Mechanisms of Platelet Function Alterations Induced by Low-Intensity Laser Radiation

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Effects of laser radiation (wavelength 630 nm, power 1 mW) on platelet aggregation in human blood *in vitro* and *in vivo* and on platelet sensitivity to thromboxane A as well as on cyclooxygenase and thromboxane synthetase activities were studied. Changes in platelet functional activity were shown to be related to platelet transformation into inactive discoid form, suppression of their sensitivity to endogenous activators, and decreased activity of the arachidonic acid cascade enzymes.

Key Words: *blood; platelets; laser irradiation*

Low-intensity laser radiation produces a positive effect on microcirculation and on rheological properties of the blood (suppression of the sludge phenomenon [7]) due to suppression of platelet function [1,8]. Platelet failure caused by laser rays results in the hypocoagulation syndrome [10]. All these factors increase the organic blood flow, in particular, in the case of vascular atherosclerotic lesions [5]. Modification of platelet function depends on the parameters of laser radiation [1]. Although the effects of laser radiation on platelet function have been thoroughly investigated, the mechanism of its action remains to be clarified.

In the present work we studied mechanisms of the platelet function suppression produced by low-intensity laser radiation.

MATERIALS AND METHODS

Blood from 17 patients with nonspecific reactive hepatitis and 14 healthy volunteers was collected from the cubital vein in 3.8% sodium citrate. Donor's blood was permanently mixed and irradiated *in vitro* for 15 min using an ALOK-1 laser (wavelength 630 nm, power 1 mW). In patients, *in vivo* blood irradiation in the cubital vein was performed through an intravenous light pipe. The same laser beam parameters were used. The 30-min irradiations were repeated daily during a 5-day period. The blood was collected before and after the course of irradiation.

Platelet-rich plasma was prepared according to [6]. Platelet aggregation was studied by the method [12] modified as in [2]. The kinetics of platelet aggregation was monitored with a 230LA dual-channel laser platelet aggregation analyzer (Biola Ltd., Russia). Platelets were activated with: ADP (5 μM, Serva), adrenaline hydrochloride (5 μM), platelet activating factor (0.01 μM, Sigma), collagen (4 μg/ml, Sigma), ristocetin (1.2 μg/ml, Sigma), and fibrinogen (3 g/l). The degree of platelet aggregation was expressed as percentage of light transmission. Aggregograms were analyzed suing an AGGR 2.20 (Biola) software [3]. Morphology and the number of platelets in aggregates were estimated with the Biola aggregation analyzer by the method [3].

Platelet sensitivity to thromboxane A_2 (Tx A_2), cyclooxygenase (COx) and thromboxane synthetase (TxS) activities were assessed indirectly in the tests with collagen (TxA_2 production inducer), aspirin (COx inhibitor), and imidasole (TxS inhibitor) by the method [4]. Platelet activity was assessed by changes in the mean size of aggregate.

The results were analyzed by the parametric statistics methods using Excel 5.0 electronic tables.

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Table 1. Effects of Low-Intensity Laser Radiation on Platelet Aggregation *In Vitro* and *In Vivo* (*M±m*)

Inducers	Irradiation *in vivo*			Irradiation *in vitro*		
	control	after irradiation	Δ, % of control	control	after irradiation	Δ, % of control
ADP, 5 μM	60.1±2.6	50.62±1.1	-15.7	73.21±1.5	47.02±2.2*	-35.52
0.05 μM	21.17±0.8	17.81±1.3	-15.8	24.10±2.3	16.81±5.2	-30.24
Adrenaline	37.2±1.5	20.33±0.9*	-45.3	45.45±6.2	34.12±3.5*	-24.92
Collagen	50.9±1.3	34.12±2.4*	-32.9	39.55±4.4	20.33±1.9*	-48.59
Platelet activating factor	76.8±2.1	49.62±1.5*	-35.39	52.1±2.2	29.12±5.7*	-44.10
Fibrinogen	65.5±3.1	40.22±2.2*	-38.59	48.2±6.3	30.22±4.7*	-37.30
Ristocetin	25.2±1.4	19.99±4.1	-20.67	27.24±2.3	20.4±4.5	-25.11
Spontaneous aggregation	2.8±2.5	1.2±0.8	-57.14	3.5±5.2	1.3±1.1	-62.65

Note. Here and in Table 2: *$p<0.05$ compared with control.

