Comparative Pathomorphological Study of Contractile Myocardium under Conditions of Increased Left and Right Ventricular Afterload

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Structural changes in the myocardium under conditions of increased left and right ventricular afterload were studied using polarization microscopy and histological, histochemical, and stereological methods. Increased afterload not complicated by heart failure was characterized by low number of damaged cardiomyocytes (3.3-6.5%) and moderate structural changes in the ventricular myocardium (contractures of different severity). Increased afterload complicated by heart failure was characterized by high ratio of damaged cardiomyocytes (5.6-19.2%) and severe reversible (grade I and II contractures) and irreversible (grade III contractures and lump degradation of myofibrils) structural changes. Irreversible damage to most cardiomyocytes included plasmatic impregnation, which was most pronounced in the subendocardial layer of ventricles operating under conditions of increased afterload. Comparative study showed that increased left and right ventricular afterload induces similar pathomorphological changes in the contractile myocardium. Our results indicate that increased afterload to the right or left ventricle is accompanied by the development of stereotypical structural changes in the myocardium. Profound and severe disturbances can cause heart failure.

Key Words: *pathomorphology; cardiomyocytes; afterload; left ventricle; right ventricle; heart failure*

Increased afterload (pressure load) leads to heart overstrain and can result in heart failure. This disorder is related to structural and metabolic changes in contractile cardiomyocytes (CMC). Polarization microscopy is the most adequate method for detection of early pathological changes in the contractile apparatus of CMC [5,9]. Modern morphofunctional study of the myocardium involves polarization microscopy and histological, histochemical, and stereological techniques [5].

Here we performed a morphofunctional study of contractile CMC under conditions of increased afterload in the left (LV) or right ventricle (RV) and determined changes prognostically unfavorable for heart failure.

MATERIALS AND METHODS

Experiments were performed on 45 guinea pigs weighing 500-700 g. Open-chest surgery was performed under conditions of artificial ventilation. Inducing and maintaining narcosis involved intraperitoneal injection of 25 mg ketamine and 60-80 mg/kg sodium thiopental, respectively.

Functional activity of the cardiovascular system was studied electrocardiogram (ECG), LV pressure, RV pressure, and its first derivative (*dP/dt*). The data

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were recorded and analyzed on a hardware-software complex (personal computer and Mingograpf-82). Increased afterload was modeled by narrowing of the aorta or pulmonary artery leading to a 100% increase in ventricular systolic pressure compared to the basal level. The duration of vascular stenosis was 30 min.

The animals were divided into 5 groups. Group 1 included control sham-operated guinea pigs without vascular stenosis (n=8). The animals with stenosis of the aorta (n=15) and pulmonary artery not accompanied by heart failure (n=15) entered groups 2 and 3, respectively. Stenosis of the aorta (n=3) and pulmonary artery (n=4) was complicated by heart failure in guinea pigs of groups 4 and 5, respectively. The animals were euthanized by intraperitoneal injection of sodium thiopental in a lethal dose 45 min after catheterization.

A morphological study was performed on samples from the middle third of the wall in LV and RV. The samples were fixed with 10% neutral paraffin (Lilly method) and embedded in paraffin. Sections (5-7 μ) were stained with hematoxylin and eosin, Schiff reagent (amylase control), and Rego's iron hematoxylin. The relative volume of the interstitium, intact CMC, and damaged cells was estimated in Rego's stained preparations using a MEKOS-Ts television image analyzer and computer software for an optical and histochemical studies of morphological objects. Polarization microscopy was performed on a MBI-15 universal microscope. The results were analyzed by Student's t test.

RESULTS

Rego's staining revealed damage to a small number of CMC in the subendocardial and subepicardial layers of cardiac ventricles in control animals (1.4% myocardial volume, Fig. 1). Polarization microscopy (Table 1) detected only individual CMC with reversible contractures of grades I (increase in anisotropy of A-discs, no shortening of I-discs) and II (increase in anisotropy and nearness of A discs, shortening of I-discs) [5,9]. The periodic acid-Schiff (PAS) reaction with amylase control did not reveal plasmatic impregnation of CMC (Table 2), which illustrates the reversible nature of damage [5,9,12]. Our results are consistent with published data on contracture changes in several CMC even in control animals [5].

The relative volume of damaged CMC significantly increased in various areas of the heart under conditions of high afterload not complicated by heart failure (Fig. 1). In animals with aortic stenosis the relative volume of damaged CMC in LV was higher than in RV. These differences were observed in the subendocardial (6.5±0.3 and 3.3±0.2%, respectively, p<0.01) and subepicardial layer (5.2±0.2 and 3.3±0.2%, respectively, p<0.01). In animals with pulmonary artery stenosis the ratio of damaged CMC increased similarly in LV and RV and insignificantly differed in the subendocardial (4.4±0.2 and 4.2±0.1%, respectively) and subepicardial layers (4.3±0.2 and 3.4±0.2%, respectively, p<0.01).

