EFFECT OF UV IRRADIATION ON FLUORESCENCE AND ANTIOXIDANT PROPERTIES OF Γ-GLOBULINS

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Ultraviolet blood irradiation is an alternative approach for the treatment of infectious diseases of various etiologies and is a new immunomodulatory method. To date, there are no data in the literature on the precise mechanisms of the effects of UV radiation on various blood components, including proteins. The aim of the present work was to analyze the fluorescent properties of γ-globulins after UVI exposure (254 nm) with simultaneous registration of their antioxidant profile by chemiluminescent method in a model with alkylperoxyl radicals. We obtained that exposure of γ-globulins to UV leads to a dose-dependent decrease in fluorescence and a more complex kinetic curve of chemiluminescence due to the formation of products with stronger antioxidant properties.

Key words: *mercury lamp UV; Ultraviolet blood irradiation; blood plasma; γ-globulins; antioxidants; fluorescence, chemiluminescence*

Due to the emergence of new infections, including SARS-CoV-2, and their resistance to existing drugs, there is currently an increased interest in the therapeutic potential of ultraviolet blood irradiation (UVBI). The efficiency of UVBI in the therapy of a number of diseases, bacterial and viral etiology, has long been demonstrated.

To understand the mechanisms of phototherapy, it is necessary to study the effect of UV irradiation (UVI) on various targets in the blood, including the plasma proteins. Globulins are an important component of blood plasma involved in providing humoral

immune protection. Earlier studies showed the effect of UVI on protein metabolism, namely on the levels of immunoglobulin (Ig) in the serum before and after exposure to UVI [1], as well as their tendency to aggregation and immune reactivity [2]. Thus, the aim of the present work was to analyze the fluorescent properties of γ-globulins after UVI exposure (254 nm) with simultaneous registration of their antioxidant profile by chemiluminescent method in a model with alkylperoxyl radicals.

Materials and Methods. UV Irradiation of samples (mercury lamp UV). Concentrated solutions (optical density not more than 0.2) of γ-globulins were irradiated in a quartz cuvette (volume 3.000 ml) using a Bio-Link UV irradiation system (Vilber Lourmat) at 254 nm (UV source 5 ×8-watt lamps).

Antioxidant capacity assay based on luminol enhanced chemiluminometry. The antioxidant activity of substances was quantified with the enhanced chemiluminescence protocol. The chemiluminescent system consisted of a source of free radicals 2,2'-azobis (2-amidinopropane) dihydrochloride (ABAP, Sigma) and a chemiluminescent probe luminol (Sigma). The method was described elsewhere [3]. A luminol solution of 1 mmol/L (Sigma) and ABAP solution of 50 mmol/L (Sigma) was prepared by dissolving the weighed samples in phosphate buffer solution (100 mM KH_2PO_4, pH 7.4, Sigma). The total volume in a cuvette was 1.000 mL. A mixture of ABAP and luminol (final concentrations were 2.5 mM and 2 μM, respectively) was added to a buffer solution (pH 7.4) at 37°C. The chemiluminescence was recorded until a stationary level had been achieved, then an aliquot of the sample was added. The registration was performed until the new steady-state level was achieved.

Results and discussion. Globulins are present in blood plasma in amounts comparable to those of human serum albumin (HSA, 30–60 g/L). Similar to albumin, the structure of globulins includes the natural fluorophore tryptophan, which makes it possible to study its phototransformation by spectrofluorimetry. Here, we have examined irradiation of γ-globulins fraction by different doses of UV (mercury lamp) with registration of fluorescence spectra (Fig. 1A, B):

