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ANTITHROMBOTIC EFFECTS OF TISSUE-TYPE PLASMINOGEN ACTIVATOR AT PHYSIOLOGICAL BLOOD LEVELS. W. Witt, B. Baldus, and P. Donner. Research Laboratories of Schering AG Berlin (West) and Bergkamen, D-1000 Berlin 65, FRG

Effective thrombolysis in human patients and experimental animals by tissue-type plasminogen activator (t-PA) usually requires t-PA plasma levels in the microgram range. Compared to that physiological plasma levels of t-PA are about 100 - 1000 times lower. To investigate the effects of t-PA at physiological blood levels rat studies were performed in vitro and in vivo employing highly purified recombinant single-chain t-PA (sct-PA; 500,000 IU/mg).

t-PA activity in rat whole blood as assessed by dilute blood clot-lysis time (DBC-LT) was increased by addition of sct-PA as low as 3 ng/ml (20 % decrease in DBC-LT). Injection of bradykinin 10, 100 and 1000 µg/kg i.v. shortened DBC-LT to 54, 23, and 10 % of controls corresponding to the effect of about 10, 30, and 100 ng/ml sct-PA added in vitro. Infusion of sct-PA 15 - 450 µg/kg/h i.v. shortened DBC-LT ex vivo dose-dependently by 20 - 90 % at steady state levels (n = 5). In the same dose range sct-PA inhibited thrombus formation along a silk thread introduced into an arteriovenous shunt in anaesthetized rats. The reduction in thrombus dry weight was dose-dependent amounting to 33 - 67 % of preapplication values (n = 5 - 8) at 15 - 450 µg/kg/h i.v. sct-PA. Already 50 µg/kg/h sct-PA corresponding to a sct-PA activity of about 15 ng/ml displayed a significant (α = 0.05) effect in this model.

The results of this study suggest that t-PA present at physiological resting or activation (bradykinin) levels during acute clot formation may have potent antithrombotic efficacy. This study provides further evidence for the importance of a balance coagulation-fibrinolysis which can be influenced on both sides towards thrombophilia as well as to achieve antithrombotic therapy, e.g. by elevating plasma fibrinolytic activity with low-dose t-PA treatment or with drugs which stimulate the endogenous fibrinolytic potential.

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SECONDARY FIBRINOLYSIS, PROTEIN C ACTIVATION, PLATELET DECREASE, BUT NOT CONTACT ACTIVATION CAN BE CORRELATED TO FREE THROMBIN ACTION IN EXPERIMENTAL DIC.
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We have studied the influence of activated coagulation on antithrombotic defence mechanisms in vivo. Conventional coagulation variables, platelets (Tcy), plasminogen (Plg), α_2-antiplasmin (AP), protein C (PC), factor VIII C, factor XII and C1-inhibitor have been measured before and during reversible tissue thromboplastin-induced DIC in the dog. Free thrombin action as derived from fibrinogen (Fbg) decrease has been expressed as integral of active thrombin concentration over time (Ψ). Protection by heparin H, pentosan polysulfate PPS or dermatan sulfate DS was studied. DIC had no consistent effect on the behaviour of factor XII and C1-inhibitor, but led to the consumption (Δ) of the following variables:

	pure DIC (10)	PPS-DS-DIC (6)	H-DIC (7)
ΨnMmin	3.87±2.46	2.67±1.10	1.31±1.44
ΔFbg g/l	1.04±0.49	0.80±0.29	0.39±0.32
ΔTcy $10^{-3}/\mu l$	178±95	180±76	159±118
ΔPlg %	7.7±10.4	10.2±4.5	4.6±4.3
ΔAP %	26.8±16.8	20.3±11.3	8.6±8.9
ΔPC %	33.8±13.9	31.2±11.0	18.6±5.2
ΔVIIIC %	58.5±27.3	40.8±29.4	39.0±17.2

The Spearman correlation coefficients between Ψ and Δ in the whole group were all statistically significant and ranged from r_s=0.51 (ΔPlg) to r_s=0.94 (ΔFbg). The response of major defense mechanisms in vivo quantitatively depends on active thrombin.

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DIPYRIDAMOLE ALONE OR WITH LOW DOSE ASPIRIN DOES NOT PREVENT ACUTE PLATELET THROMBUS FORMATION IN STENOSED DOG CORONARY ARTERIES. J. D. Folts, S. R. Smith. Cardiology Section, University of Wisconsin, Madison, WI, U.S.A.

