Participation of IIb-IIIa Glycoprotein in Spontaneous Platelet Aggregation

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In some patients with stable and unstable angina pectoris and in some donors without clinical manifestations of cardiovascular diseases and other pathologies, spontaneous platelet aggregation was completely suppressed by glycoprotein IIb-IIIa antagonists blocking the interaction of this glycoprotein with fibrinogen. Antibodies inhibiting binding of glycoprotein Ib with von Willebrand factor had no effect on the level and rate of spontaneous platelet aggregation. In the donor group, the level of spontaneous aggregation was almost 1.5-fold higher in persons with a certain genetic polymorphism (Leu→Pro substitution in position 33 of glycoprotein IIIa). The level of spontaneous aggregation correlated with the amount of glycoprotein IIb-IIIa on the platelet surface (r=0.41)

Key Words: *platelets; spontaneous aggregation; glycoprotein IIb-IIIa; glycoprotein Ib; genetic polymorphism*

Fibrinogen receptor glycoprotein (GP) IIb-IIIa (α_{IIb}/β_3 integrin) plays a key role in platelet aggregation. This receptor gains the capacity to bind fibrinogen after activation of platelets with thrombin, ADP, and other agonists. Fibrinogen forms molecular bridges between activated platelets, which leads to the formation of platelet aggregates [9]. During the disturbances in laminar blood flow and under conditions of high shear rates (a parameter reflecting differences in the rates of liquid layers), platelet aggregation is triggered by interaction of von Willebrand factor (vWF) with its receptor GP Ib. However, the interaction of GP Ib with high-molecular-weight vWF-multimers present in the blood can also stimulate platelet aggregation at low shear rates [10].

Spontaneous platelet aggregation (SPA), *i.e.* aggregation observed *in vitro* without addition of exogenous inductors, is a risk factor of acute coronary syndrome [5,11]. The mechanisms underlying SPA are little known. Specifically, the role of the major platelets receptors (GP IIb-IIIa and Ib) in this reaction is not quite clear. The correlations of SPA parameters with genetic polymorphism of IIb-IIIa and its individual expression were not evaluated.

We studies the role of GP IIb-IIIa and Ib in SPA and the effects of concentration of GP IIb-IIIa on the platelet surface and major genetic polymorphism of this receptor caused by Leu→Pro substitution in position 33 of GP IIIa amino acid sequence on this process.

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MATERIALS AND METHODS

We examined patients with coronary heart disease (CHD, 15 patients with stable and 6 patients with unstable angina pectoris) and healthy donors without symptoms of cardiovascular diseases or other pathologies, in whom SPA was detected during the study of platelet aggregation (n=24). The majority of patients (n=19) received aspirin, and donors received no drugs affecting platelet functions.

For evaluation of the effect of monafram, an antagonist to GP IIb-IIIa (Experimental Production of Medical and Biological Preparation, Russian Cardiology Research-and-Production Complex) on SPA, the drug was injected intravenously (0.25 mg/kg) to patients with unstable angina prior to coronary angioplasty. Monafram is an F(ab')2 fragment of anti-GP IIb-IIIa monoclonal antibody CRC64, which inhibits binding of IIb-IIIa to fibrinogen [3].

The blood for evaluation of platelet aggregation was stabilized with 3.8% sodium citrate (9:1 blood citrate ratio). Platelet-rich plasma (PRP) was obtained by blood centrifugation at 150g for 10 min. SPA and platelet concentration were measured on an LA-220 aggregation analyzer (Biola, Moscow). The formation of microaggregates in PRP was recorded using a highly sensitive method based on the analysis of optical density fluctuations in platelet suspension. Aggregation was measured at 37°C and constant stirring (800 rpm) over 5 min after placing the cuvette with PRP into the analyzer cell and start of stirring. The maximum size of platelet aggregates R (level of aggregation) and maximum rate of aggregate formation (ΔR/min) were determined. The device was calibrated by the size of individual platelets assumed for 1. Monoclonal antibodies CRC64 and AK2 against GP IIb-IIIa and Ib, respectively, blocking binding of these receptors with their ligands [2] were added to PRP in a concentration of 20 μg/kg 3 min before the start of aggregation measurement.