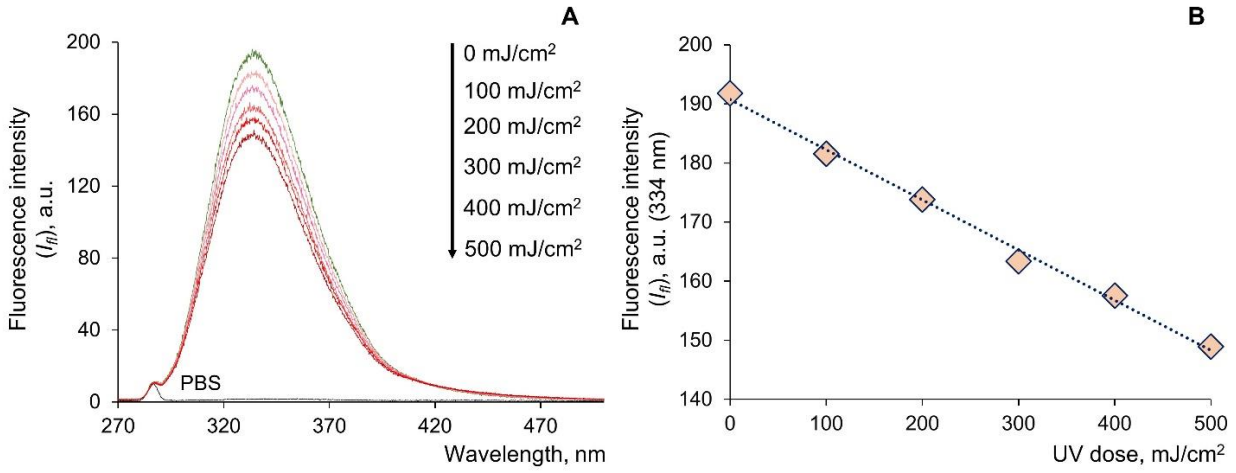

Figure 1. A — Fluorescence spectra (λ_{ex} = 260 nm) of γ-globulins solutions (c = 0.66 μM) after exposure to UV (254 nm), B — change in fluorescence intensity (I_{fl}) versus UV dose for γ-globulins solution (c = 0.66 μM) at wavelength of 334 nm.

A dose-dependent decrease in the fluorescence intensity of the γ-globulins solution was obtained with increasing UV irradiation dose. It should be noted that, according to our data obtained earlier that at comparable concentrations of γ-globulins and HSA, the tryptophan fluorescence of albumin appears to be more sensitive to UV exposure. Half quenching of fluorescence for globulins occurs at a dose of 1116 mJ/cm^2, while for albumin this value is 450 mJ/cm^2 [4]. In a similar study, UVA exposure to γ-globulins and bovine serum albumin in an *in vitro* model caused the oxidation of sulfhydryl groups [5]. According to the literature approximately 10%–30%, of protein bound homocysteine (Hcy), cysteine (Cys) and cysteinylglycine (CysGly) are disulfide-linked to globulins [6]. However, from the different rates of exchange of disulfide- linked amino acids the pools of Hcy, Cys, and CysGly bound to albumin and globulin may represent kinetically and functionally distinct pools [6].

Changes in the antioxidant potential were also evaluated. The chemiluminograms (Fig. 2) are fully different from the three-phase chemiluminograms of albumin [4].

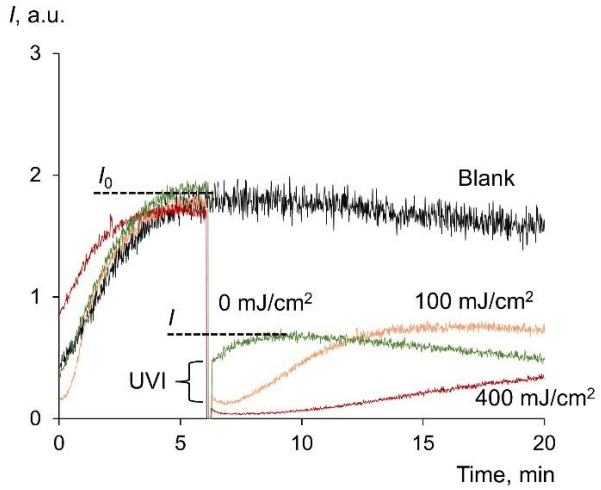

Figure. 2. Chemiluminograms of γ-globulins solutions (c = 0.66 μM) in the analytical system PBS (100 mM, pH 7.4) + ABAP (2.5 μM) + luminol (2.0 μM).

For globulins, the signal (I_0) decreases to a new stationary level (I). This behavior is characteristic of weak antioxidants. After irradiation of globulins, however, there is a marked depression of chemiluminescence, up to almost zero values, with a slow return to the initial level, indicating the formation of products with stronger antioxidant properties as a result of UV-irradiation of globulins.

Conclusions. The study of the effect of UVI on plasma proteins is an important task for using the potential of UVBI as a new immunomodulatory method. A dose-dependent decrease in the fluorescence intensity of γ-globulins with increasing UV irradiation dose was obtained. The antioxidant profiles of UV-oxidized γ-globulins in the system with alkylperoxyl radicals were recorded. It was shown that native γ-globulins are characterized by their kinetics as prolonged-acting antioxidants. A more complex kinetics was observed after UV exposure. New phases on the kinetic curve indicate the formation of products with stronger antioxidant properties as a result of UV exposure of the γ-globulins.

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