hyper-osmium EC was negligible.

The majority of the main type EC, clear and dark EC, differed significantly by the electron density of the cytoplasm; structural and functional zones were clearly differentiated in them (Fig. 1, *a*). Fenestra were sometimes seen in the peripheral regions of EC. On the other hand, irrespective of the electron density of the cytoplasm, no differences in the thickness of the organelle zone and peripheral zone were noted in the endothelium of the so-called "single-suture" capillaries, sometimes detected in the myocardium of children aged under 1 year. The nuclei in the three structural functional variants of EC contained little or moderate amount of condensed chromatin, located near the nuclear membrane, which had porous complexes. The nucleoli were mainly of classical structure, with diffuse fibrillar component, located centrally, and the adjacent compact fibrillar and granular components. The mitochondria varied in size and electron density of the matrix. Some of them contained moderately electron-dense matrix, sometimes with small clarified locuses, while others looked electron transparent and retained just small fragments of the cristae. Tubules of granular endoplasmic reticulum (GER) with large ribosomes on membranes were rarely grouped around the mitochondria. The Golgi complex was hyperplastic and consisted of several complexes, each consisting of cisterns of different size. They were surrounded by numerous vacuoles, vesicles (Fig. 1, *b*) and specific granules with matrix of different density.

The status of the microvesicular system enabled us to distinguish three arbitrary subgroups in each of the "working" EC variants. In subgroup 1 the reduction of the cytoplasm electron density during transition from dark EC to EC of the main type and clear ones was paralleled by a reduction of the population of free and fixed transport vesicles, which was observed in the presence of hypertrophied organelles, participating in intracellular synthesis. In subgroup 2 the "working" EC, differing by electron density of the cytoplasm, had similarly hypertrophic organelles, involved in intracellular synthesis and transendothelial transfer of macromolecules. Subgroup 3 in the myocardial "single-suture" capillaries in infants aged under 1 year were EC containing (irrespective of electron density of the cytoplasm) nuclei with even granular karyoplasm, hypertrophic GER and Golgi complex, surrounded by numerous vesicles and granules,

TABLE 1. Quantitative Characteristics of Various Populations of Right Atrial Capillary EC in Infants Aged under 1 Year at Different Stages of Aortocoronary Bypass Surgery with Pharmaco-Cold Cardioplegia for Correction of Congenital Heart Disease (*M*±*m*)

Stage of operation	Morphological variants of EC, %				
	main type	clear	dark	edematous	hyper-osmium
Before occlusion of aorta	44.54±2.81	15.61±3.61	37.42±5.57	1.20±0.36	1.23±0.51
End of occlusion	29.18±3.32*	29.18±4.60*	25.47±4.71	13.70±3.53*	2.47±0.73
Reperfusion stage	37.50±4.90	21.86±3.62	26.34±5.47	?2.98±5.47*	1.14±0.40

Note. *$p<0.05$ compared to values before occlusion.

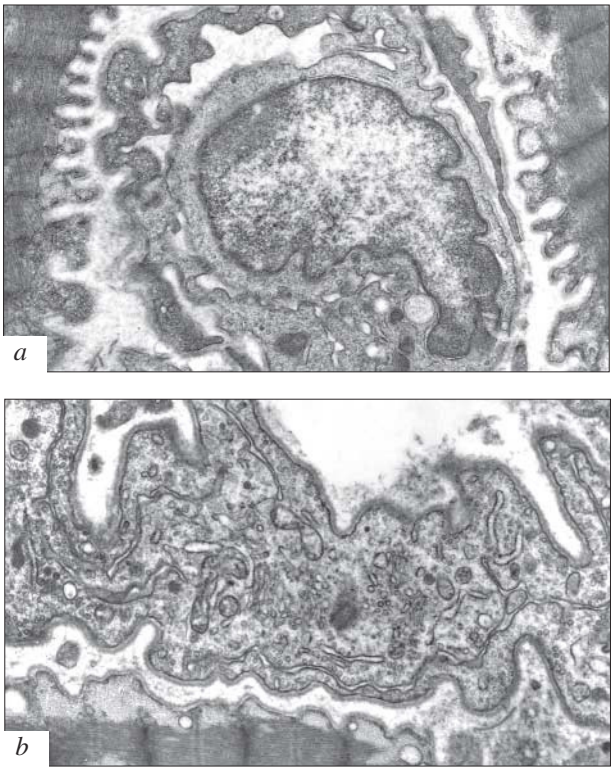

Fig. 1. Ultrastructure of right atrial capillary in a patient with congenital heart disease before correction. *a*) two morphological variants of EC (dark and main type) in a capillary with narrow lumen, ×13,200; *b*) hypertrophic and hyperplastic Golgi complex in the cytoplasm of the main type EC, ×13,200.

while the number of transport vesicles in them was the minimum.

