CLINICAL AND EXPERIMENTAL STUDY OF ENZYME HISTOCHEMICAL CHANGES IN THE VENTRICULAR MYOCARDIUM IN PULMONARY EMBOLISM

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To understand the mechanisms of the pathogenesis of acute pulmonary thromboembolism (TE) and its lethal outcome, a comparative study of structural and metabolic changes in the ventricular myocardium is essential. In view of the early development of post mortem changes the use of methods of functional morphology, including determination of enzyme activity in myocardial structures, can be done only if autopsy is undertaken within 1 h of death [1, 2, 5, 6, 14, 15]. This is possible either under experimental conditions or with urgent autopsy in hospital. The writers previously [9] studied the morphological and functional state of the ventricular myocardium in experimental pulmonary embolism.

The aim of this investigation was an enzyme histochemical study of material obtained during early autopsies on patients dying from acute pulmonary TE and also to compare the results of the autopsy and experimental studies.

EXPERIMENTAL METHOD

Autopsy material was obtained during early autopsies on 16 patients dying in the surgical departments of the N. I. Pirogov No. 1 General Hospital, from acute pulmonary TE. The distribution of the patients among clinical groups is shown in Table 1. Group 1 included patients with TE of the trunk and main pulmonary arteries. Group 2 consisted of patients with TE of the lobar and segmental branches of the pulmonary arteries. Group 3 consisted of patients with TE but without any accompanying cardiovascular diseases. Patients with a history of chronic ischemic heart disease (CIHD) and with evidence of diffuse or focal cardiosclerosis and atherosclerosis of the coronary arteries were placed in group 4. Group 5 consisted of patients with a history of CIHD and of chronic nonspecific lung diseases (CNLD), and in whom coronary cardiosclerosis and signs of chronic bronchitis, pulmonary emphysema, and pneumosclerosis were found at autopsy. Experimental material was obtained from 11 dogs in which a model of acute massive pulmonary embolism was created, and from six control dogs. The experiments were conducted under closed chest conditions with natural respiration. Premedication consisted of intramuscular injection of trimeperidine (10 mg/kg). General anesthesia was used, with intravenous drip injection of thiopental sodium (20 mg/kg). Detailed descriptions of the method of creating a model of massive pulmonary embolism, the characterization schedule, and recording methods were given previously [4]. The animals were subsequently sacrificed by intravenous injection of a lethal dose of thiopental sodium 6 h after the beginning of the experiment. Material for morphological and histochemical investigation was collected within 1 h after death of the patients or sacrifice of the animals, from the right ventricle (RV), left ventricle (LV), and anterior papillary muscle (APM), of LV, and also from the affected and intact parts of the lungs. The test material was fixed in buffered 10 neutral formalin, according to Lillie, and embedded in paraffin wax. Sections 5-7 μm thick were stained with hematoxylin and eosin, by Van Gieson's method, and

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TABLE 1. Distribution of Patients with Pulmonary TE Among Clinical Groups

Clinical group	Number of cases
By volume of embolic lesion	
1. Massive TE	8
2. TE Of branches	8
By existence of concomitant diseases	
3. TE without concomitant cardiopulmonary diseases	3
4. TE with concomitant CIHD	5
5. TE with concomitant CIHD and CNLD	5
By sex:	
6. Men	5
7. Women	11
By age:	
8.31-50 years	5
9.51-70 years	7
10. Over 71 years	4

TABLE 2. Comparison of Results of Enzyme-Histochemical Study of Experimental and Autopsy Material

Groups compared	Parameters of activity (M ± m) in percentage of experimental control values, taken as 100%					
	SDH			LDH		
	RV	LV	APM	RV	LV	APM
Massive pulmonary embolism, experimental material	129.0±5.0*	67.0±3.0*	75.0±3.0*	108.0±3.0	96.0±4.0	104.0±4.0
Massive pulmonary embolism, autopsy material	95.0±4.0	63.0±3.0*	62.0±3.0*	91.0±4.0	92.0±4.0	98.0±3.0

Legend. Asterisk indicates significant differences compared with control at the $p < 0.05$ level.

