SPECTROSCOPIC INVESTIGATIONS OF THE PHOTOCHEMICAL PROPERTIES OF THE CERULOPLASMIN

Yakovleva T.Yu., Barmasov A.V., Kholmogorov V.Ye.

Institute of Physics, St.Petersburg State University, ul. Ulianovskaya 1a, St.Petersburg, 198904 Russia

Investigations were carried out to study the action of He-Ne irradiation applied in diffuse stream onto blood ***in vitro***. Results seem to reflect the photoactivation of ***ceruloplasmin*** as one of the factors of laser therapy positive effect in pathological processes. Bioantioxidant effect of blood irradiation with He-Ne laser is regarded as one of the positive factors of photobiological action.

Ceruloplasmin (CP, ferrooxidase) - blue multifunctional copper-containing protein of the mammalian blood plasma from the family of blue oxidases. 6 atoms of copper are the integral part of the molecule of the ***CP***, due to various optical, magnetic, and redox properties copper-containing active centres in the ***CP*** are referred to three or, probably, four basic types. Copper-containing active centres of the ***CP*** are associated with such vital biological functions, as electron transport, metabolism of iron, and others.

Method of photomodification of blood by action of UV- and laser irradiation is used in clinical practice with the purposes of prophylaxis and treatment of various diseases. Immune and stimulating effects have appeared to be especially valuable in treatment by low-energy lasers of inflammatory diseases and sequelae. The condition of antioxidant system (AOS) mainly defines the expression of damaging action of products of lipoperoxidation (POL). Strong antioxidant activity of blood plasma of the man depends mainly on the copper-containing protein ***CP*** and iron-combining protein ***transferrin***. Unusual as compared with other proteins in similar systems, antioxidant properties of ***CP*** can have great importance ***in vivo***, but the mechanism of action of ***CP*** in such complex systems is not clear since antioxidant action of ***CP*** can depend on ferrooxidase activity, ascorbatoxidase activity, and activity on $O_2^{\bullet -}$ removal.

Spectroscopic and fluorescent investigations of the ***CP*** were conducted. Complicated character of the absorption and fluorescent spectra of ***CP*** in the visible and near UV-regions is conditioned by the presence of great number of aromatic amino acids residues in this protein. Fluorescent, excitation absorption and ESR spectra have been registered. 1 type

copper centres state changes were estimated by the absorption and ESR spectra, 2 type centres – by the ESR spectrum, 3 type centres – by the fluorescence spectrum.

Investigations were carried out to study the action of low-energy He-Ne laser irradiation (λ = 632.8 nm) of various dozes on POL processes, and on activity of enzyme system of antioxidant protection in blood. As a result of irradiation of blood by He-Ne laser in diffuse stream with a total doze of irradiation equal to 2.7 and 5.4 J to 20 cm^3 of blood ***in vitro*** (0.14 – 0.27 J cm^{-3}), reduction of a stationary level of primary metabolites of POL, ***dienic conjugates*** (DC), as well as one of final products - ***malonic dialdehydes*** (MDA) in blood plasma was observed. Slight hemolysis of blood began with the increase of a total doze of irradiation, and became sharply expressed at a doze of 27 J and above. It was shown that the promotion of tolerance of lipid fraction of the irradiated plasma towards lipoperoxidation, induced by Fe(II) ions, reflected the increment in total plasma antioxidant status. The study of activity of copper-containing "blue" ferments has shown that the specific activity of SOD erythrocytes (in account on milligrams of protein) authentically did not change at dozes of irradiation from 0.9 up to 5.4 J. Oxidase activity of ***CP*** raised insignificantly. The SOD-activity of plasma authentically increased on 16% beginning from the doze of irradiation equal to 2.7 J, and remained at this level with the further increase of irradiation dose. The obtained data testify that the irradiation of blood by He-Ne laser in dozes equal to 2.7 and 5.4 J reduces POL intensity, that increases lipid stability of plasma to induced oxidation, influences on fermentative system, raising it’s antioxidant activity, so that the POL processes are inhibited.

Since UV-light initiates free radical processes of POL in blood, and since irradiation by He-Ne laser, transforming of molecular oxygen into singlet state, also initiates processes of POL, and since the photoexcited ***CP*** operates as antioxidant, so, it becomes possible to obtain desirable photobiological effect by the changing of length of waves and dozes of irradiation.

Antioxidant properties of "blue" oxidases, including ***CP***, are studied intensively. But still there are lots of obscurities in structure and functions of ***CP*** active centres and their interaction.