Dipyridamole (Dip) is reputed to inhibit (I) platelet aggregation (PA) and acute thrombus formation (ATF) by two mechanisms including inhibiting 1.) platelet (Pt) phosphodiesterase, 2.) adenosine (A) reuptake by red cells, which should raise plasma A. Both effects should raise Pt cyclic AMP and thus be a potent platelet inhibitor (PI). Because aspirin (AS) inhibits Pt thromboxane A_2 production, a synergistic (S) PI effect for ASA and Dip given together has been postulated and used in clinical trials but this S has never been shown to I ATF in any in vivo model, which reasonably mimics human arterial stenosis. We have shown that ATF followed by embolization, occurs periodically in mechanically stenosed (MS) monkey and rabbit carotid arteries, and dog (D) and pig coronary arteries (CA), causing cyclical reductions in coronary blood flow (CRF) (measured with EMF probes) and periodic acute ischemia, and that these CRF can be abolished with a variety of PI including 3.0 mg/kg of ASA. To determine if there is a S effect between ASA and Dip, in open chest D, Dip was given, 2.0 mg/kg IV to D with a MS circumflex CA and having 14±5 CRF's per hour, due to periodic ATF; and simultaneously flow measured in an unstenosed normal LAD CA. The frequency and size of CRF's were not changed by Dip, although ABP decreased 21±9 mm Hg and blood flow in the unstenosed LAD increased 259±47%. A low dose of ASA, 1.0 mg/kg, which by itself diminishes but does not abolish CRF's in this model was given IV 10 min. after Dip and CRF's continued unchanged. When a second dose of ASA 1.0 mg/kg was given IV to reach the minimum effective dose of ASA in this model, CRF were abolished in all D. Thus Dip was not effective alone or in combination with low dose ASA to I CRF in this model which simulates the patient with stenosed CA. The majority of clinical trials that show inhibition of ATF, used ASA and Dip together without 3 separate patient groups on Dip alone, ASA alone and ASA plus Dip. The widespread use of Dip with ASA to prevent ATF in man needs to be reevaluated.

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THE BEHAVIOUR OF RABBIT PLASMINOGEN AT THE LUMINAL SURFACE OF RABBIT AORTA IN VIVO BEFORE AND AFTER BALLOON-CATHETER INJURY. **Mark W.C. Hatton, Susan Moar and Mary Richardson. Department of Pathology, (4N67), McMaster University Health Sciences Centre, Hamilton, Ontario, Canada L8N 3Z5**

A previous study from this laboratory has identified the susceptibility of the de-endothelialised aorta, particularly the proteoglycan (PG) components of the extracellular matrix (ECM), to proteolytic damage if exposed to plasmin in vitro. To explore the possiblity that this occurs in vivo, a possible association between ^{125}I-plasminogen (PLG) binding to the arterial wall, its activation to plasmin (PLN) and, subsequently, proteolytic damage to the intimal ECM has been studied. Intravenous injection of ^{125}I-PLG in healthy N.Z. white male rabbits showed that PLG associated minimally (<0.01% of circulating PLG/cm^2/ml blood at 1 h) with the thoracic aorta endothelium, measured after Hautchen preparation from 1-cm vessel segments. Transendothelial passage, measured as ^{125}I-PLG associated with the subendothelium (intima-media), progressed to 0.015%/cm^2/ml blood at 1 h. In contrast, the process of de-endothelialisation by balloon catheter led to a rapid uptake of ^{125}I-PLG by the denuded vessel surface. At saturation (approx. 10 min after injury), 0.7 - 0.8% of circulating PLG/cm^2/ml blood was adsorbed by the entire de-endothelialised intima-media: Of the adsorbed PLG, 2-3% was associated with the platelet layer. Uptake was not inhibited by eACA (dose: 200 mg/kg) given i.v. before ^{125}I-PLG. Adsorbed PLG was not released significantly from segments incubated in MEM containing 4% (w/v) RSA in vitro. PLN activity was not detected. Furthermore, assessment of the ECM by transmission electron microscopy, after ruthenium red staining, showed that uptake of PLG by the de-endothelialised vessel in vivo was not associated with obvious damage to the PG components.
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