Activity and Latent Activity of Lactate Dehydrogenase Isoenzymes from Patients with Disadaptation

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Spontaneous proliferation and structural and functional properties of the cell membranes were studied by measuring activity and latent activity of lactate dehydrogenase isoenzymes in peripheral blood leukocytes from patients with disadaptation at admission to the hospital and during therapy. The spectrum of lactate dehydrogenase isoenzymes in patients with disadaptation at admission did not differ from the corresponding values in the control group. Therapy was accompanied by a shift in isoenzyme spectrum of lactate dehydrogenase toward the prevalence of one isoform, which probably reflected metabolic changes in immunocytes under conditions of adaptive immune response to psychoemotional stress. Latent activity of lactate dehydrogenase isoenzymes in patients at admission was below the control. Latent enzyme activity returned to normal during therapy. The observed changes in latent enzyme activity were probably associated with recovery of structural and functional properties of leukocyte membranes.

Key Words: *leukocyte; lactate dehydrogenase; latent enzyme activity; cell membrane; disadaptation*

Reversible extreme strain of adaptive mechanisms, specified as psychoemotional stress, underlies the development of neuroses and social or stress-related disorders, the most prevalent psychopathology in the present time. In light of this, adequate evaluation of adaptive capacities and effectiveness of psychopharmacological and psychotherapeutic correction is an urgent problem. Adaptive capacity is estimated by shifts in structural and functional characteristics from the normal. Previous studies showed that the immune system is involved in the adaptive response of the organism to psychoemotional stress. A variety of changes in cell and humoral immunity (including changes in phytohemagglutinin-stimulated proliferation of immune cells) were studied in details [6]. Stimulated proliferation of lymphocytes serves as a biological criterion of the severity of stress [5].

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Isoenzyme spectrum of lactate dehydrogenase (LDG) is a biochemical marker of cell proliferation and differentiation [4], and it seems appropriate to study it as a parameters reflecting basal proliferative activity of immunocompetent cells. This parameter can be used in the complex study of the immune system during adaptation to psychoemotional stress and for evaluation of the effectiveness of therapy.

Here we measured activities of LDG isoenzymes from patients with disadaptation (DA) during therapy.

MATERIALS AND METHODS

We examined 15 patients with DA (F43.2, 7 men and 8 women, 24-46 years). Blood samples were taken at admission to hospital and during therapy (14 days after admission). The control group included 10 healthy donors of comparable age (4 men and 6 women).

The experiments were performed with leukocytes isolated from heparinized blood by centrifugation in a density gradient. Leukocytes were destructed by hypo-

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tonic shock in distilled water (500 μl) and vibration on a vortex device (3 min). We showed that 500 μl lysate contain products of destruction of 20 million cells.

Two aliquots (50 μl) were taken from the leukocyte mass (biomaterial was also used in other studies). Aliquot 1 was mixed with 50 μl incubation solution containing 0.25 M Tris-HCl (pH 6.8) and 20% glycerol. An incubation solution with 2% triton N101 (detergent) was added to aliquot 2. This detergent ensured further destruction of cell membranes and loss of latent activity (LA) of LDG. The concentration of triton N101 was chosen in previous experiments.

Aliquots were incubated at 37°C for 15 min. LDG was isolated by native electrophoresis in polyacrylamide gel using a discontinuous buffer system. Activity of LDG isoforms in gel was evaluated by nitroblue tetrazolium reduction with the formation of intensively colored formazan. The gels were subjected to black-white scanning after development of isoforms.

LDG isoenzyme distribution was determined as a totality of individual enzyme isoforms taking into account image density per unit area. LA was calculated as the difference between activities of the corresponding isoforms in samples incubated with and without detergent.

The results were analyzed by Student's *t* test.

RESULTS

The developed gels included 3 clear-cut LDG isoforms (LDG-1, LDG-2, and LDG-3, Fig. 1). LDG-4 was detected in only 5% samples. LDG-5 was absent. Activity of the corresponding isoenzymes in samples incubated with detergent was higher than in samples incubated without detergent.

The isoenzyme spectrum of LDG in DA patients at admission to hospital did not differ from the control (Table 1). LDG activity significantly decreased on day 14 of therapy and did not differ from the pretreatment and control level.

The isoenzyme spectrum of LDG in differentiating cells is shifted toward anaerobic isoforms LDG-4 and LDG-5 [4]. The ratio of "intermediate" LDG isoenzymes (LDG-2 and LDG-3) in leukocytes from DA patients increased during therapy, which was associated with the increase in spontaneous proliferation of peripheral blood cells.

