Effect of a Micellar Preparation of Polyunsaturated Phosphatidylcholine on Platelet Aggregation *In Vitro*

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Platelet aggregation was studied after incubation of cells with polyunsaturated phosphatidylcholine in platelet-rich plasma from healthy donors and coronary patients. The aggregation capacity of cells was found to be reduced after preincubation with the above drug. Statistical processing of the results using Student's and Van der Varden's tests showed more expressed effects of polyunsaturated phosphatidylcholine on cell aggregation in coronary patients than in donors.

Key Words: *platelet aggregation; platelet-rich plasma; polyunsaturated phosphatidylcholine; coronary disease*

Preparations of polyunsaturated phospholipids long have been used in medicine as agents for the treatment of atherosclerosis, as they normalize the status of blood lipoproteins and improve the clinical status of patients [6]. One of the aspects of such an effect is their influence on platelet aggregation, which promotes the formation of atheromas due to the release of inductors of vascular wall injury in the course of aggregation. Treatment of patients with these drugs, specifically, lipostabil, caused a reduction of aggregation [9]. Scientists disagree on the mechanism of the antiaggregation action of polyunsaturated phospholipids: it is either mediated by other blood components - plasma lipoproteins or prostaglandin system synthesis, or is due to alteration of the properties of the platelets proper, which are enriched with phospholipids during such treatment [9]. Previously a cholesterol-extracting and membrane-normalizing effect of a polyunsaturated phosphatidylcholine (PUPC) preparation on the red cells of atherosclerosis patients was demonstrated *in vitro,* as well as on fibroblasts in a cell culture [12]. In the search for possible methods of PUPC application, a micellar PUPC preparation has been developed, making use of a plant glycoside conducive to obtaining stable phospholipid micelles. In order to investigate the potentialities of this agent and study the mechanism of the antiaggregation effect of PUPC, we studied its effect on platelet aggregation induced by ADP or platelet activation factor (PAF) *in vitro.*

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MATERIALS AND METHODS

Blood samples from 17 healthy donors and 12 coronary patients with functional stage III and IV were used in the experiments. Donor blood was collected in anticoagulant containing citrate (130 mM) in a 1:10 ratio or heparin (10 U); patients' blood was collected in anticoagulant with heparin. Platelet-rich plasma (PRP) was prepared by 15-min centrifuging of donor blood at 150 g. PUPC was prepared as described previously [12].

The optimal conditions for incubation of PRP with the agent were selected so that the cellular activity in the control would change by no more than 50% over the course of the experiment. The final concentration of alcohol in the incubation solution should not exceed

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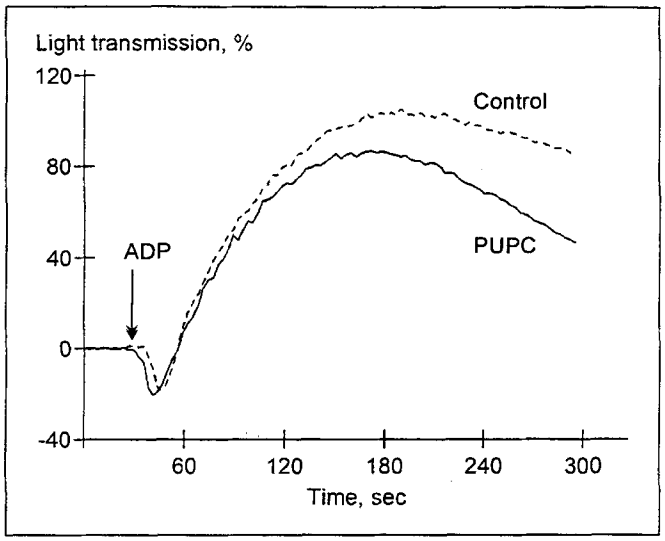

Fig. 1. Relationship between light transmission of PRP and the time elapsing after the addition of 5×10^{-6} M ADP. *Control*: 20 μl of 30% alcohol solution/ml PRP; *PUPC*: 20 μl PUPC solution/ml PRP. Incubation and measurements at 37°C.

2%. The share of PUPC was no more than 40 μl per ml of plasma. PRP was incubated with the agent for 1-2.5 h at 30 and 37°C.

For the control, plasma incubated under the same conditions but without PUPC, with an equivalent amount of alcohol, was used.

