Effect of Calcium Antagonists on Ultrastructure of Myocardial Microvessels during Open Heart Surgery under Conditions of Pharmacological and Cold Cardioplegia

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Ultrastructure of myocardial microvessels was examined during surgical correction of congenital heart disease (ventricular septal defect) under conditions of cold and pharmacological low-potassium cardioplegia without perfusion. Addition of calcium antagonist verapamil to cardioplegic solution prevented postischemic damage to vascular and perivascular structures occurring during reperfusion and body temperature rise.

Key Words: *congenital heart diseases, myocardium; microvessels; Ca^{2+}-channels; ultrastructure*

Correction of congenital heart diseases is associated with both compensatory and pathological changes in the myocardium [9], which decreases heart resistance to surgical stress leading to disturbances in calcium homeostasis. Increased Ca^{2+} permeability of cell membranes impairs structural and functional integrity of cardiomyocytes, which most markedly manifests during reperfusion of ischemic myocardium [2]. The possibility of preventing and correcting ischemic and reperfusion damages to the heart with drugs inhibiting Ca^{2+} entry was demonstrated [3,4], however the data on the use of these blockers as components of cardioplegic solutions are insufficient.

The aim of the present study was to examine ultrastructural changes in coronary microvessels (MV) during correction of ventricular septal defect using cardioplegic solutions containing Ca^{2+} antagonists.

MATERIALS AND METHODS

Forty-four myocardium specimens obtained by knife biopsy from the right atrium of 15 patients with congenital ventricular septal defect (mean age 10.7±1.1 years) were examined under an electron microscope. The surgery was carried out under conditions of deep hypothermia (26-24°C) with cold and drug cardioplegia. Cardioplegic solution (2-4°C) was infused once into the aorta root (5-8 ml/kg body weight). Infusion pressure did not exceed 60-80 mm Hg.

In group I patients, the main surgical stage was performed by standard method of drug and cold cardioplegia routinely used in the Institute of Circulatory Pathology [5]. Group II patients received injections of standard cardioplegic solution containing 2.25 mg/liter Ca^{2+}-channel blocker verapamil (Knoll AG).

In both groups, specimens were obtained before occlusion at myocardium temperature of 26.10± 0.14°C (n=8 and n=7 in groups I and II, respectively), at the end of ischemic period lasting for 22.64±2.24 min (n=7 in both groups), and during reperfusion 36.53±5.17 min after resumption of cardiac activity at myocardium temperature of 31.16±0.24°C (n=8 and n=7 in groups I and II, respectively). The material for electron microscopy was processed routinely.

All MV in a section independently on their size were subjected to ultrastructural analysis. The total number of MV profiles and the number of profiles with open and slit-like lumens were determined. The

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latter were divided into two types depending on hemodynamic changes. Type I included MV with preserved wall ultrastructure and slit-like lumens and MV with sharply narrowed lumens due to focal swelling of the perinuclear zone of endothelial cell (EC). These changes in vascular wall architectonics and MV endothelium reflect adaptive rearrangements of heart hemodynamics caused by blood flow redistribution and regulation of MV filling. Type II included MV with pathological changes in blood cells, noncellular components, and EC. These MV contained erythrocyte, leukocyte and platelet aggregates; edema, swelling, and alteration of EC were seen in some MV, EC formed cytoplasmic outgrowths partially or completely obstructing the MV lumen. Additionally, the volume density of connective tissue and interstitial fluid [6] were determined in specimens obtained during reperfusion. The data were processed statistically using Student *t* and Fisher tests.

RESULTS

In the specimens obtained before aorta occlusion, type I MV with signs of adaptive microcirculatory changes prevailed (Table 1). Type II MV presented a minor part of all MV and contained erythrocytes with preserved ultrastructure, uniformly granular plasma with EC fragments formed due to microclasmatosis, and middle-size membrane structures filled with electron transparent or granular matrix. Sclerosis and focal edema were seem in the myocardial stroma.

During the occlusive (ischemic) period of operation the number of closed MV of both types increased compared to the preischemic period and the open/closed MV ratio was shifted towards closed MV. In group I type I MV dominated (Table 1, Fig. 1, *a*), while in group II the number of narrowed MV closed by swollen EC or their cytoplasmic outgrowths was considerably increased (Fig. 1, *b*). Sludge of blood cells was only occasionally seen in both groups.

