

EFFECTS OF THE THROMBOXANE SYNTHETASE INHIBITOR UK37248 ON MONOLAYER ADHESION OF RABBIT PLATELETS. J.A. Davies and V.C. Menys, University Department of Medicine, General Infirmary, Leeds LS1 3EX, U.K.

Vasoconstriction and platelet aggregation in response to thromboxane A₂ are thought to be important in thrombogenesis. We assessed the effects of inhibition of thromboxane synthesis using UK37248 on platelet monolayer adhesion. This was quantitated as uptake of washed, Na ⁵¹Cr-labelled platelets onto collagen-coated glass or damaged rabbit aorta, using a perfusion device. UK37248 did not inhibit adhesion to collagen-coated glass. At 1 μM final concentration and in the presence of 40% washed rabbit red cells, UK37248 caused a significant ($p < 0.001$) reduction in platelet adhesion to damaged rabbit aorta from $122,000 \pm 17,000$ to $65,600 \pm 4,500$ platelets per mm² (mean \pm SEM). In the absence of red cells platelet adhesion was much less but addition of 1 μM UK37248 similarly reduced adhesion to about 50% of control value ($p < 0.05$). Dose-ranging experiments at concentrations from 0.3 to 10 μM showed maximal inhibition of adhesion (52%) to damaged aorta at 1.0 μM concentration. Failure to inhibit adhesion to a non-vascular surface suggested that PGI₂ might be involved in the reaction. We are investigating the possibility that UK37248 inhibits platelet adhesion to damaged aorta due to synthesis of PGI₂ by vascular cells from endoperoxides accumulating in platelets.

1364

PLATELET AGGREGATION INDUCED BY FORMALDEHYDE. J.A. Zeller, K. Eurenus, R.E. Dayhoff, R.S. Ledley, L.S. Rotolo. Platelet Research Laboratory, Veterans Administration Medical Center and Georgetown University Medical Center, Washington, D.C. 20422 U.S.A.

Formaldehyde causes platelet aggregation (or agglutination) which varies with dosage. Aggregation was studied in ten blood samples drawn from normal volunteers. A small volume of formaldehyde was added to platelet rich plasma in a light transmission aggregometer to produce final formaldehyde concentrations between 0.1% and 8%. Measurements were made by three methods: (1) light transmission aggregometry, (2) visual semi-quantitative microscopic analysis, and (3) quantitative image analysis (Computerized Platelet Aggregation Analysis). Using the visual semi-quantitative microscopic method, the dose response curve (expressed as the percentage of platelets involved in aggregates) increased from 11% at a formaldehyde concentration of 0.1% to 41% at a 0.5% formaldehyde concentration; it then decreased to 11% at a formaldehyde concentration of 8%. This curve may reflect the influence of two different formaldehyde effects: an aggregating effect which increases until about 0.5% concentration, whereafter a fixative effect may predominate. Light transmission aggregometry recordings did not provide a reliable indicator of the presence and degree of aggregation. A comparison of the visual semi-quantitative method and CPAA show similar detection of aggregating effects. In summary, formaldehyde has an aggregating effect on normal platelets. The physiologic significance of this effect is unknown; however, because formaldehyde is used to process platelets in studies of platelet aggregation, this effect may be of importance.

1363

INHIBITION OF PLATELET ADHESION TO GLASS BY HUMAN β -LIPO-PROTEIN. N.C. Sharma, S.F. Mohammad, H.Y.K. Chuang and R.G. Mason. Department of Pathology, College of Medicine, University of Utah, Salt Lake City, Utah 84132. U.S.A.

Platelets, by virtue of their seemingly innate adhesiveness to many nonbiologic surfaces, appear to play an important role in blood-material interactions that may lead to the dysfunction of extracorporeal or implanted devices that contact blood. A number of attempts have been made in the past to minimize the adhesion of platelets to artificial surfaces in order to improve their compatibility with blood by use either of pharmacologic agents or plasma protein preparations. We describe here inhibition of platelet adhesion to glass by β -lipoprotein. β -lipoprotein was purified from human serum preincubated at 37°C for 16 hr, by normal and sodium bromide density ultracentrifugations at 320,000 \times g for 24 hr at 4°C. The β -lipoprotein preparations were homogeneous when tested with specific rabbit antihuman β -lipoprotein antiserum in immunoelectrophoresis and antigen-antibody crossed immunoelectrophoresis. In electrophoresis in 1% agarose gels β -lipoprotein migrated as a single band. The band reacted with both the protein revealing stain Coomassie brilliant Blue R-250 and the Oil Red O stain for lipids. The effects of purified β -lipoprotein on the adhesiveness of gel filtered human platelets to glass were studied by the centrifugation method. β -lipoprotein at a concentration of 23 mg/ml inhibited platelet adhesion by about 61% when compared with values obtained when platelets were suspended in phosphate buffered saline alone. Delipidation of β -lipoprotein resulted in complete loss of its platelet adhesion inhibitory activity mainly due to denaturation and insolubilization of the protein moiety. These observations suggest the possibility of improving the compatibility of artificial surfaces that contact blood either by coating the foreign surfaces with β -lipoprotein or by adding concentrated preparations of the protein to blood.

1365

THE EFFECTS OF PROSTACYCLIN (PGI₂) ON INTRAVASCULAR PLATELET AGGREGATION. G.G. Duncan, G. Mallarkey and G.M. Smith. School of Pharmacy, Robert Gordon's Institute of Technology, Aberdeen, Scotland.

Intravascular aggregation can be measured by counting the number of circulating platelets before and after the injection of aggregation agents. The Technicon Auto-counter was modified to count platelets continuously and connected via a double cannula in a carotid artery to an anaesthetised animal.

Adenosine diphosphate (ADP) and collagen gave dose-dependent falls in the circulating platelet count when injected into rats, guinea pigs and rabbits. This enabled aggregation to be accurately quantitated *in vivo*.

The infusion of PGI₂ (0.25-1 ug/kg/min) in anaesthetised rats and rabbits produced a dose-dependent inhibition of the fall in platelet count produced by ADP and collagen. The formation of PGI₂ can be inhibited *in vitro* by 15-hydroperoxyarachidonic acid (15HPAA). When 20 ug/kg/min of 15HPAA was infused into rats, aggregation produced by collagen was significantly increased suggesting that PGI₂ is continuously formed by the rat vascular endothelium. This observation was confirmed by infusing 6-keto PGF_{1 α} antiserum. This antibody also prevented the inhibitory activity of PGI₂ on collagen-induced aggregation. The study of continuous platelet counting in guinea pigs has been hampered by the occurrence of thrombocytopenia in certain animals. When 2 ug/kg/min of PGI₂ was infused for 10 mins, a rise in the circulating platelet count to a steady plateau $4-5 \times 10^5$ platelets occurred.

These experiments have shown that PGI₂ will prevent aggregation by ADP and collagen and will reverse spontaneous thrombocytopenia and that PGI₂ is continuously released from the vessels of anaesthetised rats.