Aggregation was measured using a Biola platelet aggregation analyzer (Moscow) in 300 μl samples. The concentration of cells was $(2\text{-}3)\times10^8$ cells/ml of plasma, measured at 30 and 37°C. ADP and PAF were added as concentrated solutions in doses of 5 to 15 μl per sample, so that the final concentration of inductors in a sample were 5×10^{-6} M or 10^{-5} M for ADP and 5×10^{-7} M for PAF.

In the course of the experiment the curves reflecting changes of light transmission and the mean radius of platelet aggregates after the addition of the aggregation inductor to PRP were recorded [2,5] (Fig. 1). The aggregation parameters measured were the maximal velocities of changes in light transmission (A) and mean radius (R). The rate of alteration of the recorded value at time t was determined as the tangent of the slope to the curve at the point corresponding to this time. The curves were computer-processed. Each experimental point on the curves represents the mean value for three independent measurements. The mean square error in measurements did not exceed 10% of the obtained value.

The sensitivities of platelets of examinees (donors and coronary patients) to PUPC were compared at a drug concentration of 30 μl/ml and 2-hour incubation. For comparing the effect of PUPC on donor platelet aggregation the Δ parameter was used, representing the change of the cell aggregation parameter (A or R) in the case of their incubation with PUPC in comparison with the control sample (in percent): $\Delta=(A_C-A_{PUPC})/A_C\times100\%$, where A_C is the aggregation value in the

