Second Messengers cAMP, Ca^{2+}, and NO Modulate Functional Properties of Human Lymphocytes under Conditions of Exposure to UV Light

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We studied the effect of UV light (240-390 nm) in doses of 75.5-4530 J/m^2 on functional properties and level of intracellular messengers (cAMP, Ca^{2+}, NO) in lymphocytic cells of the peripheral blood. Correcting effects of UV light in a dose of 151 J/m^2 on cytotoxic activity of lymphocytes against Ehrlich ascetic carcinoma and calcium concentration in lymphocyte cytosol were observed. We found that UV light reduces the cAMP content and increases NO concentration (151-4530 J/m^2) in photomodified cells. The involvement of adenylate cyclase and phosphoinositide signaling pathway into the mechanisms of modulation of functional properties of lymphocytes after exposure to UV light was demonstrated.

Key Words: *cyclic adenosine monophosphate; calcium ions; nitric oxide; lymphocytes; exposure to UV light*

The mechanisms of the therapeutic effects of photomodified blood are still little studied [3]. It was previously found [12] that UV light modifies the proliferation and differentiation processes in lymphocytes, their cytotoxicity, reaction to foreign antigen, *etc.* At the same time, the interrelationship between structural and functional photomodifications of lymphocytes with changes in the level of second messengers is not comprehensively studied. Elucidation of the peculiarities of transduction of the external signal in lymphocytic cells will help to understand the molecular mechanisms underlying the action of physicochemical factors, *e.g.* UV light, on immunocompetent cells and to develop new methods of targeted correction of the immune status under pathological conditions.

Here we studied changes in functional properties and content of second messengers (cAMP, Ca^{2+}, and NO) in peripheral blood lymphocytes after exposure to UV light (240-390 nm) in doses of 75.5-4530 J/m^2.

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MATERIALS AND METHODS

Lymphocytes were isolated from donor peripheral blood by centrifugation in Ficoll-verografin density gradient (ρ=1.077 g/cm^3). The suspension was separated into T and B cells on Nylon-wool columns [8]. Cytotoxic activity of lymphocytes against Ehrlich ascetic carcinoma cells cultured in SHK mice were evaluated using colorimetric MTT analysis [14]. Antibody-producing capacity of lymphocytes was evaluated by method of local hemolysis in gel [4]. Lymphocyte viability was determined using standard trypan blue exclusion test [5]. cAMP content in lymphocyte cytosol before and after UV exposure was measured by radioimmunoassay using standard cAMP-I^{125} kits (Immunotech) and γ-counter in Radioisotope Laboratory of Voronezh Regional Hospital. Free cytoplasmic Ca^{2+} ($[Ca^{2+}]_i$) in lymphocyte cytosol was measured using Fura-2AM fluorescent probe on a RF-1501 (Shimadzu) in a spectrofluorometric thermocontrolled (37°C) cuvette [1]. For quantitative evaluation of NO production, colorimetric measurement of nitrite content

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in lymphocyte cytosol with Griess reagent was used [2]. The samples were exposed to UV light from a DRT-400 mercury quartz lamp through a UFS-1 filter with transmission band of 240-390 nm under conditions of constant stirring in a thermocontrolled cuvette (20±1°C). The intensity of irradiation was 151 J/m^2/min. The data were processed statistically using Statgraphics software. The significance of the differences between the control and experimental samples was verified using Student's *t* test. The differences were significant at $p<0.05$ [7]; the data were presented as mean±standard deviation.

RESULTS

After analysis of cytotoxic activity in intact and photomodified lymphocytes from the peripheral blood, the donors were divided into three groups differing by both the initial (before UV exposure) values of this parameter and its dynamics after exposure to UV light in a dose range of 75.5-1510 J/m^2.

The first group of donors (N=8) was characterized by initially high cytotoxicity 94.7±0.9%. After exposure to UV light in doses of 75.5, 151, 755, and 1510 J/m^2, this parameter considerably decreased by 9, 14, 12, and 18% compared to the control sample (Fig. 1, *a*). Group 2 donors (N=9) with medium cytotoxicity (85.3±1.5%) differed by the dependence of the test parameter on the dose of UV irradiation (Fig. 1, *b*). Exposure to UV light in doses of 75.5 and 151 J/m^2 significantly increased lymphocyte activity compared to native sample. Exposure to 755 and 1510 J/m^2 reduced cell cytotoxicity to the control level (the studied parameter did not differ significantly from the control level). In group 3 donors (N=6) with initially low activity (66.0±4.6%), cytotoxicity of lymphocytes significantly increased compared to the control in the entire dose range (Fig. 1, *c*). These results attest to correcting effect of UV light on cytotoxic activity of lymphocytes against Ehrlich ascetic carcinoma.