TABLE 3. Dependence of Myocardial SDH and LDH Activity in Ventricles on Volume of Embolic Lesion

Clinical group	Average age of patients (M ± m)	Parameters of activity (M ± m) as percentage of values in massive IE group, taken as 100%					
		SDH			LDH		
		RV	LV	APM	RV	LV	APM
Massive TE	54.0±5.0	100.0±4.0	100.0±5.0	100.0±5.0	100.0±4.0	100.0±4.0	100.0±3.0
TE of branches	65.0±3.0	81.0±3.0*	89.0±3.0	91.0±4.0	92.0±3.0	106.0±4.0	118.0±5.0*

Legend. Asterisk indicates significant differences between groups at $p < 0.05$ level.

with fuchselin-picrofuchsine, and PAS reagent, with salivary amylase control. The enzyme-histochemical investigation was conducted on frozen sections 10 μm thick. Activity of SDH and LDH was detected with nitro-BT in the usual way [3,10,11] and was determined by visual semiquantitative evaluation. A quantitative determination [8] of SDH and LDH activity was carried out on the same specimens by means of a "Microvideomat" television system (Opton, Germany) and a Wang-720 computer (USA), using a special program of photometric analysis of histologic preparations [7]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

As our previous studies [4] showed, in acute experimental massive pulmonary embolism bidirectional changes are observed in the hemodynamic lobes on RV and LV. As Table 2 shows, SDH activity in RV rises, whereas in LV it falls, correlating with changes in the energy demands of the myocardium of the ventricles reflecting the bidirectional changes in their functional loads. The results of histochemical study of early autopsy material are given in

TABLE 4. Dependence of Myocardial SDH and LDH Activity in Ventricles on Existence of Concomitant Cardiovascular Diseases

Clinical group	Average age of patients (M ± m)	Parameters of activity (M ± m) as percentages of value in group of TE without concomitant cardiopulmonary diseases, taken as 100%					
		SDH			LDH		
		RV	LV	APM	RV	LV	APM
TE without concomitant cartiopulmonary diseases	41,0±5,0	100,0±5,0	100,0±3,0	100,0±4,0	100,0±6,0	100,0±4,0	100,0±4,0
TE with concomitant CIHD	65,0±4,0*	71,0±3,0*	72,0±3,0*	77,0±3,0*	85,0±4,0	86,0±5,0	108,0±6,0
TE with concomitant CIHD and CNLD	62,0±6,0*	71,0±4,0*	56,0±4,0*	55,0±4,0*	91,0±4,0	63,0±3,0*	80,0±3,0*

Legend. Asterisk indicates significant differences compared with group of TE without concomitant cardiopulmonary diseases at the $p < 0.05$ level.

Table 2 as percentages of the experimental control values, the validity of which was confirmed by data in the literature [5, 6]. It will be noted that in virtually every case the results of the enzyme-histochemical study of experimental and autopsy material are similar, and their changes compared with the control are in the same direction. The exception is the relatively low SDH activity in RV of patients with massive pulmonary TE. In our opinion, this fact can be explained by the existence of concomitant cardiopulmonary diseases in the patients, which, as will be shown below (Table 4), can give rise to low SDH activity in the ventricular myocardium.

The severity of the hemodynamic changes in pulmonary TE and, in particular, the level of the after load on RV, are determined primarily by the volume of the embolic lesion [12]. Table 3 gives the results of histochemical investigation in clinical groups differing in the degree of embolic occlusion of the pulmonary arterial blood flow. The small volume of the embolic lesion in TE affecting branches of the pulmonary arteries is accompanied by lower SDH activity in RV. This may be due principally to the smaller after load and, consequently, the smaller energy requirement of the myocardium of RV in branched TE compared with massive TE. Besides, the existing difference may be partly age-related (Table 5). The relatively low SDH activity in RV of patients with branched TE evidently cannot be explained by the existence of concomitant-cardiopulmonary diseases (Table 4): their frequency on the whole was rather higher (86.0% and 63.0%) but the number of patients affected with CIHD and CNLD simultaneously was significantly less (14.0% and 63.0%) compared with the massive TE group.

