Effect of Ultraviolet B Irradiation on Immobilization Stress-Induced Changes in the Protective Systems of C57Bl/6 Mice

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We studied the effect of ultraviolet B irradiation on superoxide dismutase activity, ceruloplasmin level in the plasma, and steroid hormone concentration in the adrenal glands of C57Bl/6 mice subjected to immobilization stress. Ultraviolet B irradiation did not abolish the increase in superoxide dismutase activity, but decreased ceruloplasmin level in the plasma and corticosteroid concentration in the adrenal glands of mice exposed to immobilization stress.

Key Words: *ultraviolet irradiation; immobilization stress; superoxide dismutase; ceruloplasmin; steroid hormones*

The interest in studying the biological effect of ultraviolet B irradiation (UVB, 280-320 nm) is due to a rise in its intensity at the Earth's surface associated with depletion of the atmospheric ozone layer. These changes, as well as the influences of other adverse environmental factors, increase the risk of developing skin cancer.

An important mechanism of the immunosuppressive effect of UVB irradiation is photodynamic conversion of *trans*-urocanic acid into *cis*-urocanic acid, which stimulates degradation of mast cells and release of Th2 immunosuppressive cytokines. Another mechanism suggests generation of reactive oxygen species (ROS) and variations in the activity of nonenzymatic and enzymatic antioxidant systems in the skin of irradiated animals [9]. The systemic effect of UV irradiation should be studied in details to perform correction of adverse consequences. Previous studies were directed to evaluation of changes in the antioxidant and monooxygenase systems of rat liver after irradiation of the skin [6]. The systemic effect of UVB irradiation is realized via the blood. Therefore, activity of plasma antioxidant systems plays a role in the protective response of the organism. Cu/Zn-Superoxide dismutase (SOD, EC 1.15.1.1) and ceruloplasmin (CP, EC 1.16.3.1) are the major antioxidant enzymes in blood plasma that utilize $O_2^{\bullet-}$. $O_2^{\bullet-}$ initiates the formation of other ROS. SOD catalyzes dismutation of $O_2^{\bullet-}$ into H_2O_2. CP is a polyfunctional enzyme involved not only in dismutation of $O_2^{\bullet-}$, but also in transport and distribution of Cu^{2+} and Fe^{2+} stimulating ROS generation [3,10,15]. The nonspecific reaction of the organism to environmental factors is accompanied by the appearance of stress markers. They include steroid hormones regulating the balance between catabolic and anabolic processes in tissues and providing mobilization and rapid energy redistribution in adaptive systems [7,13]. The specific effect of UVB irradiation on biochemical parameters should be studied to evaluate the immunosuppressive action and perform directional correction of changes induced by this treatment.

In the present work total SOD activity, CP level in blood plasma, and steroid hormone concentration in the adrenal glands of male C57Bl/6 mice

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were measured during various periods after immobilization stress and UVB irradiation.

MATERIALS AND METHODS

Experiments were performed on 7-9-week-old male C57Bl/6 mice obtained from Andreevka nursery (Research Center of Biomedical Technologies, Russian Academy of Medical Sciences). The animals were kept in a vivarium (Biological Faculty, M. V. Lomonosov Moscow State University) under standard conditions of housing and feeding. Hair was removed from the back (8 cm^2) 1 day before irradiation. The mice were divided into 3 groups (6 animals per group). Group 1 included control animals. Group 2 animals were subjected to immobilization stress (plaster fixation for 30 min). Group 3 animals were exposed to UVB irradiation during immobilization stress. The mice were irradiated using three LE-30 UV lamps placed at a distance of 18 cm from the skin. The intensity of radiation was measured with a RTN-30S thermocouple (5.1 W/m^2). The radiation dose during 30-min exposure was 9.1 kJ/m^2. During irradiation animal's eyes were covered with a foil radiation shield. The animals were killed by cervical dislocation 1 h and 1, 3, and 5 days after treatment. The adrenal glands were removed. The blood was sampled, placed in tubes, incubated at room temperature for 2-3 h, and centrifuged at 3000 rpm for 15 min. The supernatant (plasma) was used for biochemical studies. The plasma from control animals was irradiated in *in vitro* experiments (dose 19.4 kJ/m^2, UVB irradiation dose rate 5.4 W/m^2).

Total activity of SOD in mouse plasma was estimated as described elsewhere [4]. CP level in samples was determined by oxidase activity relative to the substrate *o*-phenylenediamine at 492 nm [8]. Plasma SOD activity and CP level were expressed in percents of control values. The concentration of 11-corticosteroids in the adrenal glands of mice was estimated as described previously [5] and expressed in μg. The amount of thiobarbituric acid-reactive products (TBA-reactive products) in blood plasma was measured *in vitro* [2] and expressed in nmol/ml plasma. Fe^{2+}-induced generation of ROS was studied after incubation of blood plasma with 10 mM $FeSO_2$ for 10 min.

