Ultrastructural Analysis of Secretory Granules of Myocardial Capillary Endothelium in Cardiosurgical Stress

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In patients with chronic myocardial tissue hypoxia irrespective of the disease (Fallot's tetralogy, coronary disease) factors of cardiosurgical stress initiate a drop of secretory production in coronary capillary endothelium and obstruction of the intravascular space by blood cells. In children the peak of exocytosis of secretory granules coincides with the period of aortic occlusion, while in adults it is attained at the stage of reperfusion.

Key Words: *Fallot's tetralogy; coronary heart disease; cardiovascular surgery; secretory function of endothelium*

Evaluation of secretory activity of endothelial cells (EC), whose ultrastructural equivalents are specific endothelial granules (SEG) or Weibel—Palade bodies, is essential for understanding of the pathogenesis of various diseases. Vasoactive molecules accumulating in these granules regulate various biological processes, among which are neovasculogenesis, vasoconstriction/vasodilatation, balance in the hemostasis system, and adhesion characteristics of vascular surfaces contacting with the blood [11,15].

The early postoperative period after open heart interventions is associated with a decrease in SEG pool in coronary capillary endothelium, which positively correlates with the progress of obstruction of the intravascular space by formed elements of the blood [3]. On the other hand, some aspects in the endothelial functioning under conditions of stress exposure remain little studied.

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We compared the dynamics of degranulation of EC in the right atrium of children with chronic hypoxia during radical correction of Fallot tetralogy and in adult patients during surgical treatment of coronary disease.

MATERIALS AND METHODS

Ultrastructural analysis of coronary capillary endothelium was carried out in diagnostic biopsy specimens of right atrial myocardium from patients of two groups. Group 1 consisted of 7 patients aged 4.9±0.5 years operated for Fallot tetralogy; group 2 consisted of 8 coronary patients aged 54.2±1.8 years subjected to aortocoronary bypass surgery. Biopsy specimens in both groups were collected before occlusion of the aorta at body temperature of 36.4±0.4 and 33.64±0.30°C in groups 1 and 2, respectively, at the end of occlusion of the aorta (61.8±7.1 and 82.30±8.13 min), after recovery of the coronary bloodflow at warming temperatures of 37.0±0.3°C and 36.42±0.35°C, respectively. Biopsy specimens for electron microscopy were processed routinely.

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In order to evaluate the secretory function of the endothelium of exchange capillaries the total number of EC profiles in a section was determined for all biopsy specimens, SEG were identified, after which the number of granules per 100 EC profiles was estimated.

Morphological characteristics of the capillary bed capacity transport were studied. The total number of exchange capillary profiles in a section was counted, irrespective of the vessel diameter. The percentage of capillaries with open and slit-like lumens in the total number of vessels was evaluated. These capillaries were subdivided into functionally closed and obturated. Collapsed capillaries and capillaries blocked by EC nuclei protruding into the lumen were referred to subgroup 1. Subgroup 2 comprised capillaries blocked with aggregations of blood cells with sharply swollen EC cytoplasm and numerous bubble-shaped formations of low electron density. The percentage of capillaries belonging to each subgroup was evaluated.

Additional hematological studies of arterial blood samples collected at the same stages of intervention as right atrial biopsy specimen were carried out in patients operated for coronary disease (n=21). The concentration of Willebrand factor in formaline-teated platelets was measured by the Evans and Austen photocolorimetry method [1]. The results were statistically processed using Student's t test.

RESULTS

In patients of both groups at all stages of the operation two morphological varieties of SEG, light and dark, were distinguished in the right atrial capillary endothelium. The former were characterized by electron-clear matrix and as a rule had a clearly seen intact membrane. Dark granules were filled with electron-dense osmiophilic contents, their membrane was usually fragmented. Tubular structures oriented parallel to the long axis were seen in the matrix of the majority of granules. On transverse sections these tubules looked like tightly packed rings. Some granules had no tubules and contained granular substance. The