It is stated in the literature [12, 13] that the presence of concomitant chronic cardiopulmonary diseases has a marked effect on the course of pulmonary embolism. As the data in Table 4 shows, lower SDH activity was observed in both ventricles of patients with concomitant CIHD. When this fact is analyzed, three factors must be taken into account: myocardial ischemia, age-related changes in metabolism, and the volume of the embolic lesion (in all patients with concomitant CIHD, TE of the branches of the pulmonary arteries was noted, whereas massive TE was present in patients without concomitant diseases). Whereas the relatively low level of aerobic metabolism in LV can be explained mainly by the first two factors, the lower SDH activity in RV is more likely to be connected with the second and, in particular, the third factor – the small volume of embolic occlusion (Table 3).

In patients with concomitant CIHD and CNLD changes in myocardial metabolism were more marked than in patients with CIHD alone (Table 4). These groups did not differ in age, but massive TE was present in 80% of patients with CIHD and CNLD. Thus it can be concluded from a comparative analysis of these groups that differences between them in relation to SDH and LDH activity are due to the presence of concomitant CNLD in these patients. It can be tentatively suggested that in this case the relatively low activity of catabolic enzymes in both ventricles may be due both to morphological and functional changes in their activity associated with decompensation of "corpulonal" and to the action of systemic factors, one of the most important of which is hypoxia.

The results of this investigation thus confirm that concomitant chronic cardiopulmonary diseases (CIHD and CNLD) can influence the course of pulmonary embolism and, in particular, the character of the metabolic changes in the heart. The relatively low activity of catabolic enzymes discovered in the ventricular myocardium, indicating a low level of energy provision, may be one reason why the critical value of embolic obstruction at which depression of cardiac activity takes place is significantly lower in Persons with concomitant cardiopulmonary diseases [12].

TABLE 5. Dependence of Myocardial SDH and LDH Activity in Ventricles on Age of Patients with Pulmonary TE

Clinical groups	Parameters of activity (M ± m) as percetnages of values in group of patients aged 31-50 years, taken as 100%					
	SKH			LDH		
	RV	LV	APM	RV	LV	APM
Patients aged 31-50 years	100,0±4,0	100,0±4,0	100,0±5,0	100,0±5,0	100,0±4,0	100,0±4,0
Patients aged 51-70 years	95,0±5,0	75,0±3,0*	90,0±4,0	94,0±4,0	97,0±5,0	109,0±5,0
Patients aged over 71 years	66,0±4,0*	68,0±4,0*	66,0±5,0*	82,0±4,0*	96,0±6,0	113,0±6,0

Legend. Asterisk indicates significant differences compared group of patients aged 31-50 years at $p < 0.05$ level.

TABLE 6. Dependence of Myocardial SDH and LDH Activity in Ventricles on Sex of Patients with Pulmonary TE

Clinical groups	Average age of patients (M ± m)	Parameters of activity (M ± m) as percentages of values in group of men, taken as 100%					
		SDH			LDH		
		RV	LV	APM	RV	LV	APM
Men	58,0±4,0	100,0±4,0	100,0±4,0	100,0±4,0	100,0±7,0	100,0±5,0	100,0±5,0
Women	59,0±5,0	75,0±3,0*	85,0±4,0*	81,0±4,0*	92,0±3,0	108,0±4,0	114,0±4,0*

Legend. Asterisk indicates significant differences between groups at $p < 0.05$.