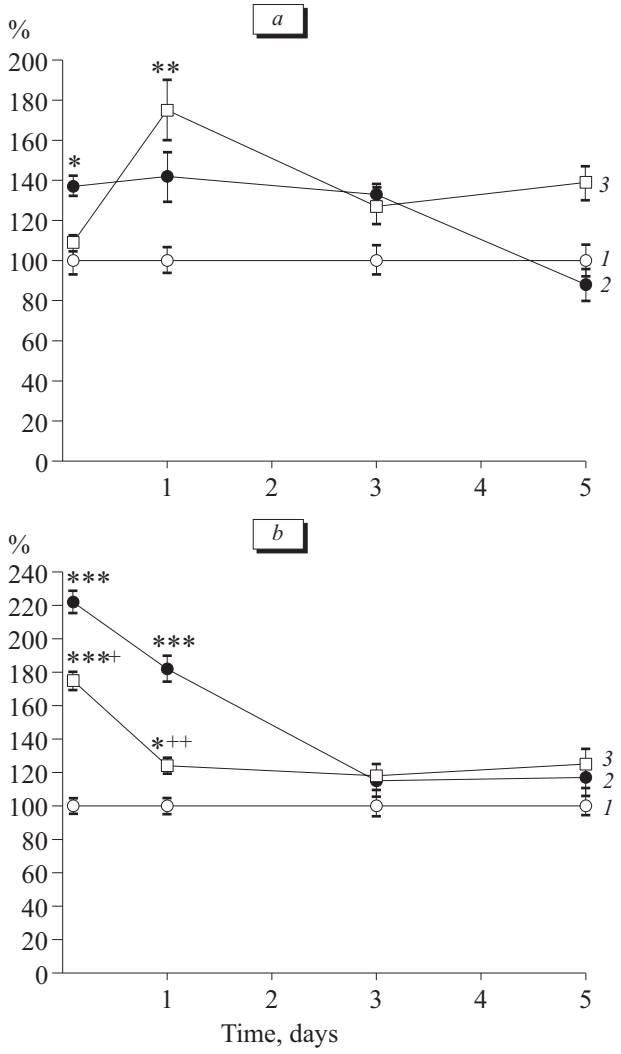
The results were analyzed by analysis of variance (ANOVA) using GraphPad Prism 4 software. The differences were significant at $p<0.05$.

RESULTS

SOD activity in animals 1 h after immobilization stress increased by 37% and remained unchanged for 3 days. SOD activity increased up to 175% on day 1 after UVB irradiation and immobilization, but did not differ from the control 1 h after treatment. It should be emphasized that enzyme activity tended to increase over 5 days after combined exposure to irradiation and immobilization. These changes reflect the compensatory reaction to stress (Fig. 1, *a*). Variations in plasma SOD activity reflect increased generation of $O_2^{\bullet-}$, which induces SOD activity [11]. Enzyme activation is not related to increased concentration of cytokines migrating from the skin to the blood after UVB irradiation. Published data show that cytokines responsible for changes in Mn-SOD activity undetected in the plasma do not modulate the increase in Cu/Zn-SOD activity in UVB-irradiated keratinocytes [13]. It was hypothesized that Cu/Zn-SOD contributes to detoxification of ROS during the early period after UVB irradiation [12]. The concentration of another copper-containing enzyme in blood plasma (CP) 2-fold increased 1 h and 1 day after immobilization, but decreased to normal on day 3 (Fig. 1, *b*). Combined exposure to immobilization and UVB irradiation abolished the stress-induced increase in plasma CP level, which returned to normal 1 day after treatment. Glycoprotein CP is an acute-phase protein and polyfunctional antioxidant enzyme. Increased level of CP after immobilization stress can be considered as a protective response of the organism to adverse factors. The decrease in CP level after combined exposure to immobilization and UVB irradiation suggests that activity of this enzyme is modulated by other mechanisms. These changes play a negative role in the organism. The decrease in plasma CP level probably contributes to damage to skin cell membranes and development of UV-induced malignant neoplasms in the skin [14].

The concentration of 11-corticosteroids increased 3 days after immobilization stress (by 30%), but remained unchanged in animals exposed to stress and UVB irradiation (Fig. 1, *c*). These changes attest to decreased adaptive capacity of the organism.