Open MV contained heterogeneous plasma independently on the composition of cardioplegic solution. In both groups, some MV were filled with uniformly granular plasma, other MV contained floccular or osmiophilic plasma. Blood cells were presented by erythrocytes with normal ultrastructure typical of native cells. Some MV contained erythrocytes with abnormal shape and transparent granular matrix. Open MV with small EC fragments and bubble-shaped structures in the lumen were more abundant compared to the previous stage. Moderate interstitial edema, myelin-like structures, erythrocytes and transparent bubbles similar to those in vascular lumens were found in all specimens.

After resumption of cardiac activity (reperfusion), the number of open MV increased in group II, but not in group I (Table 1). It should be emphasized that in closed MV of group II patients the compensatory reactions in the regulation of microcirculation prevailed. On the contrary, in group I both the compensatory and alterative reactions occurred with equal frequency.

The structure of the plasma in open MV during reperfusion was different. In group II, practically all MV had normal plasma ultrastructure (Fig 2, *a),* while in group I it was loosened or contained electron-transparent loci of various sizes and shapes not surrounded with membranes (Fig. 2. *b*). In both groups, erythrocytes formed coin columns; however, in group I some cells had abnormal shape and electron transparent matrix.

During reperfusion the initial structure of the stroma most markedly recovered in group II patients. Thus, volume density of interstitial fluid in this group was significantly lower than in group I (0.071±0.006 and 0.164±70.010, respectively, $p<0.05$). In both groups the interstitial connective tissue contained destroyed cardiomyocytes, bubble-like membrane structures, free and aggregated erythrocytes; however, in group II the relative volume of cell debris in the interstitium was significantly lower.

TABLE 1. Number of Myocardial MV (% of Total Number in Section) with Different Perfusion Capacities in Patients with Ventricular Septal Defect Operated under Conditions of Cold and Drug-Induced Cardioplegia with Various Solutions (*M±m, n*=7-8)

MV characteristics	Before occlusion		At the end of occlusion		Reperfusion	
	control	verapamil	control	verapamil	control	verapamil
Open	41.5±7.1	38.0±10.9	16.6±73.6	10.4±1.1	27.8±6.7	59.3±6.2*
Closed	58.5±7.1	62.0±10.9	83.5±3.6	89.6±1.1	72.3±6.7	40.7±6.2*
including:						
physiologically	47.8±7.4	53.7±9.6	73.1±4.6	57.9±5.4**	39.0±7.3	29.6±7.5
pathologically	10.8±4.1	8.4±2.8	10.5±3.1	31.8±5.8*	33.3±9.1	11.1±2.5**

Note. *$p<0.01$, **$p<0.05$ compared to the control.