Edematous EC had electron-transparent cytoplasmic matrix with sharply swollen nuclei and mitochondria and vacuolated and reduced GER cisterns and Golgi complex. The organelles virtually could not be discerned in osmiophilic cytoplasm of hyper-osmium EC.

A significant ($p<0.05$) reduction in the number of the main type EC in the capillary endothelium was registered at the end of long total myocardial ischemia (Table 1). This was paralleled by a statistically significant increase in the percentage of clear and edematous EC, while the counts of dark and hyper-osmium EC remained at the level of the pre-occlusion stage of the operation.

Subcellular organization of the main type, clear, and dark EC varied, but on the whole, 2 subgroups could be distinguished in each of the three "working" variants. Subgroup 1 included EC with fine organization, similar to that at the beginning of surgery, though their secretory system contained granules characterized by much greater variety of shapes and sizes than before occlusion of the aorta (Fig. 2, *a*). The nuclei in subgroup 2 contained high amounts of osmiophilic heterochromatin. Different organization of chromatin, including its significant loosening observed in some clear EC, or, by contrast, pronounced condensation of euchromatin structure intrinsic of the dark EC, was observed in the karyoplasm zones free from compact lumps. Scanty mitochondria and small GER tubules with few attached ribosomes were grouped in the perinuclear space. The cristae in the majority of mitochondria were reduced and packed in locally or completely clarified matrix. Golgi complex was presented by short fragments of cisterns and accumulation of vacuoles. Visually all "working" varieties of EC contained less transport vesicles than at the beginning of surgery.

The ultrastructure of hyper-osmium EC was typical, but myelin-like structures were more often accumulated on large protrusions of the luminal surface of edematous EC, than during the previous stage of the operation (Fig. 2, *b*).

During myocardial reperfusion, the percentage of the main type, clear, dark, and hyper-osmium EC changed negligibly in comparison with the ischemic period and the pre-occlusion stage of the operation; the number of edematous EC was more than before occlusion of the aorta (Table 1).

The ultrastructure of the main type and clear EC was characterized by polymorphism. The common architectonics and subcellular morphology of a minor part of EC was the same as before occlusion. The profiles of other EC were unevenly dilated. Sites without organelles and granules appeared in their cytoplasm; the nuclei had clarified karyoplasm. The mitochondria looked swollen and had virtually no cristae (Fig. 3, *a*), while GER and Golgi complex were vacuolated. Dark EC were clearly subdivided into 2 subgroups. One of the subgroups did not differ from the typical dark EC, while in the other EC were close to the hyper-osmium cells, their fine pseudopodias were often rejected into the capillary lumen (Fig. 3, *b*). The micropinocytous activity of the cells varied. For example, cells with cytoplasm hypersaturated by micropinocytous vesicles were detected in all three "working" variants of EC, while in other EC the number of vesicles was sharply reduced. In addition, there were cells in which the cytoplasmic areas, densely packed with micropinocytous vesicles, alternated with sites completely devoid of transport vesicles. The morphology of edematous and hyper-osmium EC virtually did not differ from that at the previous stages of the operation.

Our findings indicate that all morphological variants of EC, previously detected in animal experiments [6] and in cardiovascular patients aged more than 1 year [5], were present in the right atrial

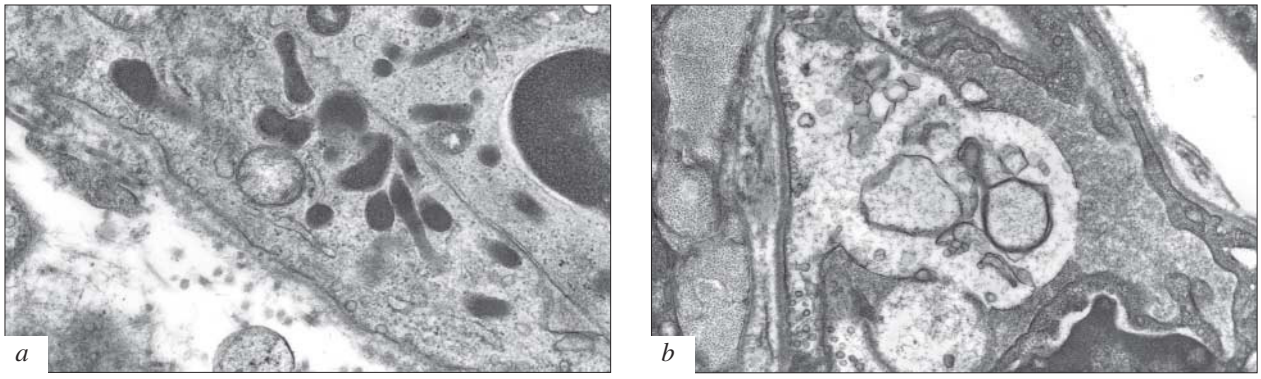

Fig. 2. Ultrastructure of right atrial capillary in a patient with congenital heart disease at the end of aortic occlusion. *a*) accumulation of specific endothelial granules, sharply differing by size and shape, in the cytoplasm of the main type cells, ×26,000; *b*) large protrusion of an edematous EC, containing myelin-like bodies, deep into the capillary lumen, ×16,600.