Previous experiments with pure solutions of CP showed that UVB irradiation causes photoinactivation of the enzyme [1]. Our study showed that 40% decrease in CP content is accompanied by an increase in the concentration of TBA-reactive products in irradiated plasma (Fig. 2, *a*, *b*). Threefold increase in the amount of ROS did not change plasma CP level. Therefore, ROS are not involved in the decrease in plasma CP level during UVB irradiation (Fig. 2, *a*, *b*). It can be hypothesized that decreased plasma level of CP in animals after *in vivo* UVB irradiation is mainly related to photoinactivation of the enzyme. Penetration power of



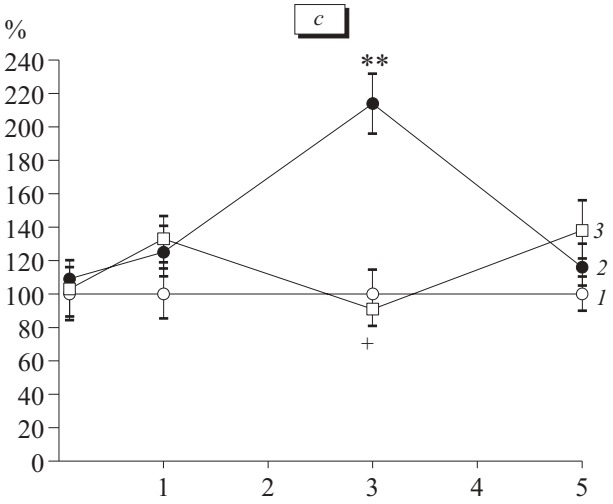


Fig. 1. Effect of UVB irradiation on SOD activity (*a*), ceruloplasmin (CP) level in blood plasma (*b*), and corticosteroid concentration in the adrenal glands of mice (*c*): control animals (*1*); immobilization (*2*); and immobilization and UVB irradiation (*3*). **p*<0.05, ***p*<0.01, ****p*<0.001 compared to the control; ^{+}p<0.01 and ^{++}p<0.001 compared to immobilization stress.

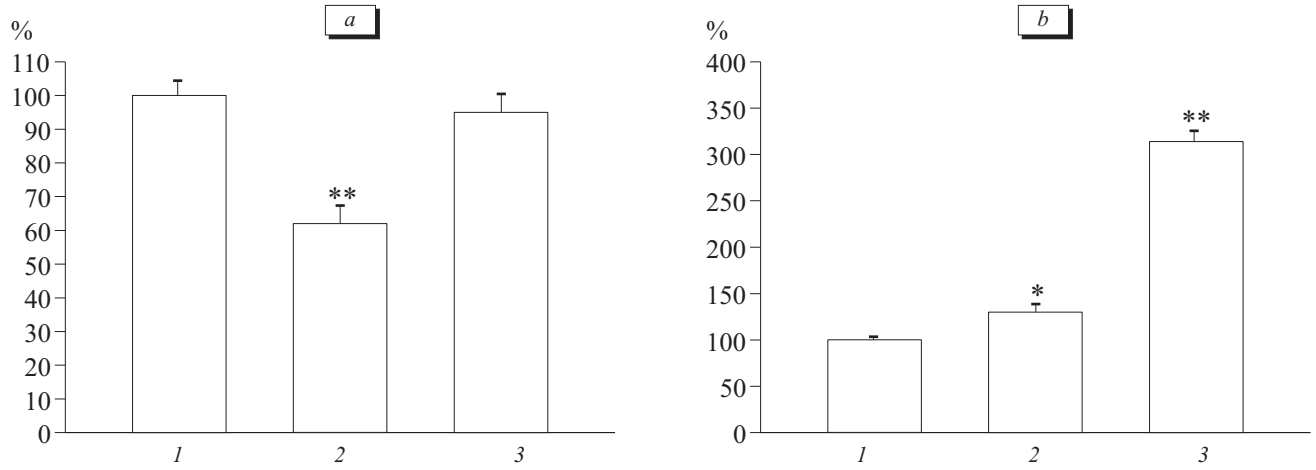


Fig. 2. Amount of TBA-reactive products (*a*) and concentration of CP (*b*) in the plasma under control conditions (*1*) and after *in vitro* UVB irradiation (*2*) or Fe^{2+}-induced generation of ROS (*3*). **p*<0.05 and ***p*<0.001 compared to the control.

UVB irradiation is sufficient to affect the blood in skin capillaries.

Our findings demonstrate the specific effect of UVB irradiation on CP level in blood plasma and steroid hormone concentration in the adrenal glands. These changes reflect the suppressive effect of UVB irradiation.

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