PLATELET AGGREGATION

TISSUE PLASMINOGEN ACTIVATOR INHIBITS THROMBIN-INDUCED AGGREGATION AND SHAPES PLATELETS, BUT FACILITATES SEQUESTRATION IN CRYSTALLOGRAPHIC INVESTIGATION OF PLASMINOGEN. M. Maman is deeply appreciated.

Folowed administration of tissue plasminogen activator (t-PA) has caused bleeding problems in some patients, that did not necessarily correlate with a significant drop of fibrinogen levels. We have therefore evaluated the effect of t-PA on platelet function in vitro.

Incubation of gel-filtered platelets for one hour at 37°C with 180 µg/ml plasminogen and increasing concentrations of t-PA (50-1600 ng/ml) significantly inhibited shape change and aggregation induced by thrombin and the thromboxane mimetic U 46619 in a dose-dependent manner. In an EDTA milieu, which abolishes aggregation, a dual effect of t-PA and plasminogen was observed in the aggregometer: the thrombin- or U 46619-elicited initial decrease in light transmission, reflecting the disc-to-sphere transformation of platelets, was almost completely inhibited from 50 ng/ml t-PA upwards; the subsequent increase in light transmission, reflecting granule secretion, was however enhanced by small amounts of t-PA (up to 200 ng/ml). The latter finding was confirmed by direct measurement of secreted ATP: t-PA at concentrations up to 200 ng/ml enhanced thrombin- or U 46619-induced secretion. The amount of plasmin generated in the gel-filtered platelet/plasminogen-t-PA mixtures was quantified.

The same amounts of plasmin, while also inhibiting the disc-to-sphere transformation of the platelets, did not enhance thrombin- or U 46619-induced ATP secretion, when whole blood or platelet-rich plasma was used instead of gel-filtered platelets. For constant thrombin concentrations, the amount of released plasmin increased with rising t-PA concentrations up to 200 ng/ml. No enhancement of secretion took place with t-PA concentrations higher than 200 ng/ml. Similarly, the diminution of the disc-to-sphere transformation of platelets by t-PA was paralleled by a decrease in light transmission, reflecting the disc-to-sphere transformation of platelets, but was not observed in an EDTA milieu.

We have purified from human placenta an anticoagulatory protein (VAG, Mr=32,000), which inhibits phospholipid dependent procoagulant reactions through a calcium dependent high affinity binding to phospholipid membranes. Antagonistic action of VAG on platelet rich plasma (cPRP). VAG does not affect platelet aggregation and secretion in response to ADP, collagen and thrombin.

For VAG (0.5-5 µg/ml) the concentration of cPRP required to produce 50% aggregation was increased by 10- to 20-fold in an antigenic preparation of VAG to produce 50% aggregation. The aggregation obtained in the presence of VAG did not enhance thrombin- or cPRP-induced secretion. The amount of secreted ATP was the same in platelets in the presence of VAG or in control platelets.

We have demonstrated that VAG is a heparin antagonist. Since VAG is unable to inhibit platelet aggregation, it has no effect on platelet aggregation and secretion in response to ADP, collagen and thrombin.

We have therefore evaluated the effect of VAG on platelet aggregation and secretion in response to ADP, collagen and thrombin. We have also measured the amount of plasmin generated in the platelet rich plasma in the presence of VAG.

The response of ADP-stimulated platelets in cPRP differs strikingly from that in cPRP. In the latter, platelets react with a dose-dependent primary aggregation, followed by a thrombin (IIa) independent second wave of aggregation associated with SHT-secretion. Platelets in cPRP, however, demonstrate an increased primary aggregation in response to ADP, which is followed by a IIa-mediated second wave of aggregation and SHT-secretion. Increasing the VAG concentration does not affect the primary aggregation response, but delayed the IIa-dependent secondary events in a dose-dependent way. At 0.5 µg/ml VAG, platelets react to ADP (10 µM f.c.) with reversible aggregation only. No matter how high ADP-dose, secretion reaction does not occur. At this VAG concentration, ephrinphrin (50 µM f.c.) does not cause aggregation and SHT-release at all, indicating that the secretion reaction is completely quenched. Although in cPRP, ephrinphrin retains its synergistic effect on ADP to aggregate platelets, no SHT release was ever observed and the resulting aggregation was always reversible.

It is concluded that VAG is a suitable antagonist to investigate platelet function in the presence of physiological calcium concentration. Since platelet aggregation and release appear very different from results obtained in the usual way (cPRP, low calcium concentration) the physiological meaning of this latter work and needs re-evaluation. Finally, our results cast severe doubt on epinephrin as an important platelet stimulant under physiological conditions.

Inhibition of Factor Xa and thrombin by antithrombin III and heparin during human prothrombin activation. S. Schoen, R. van Gool, I.B.M.C., University of Leuven, Belgium.

Prothrombin-catalyzed human prothrombin activation results in the generation of thrombin and meizothrombin-des F1 (MDFl) as well as factor Xa (Xa) when unfractionated heparin (UFH) or sodium heparin is added. The occurrence of MDFl and Xa, which may be divided into two distinct populations, is enhanced by the presence of heparin. Heparin also inactivates thrombin and MDFl, and enhances thrombin dependent second order rate constants of inhibition of both thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl. The presence of heparin decreases the rate of thrombin and MDFl formation, and enhances the inactivation of thrombin and MDFl.