Realization of the cytotoxic activity of lymphocytes *in vitro* is related to T cell-dependent antibody-independent and antibody-dependent mechanisms [12]. For evaluation of the contribution of T cell-dependent antibody-independent mechanism into realization of the total cytotoxic activity of lymphocytes, we measured activity of T cell suspensions against tumor cells.

We found that the dynamics of UV-induced changes in cytotoxicity of lymphocyte mixture and T-cell suspension (T cell content 87±3%) were similar, but in case of cell mixture the observed effects were more pronounced.

Antibody-dependent cytotoxicity is related to B cells producing antibodies. If the whole cell (target) is the antigen, G-class antibodies bind Fc-receptors of natural killers (NK) carrying FcγRIII receptor. In the formed target cell–antibody–NK complex NK realizes its killer function against the target cell.

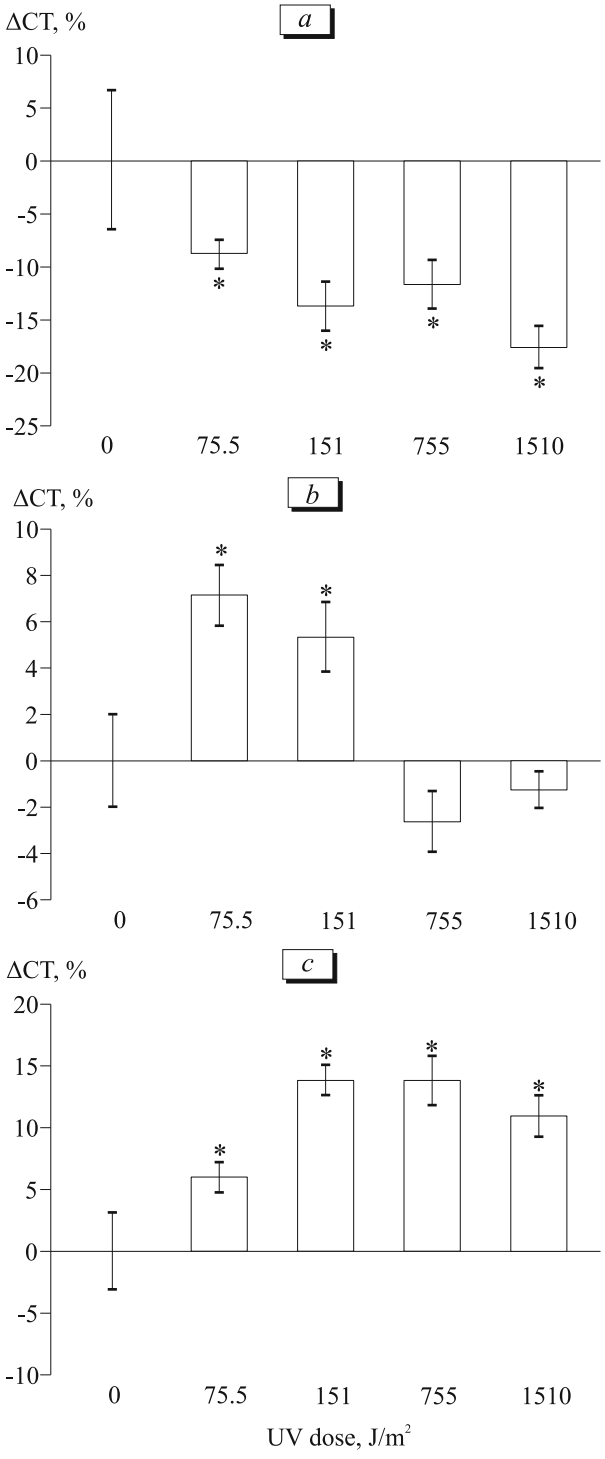

Fig. 1. Effect of UV exposure on cytotoxic activity (ΔCT) of human lymphocytes against Ehrlich ascitic carcinoma cells. *a*) group 1 donors; *b*) group 2 donors; *c*) group 3 donors. Here and in Figs 2 and 3: *$p<0.05$ compared to the control.