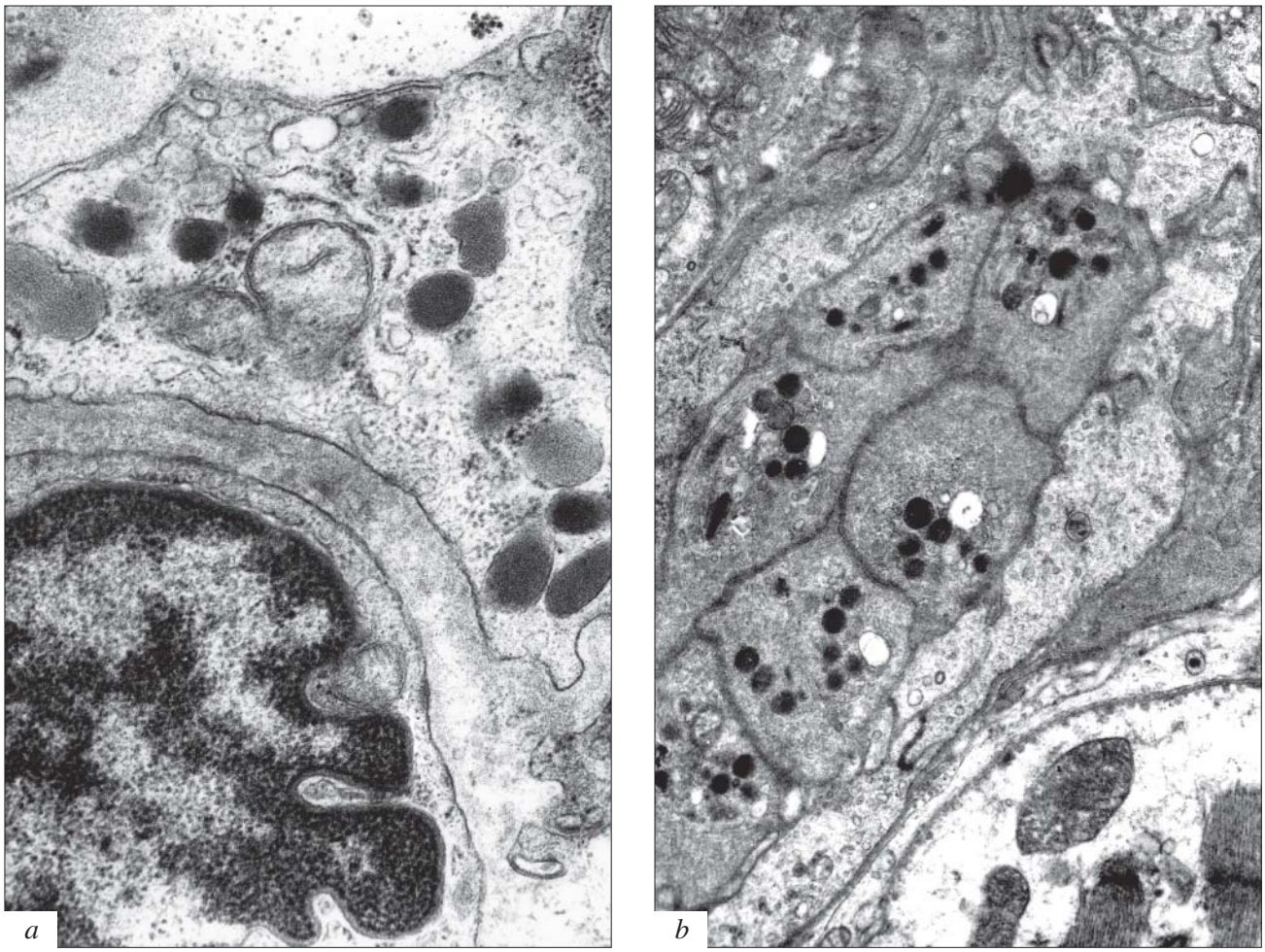

Fig. 1. Ultrastructure of right atrial capillaries in patients with Fallot tetralogy. *a*) before aorta occlusion (group of secretory granules differing by the shape and electron density of the matrix in endothelial cell cytoplasm), ×26,000; *b*) at the end of aorta occlusion (platelet aggregation obturates capillary lumen), ×15,000.

most prevalent shapes of SEG were round, oval, and irregular; stab-like granules were rare (Fig. 1, *a*). The size of SEG varied within 0.1-0.4 μ (width) and 0.4-1.5 μ (length).

