BIOPHYSICS AND BIOCHEMISTRY

Mechanism of Hypocoagulative Effect of Low-Intensity Laser Radiation

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Aggregation of platelets and their role in the hypocoagulation syndrome was studied after *in vitro* irradiation of blood with a laser. Thromboelastography was performed in platelet-rich and platelet-free plasma. Low-intensity laser radiation affected the coagulation system via platelets. It decreased platelet aggregation induced by ADP, collagen, epinephrine, ristocetin, platelet activating factor, and fibrinogen.

Key Words: *low intensity laser radiation; blood coagulation; blood platelets*

Low-intensity laser radiation (LILR) improves rheological parameters of the blood and provokes hypocoagulation syndrome [6]. LILR affects blood coagulation system and fibrinolysis as evidenced by blood decreased blood coagulability and retards clot formation. This phenomenon is explained by inhibition of thromboplastin activation and delay of the prothrombin-thrombin conversion without any significant effect on fibrinogenesis [7]. There is evidence arguing these findings [10]. This discrepancy is probably related to different methods of LILR. Our data obtained in large-scale clinical studies show that irradiation of blood by a low-intensity laser light (λ=630 nm, beam power 1 mW) decreases functional activity of platelets [9]. Our aim was to study the role of platelets in the hypocoagulation syndrome after irradiation of blood with a laser light.

MATERIALS AND METHODS

Blood was obtained from 10 donors. It was drawn from the cubital vein and stabilized with 3.8% sodium citrate, and irradiated *in vitro* for 15 min using an ALOK-1 He-Ne laser (l=630 nm, beam power at the light guide tip 1 mW). Platelet-rich and platelet-free plasma was prepared by the method described elsewhere [8].

Platelet-rich plasma and platelet-free plasma prepared from irradiated and native blood were used for thrombelastography (TEG), which was performed out with a GKGM-4-02 thromelastograph-hemocoagulograph (Russia) by a modificed [2] method [11]. The following parameters were determined: period of reaction, clotting time, maximum amplitude, clot elasticity index, clot retardation index, and specific coagulation index.

Platelet aggregation was studied by the method [12] with modifications [4] in a 230LA dual-channel laser platelet aggregation analyzer (Biola, Russia). Platelet aggregation was induced with ADP (Serva) diluted to a final concentration of 5 μM, epinephrine 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/liter). Induced platelet aggregation was characterized by the aggregation and desaggregation slopes in degrees and by the percentage of the maximum amplitude. Spontaneous platelet aggregation was taken into account. The aggregation plots were processed with an AGGR-2.20 original software [4].

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TABLE 1. Platelet-Rich and Platelet-Free Plasma Indices before and after LILR (*M±m*)

Indices	Platelet-rich plasma			Platelet-free plasma		
	before irradiation	after irradiation	Δ, %	before irradiation	after irradiation	Δ, %
Period of reaction, min	4.7±0.3	5.9±0.2*	25.53	5.5±0.3	6.6±0.6	15
Clotting time, min	3.5±0.2	4.7±0.4*	34.28	5.3±0.4	5.9±0.5	11.32
Maximum amplitude, min	56.1±0.6	43.1±2.4*	-23.17	22.1±2.1	20.6±1.2	-6.78
Clot elasticity index	127±0.5	75.43±1.4	-40.60	27.65±1.4	27.83±0.8	0.65
Clot retardation index	9.0±1.2	11.8±2.2	31.11	12.9±0.9	13.8±2.5	6.97
Specific coagulation index, min	5.5±0.7	7.1±0.4*	29.09	7.6±1.4	7.9±0.9	3.94

Note. Here and in Table 2: *$p<0.05$ compared with the indices before irradiation.

The results were statistically analyzed with Excel 5.0 parametric tables.

RESULTS

Irradiation of the blood of healthy subjects provoked the hypocoagulation syndrome detected by TEG (Table 1). It is characterized by a significant increase in the specific coagulation index. Analysis of TEG of platelet-rich plasma revealed and apparent decrease in the rate of thromboplastin and thrombin formation, which results in inhibition of fibrinogen conversion into fibrin. It is corroborated by 25% and 34% increase in the reaction and clotting times, respectively. The maximum amplitude of TEG markedly decreased; the clot elasticity index decreased by 40%. These two parameters are crucial for the characterization of platelet function [5]. LILR may provoke the hypocoagulation syndrome by affecting both the coagulation system and functional activity of the platelets.

