# BIOPHYSICS AND BIOCHEMISTRY

# Effect of He-Ne Laser Radiation on Lipids in Platelets

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Low-intensity He-Ne laser radiation induces metabolic rearrangements in platelet lipids and activates some lipid-dependent systems of cell regulation as well as the synthesis of lipid-like second messengers. Modification of the lipid phase and physicochemical state of biological membranes are the molecular basis of biochemical adaptation of the platelets to laser radiation.

**Key Words:** *platelets; He-Ne laser; esterified fatty acids; 1,2-diacylglycerole); myoinositol-1,4,5,-trisphosphate*

The molecular basis of biological effects of He-Ne laser irradiation is the photo-induced rearrangements in biological membranes [4,5]. The membrane lipids arranged in the functionally active matrix integrate the external influences and participate in activation of the cell self-regulation programs [1,8]. Investigation of lipid role in cellular response to laser stimulation is important. This paper focuses on molecular analysis of photo-induced rearrangements in platelet lipids and is an attempt to elucidate the role of lipids in the biochemistry of cell adaptation to low-intensity laser radiation.

## MATERIALS AND METHODS

Platelets were isolated by fractional centrifugation of bovine venous blood stabilized with EDTA (0.1 M) in the ratio 10:1 and suspended in Tirode buffer, pH 7.4 [6]. Irradiation was performed with an LG-75 He-Ne laser (l=632.7 nm, beam power 2 mW, exposure time 5 min, dose 6 J/cm$^2$). The lipids were extracted in a chloroform-methanol system (1:2, v/v). Phospholipids were separated from neutral lipids by sedimentation in cold acetone [3]. Methyl ethers of fatty acids were analyzed by gas-liquid chromatography using a Chromaton-N-super column with OV-101 liquid phase (5%) in an isothermal mode at 190°C [3,10]. Membrane microviscosity of the photomodified platelets (PMP) were evaluated according to the degree of pyrene excimerization [2]. The myoinositol phosphates were separated by ion-exchange chromatography on Dowex 1×8 (200-400) in the formiate form [7]. Phospholipase $A_2$ ($PLA_2$) activity was determined in the medium containing (mM) Tris-HCl (10, pH 8.0), Triton X-100 (15), $CaCl_2$ (10), and 1/2 mM phosphatidylcholine according to the production rate of free fatty acids. The data were statistically analyzed using the variation analysis and Student's t test.

## RESULTS

Low-intensity laser radiation induces various biochemical rearrangements in the lipid phase of platelet membrane, involving both phospholipids and neutral lipids. The fragments of lipid hydrophobic moiety, which determine the microviscosity of the membrane and are most labile to photostimulation, are long-chain unsaturated fatty acids (18:1, 18:2, 18:3, 20:1, 20:2, 20:4, and 22:1) and saturated acid 22:0 (Table 1).

Diverse modifications of the fatty acid composition (16:0, 18:1, 18:2, 20:1, 20:4, 22:0, and 22:1) in

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**TABLE 1.** Effect of Laser Radiation on the Composition of Phospholipid Fatty Acids and Neutral Lipids in the Platelets (%, *M±m*)

| Fatty acids | Phospoholipids | | Neutral lipids | |
|---|---|---|---|---|
| | control | radiation | control | radiation |
| 12:0 | 4.9±0.2 | — | — | 5.0±0.2 |
| 14:0 | 11.2±1.1 | 2.9±0.1 | 14.5±0.4 | 5.1±0.2 |
| 15:0 | 11.8±0.8 | 3.7±0.2 | 11.7±0.4 | 6.1±0.3 |
| 16:0 | 13.7±0.7 | 1.2±0.1 | 4.6±0.1 | 22.2±0.9 |
| 16:1 | 13.0±0.8 | 1.5±0.1 | 9.9±0.3 | 7.5±0.7 |
| 18:0 | 2.7±0.1 | — | 2.4±0.3 | 1.0±0.1 |
| 18:1 | 8.4±0.6 | — | 1.1±0.1 | 1.5±0.1 |
| 18:2 | 10.2±0.7 | 70.8±5.4 | 6.8±0.2 | 2.6±0.1 |
| 18:3 | — | — | 10.9±0.3 | 16.2±0.9 |
| 20:1 | 2.7±0.1 | 7.0±0.8 | 15.9±0.6 | 2.7±0.1 |
| 20:2 | — | 1.3±0.1 | — | — |
| 20:4 | — | 0.9±0.1 | 3.5±0.2 | 3.0±0.1 |
| 22:0 | 21.6±1.2 | 8.3±0.4 | 14.2±0.5 | 24.9±1.8 |
| 22:1 | — | 2.4±0.3 | 4.8±0.3 | 2.2±0.1 |

phospholipids and neutral lipids indicate photo-induced activation of the exchange processes that underlie metabolic interrelations between various classes of lipids. Specifically, this is observed in the phosphoinositide cycle, whose components (phosphoinositides, phosphatidic acids, and 1,2-diacylglyceroles) have a characteristic and relatively specific set of fatty acids.

Modification of the lipid component is the molecular basis for physicochemical rearrangements in PMP biomembranes resulting in a decrease in their microviscosity. In addition to specific changes in the content of lipid fatty acids, this is indicated by a 28% increase in excimer fluorescent peak of the hydrophobic probe pyrene ($p<0.001$) relative to the control. Molecular rearrangements in the platelet lipid component substantiate the view on the photo-induced rearrangements in biomembranes as integrate biochemical response of the structures underlying the long-term adaptation.

PMP demonstrate an increase in activity of $PLA_2$ by 90% ($p<0.001$) compared with control level (1.22± 0.18 μmol/min/$10^9$ platelet). $PLA_2$ (in platelets this enzyme is specific for the arachidonate-containing phospholipids) catalyzes the formation of arachidonic acid and lysophosphatidylcholine, which, together with their metabolites, participate in the regulation of functional platelet activity [11]. High activity of the phosphoinositide-specific phospholipase C (a key enzyme of the phosphoinositide cycle) manifests itself in increased myo-inositol-1,4,5-trisphosphate level (27.58±2.24 pmol/$10^9$ platelet, which is 120% higher than the control level) and 1,2-diacylglycerol level (0.15 μmol/$10^9$ platelet surpasses the control value by 45%, $p<0.001$). However, this rise of myo-inositol-1,4,5-trisphospate is insufficient for the maximum release of stored calcium because its content in activated platelets increases by dozens of times [10]. At the same time, the dynamics of 1,2-diacylglycerol level (another second messenger) attests to its high metabolic activity in PMP. Our findings are indicative of activation of the lipid-dependent systems of cellular control that participate in the control of platelet functions.

The He-Ne laser low-intensity radiation can be characterized as an informational factor whose effect is mediated by a complex biomembrane reaction. This reaction includes qualitative and quantitative rearrangements in the membrane lipid phase, modification of the lipid-dependent system of the cellular control, and formation of the second messengers which play a key role in the biochemical processes of cellular adaptation. Hopefully, analysis of the transmission ways for the external electromagnetic radiation in the cellular structures that are not specialized for reception of such signals may reveal the general principles of photobiostimulation and their realization in clinical practice.

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