It was shown that UV exposure in doses of 75.5-1510 J/m^2 suppresses antibody-forming capacity of B cells (production of IgG) in the entire dose range (n=5) (Fig. 2).

Hence, UV exposure in doses of 75.5-1510 J/m^2 primarily stimulates the antibody-independent mechanism of lymphocyte cytotoxicity mediated by B cells.

It is known that immunological activity of lymphocytes and T cell proliferation are related to modulation of the level of cyclic nucleotides, in particular, cAMP [6,9]. The increase in cAMP concentration is accompanied by considerable changes in T cell activity: suppression of proliferation and antibody-dependent cytolysis and modulation of cytokine synthesis and secretion. Antiproliferative effects of cAMP can be a result of inhibition of tyrosine protein kinases p56^{lck} and ZAP-70, membrane phospholipase C, and elements of mitogen-activated kinase cascade (MAPK-cascade) and regulation of IL-2 gene transcription. At the same time, the humoral immune response is stimulated by this second messenger.

After UV exposure in doses of 151, 1510, and 9060 J/m^2, the intracellular cAMP concentration decreased by 65, 62, and 40%, respectively, compared to that in intact lymphocytes (Fig. 3). Thus, activation of T cells responsible for antibody-independent mechanism of cytotoxicity is related to a decrease in cAMP content in the cytosol of the studied cells.

It is known that different signal transduction systems in the cell interact via modulation and mutual regulation of second messenger concentrations [6]. In light of this it was interesting to study the levels of intracellular calcium in human lymphocytes after UV exposure in different doses.

Calcium concentration in intact cells is 137±20 nM, which agrees with published data [11]. Evaluation of intracellular calcium concentration in lymphocytes exposed to UV light in a dose of 151 J/m^2 showed that all donors can be divided into two groups differing by the initial values and the dynamics of the studied parameter after UV exposure. In group 1 (N=7), Ca^{2+} content in native lymphocytes was 266.7±26.9 nM. After UV exposure, Ca^{2+} content in the cytosol significantly decreased to 135.8±27.0 nM compared to that in intact cells. In group 2 (N=7), Ca^{2+} content in native lymphocytes was 87.0±16.6 nM and after UV exposure it increased to 149.01±15.5 nM. Thus, UV exposure in a dose of 151 J/m^2 normalizes Ca^{2+} content in donors with its initially reduced or elevated levels.

For evaluation of the mechanisms of modification of Ca^{2+} content in lymphocytes after exposure to UV light we studied changes in the concentration of this intracellular messenger after UV exposure (151 J/m^2) in immune cells suspended in a calcium-free medium.

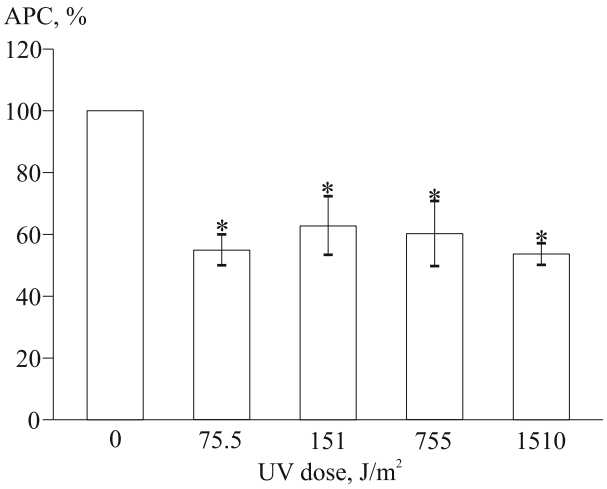

Fig. 2. Antibody-producing capacity (APC) of lymphocytes after UV modification.

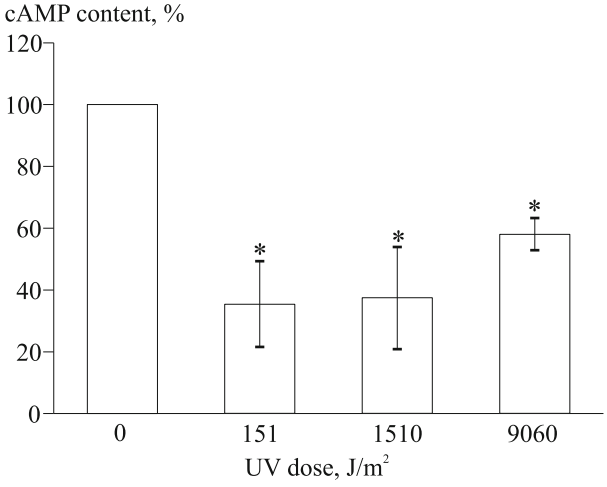

Fig. 3. cAMP content in cytosol of UV-exposed lymphocytes.