Analysis of platelet-free plasma TEG showed that LILR did not change the specific coagulation index (Table 1). It produced an insignificant increase in the coagulation period and virtually did not affect the maximum amplitude and the elasticity of the blood clot. Thus, in the absence of platelets, LILR practically did not affect the blood coagulation system. It can be proposed that plasma irradiation inhibits the release of platelet factors that trigger the coagulation mechanisms.

We suppose that LILR affects the coagulation system of the whole blood via platelets, because hypocoagulation is observed only in their presence.

The effect of LILR on functional activity of platelets was studied. An insignificant decrease in spontaneous platelet aggregation was found. More pronounced changes were observed when platelet functional activity was assessed in response to various activators (Table 2). Thus, LILR led to a more than two-fold decrease in platelet aggregation. This tendency was observed with all activators used in the study: platelet aggregation decreased by 49% response to ADP, 35% to epinephrine, 52% to collagen, 33% to ristocetin, 30% to fibrinogen, and 37% to platelet activating factor. The aggregation plots

TABLE 2. Effect of LILR on Human Platelet Aggregation Indices (*M±m*)

Aggregation plot indices	Aggregation inducers					
	ADP, 5 μM	collagen, 4 μg/ml	epinephrine, 5 μM	ristocetin, 1.2 μg/ml	platelet activating factor, 0.01 μM	fibrinogen, 3 g/l
Intact plasma						
maximum amplitude, arb. units.	60.1±1.2	50.9±3.3	37.2±2.5	25.2±2.7	76.6±0.9	65.5±2.1
aggregation slope, °	61.3±2.3	44.1±1.7	34.1±3.4	24.1±2.2	85.3±1.6	80.9±1.4
desaggregation slope, °	0	5.5±0.9	6.1±2.5	0	0	0
Plasma+laser irradiation						
maximum amplitude, arb. units.	33.6±1.4*	22.2±1.5*	21.6±2.3*	16.7±2.4*	43.6±1.5*	41.6±2.6*
aggregation slope, °	53.6±3.2	25.6±4.1*	17.6±1.8*	16.6±1.1*	53.6±2.7*	44.7±1.3*
desaggregation slope, °	8.6±2.2	9.6±2.6*	9.9±1.3	0	0	0

revealed no desaggregation in all cases. In control experiments with native plasma desaggregation was induced only by collagen and epinephrine. After LILR, desaggregation induced by these factors increased and there were signs of desaggregation in response to ADP. Since irradiation decreases platelet activation by all examined activators, it probably disturbs the processes of coagulation system that are common to all receptors of these activators. This may be calcium release or cAMP synthesis in the platelets. There is evidence that platelet aggregation is accompanied by activation of IIb/IIIa glycoprotein receptor. It is reasonable to suppose that nonspecific action of LILR on platelets results from a change in the activity of glycoproteins on the platelet surface.

Reversibility of platelet aggregation is of profound biological importance. It is regulated by the balance between prostacyclin and prostaglandin systems and depends on the intensity and duration of stimulation. When stimulation of the platelets is sufficiently strong and long, the dense tubular system releases calcium in amounts activating phospholipase, and stimulates secretion of thromboxane A_2 [5]. Presumably, irradiated platelets do not release sufficient amount of secondary activators of aggregation in response to activators which results in desaggregation of platelet complexes. It cannot be excluded that subsequent thrombocytastenia determines the decrease in the sensitivity of platelets to activators.

Thus, LILR affects blood coagulation system via the platelets. LILR directly affects platelets, which is manifested not only in moderation of response to the inducers, but also in probable suppression of secretion of platelet factors and activity of prothrombinase, as well as in retardation of fibrin clot formation. Evidently, a drastic decrease in platelet functional activity is responsible for the hypocoagulative effect of LILR.

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