The blood for genotyping was collected in 0.5 M EDTA solution (100:1). DNA was isolated by phenol—chloroform method. Leu33Pro mutation was detected by PCR followed by restriction analysis with MspI endonuclease.

The amount of GP IIb-IIIa on platelet surface was determined on a flow cytometer (Partec PAS) using a GPIIb/IIIa Flow Cytometry Kit (American Diagnostica ADIAflo) according to manufacturer's instructions.

The data were processed statistically using nonparametric Mann—Whitney U test for independent samples and Wilcoxon test for paired samples. Correlation analysis was performed using Spearman's test.

RESULTS

SPA was revealed in some patients with CHD and in some healthy donors, which had no clinical manifestations of cardiovascular diseases and other pathologies. CRC64 (an antagonist preventing interaction of GP IIb-IIIa with fibrinogen [2]) added to PRP eliminated SPA in patients with stable angina and in healthy donors (Fig. 1, a, b; Table 1). In patients with unstable angina, SPA was also suppressed by intravenous injection of IIb-IIIa antagonist monafram (Fig. 1, c; Table 1). In contrast to IIb-IIIa blockers, AK2 blocking GP Ib binding with vWF had no effect on the level and rate of SPA in patients with stable angina pectoris (Fig. 1, a; Table 1). Similar data were obtained previously: SPA revealed in tobacco smoker males was eliminated by antibodies against GP IIb-IIIa, but not against GP Ib [8]. These findings suggest that at least in some patients with CHD and some healthy donors without clinically documented pathologies, induction and the development of SPA is determined by binding of fibrinogen to activated GP IIb-IIIa and not by the presence of high-molecular vWF multimers capable to trigger SPA by interaction with GP Ib.

TABLE 1. Effect of GP IIb-IIIa and GP Ib Inhibitors on SPA (M±m)

Group	Inhibitor	Maximum SPA level	Maximum SPA rate
Donors (n=5)	Without inhibitors	1.58±0.07	0.46±0.01
	CRC64	1.02±0.09*	—
CHD patients with stable angina (n=15)	Without inhibitors	2.44±0.18	0.90±0.15
	CRC64	0.98±0.06**	—
	AK2	2.17±0.14	0.92±0.11
CHD patients with unstable angina (n=6)	Without inhibitors	2.32±0.37	0.77±0.23
	Monafram	1.02±0.02*	—

Note. *p<0.05, **p<0.01 compared to the corresponding parameter in the absence of inhibitors.