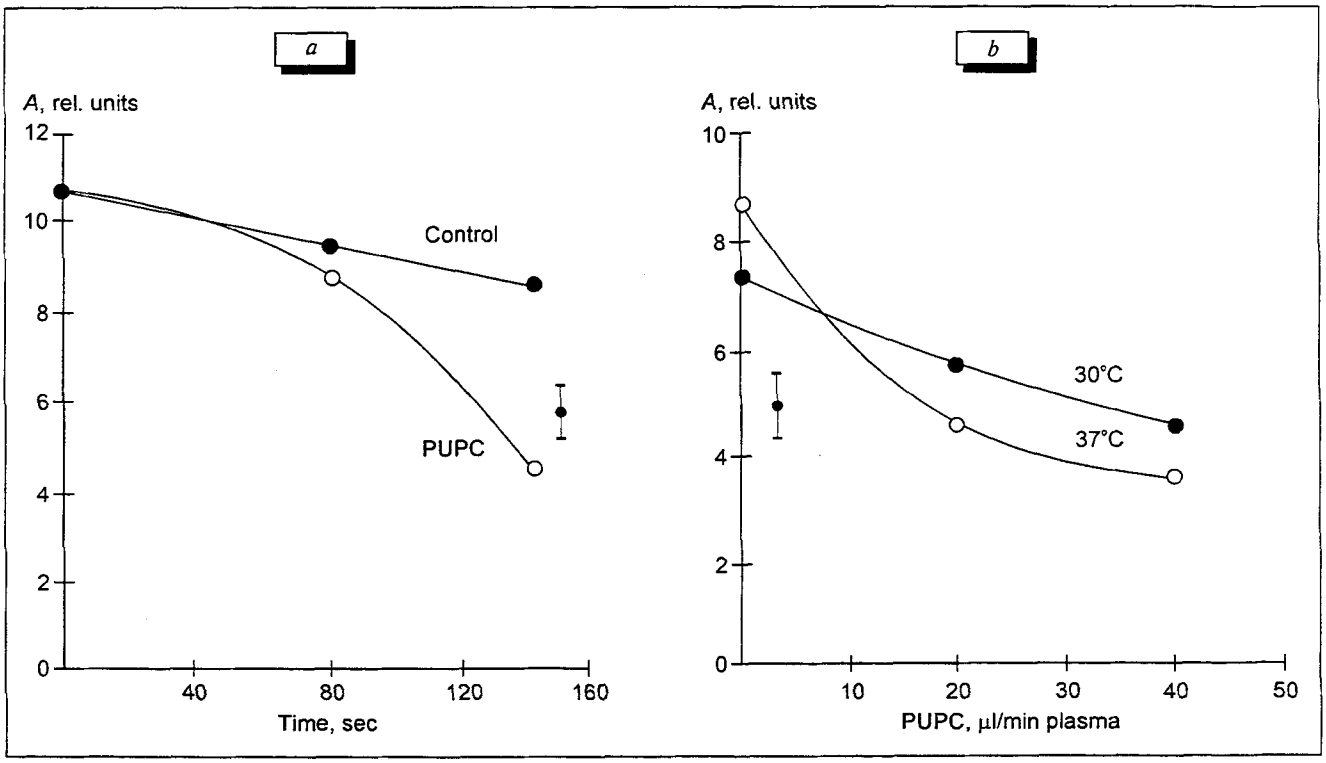

Fig. 2. Relationship between the maximal rate of light transmission change and incubation parameters (10^{-5} M ADP). *a*) relationship to incubation duration (20 μl PUPC/ml PRP, 37°C); *b*) relationship to PUPC concentration (time of incubation 2 h 20 min).

control sample after incubation and A_{PUPC} is the value in a sample incubated with PUPC.

RESULTS

The curves of light transmission of cells after the addition of aggregation inductor to PRP are presented in Fig. 1. Similar curves were obtained for changes of the mean radius of aggregates. Incubation of cells with PUPC led to alteration of all parameters of the curves: the maximal value of a recorded parameter, the time needed to attain the maximum, and hence the rate of changes, which was selected as a parameter of aggregation because of the small error in its measurements. The type of changes in the light transmission curve for PUPC-incubated plasma indicates a decrease of cell activity (the response to aggregation inductor) as a result of incubation.

An example of the relationship between the maximal rate of light transmission alteration and the time of incubation of cells with PUPC (3×10^8 cells/ml plasma) is presented in Fig. 2, *a*. Incubation of cells in a control sample without the agent led to just a negligible loss of cellular activity. Changes of parameter *A* during incubation of cells with PUPC depended on the duration of incubation: appreciable (more than 10%) changes were observed after at least a 1.5-hour incubation.

Relationships between parameter *A* and the drug concentration for one donor, 2.3×10^8 cells/ml plasma, are presented in Fig. 2, *b*. Adding just a little PUPC to PRP is enough to cause appreciable changes of cellular activity: the principal changes of the rate of light transmission occur at drug concentrations below 20 μl/ml plasma. Starting from the concentration of 30 μl PUPC/ml PRP it is possible to speak about cell saturation with the agent. A further increase of the concentration does not affect the rate of light transmission. The curves demonstrate differences in the concentration relationships for various incubation temperatures and subsequent measurements of cell activities. Similar results were obtained in experiments when PAF was used as aggregation inductor (not shown in the figure).

Hence, incubation of platelets with PUPC leads to a reduction of cellular activity. The data suggest that these changes in activity are determined by changes of membrane lipid composition. The effect is observed after a long (more than 1 h) incubation of cells with PUPC and depends on the duration of incubation; the effect of the drug on cellular activity is the most expressed if the incubation and measurements are carried out at 37°C (at this temperature lipid exchange between cells and drug is maximal, as is the cellular activity). The effect does not depend on the aggregation inductor used (although ADP and PAF bind to different membrane receptors of platelets [7]).