RESULTS

Blood irradiation both *in vitro* and *in vivo* caused a nearly 2-fold reduction in spontaneous aggregation and decreased the response to all platelet activators listed above (Table 1). Platelet sensitivity to collagen, platelet activating factor, and fibrinogen decreased to a greater extent than to ADP, adrenaline, and ristocetin. Effect of laser radiation of the blood *in vivo* and *in vitro* was unilateral and of the same order. The decrease in platelet activation induced by collagen and fibrinogen is of particular interest in view of thrombogenesis. It is likely that laser light modifies platelets both by membrane reconstruction and by changing platelet receptor sensitivity. A decreased sensitivity to activators may be due to the harmful effect of low-intensity laser radiation on the mechanism of platelet activation and, in particular, due to modification of glycoprotein membrane receptors. After irradiation, platelet counts in samples did not change, but the count of activated forms of platelets decreased, since the proportion of spheric platelets decreased, while that of discoid platelets increased (Table 2). This may account for decline in spontaneous platelet aggregation (Table 1).

Among the main factors that regulate platelet activity are TxA_2, Cox, and TxS contained in platelets [4]. It was shown that the addition of collagen-treated platelets to intact ones increases aggregation by 56.4% (Table 3). This was due to TxA_2 release by collagen-activated platelets [4]. The addition of collagen-treated platelets to irradiated ones decreased aggregation 4-fold. It is likely that laser radiation reduces platelet sensitivity to TxA_2, which results in platelet activity failure.

In experiments with aspirin and imidasole, nearly complete inhibition of platelet aggregation by laser radiation was observed (Table 3). Presumably, COx and TxS (the key enzymes of the arachidonic acid cascade) play an important role in platelet activity failure caused by laser light. However, direct measurements of enzyme activities are necessary to confirm this hypothesis because the addition of TxA_2 (collagen-activated platelets) did not restore aggregability.

Laser radiation suppresses the release of endogenous proaggregants by collagen activated platelets. In our experiments intact platelets activated by collagen released biologically active substances, which resulted in platelet aggregation (30% of the maximum). Laser suppresses this release by more than 10-fold.

Thus, laser radiation reduces the activity of platelet receptors. However, there is a second wave of aggregation induced by certain substances due to release of endogenous aggregants. For example, in experiments with adrenaline, the early peak of aggregation was followed by a second peak after irradiation. When COx and TxS were inhibited and collagen-induced aggregation was completely suppressed, the addition of TxA_2 restored platelet func-

Table 2. Effects of *In Vitro* Laser Irradiation on Platelet Morphology (*M±m*)

Values	Platelet counts $\times 10^9$/l	Platelets, %	
		spheric	discoid
Control	250±1.4	10.71±0.3	89.07±1.3
After irradiation	250±2.2	3.71±0.7*	96.27±0.6*

Table 3. Effects of *In Vitro* Laser Radiation on Platelet Sensitivity to TxA_2 and on Activities of COx (Test with Aspirin) and TxS (Test with Imidasole, *M±m*)

Platelets	Aggregation induced by collagen-activated platelets (ACAP), arb. units	Maximum platelet aggregation induced by collagen (MAC), arb. units	MAC/ACAP
Intact	5.01±1.53	8.24±2.02	1.64
Laser radiation	1.17±0.05	7.7±0.94	6.58
Aspirin	1.2±0.01	4.5±0.82	3.75
Aspirin treatment+laser radiation	1.1±0.06	1.9±0.2	1.72
Imidasole	1.9±0.1	4.10±0.71	2.15
Imidasole+laser radiation	1.25±0.09	1.23±0.02	0.9

tion. In our experiments, exogenous TxA_2 did not restore platelet aggregation after laser irradiation.

Our results indicate that laser radiation increases the proportion of discoid platelets, decreases that of spheric platelets without changing the total counts, and makes them insensitive to various activators. Platelet function failure is associated with decreased aggregation and sensitivity to endogenous aggregants and inhibition of the arachidonic acid cascade enzymes.

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