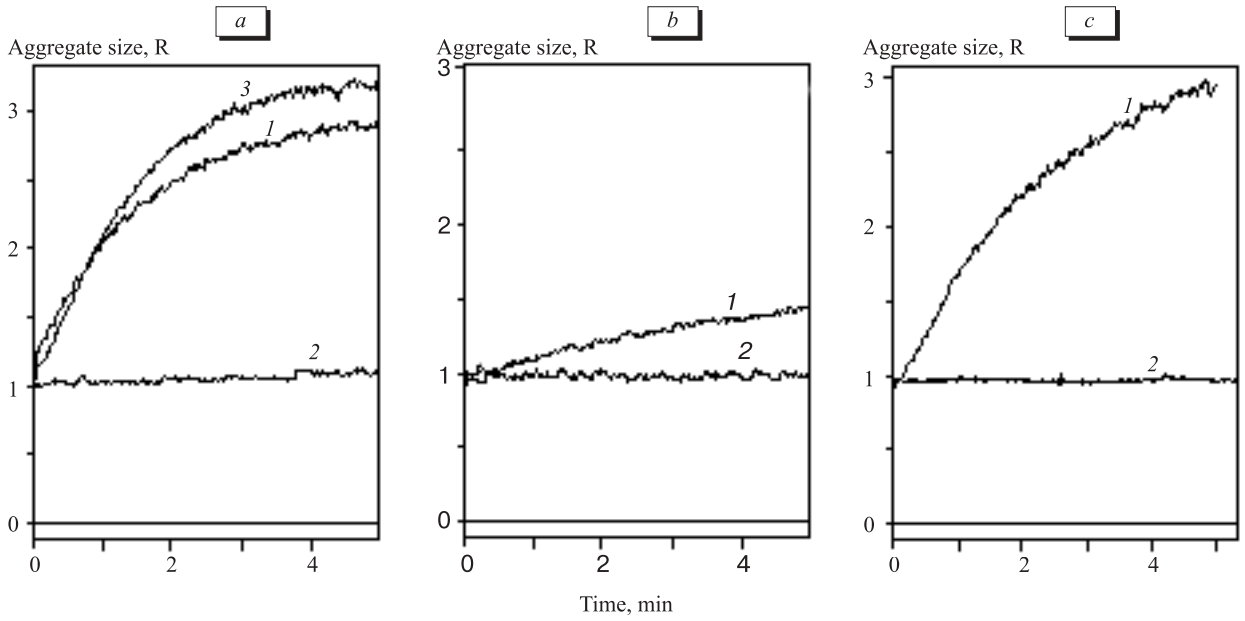

Fig. 1. Inhibition of SPA by GP IIb-IIIa antagonists. *a*: PRP from a patient with stable angina in the absence of antibodies (*1*) and in the presence of 20 μg/kg CRC64 (*2*) or 20 μg/kg AK2 (3); *b*: PRP of a donor in the absence of antibodies (*1*) or in the presence of 20 μg/kg CRC64 (*2*); *c*: PRP of a patient with unstable angina pectoris before (*1*) and 1 h after (*2*) intravenous injection of 0.25 mg/kg monafram.

Mutation Leu33Pro in GP IIIb is prevalent in European populations (10-20%). This mutation increases the risk of arterial thrombosis in young individuals [1,4,6,13] and is associated with enhanced platelet aggregation under the action of some agonists [1,7]. SPA was found in some donors with GP IIIa genotypes Leu33Leu33, Pro33Leu33, and Pro33Pro33. However, in persons carrying GP IIIa allele Pro33 (genotypes Pro33Leu33 and Pro33Pro33), the maximum SPA level was almost 1.5-fold higher than in donors with Leu33Leu33 genotype (Table 2), although SPA rates in these groups were similar. The content of GP IIb-IIIa on platelet surface and platelet concentration in PRP were similar in groups with different GP IIIa genotype (Table 2). These data suggest that enhanced SPA in donors with Leu33Pro mutation in GP IIIa is caused by changes in functional activity of GP IIb-IIIa. In model cells, Leu33Pro substitution in GP IIIa enhanced signal and adhesive functions of GP IIb-IIIa [12]. These effects can underlie elevated SPA in persons with GP IIIb Pro33 receptor variant. At the same time, individual variations in the content of GP IIb-IIIa ($44.8–82.7\times10^3$ molecules per platelet) also affected SPA parameters. Analysis of the total donor group revealed significant correlation between SPA intensity and the amount of GP IIb-IIIa on the platelet surface (r=0.41, p<0.05).

Our findings suggest that the development of SPA in some CHD patients and some healthy donors without overt clinical pathologies is determined by interaction between GP IIb-IIIa and fibrinogen, while its intensity depends on genetic polymorphism caused by Leu33Pro mutation in GP IIIa

TABLE 2. SPA Parameters, GP IIb-IIIa Content, and Platelet Concentration in PRP in Donors with Different GP IIIa Genotypes ($M\pm m$)

Parameter	GP IIIa genotype	
	Leu33Leu33 (n=13)	Pro33Pro33 (n=1)+ Pro33Leu33 (n=10)
Maximum SPA level	1.33±0.06	1.87±0.20*
Maximum SPA rate	0.60±0.09	0.67±0.08
GP IIb-IIIa content, 10^3 molecules per platelet	55.8±1.6	61.6±3.2
Platelet concentration in PRP, 10^3/mm^3	219±10	224±15

Note. *p<0.05 compared to Leu33Leu33.

and the level of expression of this receptor on the platelet membrane. It can be hypothesized that SPA in these persons is caused by elevated plasma concentration of endogenous platelet agonists such as thrombin, ADP, *etc*. The agonists induce GP IIb-IIIa activation and its binding with the ligand followed by aggregation under conditions of persistent stirring in the aggregometer cell in the absence of exogenous inductors.

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