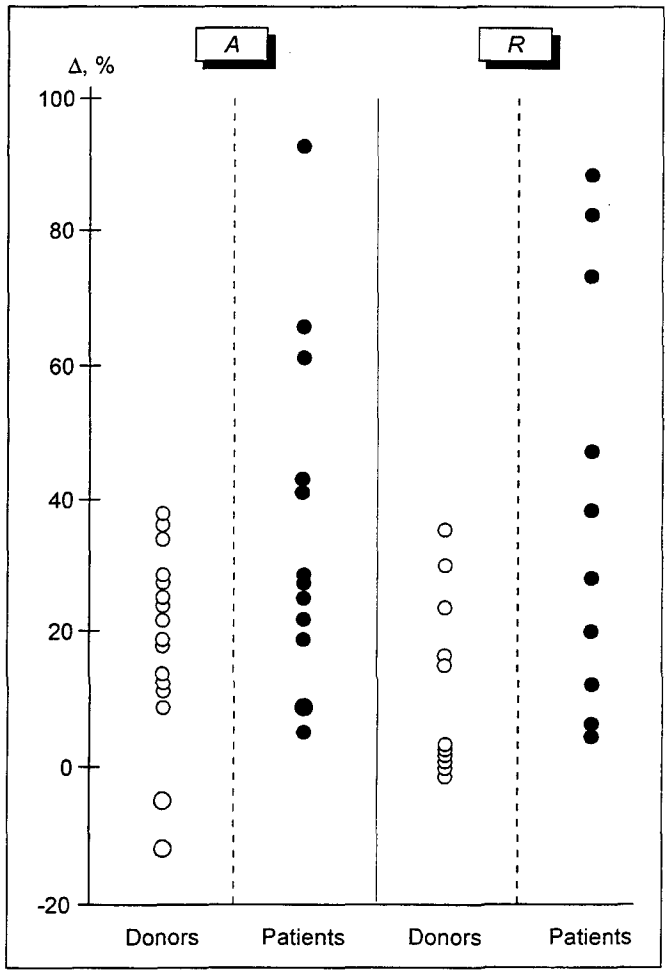

Fig. 3. Calculated parameters for healthy donors and coronary patients.

Since some scientists report alteration of the aggregation and chemical properties of platelets in atherosclerosis patients [1,3], we deemed it interesting to investigate the possible specificity of the effect of PUPC on such platelets. With this in mind, we compared the effect of PUPC on platelet aggregation in healthy donors and coronary patients. Figure 3 presents the values of parameter Δ calculated on the basis of experimental values and characterizing the changes in the rate of light transmission decrease, *A*, or aggregate radius *R* in comparison with the control. Since these values vary within a wide range in different individuals, we present the initial values (Fig. 3) in addition to those calculated on the basis of their mean values (Table 1). Table 1 presents the mean square errors in the mean values and the parameters of Student's *t* test [4]: $(\Delta_i-\Delta)^2$, and *t* calculated, and *t* tabulated for 5 and 1% levels of significance. Analyzing the data presented in the table, we can draw the following conclusions: the difference between the mean values

TABLE 1. Calculated and Theoretical Values of Student's *t* Test

Parameter	*n*	Δ	σ	$(\Delta_i-\Delta)^2$	t_{calc}	$t^{5\%}_{tab}$	$t^{1\%}_{tab}$
A							
Healthy donors	17	18.24	3.31	2975.06	2.52	2.06	2.78
Patients	12	36.75	7.42	7250.25			
R							
Healthy donors	16	9.13	2.88	2115.75	3.37	2.06	2.79
Patients	12	37	8.76	10098.0			

in the control (healthy donors) and experiment (coronary patients) is reliable for the results of measuring the changes in light transmission with the 5% level of significance and for the mean radius of aggregates with the 1% level of significance. Using the nonparametric Van der Varden's test to analyze the experimental values made us reject the initial hypothesis (about the equality of the general parameters of the groups compared) with the 1% level of significance for both aggregation parameters. Hence, we may assert with a high degree of certainty that the effect of the drug on platelet aggregation is stronger in patients than in healthy donors.

Such a difference means that the mechanism of aggregation inhibition under the effect of PUPC is due to the effect of PUPC on the microviscosity of platelet membranes from which it derives cholesterol; this process is naturally more intensive in atherosclerosis patients with excessive accumulation of cholesterol in the platelets [3]. The binding capacity of thrombin receptors is increased in such platelets with increased microviscosity of membranes, due to their possible association with the formation of oligomer groups [11]. It is possible that the liquefying action of PUPC on the membrane [12] reduces the aggregation by acting on the receptor. On the other hand, the problem of the microviscosity of platelet membranes in atherosclerosis patients and its relationship with aggregation is a dubious one because of the heterogeneity of membrane lipids with different properties of individual lipid domains: both an increase [3] and a decrease [10] of platelet membrane microviscosity in atherosclerosis, or no changes at all [8] were observed when this parameter was assessed by different methods. Therefore, our data on the more intensive effects of PUPC on platelets from atherosclerosis patients, as well as another report [3] of an inverse correlation between the time of aggregation and the orderliness of membrane lipids, may indicate that the most rigid microsites of platelet membranes rich in cholesterol because of its disproportionate distribution in the membrane are responsible for the processes of aggregation. PUPC action in such a case, similarly as during incubation with other cells, simulates the properties of high density lipoproteins, *in vitro* incubation with which also leads to a reduction of platelet aggregation. Hence, PUPC is an effective antiaggregation agent acting at the membrane level. This supplements a previously demonstrated effect on the erythrocyte membranes of atherosclerosis patients and on the aortas of experimental animals [12] as one more example of the nonspecific normalizing effect of PUPC on reverse transport of cholesterol.

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