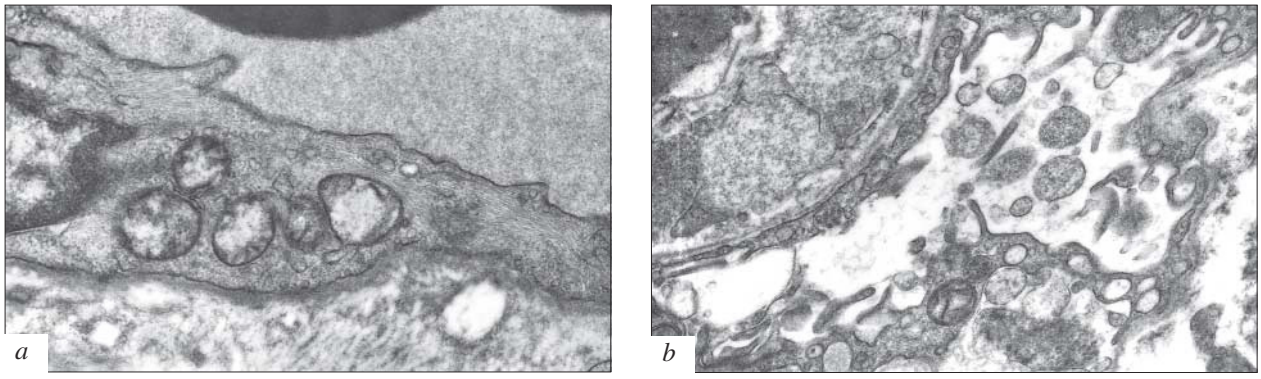

Fig. 3. Right atrial capillary ultrastructure in a patient with congenital heart disease at the stage of myocardial reperfusion. *a*) sharply swollen mitochondria with few cristae and clarified matrix in the cytoplasm of the main type EC, ×20,000; *b*) pseudopodias of a dark EC, budding into the capillary lumen, ×16,600.

exchange capillary endothelium of infants aged under 1 year. However, precise classification of EC appurtenance to one of the 5 morphological variants is difficult. This is largely explained by the possibility of using the totality of suggested ultrastructural criteria for just a part of the total cell population. "Leveling" of the degree of development of organelles, involved in intracellular synthesis and transendothelial transfer of macromolecules, was observed in some EC irrespective of the cytoplasm density. The main cause of this phenomenon is, no doubt, hypertrophic processes in the myocardium of patients with congenital heart disease even in so young age [3]. It is noteworthy that the severity of hypertrophic and hyperplastic changes in the structural elements of the right heart compartments (including the atrial capillary endothelium) in ventricular septal defect complicated by pulmonary hypertension and in Fallot's tetralogy is determined by the pressure gradient between the right ventricle and pulmonary artery.

In addition, EC with morphology differing from the definitive one are detected in infants aged under 1 year. These cells are characterized by the absence of clear-cut interface between the structural and functional zones and little number of micropinocytous vesicles, which is a sign of incomplete differentiation of vascular endothelium. The progress of postnatal functional specialization of myocardial cells in congenital heart disease is nonspecific, which was confirmed by previous studies, demonstrating high degree of myofibrillogenesis in right ventricular cardiomyocytes in patients with Fallot's tetralogy aged about 9 months [2].

Cardiosurgical stress induces coronary capillary endothelial damage by the colliquation necrosis type without causing activation of the coagulation necrosis. This is in line with the opinion of many authors, considering that the newborns and infants aged under 1 year are characterized by high tissue hydrophilia [7] and higher (in comparison with adults) sensitivity of the specific membrane pumps regulating the ionic flows outside and inside the cell, to pathological stress associated with circulation arrest under conditions of artificial circulation [4]. On the other hand, tolerance of dark EC to cardiosurgical stress factors proven at the ultrastructural level by the absence of changes in their

population percentage at different stages of the operation in our study, can be regarded, according to some authors [5], as an evidence of adequate anesthesiological measures aimed at protection of the myocardium from ischemic and reperfusion injuries.

Labile intraoperative response of the main type and clear EC seems to be an additional morphological sign of adequate cardioprotection. Blood-flow recovery in the myocardium under conditions of pronounced sensitivity of these morphological variants of EC to ischemic stress promotes restoration of the population percentage of each of these variants to the level, just slightly differing from the pre-occlusion level.

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