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ENZYME ACTIVITY OF PERIPHERAL BLOOD CELLS IN EXPERIMENTAL CHRONIC MYOCARDITIS LINKED WITH PERSISTENCE OF COXSACKIE A VIRUS

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Cytochemical parameters of peripheral blood cells reflect the characteristics of metabolism in the tissues of the internal organs [2, 5, 7, 9] and they can accordingly be used to assess the severity of a pathological process. The enzyme profile of the blood leukocytes has been studied experimentally only in acute virus diseases [3, 6]. It has been shown that a chronic virus infection develops in animals infected with strains of Coxsackie viruses A13 and A18, isolated from patients with rheumatic carditis and myocarditis [1, 4, 11].

In human cardiopathies associated with chronic virus infection, intravital determination of the character of the pathomorphological changes in the myocardium may be very difficult, and accordingly the aim of the present investigation was to look for informative criteria for assessing activity of the process and the degree of damage to the internal organs in experimental chronic virus myocarditis.

EXPERIMENTAL METHOD

Experiments were carried out on 18 noninbred male albino rats weighing 200-260 g. The experimental animals (n = 12) each received an intraperitoneal injection of 1.0 ml of culture fluid containing $10^{5.6}$ TCD_{50} of Coxsackie A13 virus (strain 4523) isolated from a child with rheumatic carditis [4]. The virologic methods included investigation of the thymus and blood clots from all animals for presence of the virus 60 days after infection. The virus was reisolated by infection of primary trypsinized tissue cultures of human embryonic fibroblasts (HEF) with the test material. The titer of antibodies against Coxsackie A13 virus was determined in the blood sera by the neutralization of cytopathic activity of the virus test. Blood was taken for cytochemical investigation from the caudal vein 8 days and 2 months after infection, and succinate dehydrogenase (SDH) activity of the lymphocytes and platelets, α-glycerophosphate dehydrogenase (α-GPDH) activity of the lymphocytes [8], and alkaline phosphatase (AlP) activity of the neutrophils were determined by the azo-coupling method [10]. For the histopathological investigation sections from preparations of the heart, fixed in Carnoy's fluid, were stained with hematoxylin and eosin. Statistical analysis of the results was carried out by the Student and Kolmogorov–Smirnov tests. Correlation analysis was done by Nairi-2 computer.

EXPERIMENTAL RESULTS

During virologic investigation Coxsackie A13 virus was reisolated from the thymus or blood of eight of the 12 animals 60 days after infection. Antibodies of the appropriate specificity were present at this time in the blood in titers of 4 to 6 $\log_2$ in all infected animals. On histologic investigation the degree of heart damage was estimated from the presence of signs of virus myocarditis such as the formation of perivasuclar and subendocardial granulomas, infiltration of the myocardium with lymphocytes, destructive changes in the cardiomyocytes, cardiosclerosis, and petrification. The intensity of myocardial damage was pronounced in animals in whose thymus no virus was present (Table 1). Significantly strong correlation was found

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TABLE 1. Myocardial Lesions Associated with Virus Infection

Tissue from which virus was isolated	Number of animals	Antibodies against virus (geometric mean) ($\log_2$)	Number of animals with signs of damage				
			Destruction of cardiomyocytes	Infiltration by cells	Granulomas	Sclerosis	Petrification
Thymus and blood	4	4,5	3	3	4	1	1
Thymus	2	4,5	2	1	2	1	1
Blood	2	4,5	2	1	1	1	0
Virus not isolated	4	5,75	0	0	1	0	1

TABLE 2. Changes in Enzyme Activity of Lymphocytes, Neutrophils, and Platelets during Course of Infection (M ± m)

Group of animals	Time after infection, days	α-GPDH of lymphocytes	AlP of neutrophils	SDH of lymphocytes	SDH of lymphocytes
Experimental	8	12,01±0,17	100,52±10,61	18,93±1,61	1,74±0,31
	60	9,06±0,18*	153,87±7,62*	14,62±1,53	1,12±0,23
Control	8	11,76±0,19	115,83±9,93	15,21±1,08	1,43±0,35
	60	12,12±0,13	116,17±8,32	13,82±1,03	1,28±0,08

Legend. *) Coefficients of correlation (r) at $P < 0.02$ level.

TABLE 3. Correlation between Enzyme Activity of Peripheral Blood Cells and Activity of Infectious Process and Myocardial Damage in Chronic Virus Myocarditis

Parameter of activity of infectious process and of myocardial damage	Lymphocytes		AlP of neutrophils	SDH of platelets
	α-GPDH	SDH		
Virus reisolated from thymus	—0,686*	—0,157	0,420	—0,438
Titer of antiviral antibodies	0,087	0,185	—0,114	0,648*
Destruction of cardiomyocytes	—0,593*	—0,170	0,590*	—0,489
Infiltration of myocardium by small round cells	—0,210	—0,586*	0,229	—0,261
Petrification in myocardium	—0,339	—0,585*	0,302	—0,370

Legend. *) Coefficient of correlation (r) at $P < 0.01$ level.

between persistence of virus in the thymus and the presence of granulomas in the myocardium of the infected animals ($r = 0.707$). In the course of development of the infection the level of SDH activity of the lymphocytes and platelets fell until the 60th day after infection. α-GPDH activity of the lymphocytes, which was close to the control values in the acute stage fell statistically significantly in the chronic phase of the infections (Table 2). Correlation analysis of levels of enzyme activity of the blood cells and the results of the virological and pathomorphological tests revealed significant correlation between the decrease in α-GPDH activity of the lymphocytes and the presence of virus in the thymus, and also destruction of cardiomyocytes (Table 3). Inflammatory-dystrophic changes in the affected organ and, in particular, infiltration of the myocardial tissue by lymphocytes and plasma cells, with the development of petrification, were linked with lowering of SDH activity. Meanwhile the increase in AlP activity of the neutrophils during the development of chronic myocarditis correlated with the extensive destructive changes and disintegration of the muscle fibers.

Changes in enzyme activity of the lymphocytes, neutrophils, and platelets which, taken together, reflect activity of the infectious process and of the associated destructive and inflammatory changes in the affected organ, were thus discovered by the use of this model of chronic myocarditis. The reduction in dehydrogenase activity accompanied by activation of AlP of the neutrophils reflect the degree of toxic damage and the intensity of the morphological disturbances in the myocardium, as is confirmed by correlation analysis. Changes in the enzyme profile thus revealed can be regarded as informative criteria characterizing the degree

of pathomorphological damage in chronic and subschronic cardiopathies of virus etiology.

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