BIOPHYSICS AND BIOCHEMISTRY

Effect of UV Irradiation on Functional Activity of Donor Blood Neutrophils

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Activation of NADPH-oxidase enzymatic complex was observed after UV irradiation of the blood and neutrophil suspension in a dose of 151 J/m^2. UV irradiation in doses of 75.5 and 151.0 J/m^2 corrected myeloperoxidase activity and intensity of LPO processes in donor blood.

Key Words: *NADPH-oxidase; neutrophils; myeloperoxidase; LPO; UV light*

The blood exposed to UV and visible light is characterized by an appreciable therapeutic potential, specifically, by immunomodulating activity [4]. Photomodified blood "translates" light-induced changes to the entire volume of the autologous blood, which was demonstrated for its various characteristics and functions [3].

Effects of UV light depend on the presence of O_2 and the formation of AOF, specifically $^1O_2$, $O_2^{\bullet-}$, $^\bullet OH$ and H_2O_2. Exposure of cells to visible light in the presence of SOD, catalase, peroxidase, and nonenzymatic antioxidants prevents photostimulation of functional activity: proliferation, oxidative burst, phagocytosis in leukocytes [3]. Active O_2 metabolites not only induce phagocyte priming [8], but also trigger the synthesis of some proteins and cytokines [3].

The effects of UV irradiation on the blood of donors and patients with various pathologies were studied not once, but complex studies of the effect of UV irradiation on AOF production by neutrophils (NP) and content of LPO products in donor blood were never carried out. It is known that activation of phagocytosis and enhanced formation of AOF lead to intensification of LPO and vice versa, LPO products sensitize NP [6,7].

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We studied the effects of UV irradiation on NP function by evaluating activities of myeloperoxidase (MPO) and NADPH-oxidase system and LPO intensity in donor blood.

MATERIALS AND METHODS

Heparinized donor (n=25) blood and NP suspension (10^6 cell/ml) were exposed to light emitted by a DRT-400 lamp (irradiation intensity 151 J/m^2/min) through an UFS-1 photofilter with a 240-290 nm pass band in doses of 75.5-1510.0 J/m^2. The content of dienic conjugates (DC) and ketodienes (KD) was measured by optical density of heptane extracts on an SF-46 spectrophotometer at 232 nm (DC) and 273 nm (KD) [2]. NP were isolated in ficoll-urograffin double density gradient (ρ=1.077 and 1.119 g/ml) [10]. Functional activity of NP was evaluated by measuring luminol- and lucinogenin-dependent chemiluminescence on a BCL-06 M device. Myeloperoxidase activity was evaluated by the degree of tetramethylbenzidine oxidation [1]. The significance of differences was evaluated by paired comparisons.

RESULTS

UV-induced priming of NP was observed at irradiation doses of 75.5 and 151.0 J/m^2 (Fig. 1).

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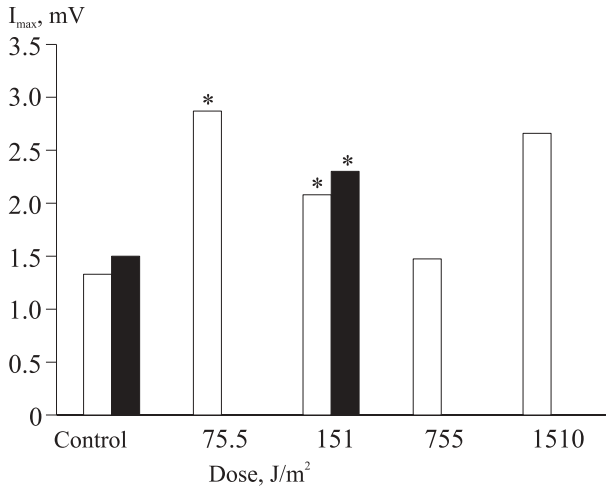


Fig. 1. Effect of UV light on AOF production by donor blood neutrophils. Light bars: luminol chemiluminescence; dark bars: lucinogenin chemiluminescence. Here and in Figs. 2, 3: *$p<0.05$ compared to the control (before irradiation).