Our results are consistent with published data that psychoemotional stress decreases stimulated proliferative activity of immune cells [6]. These data agree with the isoenzyme distribution of leukocyte LDG in untreated patients and healthy donors. It can be hypothesized that functional changes in leukocytes accompanied by inhibition of stimulated proliferation *in vitro* does not modulate spontaneous proliferative activity of these cells.

Previous studies showed that adaptive immune changes in recovering patients with chronic psychoemotional stress are accompanied by a compensatory increase in stimulated proliferative activity of immune cells above the control level [3]. These data are consistent with changes in the isoenzyme spectrum of leukocyte LDG, which reflect the increase in spontaneous proliferative activity on day 14 of therapy.

The increase in stimulated proliferation of immune cells probably results from high proliferative readiness of these cells. It can be hypothesized that the increase in spontaneous proliferation of leukocytes reflects high proliferative capacity of these cells.

Adaptive processes at the cellular level are accompanied by changes in activity of energy-producing enzymes and functional state of cell membranes. Any significant stress factor activates peroxidation processes (oxidative stress). Oxidative modification of membrane phospholipids and proteins modulates their physicochemical properties. In the present study functional activity of leukocyte membrane was determined by LA of LDG isoenzymes in these cells. LA of LDG isoenzymes was low in DA patients at admission to

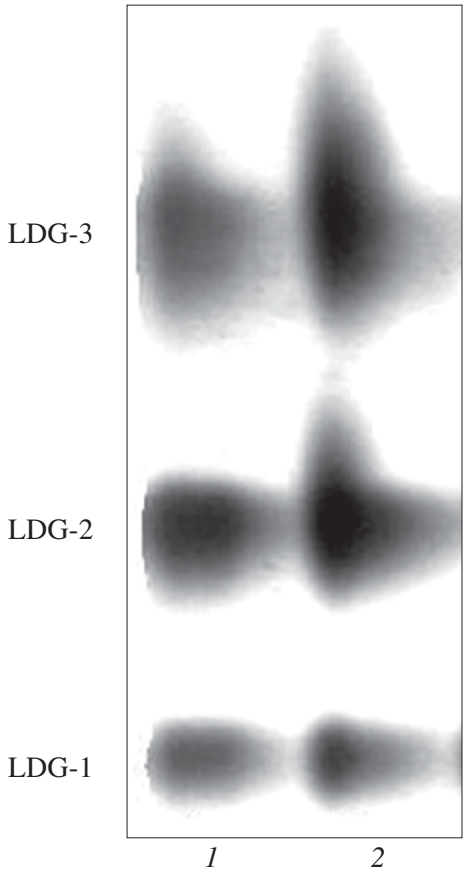

Fig. 1. Enzymogram of leukocyte lactate dehydrogenase isoenzymes. Isoenzymes detected without preincubation (*1*) or after 15-min incubation of the cell lysate with detergent (1% N101, *2*).

TABLE 1. Isoenzyme Spectrum of Leukocyte LDG (*M*±*m*)

Group		Ratio, %		
		LDG-1	LDG-2	LDG-3
Control		21.5±8.1	35.4±8.9	31.2±7.9
DA patients	at admission	22.15±6.8	39.7±5.6	34.45±4.9
	day 14 of therapy	16.2±6.7*+	35.2±8.7	31.8±6.4

Note. Here and in Table 2: $p<0.05$: *compared to the control; +compared to the state at admission.

TABLE 2. LA of LDG Isoenzymes in Leukocytes (*M*±*m*)

Group		Ratio, %		
		LDG-1	LDG-2	LDG-3
Control		51.1±15.7	32.8±5.9	49.2±13.5
DA patients	at admission	24.1±6.8*	19.3±6.8*	29.0±11.3
	day 14 of therapy	51.6±10.9	37.3±4.3	54.2±12.4

hospital, but increased to normal during therapy (Table 2).

LA is the difference between activities of the corresponding isoenzymes measured in media with various aggregate states. The suspension of destructed cells contains vesicles and protein-lipid or protein-protein aggregates. In this system enzyme is partially located within vesicles and bound to the membrane and other proteins [1,2]. The estimated activity of isoenzymes would be underestimeted, since protein cannot completely permeate the gel during native electrophoresis. The immobilized enzyme is released after incubation of destructed cells with the detergent. In this case, the estimated activity would correspond to the total concentration of isoenzyme.

In our study the aggregate state of the incubation medium depends on physicochemical properties of the leukocyte membrane. Therefore, changes in LA of LDG isoenzymes are associated with the recovery of physicochemical properties of leukocyte membranes in DA patients during therapy.

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