Apoptosis in Peripheral Blood Leukocytes Induced by Hyperthermia and Prednisolone in Patients with Dysadaptation

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We studied morphological changes in blood leukocytes produced by prednisolone and hyperthermia. Patients with dysadaptation were characterized by activation of spontaneous apoptosis and inhibition of induced apoptosis in neutrophils and lymphocytes compared to healthy donors.

Key Words: *apoptosis; neutrophils; lymphocytes; dysadaptation*

The mechanisms underlying pathophysiological states of subjective distress and emotional disorders that decrease social activity and reduce the quality of life are poorly known. Psychoemotional disorders are associated with the effect of strong stress factors and profound changes in life, which results in dysadaptation (ICD-10).

Stress reactions are accompanied by the release of catecholamines and glucocorticoids (GC) into the circulation. The adaptive response of hematopoietic cells in tissues manifests in their regeneration and triggers genetically programmed cell death. Intensification of apoptosis is typical of long-term stress [2].

Apoptosis of polymorphonuclear leukocytes can be easily induced and serves as a convenient model for studying the mechanisms of cell death under the influence of various factors. Hyperthermia rapidly produces cell death under conditions of blood culturing. This is a strong factor inducing apoptosis. GC produce different effects on leukocytes depending on cell maturity. GC markedly increase viability of neutrophils [2,10], while in high doses these hormones *in vivo* induce T cell death [7]. The effect of GC increases from hydrocortisone to its synthetic analogues (prednisolone and dexamethasone) [3]. The effect of dexamethasone in doses of 10-100 mM is most pronounced after 4-h incubation [9]. *In vivo* immunomodulatory activity of GC increases under conditions of artificial hyperthermia [6]. *In vitro* effects of combination exposure to hyperthermia and GC remain unknown.

Here we studied the influence of hyperthermia and prednisolone on apoptosis in peripheral blood neutrophils and lymphocytes from patients with dysadaptation.

MATERIALS AND METHODS

Morphological changes in leukocytes typical of apoptosis were determined by light microscopy of blood smears from patients with dysadaptation (F-43, ICD-10, n=22) and healthy donors (n=12). Diagnostic examination and blood sampling were performed during hospitalization at the Research Institute for Mental Health before the start of pharmacotherapy. The groups of patients and healthy donors were age- (32.6±2.1 and 32.2±3.2 years) and sex-matched.

Heparinized blood samples were incubated in the presence or absence of 10 μM prednisolone (Gedeon Richter) at room temperature or 41°C for 4 h. Blood smears were stained by the method of Romanovsky. The number of apoptotic cells was expressed in percents of white blood count (number of neutrophils or lymphocytes).

The results were analyzed by nonparametric Kruskal—Wallis test.

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TABLE 1. Effects of Hyperthermia and Prednisolone (10 μM) on Apoptosis in Neutrophils and Lymphocytes during *in Vitro* Culturing (*M*±*m*)

Experimental conditions	Apoptotic neutrophils, %		Lymphocytes with fragmented nuclei, %	
	healthy donors	patients with dysadaptation	healthy donors	patients with dysadaptation
Without incubation	0±0 (12)	0.66±0.28** (22)	0.18±0.18	0.26±0.18
Incubation at 20°C	0.79±0.27 (12)	0.86±0.47 (15)	5.24±1.38	0±0*
+prednisolone	1.40±0.65 (11)	0.83±0.83 (8)	7.26±2.25	0.47±0.31*
Incubation at 41°C	33.26±8.13^{+} (12)	7.24±2.40** (12)	13.14±2.65^{++}	0.25±0.17*
+prednisolone	26.18±9.28^{+} (12)	4.72±2.24** (10)	9.94±2.30	0.14±0.14*

Note. *p<0.001 and **p<0.05 compared to healthy donors; ^{+}p<0.001 and ^{++}p<0.05 compared to incubation at 20°C under similar conditions. Number of measurements is shown in brackets.

RESULTS

Polymorphonuclear leukocytes with morphological signs of apoptosis were found in blood smears. These neutrophils were smaller and had a round shape. In individual cells the cytoplasm included several large vacuoles that were localized unipolarly. Morphological changes in the nuclear substance manifested in a decrease in the size of nuclei and condensation and granulation of chromatin in the peripheral zone. The intensity of spontaneous apoptosis in neutrophils differed in blood smears from patients with dysadaptation and healthy donors (Table 1). Incubation of the blood at room temperature increased the count of apoptotic cells (Table 1).

Long-term heating sharply reduced viability of neutrophils (Table 1). After high-temperature culturing of blood samples with prednisolone the ratio of cells with granulated nuclear chromatin decreased. However, intragroup differences were statistically insignificant.

Probably, induction of heat-shock proteins impairs intracellular mechanisms due to the formation of disulfide bonds in GC receptors [5] and attenuates the antiapoptotic effect of GC on neutrophils. Published data show that apoptosis in thymocytes induced by hyperthermia of the cell suspension at 43°C for 1 h does not depend on protein synthesis and ATP generation [8]. The effects of these factors are probably realized via different metabolic pathways.

Prednisolone and hyperthermia caused degradation of the nuclear substance in lymphocytes. Fragmentation of chromatin resulted in the formation of 2-6 fragments. In healthy donors changes in lymphocytes and neutrophils were similar. Incubation of the blood even at room temperature caused apoptosis in lymphocytes. It should be emphasized that hyperthermia produced most pronounced changes (Table 1). Patients with dysadaptation displayed different reactions to test factors. In blood samples subjected to hyperthermia and treatment with prednisolone, lymphocytes displayed considerable resistance. The number of cells with pronounced morphological changes was insignificant (Table 1). Probably, chronic stress inducing hormone imbalance and long-lasting increase in GC level reduces individual sensitivity to various stimuli. On the one hand, GC are responsible for the realization of the stress reaction. On the other hand, these hormones are elements of the antioxidant system that increases protective reserves by the negative feedback mechanism [1]. Intensification of spontaneous apoptosis in neutrophils from patients with dysadaptation is probably related to the activation of lipid peroxidation and increase in blood content of active products impairing membrane integrity and inducing apoptosis [4].

Our findings suggest that prednisolone and hyperthermia modulate the reaction of neutrophils and lymphocytes from patients with dysadaptation. Blood cells from healthy donors display high reactivity. Patients with dysadaptation are characterized by the activation of spontaneous apoptosis and inhibition of induced apoptosis in neutrophils and lymphocytes compared to healthy donors.

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