

OPTICAL IRRADIATION EFFECT ON BLOOD AND ITS COMPONENTS

Vladimir E. Kholmogorov^a, Tat'yana Yu. Yakovleva^b, Alexander V. Barmasov^a

^aSt.Petersburg State University, Institute of Physics, St.Petersburg, Russia

^bRussian State Hydrometeorological University, St.Petersburg, Russia

The plasma after centrifuging of whole blood is characterised by very strong absorption in the region of 250 nm, intensive band with maximum at 280-285 nm and weak bands with maxima in the region of 370 and 420 nm. Blood plasma is extremely photosensitive system. Photoprocesses under UV light with wavelength 253.7 nm are especially effectively carried out. At photoirradiation of plasma at temperature 300 K new band appears on its absorption band long-wave slope in region of 325-340 nm, which continues to grow with increase of time of irradiation.

The EPR spectra of UV-irradiated plasma of blood at 77 K correspond to EPR signals of UV-irradiated proteins. To EPR spectrum bring in the contribution cation-radicals of tryptophan, anion-radicals of peptide linkage, acetylic free radicals of fragments of protein molecules, sulphur-containing free radicals, free radicals of low molecular compounds. It was possible to identify them by methods a photo- and thermoselection, having used spectral photosensitivity of some free radicals and their chemical activity in dark reactions.

In presence of molecular oxygen the part of free radicals enters with it into reaction, that results in appearance of active peroxide radicals: $R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$.

Main photoreaction in proteins is tryptophan inactivation. The primary step of this photoreaction - phototransfer of electron from amine group of tryptophan to electron acceptor, as which can act peptide group of this or near protein.

From non-protein compounds the highest concentration in blood plasma have common lipids, phosphoglycerides, cholesterol and fatty acids. However lipids absorb UV-light in short-wave area of a spectrum ($\lambda < 230$ nm), and the maxima of absorption of thin fatty acids are in the region $\lambda < 220$ nm.

UV-light with wavelength 253.7 nm, absorbed only by proteins (fragments of aromatic amino acids) and longwave slopes of absorption bands of lipids, initiates a chain of free radical reactions in lipids. The concentration of chemically active free radicals and peroxide radicals at 300 K is not high because of their constant participation in reactions, so in normal conditions it is impossible to find out them by absorption and EPR spectra. Besides this chain of reactions is broken by native antioxidants (α -tocopherol). Peroxidation of lipids (POL) efficiency was judged by accumulation of dienic conjugates (DC) ($\lambda_{\text{abs}} = 233$ nm) and carbonyl-containing compounds, in particular, malonic

dialdehyde (MDA), which enters colour reaction with thiobarbituric acid ($\lambda_{\text{abs}} = 353 \text{ nm}$), or by the level of chemiluminescence, being a consequence of the reaction of disproportioning of peroxide lipid radicals.

Photochemical conversions of membrane proteins and lipids lay in the basis of photomodification of biomembranes of blood cells.

It was established, that UV light induces free radicals in erythrocytes and lymphocytes, the significant part of those free radicals is stabilised at 77 K. EPR signals of those free radicals overlap creating a complex resulting EPR spectrum. To separate this spectrum into individual EPR signals a technique of step thermoannealing of samples was applied at various values of temperatures within 5 minutes. At 300 K free radicals do not find out due to their participation in secondary photo- and dark reactions, resulting in photomodification of properties of biomembranes.

By one of primary photoacceptors, responsible for initial photophysical steps, has appeared molecular oxygen, contained in rather high concentration in all live biological systems. The experimental proofs of this were obtained by study of biological action spectra. The experiments with human erythrocytes and lymphocytes have shown, that direct photoexcitation of singlet molecular oxygen ($\lambda_{\text{abs}} = 586, 638, 762 \text{ and } 1264 \text{ nm}$) causes updating of their biological membranes.

In this connection it was important to study influence of various doses of radiation of He-Ne laser on POL processes and on the activity of fermentative system of antioxidant protection in blood.

In result of irradiation of blood by He-Ne laser decrease of a stationary level of primary metabolites of POL, DC, as well as one of final products - MDA in plasma of blood was observed. Irradiation of blood *in vitro* by He-Ne laser radiation in used radiant energies (0.9 - 5.4 J) reduces POL intensity, that increases stability of plasma lipids to induced oxidation.

As well known, the spectrum of He-Ne laser radiation coincides maxima of absorption of hemoproteins (met-form) and copper-containing ferments (superoxidedismutase and ceruloplasmin (CP)), that assumes their participation in photochemical reactions. Study of CP activity - peculiar target for display of photobiological action of laser radiation with $\lambda = 632.8 \text{ nm}$, has shown, that not only antioxidant, but also the SOD activity of plasma authentically increased, beginning from radiant energy 2.7 J, on 16 % and remained at this level at further increase of radiant energy.

It is possible to assume, that irradiation of integral blood by red light with wavelength, continuous to a maximum of CP absorption in visible region of spectrum, caused by Cu ions of I type, responsible for oxidase activity, can bring to conformational changes of CP molecules, in result of which its dismutase activity was increased. The obtained data testify that irradiation of blood by He-Ne laser radiation with radiant energies 2.7 and 5.4 J renders photoactivating action on CP.