Before occlusion of the aorta the number of SEG per 100 EC was virtually the same in both groups; the values varied within a wider range in coronary patients in comparison with those with congenital heart disease (Tables 1, 2). This suggests that the level of secretory production of capillary endothelium did not depend on the disease and patient's age. Variations in the fine structure of granules were also unspecific and were determined by accumulation of a wide spectrum of bioactive products, such as Willebrand factor [5], endothelin-I [10], and P-selectin [9]. As not all these molecules are simultaneously present in each granule, variations in electron density and fine structure of the granules can reflect SEG capacity to accumulate different sets of bioactive products.

Perfusion characteristics of coronary capillaries in patients of both groups had common features and differences. Adult coronary patients had much more open capillaries than children, the greater part of capillaries with slit-like lumen being functionally excluded from the circulation in both cases (Tables 1, 2). These differences can depend on specific features of myocardial capillary bed remodeling in this heart disease. Capillaries of patients with congenital heart disease were transformed into vessels of capacity type, true (exchange) capillaries were desolate, atrophic, and reduced [4].

The number of SEG dropped at the end of the period of aortal occlusion in both groups (Tables 1,2). In group 1 the reduction of granules was statistically significant in comparison with the preocclusion stage of the intervention ($p<0.05$), the number of closed capillaries increased at the expense of simultaneous activation of functional mechanisms of lumen stenosis and development of obturation (Table 1). In group 2 the decrease in SEG population was negligible and was not associated with exclusion of additional capillaries from the circulation. However, the percentage of closed obstructed vessels increased significantly ($p<0.05$) in coronary patients, the number of functionally closed capillaries remained unchanged (Table 2).

Obstruction of the vascular lumen seemed to be caused by exocytosis of granules, through which bioactive molecules accumulated in SEG are released into extravascular space. Massive degranulation of EC leads to induction of adhesion molecules [6], activates procoagulant factors [5] and vasoconstrictive agents, and increases vasomotor tone [10]. As a result, capillary dilatation is impaired and blood cell adhesion to vascular wall increases (Fig. 1, *b*), which deteriorates myocardial revascularization.

TABLE 1. Changes in the Perfusion Capacity of Right Atrial Capillaries and Endothelial Granule-Producing Function in Patients with Fallot Tetralogy at Different Stages of Correction under Conditions of Artificial Circulation (*M*±*m*)

Stage of operation	Capillaries, %		Mechanisms of capillary exclusion from bloodflow, %		Specific endothelial granules, number per 100 cells
	open	closed	functional stenosis	obturation	
Before occlusion of aorta (T_n=36.4±0.4°C)	36.44±3.74	63.56±3.74	52.87±4.25	10.69±2.04	206±20
At the end of occlusion of aorta, 61.8±7.1 min	22.19±4.58*	77.81±4.58*	51.24±5.67	26.57±2.98*	95±21*
Reperfusion (T_n=37.0±0.3°C)	31.85±6.51	68.15±6.51	34.25±6.53*	33.90±5.16*	90±23*

Note. Here and in Table 2: *$p<0.05$ *vs.* the value before occlusion.

TABLE 2. Changes in the Perfusion Capacity of Right Atrial Capillaries and Endothelial Granule-Producing Function in Coronary Patients at Different Stages of Aortocoronary Bypass Surgery under Conditions of Artificial Circulation (*M*±*m*)

Stage of operation	Capillaries, %		Mechanisms of capillary exclusion from bloodflow, %		Specific endothelial granules, number per 100 cells
	open	closed	functional stenosis	obturation	
Before occlusion of aorta (T_n=33.6±0.3°C)	53.81±2.84	46.91±2.84	35.62±2.64	10.57±1.88	219±47
At the end of occlusion of aorta, 82.3±8.1 min	43.48±5.42	56.52±5.42	26.78±4.99	29.74±6.28*	123±29
Reperfusion (T_n=36.4±0.4°C)	40.71±5.41	59.29±5.41	15.66±4.18*	43.63±4.58*	79±12*

