
In view of the therapeutic applications of rt-PA it is of interest to investigate whether there is any difference in the lysisability between fresh and aged thrombi. The efficiency of fibrinolysis by rt-PA was studied in 3 different ways: in vivo, by measuring the thrombus weight of fresh (1 h) or aged (24 h) thrombi in the carotid artery of rabbits which had been treated with rt-PA (0.4 mg/kg) or saline for 1 h. In vivo, by measuring 14C-release of in vivo fresh (1 h) and aged (24 h) thrombi (labelled with 125I-fibrinogen) suspended in vitro in plasma containing albumin as a space marker. The membrane GPIIb-IIIa complex was formed and lysed in vitro with rt-PA (0.4 U/ml). In vivo, by measuring 125I-release of fresh (1 h) and aged (6 or 24 h) human native whole blood clots, PPP-clots, PRP-clots and squeezed PPP-clots which were formed and lysed in vitro with rt-PA (1 μg/ml). In vivo, as ex vivo rt-PA lysed fresh (1 h) thrombi much better than aged (24 h) thrombi. This difference was more pronounced immediately after the onset of fibrinolysis, but decreased with time. However, in vitro relatively little difference was observed in fibrinolysis efficiency between fresh (1 h) and aged (6 or 24 h) clots; fibrinolysis of these clots was decreased (PPP > whole blood > PRP) with increasing clot retraction, which was almost complete within 1 h. This result was in line with another confirmed that rt-PA-clots were "extracted" by squeezing them just before lysis. Therefore we conclude that a considerable difference in lysis efficiency between fresh and aged thrombi in vivo was obtained only when thrombi were examined both in vivo and in vitro. This difference was less pronounced with increasing fibrinolysis time.

PLATELET INHIBITION (1)

THE THIENOPYRIDINE PCR 4099 INHIBITS THE ADP AGGREGATION PATHWAY OF HUMAN PLATELETS BY INTERFERING WITH THE BINDING OF FIBRINOGEN TO THE GLYCOPROTEIN IIb-IIIa COMPLEX. C. Gachet (1), A. Sitarl (1), G. Bouloux (2), L.-A. Maffrand (3) and J.-P. Hummel (3). INSERM U.311, Centre Régional de Transfusion Sanguine, Strasbourg, France (1) and Sanofi Recherche, Toulouse, France (2).

The thienopyridine, PCR 4099, is a synthetic structural analog of ticlopidine. After oral administration in man, it prolongs the bleeding time (BT) and inhibits aggregation on stimulation with ADP, collagen, thrombin or 5 μM PAR. It inhibits the effect of low concentrations of thrombin (< 0.05 U/ml) on platelets, measured after centrifugation at 17,000 g for 1 min in the presence of 5 μM collagen as a stable platelet aggregating agent. The prolonged administration of PCR 4099 inhibited almost completely platelet aggregation induced by 0.5 to 10 μM ADP. Although the effect of ADP on aggregation was blocked at high concentrations, PCR 4099 did not modify ADP-induced aggregation. It also antagonized the effects of high concentrations of thrombin (< 0.05 U/ml) were inhibited by PCR 4099 administration. The binding of 125I-fibrinogen to human platelets was measured by crossed immuno-electrophoresis (IE) in the presence of rabbit anti-human platelet antiserum. The prolonged administration of PCR 4099 inhibited almost completely platelet aggregation induced by 0.5 to 10 μM ADP. Although the effect of ADP on aggregation was blocked at high concentrations, PCR 4099 did not modify ADP-induced aggregation. It also antagonized the effects of high concentrations of thrombin (< 0.05 U/ml). The effect of PCR 4099 did not modify the pattern of immunoprecipitates as revealed by IE. In particular, the GPIIb-

CD63 antigen complex was identified as the site of interaction of PCR 4099, whereas the GPIb-IIIa complex was not changed. In conclusion, PCR 4099, which is more potent than ticlopidine in man, inhibits specifically the ADP aggregation pathway by interfering with the binding of fibrinogen to the GPIb-IIIa complex in platelets having undergone shape change.

INTERACTIONS BETWEEN PGEl AND INHIBITORS OF PLATELET AGGREGATION THAT ACT THROUGH CAMP. S.J. Gray and S. Hepinstall. Department of Medicine, University Hospital, Nottingham, NG7 2UH, U.K.

PGEl has a biphasic effect on platelet aggregation with low concentrations of the prostaglandin potentiating aggregation and high concentrations inhibiting it. In this investigation we have studied the interaction of PGEl with agents that inhibit platelet aggregation through an effect on CAMP. The agents considered raise the levels of CAMP in platelets by different mechanisms: PGI2, PGF2α, and adenosine combine with specific surface-located receptors and stimulate adenylate cyclase (AC) via the guanine nucleotide binding protein (GNBP). Forskolin stimulates AC directly, and AH-P 719 and DN 6963 inhibit CAMP phosphodiesterase (PDE). ADP-induced platelet aggregation was measured in platelet-rich plasma and CAMP was measured in platelets labelled with 3H-adenine.

PGEl, alone potentiated platelet aggregation at concentrations from 10-8 to 10-10 M and inhibited aggregation at 10-6 M. PGEl did not reduce CAMP levels at any concentration and increased CAMP levels at concentrations > 10-6 M, probably by stimulating AC. PGEl (10-8 to 10-6 M), PGF2α (10-6 to 10-8 M) and adenosine (8x10-6 to 2x10-5 M) increased the levels of CAMP in platelets and inhibited aggregation. These changes were reversed by low concentrations of PGEl (10-9 to 10-8 M) and AH-P 719 (10-7 to 10-5 M) and DN 6963 (5x10-6 to 10-5 M) increased the level of CAMP in platelets and inhibited aggregation. However, PGEl did not reverse the inhibitory effects of these particular agents. In contrast, PGEl potentiated the effects of the agents at all the concentrations of PGEl that were tested (10-8 to 10-6 M). These different results obtained with PGEl, in combination with agents that act via surface-located receptors compared with agents that stimulate AC directly or act through CAMP, suggest that PGEl may potentiate platelet aggregation by acting at a point between the platelet receptor and AC i.e. GNBP.

PGEl is one of the major prostaglandins synthesized by human mononuclear cells and is the most important of the prostaglandins in the micro-circulation. It increases cAMP in a variety of cells. However, it is not known whether PGEl also has a role in the micro-circulation. It is possible that PGEl is involved in the pathogenicity of thrombosis by interfering with the binding of fibrinogen to the surface-located receptors and stimulating adenylate cyclase (AC).

THE INHIBITION OF PLATELET AGGREGATION OF FRESH AND AGED HUMAN PLATELETS BY THROMBIN IN THE PRESENCE OF ADP. C. Bouloux (2) and J.-P. Hummel (3). INSERM U.311, Centre Régional de Transfusion Sanguine, Strasbourg, France (1) and Sanofi Recherche, Toulouse, France (2).

Platelet aggregation was measured in PRP with increasing clot retraction, which was almost complete within 1 h. This result was in line with another confirmed that rt-PA-clots were "extracted" by squeezing them just before lysis. Therefore we conclude that a considerable difference in lysis efficiency between fresh and aged thrombi in vivo was obtained only when thrombi were examined both in vivo and in vitro. This difference was less pronounced with increasing fibrinolysis time.

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