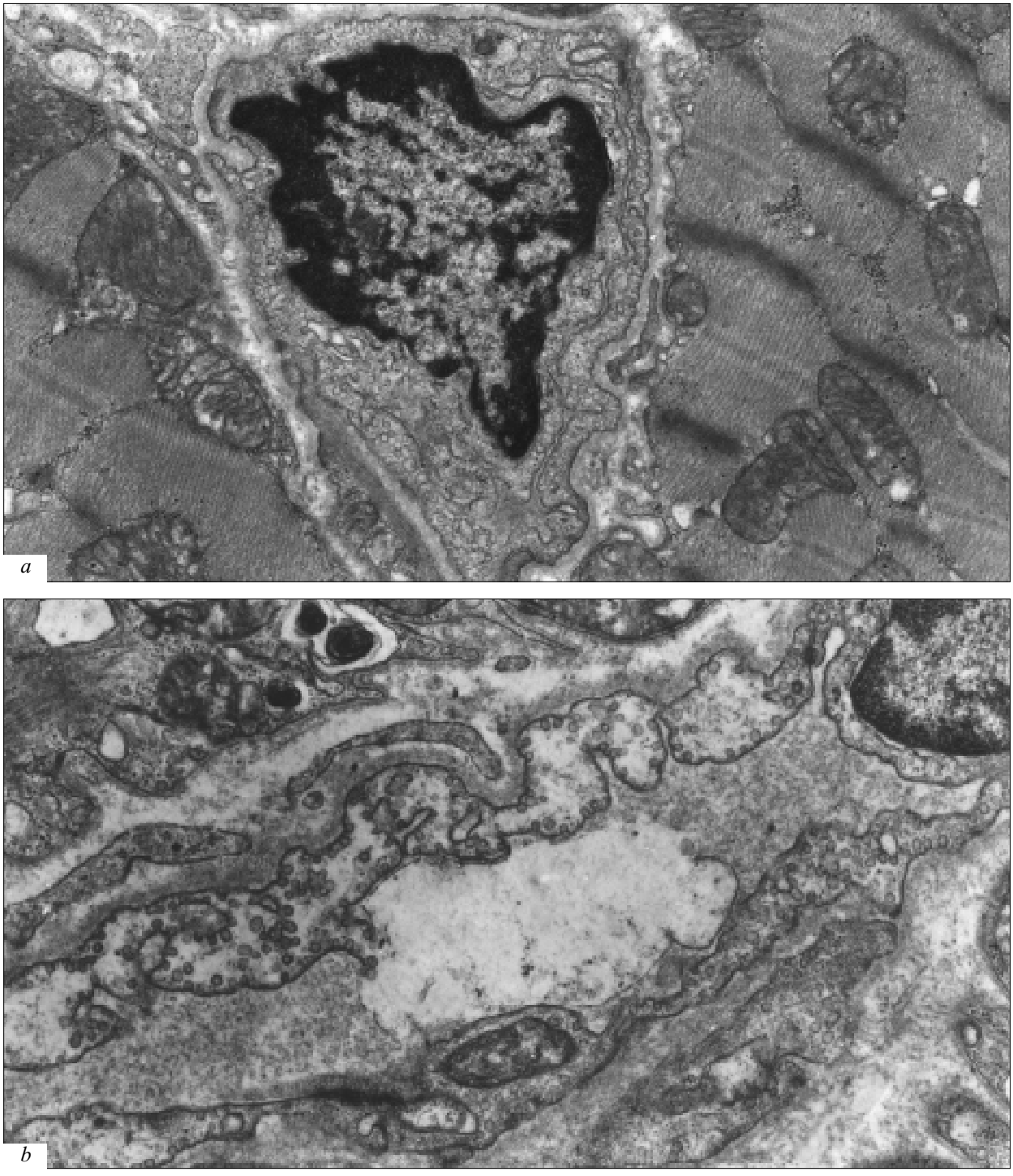

Fig. 1. Ultrastructure of microvessels in the right atrium in patients with ventricular septal defect receiving standard (*a*) and verapamil-containing (*b*) cardioplegia solution during occlusion of the main cardiac vessels. *a*) large endotheliocyte nucleus protruding into microvessel lumen, ×21,500; *b*) swollen outgrowth of endothelial cell obstructing microvessel lumen, ×23,500.

The obtained results suggest that cardiac arrest with cardioplegic solutions was accompanied by changes in coronary MVnot depending on the composition of cardioplegic solutions and reactions induced by Ca^{2+} blockers. In both groups the number of open MV decreased, plasma structure was impaired, accumulation of cell debris in vessels and perivascular zone and interstitial edema were seen. These reactions were induced by deep hypothermia decreasing perfusion pressure in myocardial MV [15], cardioplegia associated with blood sedimentation and coagulation [7], and global cardiac ische-