The cytotoxicity of NP is closely related to products of oxygen-dependent metabolism. Rapid accumulation of $O_2^{\bullet-}$ starts from activation of membrane oxidases catalyzing electron transfer from NADPH to molecular O_2 with triggering hexose monophosphate shunt [11].

Free radicals and H_2O_2 released by NP into the extracellular medium induce LPO and protein denaturing, thus providing conditions for cell membrane lysis — the first stage of cytotoxic action of NP. The effect of H_2O_2 on LPO is potentiated as a result of interaction with MPO and formation of hypochlorite (OCl^-) in the MPO—galloide—H_2O_2 system. This process underlies the participation of MPO in killer activity of NP [11].

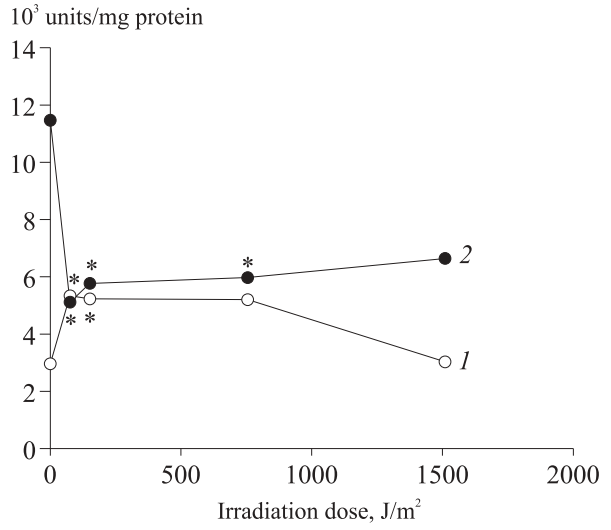


Fig. 2. Effect of UV light on myeloperoxidase activity in donor neutrophils in group 1 (*1*) and 2 (*2*).

Studies of the effect of UV light on MPO activity of blood NP showed that blood donors could be divided into two groups by the type of relationship between MPO activity and UV light dose and by the initial level of enzyme activity (Fig. 2). Initially low enzyme activity (group 1) increased under the effect of UV exposure in doses of 75.5 and 151.0 J/m^2, while in group 2 this parameter decreased. MPO activity was similar in donor groups whose blood was exposed to UV in doses of 75.5-755.0 J/m^2, which attests to a corrective effect of these doses of UV exposure on the blood. Increasing the dose to 1510 J/m^2 did not lead to significant changes in MPO activity in comparison with the control.

UV exposure in doses of 75.5 and 151.0 J/m^2 increased functional activity of NP in all donors, and hence, the decrease of MPO activity in group 2 was compensated by activation of other enzymes.

The effect of UV light (151.0 J/m^2) on functional activity of NADPH-oxidase complex was evaluated by measuring chemiluminescent response using lucinogenin (selectively reacting with $O_2^{\bullet-}$) instead of luminol [11].

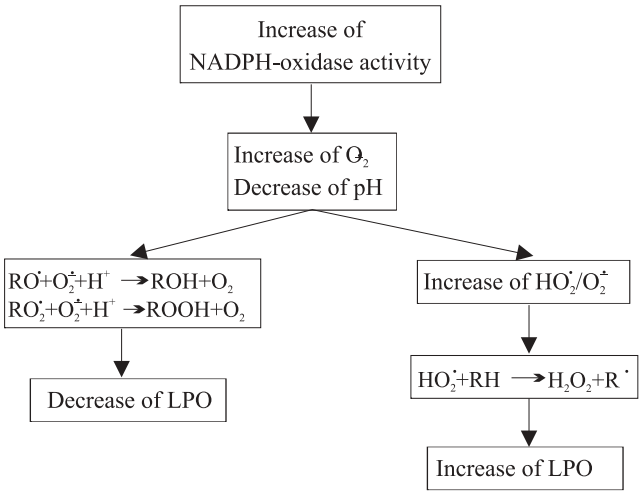
Chemiluminescence of NP suspension increased in all donors after irradiation of the blood and cell suspension. After UV exposure NADPH-oxidase activity in NP increased by 53.7 and 25% for the blood and suspension, respectively (Fig. 1).

