Effect of He-Ne Laser on Platelet Activation and Aggregation

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Irradiation of whole blood with He-Ne laser inhibits platelet adhesion and aggregation on extracellular matrix under conditions of high shear rate and reduces fibrinogen binding and expression of P-selectin on the plasma membrane induced by a thrombin analog.

Key Words: *helium-neon laser; platelets; aggregation; activation*

Intravenous and transcutaneous irradiation of the blood with helium-neon laser is widely used in the treatment of diseases accompanied by hemorheological disturbances [1,2]. At the same time, the cell mechanisms of the correcting effect of the coherent light remain unknown. Our aim was to study the modifying effect of He-Ne laser on behavior of the platelets located on extracellular matrix and the state of membrane processes reflecting platelet activation.

MATERIALS AND METHODS

The experiments were carried out on whole blood from 17 healthy donors. Adhesion and aggregation of platelets on extracellular matrix were studied with an Cone and Plate(let) Analyzer under conditions of high shear rate (1300 sec^{-1}) [3,5]. Fibrinogen binding and P-selectin expression were studied by flow cytometry on an EPICS XL Coulter (Caulter Corp.) analyzer equipped with an argon laser (λ=488 nm) using monoclonal antibodies. Aggregation was induced with thrombin receptor activating peptide (TRAP) in doses of 6.2 and 25 μM. Prostaglandin PGE_1 (100 nM) was used as the inhibitor of platelet activation. Blood specimens were irradiated for 10 min with a He-Ne laser (λ=632.8 nm, 7 mW beam power at the light guide tip); the total absorbed power was 4.2 J.

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RESULTS

He-Ne laser irradiation of the blood decreases aggregation of platelets on the extracellular matrix at high shear rate. In control blood specimens, the mean size of particles precipitated on the matrix was 37.2±1.8 μ^2, while the area occupied by these particles was 17.8± 1.3%. After laser irradiation the mean size of the aggregates decreased by 29% ($p<0.001$), while the corresponding area decreased by 40% ($p<0.001$).

The observed decrease in platelet adhesion and aggregation on extracellular matrix after laser irradiation can result from true platelet-inactivating effect of radiation and primary activation followed by exhaustion. However, in the latter case the number of individual platelets should decrease due to aggregation. In our experiments the number of the platelets in the control and after 10-min irradiation was 223±29 $\times 10^9$ and 227±21$\times 10^9$ $liter^{-1}$, respectively, i. e. laser irradiation did not change the number of platelets, which attests to the absence of preceding hyperaggregation.

Functional activation of platelets is preceded by rearrangement of their membrane structures known as platelet activation and characterized by 2 important parameters: fibrinogen binding capacity and expression of P-selectin on the plasma membrane [4].

The percent of fluorescent cells (PFC) and the mean fluorescence intensity (MFI) in intact blood were 4.3±1.17% and 0.425±0.027 arb. units, respectively.

In blood samples preincubated with PGE_1 (inhibitor of platelet activation) PFC was 2.8±0.29% and MFI 0.419±0.019 arb. units. It suggests the absence of spontaneous platelet activation in control samples. After a 10-min laser irradiation of the whole blood, PFC was 2.16±1.2% and MFI 0.419±0.029 arb. units (differences with intact blood are insignificant).

Therefore, laser irradiation does not affect spontaneous platelets activation.

In tests with TRAP-induced fibrinogen binding to glycoprotein IIb-IIIa, PFC and MFI in the control were 1.9±0.9% and 0.326±0.03 arb. units, respectively, while after incubation with TRAP (6.2 μM) these parameters increased to 14.6±4.1% ($p<0.02$) and 0.469± 0.045 arb. units ($p<0.05$), respectively. After preliminary (before incubation with TRAP) 10-min laser irradiation PFC was 3.7±1.3% ($p<0.05$ compared to incubation with TRAP without irradiation), while MFI decreased to 0.365±0.018 arb. units.

Hence, He-Ne laser irradiation of the whole blood prevents fibrinogen binding by platelets induced by 6.2 μM TRAP.

Incubation of the whole blood with 25 μM TRAP led to a pronounced increase in fibrinogen binding to platelets: PFC was 60±6% and MFI was 1.06±0.15 arb. units ($p<0.001$ compared to intact blood). Preliminary laser irradiation induced minor changes in these parameters: 65.2±7.08% and 1.18±0.19 arb. units, respectively. It proves that *in vitro* laser irradiation of the blood does not prevent fibrinogen binding by platelets induced by a large dose TRAP (25 μM).

In the next series we studied the effect of laser irradiation on the expression of P-selectin on platelet membrane. In intact blood PFC was 3.5±0.87% and MFI 0.157±0.02 arb. units. Preincubation with PGE_1 decreased PFC to 1.2±0.25% ($p<0.05$) and MFI to 0.130±0.007 arb. units, which attests to minor spontaneous platelet activation. After laser irradiation of the blood PFC became 1.27±0.23% ($p<0.05$ compared to intact blood), while MFI remained unchanged (0.134±0.01 arb. units). Thus, He-Ne laser irradiation of the blood prevents spontaneous expression of P-selectin on platelet membrane.

In experiments on activation of TRAP-induced expression of P-selectin, PFC and MFI in intact blood were 2.9±1.0% and 0.127±0.006 arb. units, respectively. Incubation of the blood with TRAP (6.2 μM) increased PFC to 24.1±3.1% ($p<0.001$ compared to control) and MFI to 0.228±0.015 arb. units ($p<0.001$). Laser irradiation strongly inhibited TRAP-induced expression of P-selectin: PFC increased only to 7.2±1.7% ($p<0.01$ compared to incubation with TRAP without irradiation) and MFI to 0.142±0.005 arb. units ($p<0.001$). Therefore, He-Ne laser irradiation of the whole blood inhibits expression of P-selectin on the platelet membrane induced by low TRAP concentration (6.2 μM).

Incubation of the blood with 25 μM TRAP considerably increased P-selectin expression on platelet membrane: PFC increased to 76.4±4.2% ($p<0.001$ compared to intact blood) and MFI to 1.12±0.2 arb. units ($p<0.001$). Preliminary laser irradiation of the blood before incubation with TRAP had no effect on these parameters (77.8±4.5% and 1.08±0.25 arb. units, respectively).

Our study shows that irradiation of the whole blood with He-Ne laser inhibits platelet activation and aggregation on extracellular matrix. These effects of low-intensity laser radiation probably underlie its beneficial clinical application in the treatment of the diseases characterized by platelet hyperfunction.

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