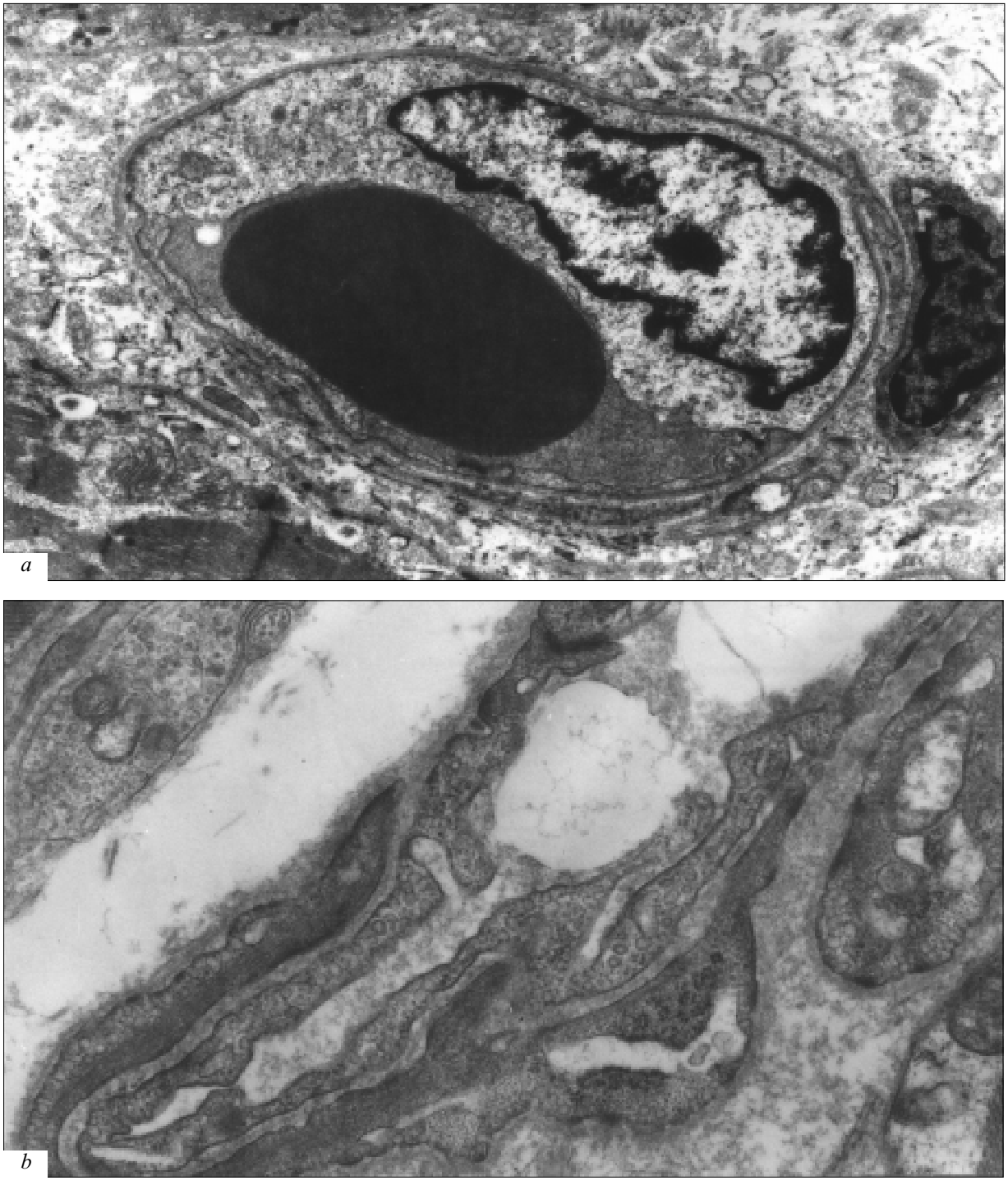

Fig. 2. Ultrastructure of microvessels in the right atrium in patients with ventricular septal defect receiving standard (a) and verapamil-containing (b) cardioplegia solution during reperfusion and warming. *a*) normal plasma structure in an open microvessel lumen, ×10,750; *b*) bubble-like structures in microvessel lumen, ×29,250.

mia. The latter was accompanied by pathological microclasmatosis of MV endothelium, edema and loosening of the perivascular connective tissue, perivascular extravasation occurring against the background of impaired barrier function of the endothelium due to oxygen deficiency [1].

Specific MV response to Ca^{2+} blockers during occlusion manifested in impaired cell volume regulation, which depend on the composition of the cardioplegic solution and its interaction with other factors: deep hypothermia and long-term cardiac ischemia. Ca^{2+} blockers are ineffective in some forms of

cardiac decompensation and even aggravate cardiac ischemia due to impaired cation transport in cardiomyocytes [8].

In patients receiving verapamil, endothelium edema and impairment of MV permeability were reversible and showed morphofunctional signs of recovery at the early stages of reperfusion. According to our and previous data, this can be mediated by several mechanisms.

First, verapamil mediates MV vasodilation after long-term ischemia/reperfusion [10] because this drug maintains high level of NO production even by ischemic EC [11] and decreases the tone of precapillary sphincters by reducing Ca^{2+} entry into vascular smooth muscle cells [12].

Second, in group II the decrease in peripheral vascular resistance can be mediated by protective effect of Ca^{2+} antagonists on EC cytoskeleton, which is confirmed by the recovery of endotheliocyte mobility and restoration of their ability to regulate the blood flow due to dynamic changes (plasticity) in their perinuclear zone. Moreover, verapamil improves microcirculation during tissue reoxygenation by reducing interstitial edema and promoting elimination of microemboli and degenerated erythrocytes from the vessels. The latter is confirmed by the ability of Ca^{2+} antagonists to prevent oxidative damage to coronary vessels associated with impaired intracellular electrolyte homeostasis [13], as well as their nonspecific cytoprotective properties (inhibition of erythrocyte hemolysis) [14].

The obtained data indicate that application of Ca^{2+} antagonists during standard cardioplegia procedure does not potentiate its inhibitory action on ischemic changes in cardiac MV, but prevents the development of microvascular abnormalities associated with reperfusion paradox producing maximum pathogenetic effect on cell resistance to ischemia.

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