# AGONIST-INDUCED PLATELET ADHESION. USE OF THE METHOD ON PATIENTS WITH MYOCARDIAL INFARCTION

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The formation of intravascular juxtamural thrombi is initiated by adhesion and aggregation of platelets on the surface of the damaged vascular wall [1, 4-6]. The key role of platelets in the development of thrombosis accounts for the urgency of the development of methods of evaluating platelet function [8, 9, 14]. The most widely used of these methods are Born's aggregometry, radioimmunoassay of specific proteins and thromboxane $A_2$, released from activated platelets, in the plasma, and determination of circulating platelet aggregates and the life span of platelets, etc. [7, 12, 13, 15]. Methods involving measurement of platelet adhesion are less widely used, and little has been published on their use so far. Vasil'eva et al. [2] recorded increased adhesive activity of platelets relative to siliconized glass in patients with coronary heart disease (CHD) and with acute myocardial infarction (AMI), whereas Sinitsyn et al. [3] found a small but significant increase in platelet adhesion to type IV collagen in patients with stable angina.

The writers have developed a method of measuring the adhesive activity of platelets stimulated by low doses of agonists (agonist-induced adhesion). ADP, adrenalin, and U46619, a stable analog of thromboxane $A_2$, were used as inducers. The suggested method was tested for determination of platelet activity in patients with AMI.

## EXPERIMENTAL METHOD

Blood (9 ml) from healthy male donors and men with AMI on the 1st day was collected in plastic tubes with 3.8% Na citrate in the ratio of 9:1. Myocardial infarction was recorded as ECG changes and a raised plasma creatine phosphokinase level.

Platelet-enriched plasma (PEP) was obtained by centrifugation at 150*g* (10 min), and platelet-free plasma was obtained at 1500*g* (15 min) at room temperature. The platelets were counted in a PL-100 automatic platelet counter ("TOA Medical Electronics," Japan); the PEP was adjusted to a platelet count of $2 \cdot 10^8$/ml by the addition of autologous plasma.

Platelet adhesion was measured in a Payton two-channel aggregometer (USA) at 37°C and with mixing at the rate of 900 rpm. Threshold doses of the inducers, stimulating irreversible aggregation, were determined by adding APD and adrenalin to the platelets.

To measure agonist-stimulated platelet adhesion 250 $\mu$l of PEP was introduced into 16-mm wells of 24-well culture plates (Multiwell, from "Cortar" USA, or "Falcon," USA). It was first established that the adhesiveness of the plastic produced by these firms relative to platelets does not differ significantly. Immediately after addition of PEP to the wells, ADP ("Sigma," USA), adrenalin ("Sigma"), or U46619 ("Upjohn Co.," USA) was added to the platelets. The corresponding volumes of buffer were added to the control samples. The platelets were incubated with plastic for 20 min at 37°C. Platelets unattached after incubation were washed off 3 times with phosphate-salt buffer and the adherent platelets were fixed with 2.5% glutaraldehyde ("Serva," West Germany) in 100 mM phosphate buffer, pH 7.4. Platelet adhesion was analyzed by scanning electron microscopy [3, 11]; preparations were obtained as described previously [10] and the number of adherent platelets was counted on a "Philips PSEM" ×500 microscope (The Netherlands) with magnification of 2500 and with tilting of the preparation through 45°. The total number

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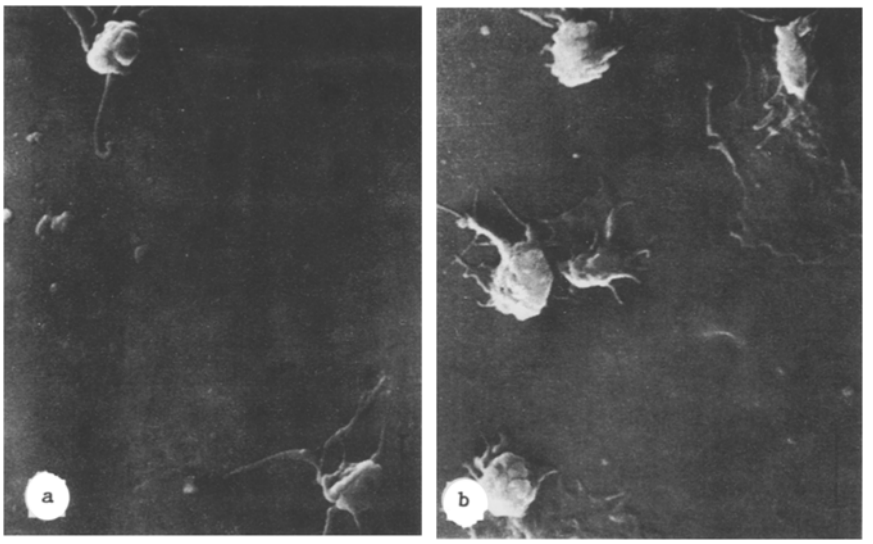


