

CORRELATION BETWEEN THE ANTICOAGULANT AND ANTIPLATELET EFFECT OF D-PHE-PRO-ARG-H (RG-2958). D. Bagdy, É. Barabás, L. Sebestyén, M. Diószegi, Zs. Fittler, S. Bajusz, E. Széll. Institute for Drug Research, H-1325 Budapest P.O.Box 82. Hungary

Anticoagulants usually have no antiplatelet effect and platelet function inhibitors do not interact with the coagulation factors. Since thrombin has a decisive role in thrombus formation (growth and stabilization), inhibitors of the effect of thrombin on platelets may be of special importance in developing a novel type of anticoagulant with antiplatelet properties.

D-Phe-Pro-Arg-H /I/ designed and synthesized in our Institute was found to be a highly specific, reversible non-competitive inhibitor of thrombin, a specific platelet agonist. ($K_i = 1 \times 10^{-6}$ M). /I/ was administered parenterally and orally to white New Zealand rabbits and to beagle dogs. The kinetics of action was recorded by measuring the WBCT, APTT, PT, TT, platelet count (PC) and platelet aggregation (PA). Optimum degree of anticoagulation was considered by the values proposed by Verstraete and Verwilghen. /I/ was shown to be a highly specific inhibitor of PA induced by thrombin. No direct interaction between the inhibitor and the platelet membrane could be detected. Aggregability of human platelets in citrated PRP and that of the gel-filtrated platelets induced by ADP or collagen did not change after incubation with /I/. The antiplatelet effect of /I/ was studied by *ex vivo* experiments where the inhibitor was the anticoagulant (30 ug/ml whole blood) instead of citrate. Comparing the aggregability caused by several inducers in citrated human PRP with that of in /I/-PRP a significant difference was observed when epinephrine was the PA-inducer. /I/ acts via formation of an enzyme-inhibitor complex that inhibits the binding of thrombin on their receptor-sites at the platelet membrane. *In vivo* experiments showed a close correlation between TT and PA induced by thrombin. /I/ proved completely harmless to platelets and red blood cells. No significant change in PC could be detected.

MICROEMBOLISATION DURING SURGICAL SHOCK: EFFECT OF PROSTAGLANDIN E1. KR Poskitt, JTC Irwin, CM Backhouse, CN McCollum. Department of Surgery, Charing Cross & Westminster Medical School, London W6 8RF.

Embolisation of microaggregates following major surgery may be a cause of pulmonary arterio-venous shunt and postoperative respiratory failure (1). Prostaglandin E1 may prevent intravascular aggregation and we studied this possibility in a pig model of surgical shock.

Following autologous platelet labelling with ¹¹¹Indium, 16 pigs (20-30kg) were randomised to receive a perioperative infusion of PGE1 (100ng/kg/min) or placebo. Arterial and Swann Ganz catheters were inserted under anaesthesia prior to surgery consisting of midline laparotomy, exteriorisation of small bowel 1.5 hours of aortic clamping and 1 hour of hypotension. On induction, during shock and at 3 days in survivors platelet and leucocyte count, blood radioactivity, venous aggregates (SFP), lung platelet uptake (LPU), pulmonary vascular resistance (PVR) and alveolar-arterial pO₂ difference (A-adO₂) were measured.

| BP mmHg | During Surgical Shock | | | At 3 days |
|--------------|-----------------------|----------|------------------|--------------------|
| | SFP | LPU | PVR dscm-5m-2 | A-adO ₂ |
| Placebo 76±7 | 2.5±0.3 | 11.7±1.8 | 1191±78 | 34.6±4.5 |
| PGE1 63±27 | 1.1±0.5* | 8.3±1.0* | 754±137* | 29.2±4.4 |

All results mean ± sem *p<0.05 Mann Whitney U-test

During surgical shock, the formation of venous aggregates, the fall in circulating radiolabelled platelets and their accumulation in lungs were reduced by PGE1 (p<0.05). BP, CVP and PWP were all lower on PGE1 and at 3 days the improvement in A-adO₂ in PGE1 pigs failed to reach significance.

PGE1 reduced platelet aggregate formation and their subsequent pulmonary microembolisation despite worsening shock due to vasodilation.

1. McCollum CN, Campbell IT. The value of measuring intravascular platelet aggregation in the prediction of postoperative pulmonary dysfunction. *Br J Surg* 1979; 66; 703-707.