The results of the histochemical investigation, shown in Table 5, demonstrate relatively low SDH activity in both ventricles and LDH activity in RV of old patients with pulmonary TE. Besides age changes, this tendency may also be associated with an increase in the frequency of cases of TE affecting branches of the pulmonary arteries (0°h, 60°h, 75%) and concomitant cardiopulmonary diseases (40°h, 80%, 100%) in groups of elderly patients (Tables 3 and 4).

The results given in Table 6 indicate lower SDH activity in both ventricles in women than in men. These groups do not differ in age composition or in the degree of embolic obstruction (frequency of cases of massive TE 60% and 50%). The frequency of concomitant cardiopulmonary diseases in women and men does not differ on the whole (70% and 75%), but the frequency of cases with a combination of CIHD and CNLD is greater in women (40% and 25%). Thus the differences present can be explained both by sex differences and by the somewhat higher frequency of combined cardiopulmonary diseases in women (Table 4). The sex-related differences discovered were taken into account during analysis of the clinical groups, but they had no effect on its results.

Comparative analysis of the clinical groups of patients with pulmonary TE thus revealed several factors which correlate with relatively low activity of catabolic enzymes in the ventricular myocardium: the volume of the embolic lesion is small (TE of lobar and segmental branches of the pulmonary arteries), concomitant chronic cardiopulmonary diseases (CIHD and CNLD), and dependence on age (elderly) and sex (female) are present. Concomitant cardiopulmonary diseases, and also age- and sex-related aspects of metabolism, lowering the level of energy provision in the ventricular myocardium of patients with pulmonary TE, can thereby promote the development of cardiac failure in these patients even if the volume of embolic obstruction is relatively small.

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DEVELOPMENT OF INTERFOLLICULAR EPIDERMIS ON SURFACE OF COLLAGEN SKELETON OF THE DERMIS: EXPERIMENTAL STUDY

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KEY WORDS: interfollicular epidermis; collagen skeleton of dermis; transplantation

Studies of healing of skin wounds in mammals [1] and experiments involving transplantation of mouse skin cells [7, 8] have revealed a phenomenon of regeneration of epidermal derivatives. A fundamental problem in this connection is the discovery of the cellular sources and conditions essential for their secondary formation, and to establish whether the keratinocytes covering the stratum papillare of the dermis can undergo transdifferentiation, with subsequent participation in the formation of derivatives.

The aim of this investigation was to study the state of keratinocytes of the interfollicular epidermis (IFE) of sexually mature rats, responsible for secondary filling of the hair follicles of the acellular collagen skeleton of the dermis (CSD) after autografting.

EXPERIMENTAL METHOD

Experiments were carried out on 34 noninbred male laboratory rats weighing 190-220 g. The transplantation technique included obtaining the CSD, preparing a receptive bed, and detachment and transplantation of the IFE.

All manipulations requiring anesthesia or immobilization of the animals (formation of the receptive bed, vacuum separation of the epidermis, wound dressing, sacrifice) were performed under ether anesthesia. To prepare the CSD a full-thickness skin graft measuring 4 × 6 cm was excised from the abdominal wall of a sacrificed rat weighing 140-170 g, fixed around the edge to a glass frame, and immersed for 24 h in a 1.5-2% solution of sodium carbonate at 36°C. The epidermis and its derivatives were then removed mechanically from one side of the graft, and remnants of the subcutaneous fatty areolar tissue from the other side. For several hours the dermal graft was washed in water and immersed for 2-24 h in medium 199 with gramicidin (20 mg/100 ml medium). This treatment of the skin graft completely removed all cells, while leaving the collagen skeleton relatively unchanged. To prepare the receptive bed, the hair was removed in the region of the anterior third of the spine. A circular skin incision is made in the interscapular region down to subcutaneous fatty areolar tissue, and 2 cm in diameter. The base of a protective chamber was sutured to the outer edge of the incision. A gauze pad was applied to the skin graft, left inside the

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