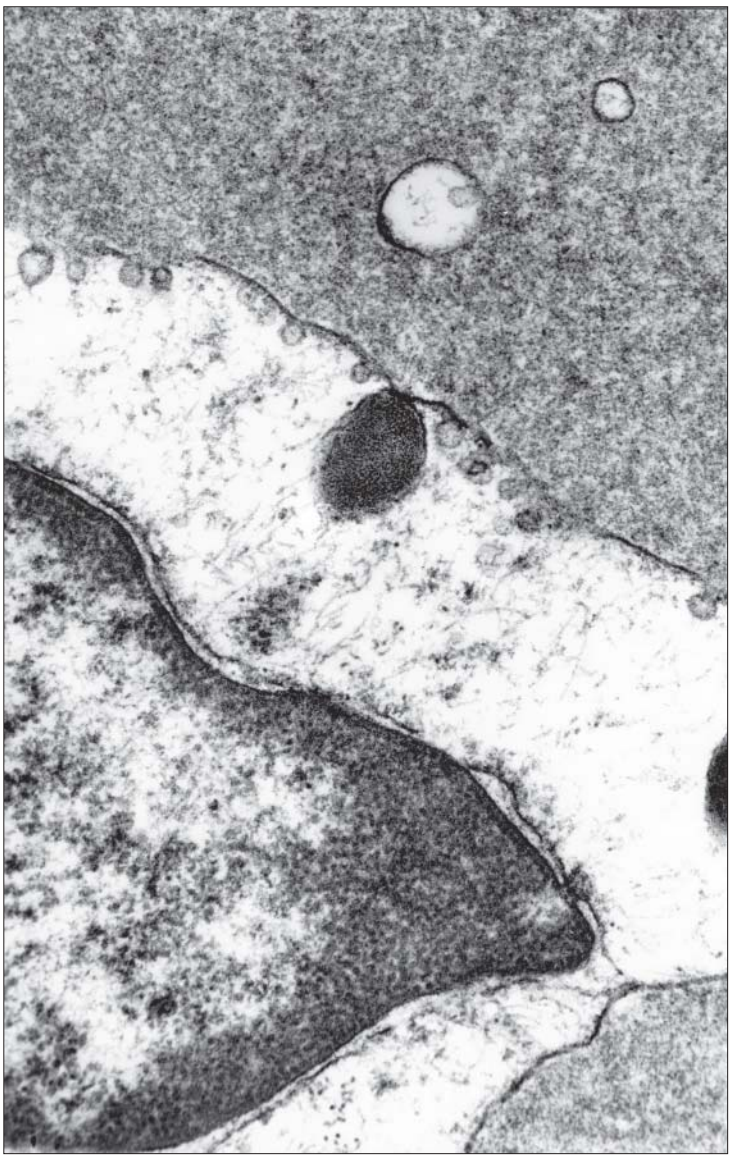

Fig. 2. Ultrastructure of right atrial capillary in a coronary patient during myocardial reperfusion. Point contact of the secretory granule membrane with luminal surface of endothelial cell, ×32,000.

In group 1 the number of SEG did not change during reperfusion of the myocardium in comparison with the occlusion period (Table 1). By contrast, in group 2 the trend to a decrease in the number of SEG was retained, their number became 2.8 times lower than at the beginning of the operation (Table 2). The number of functionally closed capillaries decreased in both groups ($p<0.05$), while the number of obstructed capillaries increased (Tables 1, 2).

The mechanisms of reactions detected in the coronary vessels of patients with congenital heart diseas, seem to be different. Bioactive molecules stored in SEG and modulating the capillary capacity of passage can be released into the lumen not only through regulation pathway, but also constitutively [7]. In the latter case accumulation of synthesized products in specific organelles of EC is ruled out and the products are released into intravascular space directly from the Golgi compartment. During reperfusion the reactions of oxygenated blood with ischemic endothelium can cause a burst in the formation of free oxygen radicals, which impair characteristics of EC plasma membranes [13] by promoting cell swelling and impeding recovery of the capillary blood flow.