INHIBITION OF THE THROMBOPENIC EFFECT OF ELLAGIC ACID BY PCR 4099, AN ANTIAGGREGATING AGENT, IN RATS. J. DAMAS, V. GREGG and G. REMACLE-VOLON. Physiologie humaine, Université de Liège, Belgium.

In the rat, intravenous injection of large doses (30 mg/Kg) of ellagic acid (EA) induced a decrease in the plasma level of fibrinogen and in the blood platelet content and an increase of the activated partial thromboplastin time. EA induced the accumulation of Cr⁵¹-labelled platelets into the lung and the liver accompanied by a 64% fall in Cr⁵¹ blood radioactivity.

The long-lasting thrombocytopenia induced by EA was inhibited by heparin (4 mg/Kg), defibrase (20 U/Kg), and cloccoumarol (4 mg/Kg). It was not inhibited by aspirin (90 mg/Kg), indomethacin (8 mg/Kg) and ketoprofen (4-10 mg/Kg). The thrombocytopenia would depend on an intravascular coagulation.

Using this model of platelet stimulation, we studied the protective effect of PCR 4099. This drug was first given as a single dose, orally four hours before the injection of EA. It reduced the thrombocytopenia for 10 mg/Kg and suppressed it for 40 mg/Kg. The thrombocytopenia was also inhibited by 3 day oral administration of PCR 4099 4 mg/Kg. PCR 4099 is thus a potent inhibitor of this kind of platelet stimulation.

| Treatment | Platelet levels x 10 ³ /ul | |
|-----------------------|---------------------------------------|-----------------|
| None | 828 ± 27 | (n = 8) |
| EA | 442 ± 35 | (n = 13) |
| EA + PCR 4099 4 mg/Kg | 475 ± 55 | (n = 4) |
| 10 mg/Kg | 579 ± 46 | p<0.05 (n = 10) |
| 40 mg/Kg | 823 ± 37 | p<0.001 (n = 8) |
| 2 x 4 mg/Kg | 558 ± 56 | (n = 6) |
| 3 x 4 mg/Kg | 695 ± 54 | p<0.005 (n = 6) |

DIFFERENTIAL INHIBITION OF THE PLATELET ACTIVATION SEQUENCE: SHAPE CHANGE, MICRO- AND MACRO- AGGREGATION, BY A STABLE PROSTACYCLIN ANALOGUE (ILOPROST). L.G. Pedvis, T. Wong, J. Wylie, and M.M. Frojmovic. Dept. of Physiology, McGill University, Montreal, Quebec, Canada H3G 1Y6.

The relative sensitivities of ADP-induced activation, and prostaglandin-mediated inhibition, were determined for rates of platelet shape change (SC), early platelet recruitment measured by electronic particle counting (PA), and turbidometrically-measured aggregation (TA). Studies were performed in stirred citrated platelet-rich plasma from 7 healthy human donors. The [ADP]_{1/2} ([ADP] giving half maximal rate) was determined for the sequence of activation steps expanding on Holmsen's classical scheme: unactivated platelets → SC → PA → TA. Distinct ADP sensitivities were obtained from log dose-response studies, with a relative dose dependency in the order of [ADP]_{1/2} TA > [ADP]_{1/2} PA > [ADP]_{1/2} SC of ~4:3:1. Sex differences in ADP sensitivities ([ADP]_{1/2}), for rates of early platelet recruitment measured at 3 seconds were studied from a pool of 20 females and 19 males. Values obtained between the two sexes were comparable (p > 0.05) and independent of hematocrit. Differential inhibition of the above activation scheme was evaluated with Iloprost (ZK 36 374), a stable carbacyclin analogue of prostacyclin (PGI₂), with similar potency as PGI₂ for the same platelet receptors. Log dose-response curves for inhibition were measured at one high [ADP] (> 1.5 μM) for all 3 parameters, or at respective [ADP]_{1/2} values for each parameter. IC₅₀ values ([ZK] causing 50% of inhibition) for inhibition of TA:PA:SC were found in the relative ratios of ~ 1:3:5, when normalized and expressed as nM ZK per μM ADP used as activator. Thus, ~3x and ~5x more ZK, and likely PGI₂, is required to respectively inhibit PA and SC, than that needed to inhibit TA. As observed above for activation, no sex differences in ZK sensitivities were observed (p > 0.1) for 6 males and 6 females. The range of ZK used in this study was below the threshold (~ 3 nM) generally reported for measurable increases in total basal cyclic 3',5'-adenosine monophosphate (cAMP). This suggests that for each parameter, any increase in cAMP may be associated with selective intracellular pools. The relationship between ZK or PGI₂ and intracellular signals remains to be determined.