The processes of neutrophil stimulation are mediated by the plasma membrane and are Ca^{2+}-dependent. Association of phosphorylated cytosol factors p47phox and p67phox with the membrane-bound components of the enzyme complex preactivates NADPH-oxidase [11]. Since LPO in the membrane increases membrane permeability (*e.g.*, for passive transport of Ca^{2+} into the cell), it can be hypothesized that NP priming after UV exposure of the blood is mediated through LPO intensification.

In the next series of experiments we evaluated the effect of UV exposure of the blood on LPO intensity. Two groups of donors were distinguished by the relationship between blood content of LPO products and exposure dose (Fig. 3). In all cases the shape of the curve was unambiguously determined by the initial content of LPO products in donor blood: UV irradiation in low doses (75.5-151.0 J/m^2) decreased initially high and increased initially low levels. When high doses of UV irradiation were used, the level of LPO did not surpass the initial values. The effects of UV exposure in doses of 75.5 and 151.0 J/m^2 can be considered as corrective. When analyzing the effects of UV light (75.5 and 151.0 J/m^2) on MPO activity and blood content of LPO products we can conclude that UV-correction was more efficient in cases when deviation of the test parameter from the mean

value was more pronounced. This relationship was discovered for infrared light and visible bands [3,5].

Analysis of our findings suggests the possible molecular mechanism of the effect of UV irradiation. In the blood and neutrophil suspension to NADPH-oxidase is one of the most important photoacceptors for UV light in phagocytes, and hence, the primary mechanisms of autotransfusions of UV-irradiated blood can be explained by increased $O_2^{\bullet-}$ concentration due to activation of this enzyme complex. UV irradiation decreases intracellular pH [9]. This is presumably due to the release of H^+ during NADPH oxidation and is caused by activation of NADPH-oxidase complex. The events can develop by two ways after activation of NADPH oxidase:



$O_2^{\bullet-}$ triggers cascade reactions leading to the formation of more reactive AOF, which is paralleled by initiation of LPO in membrane systems. In biochemical reactions $O_2^{\bullet-}$ can act as oxidant and reducing agent [12], *e.g.*, it reduces more reactive aloxyl ($RO^{\bullet}$) and perhydroxyl ($RO_2^{\bullet}$) radicals, as a result of which $O_2^{\bullet-}$ inhibits LPO under certain conditions and protects cells from damage [14].

Activation of NADPH-oxidase can be associated with intensification and attenuation of LPO, which was confirmed by our findings.

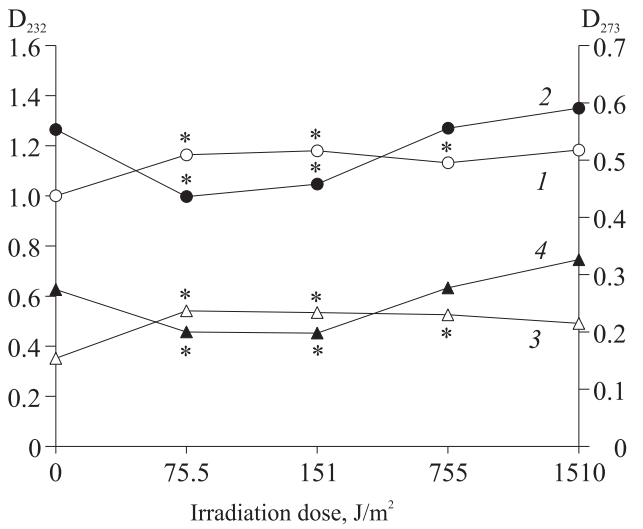


Fig. 3. Effect of UV light on blood content of dienic conjugates (*1*, *2*) and ketodienes (*3*, *4*) in groups 1(*1*, *3*) and 2 (*2*, *4*).

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