In group 1 (N=5), Ca^{2+} content in native lymphocytes was 88.6±28.9 nM. After UV exposure, Ca^{2+} content in the cytosol significantly increased to 244.1±17.7 nM compared to that in intact cells. Since the cells were suspended in a calcium-free medium, the increase in this parameter can be determined by the release of Ca^{2+} ions from intracellular depots (vesicles of the endoplasmic reticulum and mitochondria) into the cytosol. These findings attest to the involvement of the phosphoinositide signal transduction mechanism in lymphocytes into processes of modification of their functional state under conditions of UV exposure.

In group 2 (N=5), Ca^{2+} content in native lymphocytes was 252.7±48.3 nM. UV exposure significantly decreased intracellular Ca^{2+} concentration to 113.7±26.3 nM. It can be hypothesized that UV exposure activates Ca^{2+}-ATPase active transport system in the plasma membrane and membranes of the endoplasmic reticulum. This leads to Ca^{2+} pumping from the cytosol into

medium and intracellular depots. However, the possibility of Ca^{2+} leakage from lymphocytes into the medium through plasma membrane defects formed due to LPO activation during UV exposure cannot be excluded.

After UV exposure in doses of 1510 and 4530 J/m^2, intracellular calcium concentration in lymphocytes suspended in a medium with and without calcium ions significantly increased compared to that in intact cells. After UV exposure in a dose of 1510 J/m^2, Ca^{2+} content in cells suspended in a medium with and without calcium ions was 298.7±109.1 (n=5) and 314.2±105.9 mM (n=5) respectively, while after UV exposure in a dose of 4530 J/m^2 the corresponding values were 271.6±64.05 and 337.4±64.8 mM. These findings confirm the assumption on the involvement of cell calcium depots into changes of cytosolic calcium content in human lymphocytes under conditions of their UV modification.

There is an assumption that Ca^{2+} ions can act as activators of NO synthase [10]. Inducible NO synthase can be expressed in cells under the effect of various factors: toxins; inflammatory cytokines (IL-1β, IFN-γ, TNF), reactive oxygen species, γ-radiation, *etc*.

For evaluation of the possibility of induction of NO synthase in human lymphocytes under the effects of UV exposure we studied changes in the level of nitrites in the cytosol of cells modified by UV light. The levels of NO^-_2 (n=5) were 0.031±0.006, 0.036±0.004, and 0.038±0.009 μM for UV doses of 151, 1510, and 4530 J/m^2, respectively, and significantly (p<0.05) differed from the control (0.016±0.05 μM).

Hence, NO content in lymphocyte cytosol significantly increased 24 h after UV exposure compared to the control level, *i.e.* UV irradiation in doses of 151, 1510, 4530 J/m^2 acts as an inductor of inducible NO synthase and NO generation.

Thus, our experiments showed that UV exposure (240-390 nm) in doses of 151, 1510, and 9060 J/m^2 reduces the content of cAMP in lymphocyte cytosol compared to that in the control, which is accompanied by activation of T cells under conditions of exposure to UV light. We found that UV exposure in the therapeutic dose of 151 J/m^2 normalizes Ca^{2+} content in lymphocytes from donors with its initially reduced or elevated levels. Modulation of intracellular Ca^{2+} concentration in UV-exposed cells is realized via components of phosphoinositide signal transduction pathway and, probably, Ca^{2+}-ATPases of the plasma and intracellular membranes of lymphocytes. Thus, the involvement of adenylate cyclase and phosphoinositide signaling mechanism into the mechanisms of modulation of functional properties of lymphocytes after exposure to UV light was demonstrated. An increase in NO level in the lymphocyte cytosol compared to that in intact cells was observed 24 h after UV exposure in doses of 151, 1510, and 4530 J/m^2. Hence, changes in the content of second messengers cAMP, Ca^{2+}, and NO and their reciprocal influence determine modulation of functional properties of lymphocytes under conditions of exposure to UV light.

Our results should be taken into account when discussing the mechanisms underlying the effects of UV light on human immunocompetent cells.

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