Fig. 1. Stimulation of adhesion of platelets to plastic by low doses of agonists; scanning electron microscopy, scale 2 $\mu$. a) Adhesion to plastic in absence of inducers. Solitary platelets in field of vision, not spreading; b) adhesion to plastic in the presence of 0.05 $\mu$M ADP. Number of adherent platelets is increased, some can be seen to have spread, others which have not are adherent to plastic and to surface of spreading platelets.

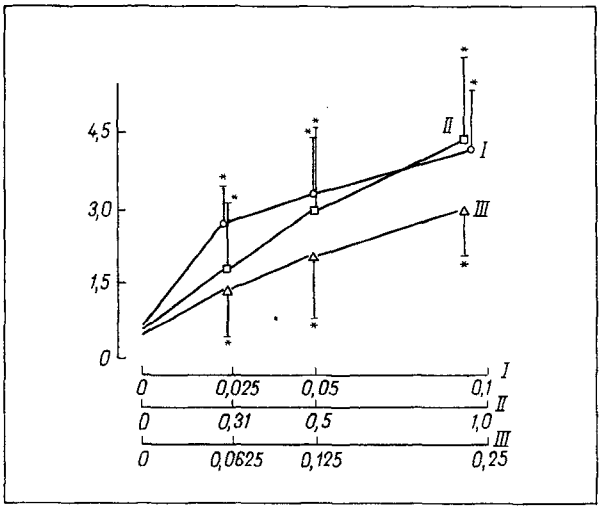


Fig. 2. Concentration curves of stimulation of platelet adhesion to plastic in the presence of ADP (I), adrenalin (II), and U46619 (III). Ordinate, number of adherent platelets per $mm^2 \times 10^{-3}$. Number of measurements at each point from 5 to 20. Asterisk indicates significant differences from control (adhesion to plastic without inducer), $p < 0.05$.

of adherent platelets was counted in 20 fields of vision, moving from the edge of the preparation toward the center with a step of 0.4 mm. The level of adhesion was calculated per $mm^2$ of substrate.

TABLE 1. Threshold Doses of Inducers Stimulating Aggregation of Platelets from Healthy Blood Donors and Patients with AMI ($M \pm m$)

| Inducer | Threshold doses for aggregation, μM | |
|---|---|---|
| | healthy blood donors | patients with AMI |
| ADP | 5,40±0,46 (21) | 4,05±0,86 (9) |
| U46619 | 0,81±0,10 (15) | 0,67±0,08 (12) |
| Adrenalin | 6,85±1,74 (13) | — |

**Legend.** Threshold doses were determined by adding ADP, U46619, and adrenalin to PEP with steps of 0.5, 0.1, and 0.5 $\mu$M respectively. Differences between healthy blood donors and patients with AMI are not significant.

## EXPERIMENTAL RESULTS

In the absence of inducers the average level of adhesion to plastic was very low in the case of healthy donors, namely 631 ± 60 platelets/mm$^2$ one or two platelets per field of vision (Fig. 1a). Activation of platelets by ADP, adrenalin, and U46619 in doses not exceeding 0.1, 1.0, and 0.25 $\mu$M respectively, stimulated platelet adhesion three-sevenfold (Figs. 1b and 2). In the presence of the agonists some of the adherent platelets spread out, whereas others that did not do so were localized both on the surface of the plastic and on the surface of the spread-out platelets (Fig. 1b).

Doses of agonists reliably stimulating platelet adhesion were significantly lower than doses stimulating irreversible platelet aggregation in a Born aggregometer. For adrenalin and U46619 concentrations stimulating a more than threefold increase of adhesion were 5-10 times lower, and for ADP 50-100 times lower, than the threshold doses stimulating irreversible platelet aggregation (Fig. 2; Table 1).