Polarization microscopy showed that the number of CMC with contractures increases in both ventricles of the heart (Table 1). We found contractures of grades I, II, and III (anisotropic conglomerates) and lump degradation of myofibrils (loss of transverse striation, numerous lumps of anisotropic substances). It reflected the development of irreversible changes [5,9]. The irreversible nature of damage to several CMC was confirmed by positive PAS reaction with amylase con-

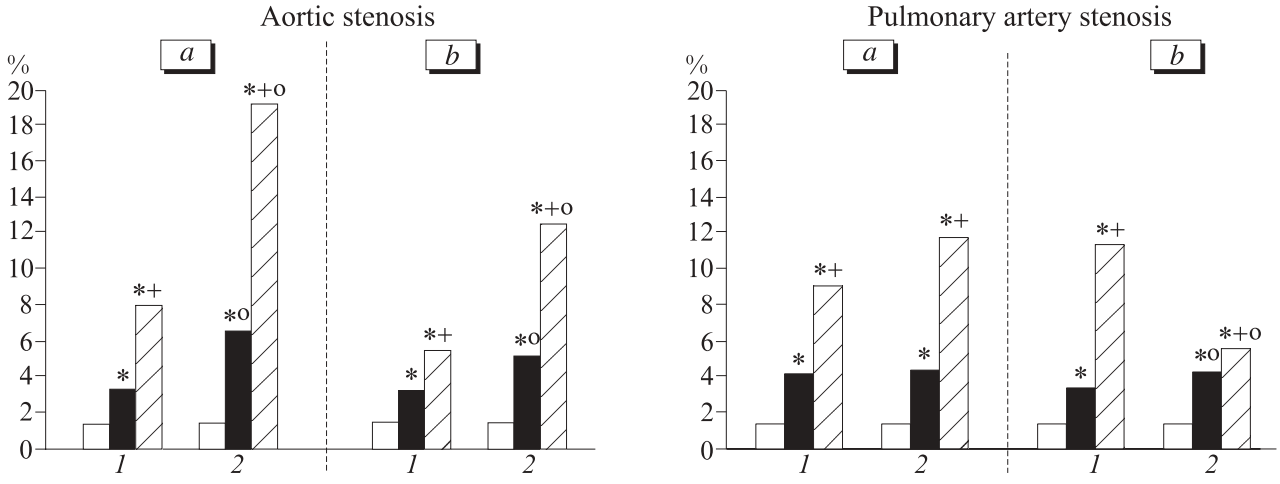

Fig. 1. Damage to cardiomyocytes in the right (*1*) and left ventricles (*2*) under conditions of increased afterload (Rego's staining). Subendocardial (*a*) and subepicardial layers (*b*). Ordinate: relative volume of damaged cardiomyocytes (%). Light bars: control. Dark bars: increased afterload without heart failure. Shaded bars: increased afterload with heart failure. p<0.01: *compared to the control; +compared to increased afterload without heart failure; °compared to the right ventricle.

trol [5,9,12]. The degree of plasmatic impregnation (Table 2) strongly correlated with the severity of damage to the contractile apparatus (grade III contractures and lump degradation of myofibrils, Table 1). Plasmatic impregnation of contractile CMC was most pronounced in the subendocardial layer of LV (grade III contractures) and RV (grade III contractures and lump degradation of myofibrils) in animals with stenosis of the aorta and pulmonary artery, respectively.

Our results show that increased afterload not complicated by heart failure is characterized by the appearance of a small number of damaged CMC (3.3-6.5%) and development of moderate structural changes in the ventricular myocardium (contractures of different severity). Previous studies showed that the increase in heart activity is accompanied by contracture of contractile CMC [3]. It could be hypothesized that the appearance of myocardial contractures in the affected ventricle is related to its hyperfunction. However, similar damage was found in the intact ventricle (relative to the site of afterload). These data indicate that the development of injury is associated with systemic alterations mediated by the sympathoadrenal system [4,8,10,13]. Our previous experiments showed that the increase in afterload during stenosis of the aorta or pulmonary artery is accompanied by activation of the sympathoadrenal system [7]. CMC in LV are more sensitive to the adverse effect of catecholamines than in RV [8]. These differences explain more extensive damage to left ventricular CMC under conditions of increased afterload in LV and RV. This conclusion is consistent with the results of our previous studies [6]. We showed that the area of myocardial damage in LV and RV is similar under conditions of increased RV afterload caused by massive pulmonary embolism (Rego's staining).