The intensity of granule exocytosis positively correlates with accumulation of Ca^{2+} in cells [12], and hence, intensification of EC degranulation process in coronary patients is most likely related to calcium overload of the myocardium, developing during reperfusion of pre-ischemized tissue. Decrease in the granule pool can be also caused by the crystalloid cardioplegic solutions; their use for rapid asystolic arrest of circulation can sharply intensify Ca^{2+} inflow into the cell [14], causing a release of numerous SEG into the blood and stimulating leukocyte and platelet adhesion to luminal surface of EC.

A specific feature of the periods of aortic occlusion and myocardial reperfusion during surgical treatment of patients of both groups is high incidence of point contacts of SEG membrane with plasma membrane of EC (Fig. 2), a morphological equivalent of exocytosis stage 1.

According to the data of hemostasis analysis, three subgroups of patients differing by the concentration of Willebrand factor in arterial blood could be distinguished in group 2. In subgroup 1 (19.05% of total number of patients) the level of this glycoprotein was normal (120-140%), in group 2 (42.5%) it was elevated (>140%), and in group 3 (38.09%) it was lowered (<120%).

At the end of total myocardial ischemia three subgroups of patients could also be distinguished: in group 1 (38.10%) the concentration of Willebrand factor increased by 55.15±14.89% compared to preocclusion stage of the operation (irrespective of the initial values of the parameter), in group 2 (28.57%) the parameter decreased by 74.28±43.33%, and in group 3 (33.33%) it remained unchanged. At the stage of reperfusion we also distinguished three subgroups of patients, differing by the dynamics of concentration of Willebrand factor in the arterial period in comparison with the period of total myocardial ischemia: in subgroup 1 (42.86%) the content of this glycoprotein decreased by 29.67±7.00%, in subgroup 2 (38.10%) its concentration sharply increased by 125.81±55.01%, and in subgroup 3 (19.04%) there were no significant changes.

Ultrastructural analysis of degranulation of coronary capillary endothelium showed a decrease in the content of endocrine products by 56.24±11.09% in 85.7% children (group 1). In group 2 three subgroups differing by the dynamics of EC degranulation in comparison with the initial stage of operation were distinguished: in subgroup 1 (62.5%) the content of SEG decreased by 52.29±7.55%, in subgroup 2 (12.5%) their content increased 1.81 times, and in subgroup 3 (25%) there were no differences. During the reperfusion stage the number of granules in capillary endothelium increased by 40.60±11.51% compared to the period of total myocardial ischemia in 80% patients of group 1. In 75% group 2 patients the number of granules in capillary endothelium decreased by 42.42± 8.65%, in 12.5% patients it slightly (1.2 times) increased, and in the rest 12.5% there were no changes in comparison with the occlusion stage.

The findings of ultrastructural analysis of the myocardium and hematological analysis showed higher functional heterogeneity of EC secretory system at all stages of the operation in group 2 compared to group 1. Since surgical treatment of coronary patients involved no global hypothermia and cardiotomy, inevitable in radical correction of Fallot tetralogy, presumably, heterogenous response of EC secretory system in both groups of patients during occlusion was stimulated by the difference in accumulation of ATP degradation products (adenosine) by the myocardium. Adenosine is a potent inductor of SEG emptying with the release of Willebrand factor from EC via activation of adenosine A_2 receptors [2].

After coronary blood flow recovery the polymorphism of EC endocrine system in patients of both groups seems to depend on the thermal regimen of reperfusion. Secretory granules are extremely sensitive to the status of EC subcortical microfilaments, which decelerate the preparatory phase of exocytosis [8]. Presumably, thermal reperfusion of the myocardium after cold cardioplegia in coronary patients causes reduction of EC actin microfilaments [7]. The activity of this process seems to vary, causing granule exocytosis in some patients, decelerating it in others, and leaving it unchanged in still others.

Hence, the number and ultrastructural variety of coronary capillary SEG in heart diseases characterized by chronic myocardial tissue hypoxia (Fallot tetralogy or coronary disease) do not depend on the disease and patient's age.

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