For standard measurement of agonist-stimulated adhesion we chose the following doses of inducers: ADP 0.05 $\mu$M, adrenalin 0.5 $\mu$M, and U46619 0.1 $\mu$M. These concentrations caused a more than threefold degree of stimulation of adhesion (Fig. 2) but did not stimulate aggregation of platelets from any of the healthy blood donors tested.

The method of agonist-stimulated adhesion was used to measure the adhesive activity of platelets from patients with AMI. Adhesive activity was measured during the 1st day of AMI. The patients' platelets were characterized by increased adhesive activity (Fig. 3). In some patients during this period, the platelets often formed small aggregates as a result of stimulation. In such cases it was difficult to count the exact number of adherent platelets, and these patients were combined into a group with 28,000 platelets/mm$^2$. The greatest increase in adhesion took place during activation of platelets by adrenalin (Fig. 3c), and a lesser degree in response to activation by ADP (Fig. 3b); when U46619 was used, an increase in adhesion compared with the healthy blood donors was observed only with some of the patients (Fig. 3d). Some increase in adhesion also was recorded in the absence of inducers, but the values obtained for adhesion were significantly lower than during platelet stimulation (Fig. 3a).

The threshold doses of ADP and U46619 stimulating irreversible platelet aggregation also were measured in the patients. By the use of this method, no significant differences in the sensitivity of platelets to inducers between healthy donors and patients could be found.

The writers showed previously [3] that the platelet adhesion test in the presence of agonists stimulates elevation of the general level of adhesion, and spreading out of the platelets, but in the case of mixing of the suspension, surface aggregates of the platelets are formed [4, 5, 13]. On the basis of this observation a new method was developed for measuring adhesive activity of platelets. Unlike approaches used previously, before each incubation with the substrate the platelets were activated by low doses of inducers. The physiologically most important agonists of platelets, namely ADP, adrenalin, and thromboxane $A_2$ (instead of the latter, its stable analog U46619 was used) were chosen as inducers.

Platelet activation by inducers added in doses 1 or 2 orders of magnitude below those stimulating irreversible platelet aggregation led to a more than threefold increase in the number of platelets adherent to plastic. The method thus developed was tested for measuring adhesive activity of platelets in patients with AMI. The level of agonist-stimulated platelet adhesion on the 1st day of AMI was significantly higher than the level of adhesion in healthy blood donors (by 3-5 times or more with some patients). Meanwhile, when the threshold doses of ADP and U46619 stimulating platelet aggregation were measured with the aid

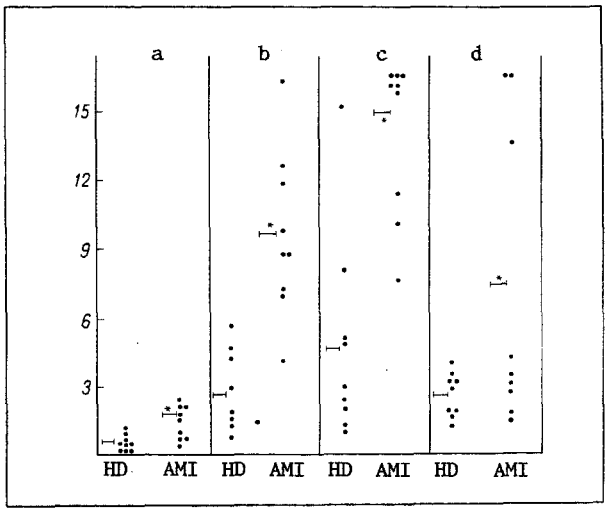


Fig. 3. Increased adhesion of platelets from patients with AMI compared with those from healthy blood donors (HD). a) Number of platelets adherent to plastic without addition of inducers, b) with addition of 0.1 $\mu$M ADP, c) 0.5 M adrenalin, and d) 0.1 $\mu$M U46619. Results of separate measurements and mean values (continuous line) are shown. Remainder of legend as to Fig. 2.

of Born's standard aggregometry method, no significant differences were found between healthy subjects and patients. These results are evidence of the greater sensitivity of the technique of agonist-induced adhesion.

The use of low, near-physiological doses of inducers to stimulate platelets, and the possibility of incubating a large number of samples simultaneously, and the very small numbers of platelets required for the measurement all suggest that this method of measuring agonist-induced adhesion can be recommended for the assessment of platelet function in patients with an increased risk of thrombus formation.

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