Under conditions of increased afterload accompanied by heart failure the relative volume of damaged CMC in various areas of the heart significantly increased and exceeded that observed in animals with uncomplicated course of the disorder (Fig. 1). In animals with aortic stenosis the relative volume of dam-

TABLE 1. Damage to Ventricular Cardiomyocytes under Conditions of Increased Afterload (Polarization Microscopy)

Type of damage, series	Aortic stenosis				Pulmonary artery stenosis			
	subendocardial layers		subepicardial layers		subendocardial layers		subepicardial layers	
	RV	LV	RV	LV	RV	LV	RV	LV
Grade I contractures								
control	—	—	—	—	—	—	—	—
stenosis	++	++	++	++	+	++	++	++
стеноз+СН	+	+	+	++	+	++	++	++
Grade II contractures								
control	—	—	—	—	—	—	—	—
stenosis	++	++	++	++	+++	++	++	++
stenosis and HF	++	+++	++	++	++	++	++	++
Grade III contractures								
control	—	—	—	—	—	—	—	—
stenosis	+	++	+	+	++	++	++	++
stenosis and HF	+++	+++	+++	+++	+++	++	++	++
Lump degradation of myofibrils								
control	—	—	—	—	—	—	—	—
stenosis	—	—	—	—	+	—	—	—
stenosis and HF	—	+	+	+	+	—	—	—
Myocytolysis								
control	—	—	—	—	—	—	—	—
stenosis	—	—	—	—	—	—	—	—
stenosis and HF	—	—	—	—	—	—	—	—

Note. —, no data or individual changes; +, minor changes; ++, moderate changes; +++, multiple changes. Here and in Table 2: HF, heart failure.

TABLE 2. Plasmatic Impregnation of Ventricular Cardiomyocytes under Conditions of Increased Afterload (Schiff Staining with Amylase Control)

Type of damage, series	Aortic stenosis				Pulmonary artery stenosis			
	subendocardial layers		subepicardial layers		subendocardial layers		subepicardial layers	
	RV	LV	RV	LV	RV	LV	RV	LV
Control	—	—	—	—	—	—	—	—
Stenosis	+	++	+	+	+++	++	++	+
Stenosis and HF	++	+++	++	++	+++	++	++	++

Note. —, no changes; +, individual changes; ++, minor changes; ++, moderate changes.

aged CMC in LV was higher than in RV. These differences were observed in the subendocardial (19.2±2.1 and 8.0±1.0%, respectively, $p<0.01$) and subepicardial layers (12.6±1.7 and 5.4±0.4%, respectively, $p<0.01$). The ratio of damaged CMC did not differ in the subendocardial layer of LV and RV in animals with pulmonary artery stenosis (11.8±1.9 and 9.1±1.4%, respectively). However, the ratio of damaged CMC in the subepicardial layer of LV was lower than in RV (5.6±0.7 and 11.4±2.0%, respectively, $p<0.01$).

Polarization microscopy showed that damage to contractile CMC includes contractures of grades I, II, and III and lump degradation of myofibrils. Aortic stenosis was accompanied by the appearance of grade III contractures and lump degradation of myofibrils in several CMC (Table 1). The increase in the ratio of irreversible damages correlated with the degree of plasmatic impregnation in CMC, which was particularly pronounced in the subendocardial layer of LV (Table 2). The animals with pulmonary artery stenosis were characterized by grade III contractures, lump degradation of myofibrils (Table 1), and pronounced plasmatic impregnation of CMC in the subendocardial layer of RV (Table 2).

Our results show that the state of increased afterload complicated by heart failure is characterized by high ratio of damaged CMC (5.6-19.2%) and severe reversible (grade I and II contractures) and irreversible structural changes (grade III contractures and lump degradation of myofibrils). Irreversible damage to most CMC includes plasmatic impregnation, which is particularly pronounced in the subendocardial layer of ventricles operating under conditions of increased afterload. Our previous studies [7] showed that this disorder is accompanied by a sharp decrease in the density of adrenergic plexes in the myocardium and activation of adrenal chromaffin cells. The development of profound and severe structural changes in the myocardium is probably associated with its desympathization and increase in the contribution of hormones to sympathoadrenal influences on the heart. Our results agree well with published data that desympathization of the myocardium increased its sensitivity to the adverse effect of circulating catecholamines [1,2,4,11].

A comparative study of the contractile myocardium under conditions of increased LV and RV afterload revealed similar pathomorphological changes. Contracture injuries and lump degradation of myofibrils were not accompanied by intracellular myocytolysis. Damage to contractile CMC was found both in LV and RV under conditions of increased afterload in one of heart ventricles. Irreversible changes and plasmatic impregnation of CMC were most pronounced in the subendocardial layer of affected ventricle (LV in aortic stenosis and RV in pulmonary artery stenosis). These data are consistent with the results of our previous experiments on the ventricular myocardium during massive pulmonary embolism [6]. Increased afterload of the cardiac ventricle under various hemodynamic conditions (stenosis of the aorta or pulmonary artery, embolic occlusion of pulmonary vessels) is accompanied by the development of stereotypic structural changes in the myocardium. Profound and severe disturbances can cause heart failure.

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