

PLATELET INTERACTION WITH BASEMENT MEMBRANE PROTEINS. L. Balleiser, J. Rauterberg, J. Risteli, H. Rohde and R. Timpl. Dept. of Medicine, Inst. for Arteriosclerosis Res., Münster, and Max-Planck-Inst. for Biochemistry, Martinsried b. München, W. Germany

Basement membranes consist mainly of two components: non-fibrillar type IV collagen and the non-collagenous glycoprotein laminin (m.w.900,000) which is capable to interact with cell surfaces. The collagenous protein was studied in form of two major fragments comprising together the total mass of the molecule. 7-S collagen which resembles the crosslinking domain of type IV collagen was isolated after collagenase digestion and consisted of four triple helices aligned in a parallel fashion (m.w.360,000). The major triple helical domain of type IV collagen (m.w.450,000) could be obtained by an acid extraction but had lost most of the 7-S domain.

Interaction with platelets was examined in aggregation, cell spreading and fibrin clot retraction assays including the determination of malondialdehyde formation. 7-S collagen was the most active component in all three assays. Aggregation was induced by as little as 25 µg/ml and was confirmed by electronmicroscopy. When compared to interstitial collagens, however, 10-20 fold higher amounts of 7-S collagen are required to produce the same effects. Acid extracted type IV collagen possessed virtually no activity. Laminin did not aggregate platelets but promoted strongly their attachment and spreading on a plastic substrate. Thus both basement membrane proteins apparently interact with platelets in different ways via distinct domains of the molecules.

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09:15 h

PLATELET-VESSEL WALL INTERACTIONS IN EXPERIMENTALLY INDUCED HYPERCHOLESTEROLEMIA. E. Tremoli, E. Agradi, A. Socini, A. Petroni and C. Galli. Institute of Pharmacology and Pharmacognosy, University of Milan, Milan, Italy.

A simple method was developed to study platelet-vessel wall interactions based on the perfusion of platelet rich plasma (PRP) through isolated segments obtained from the aorta of the same animal. The inhibition of aggregation of the perfused PRP, indicating prostacyclin (PGI₂)-like material production by aortic wall, was quantified. The effect was not present when normal PRP was perfused through the vessels obtained from aspirin-treated animals. This experimental model was used in the study of platelet-vessel wall interaction in normal (N) and hypercholesterolemic (HC) rabbits (one month on a high cholesterol - 2% W/W - diet).

In the HC animals increased aggregating response coupled with reduced platelet sensitivity to the inhibitory effect of exogenous PGI₂ was observed. When PRP of the two groups of animals was perfused through their own aortas, the inhibition of aggregation was significantly lower in HC samples, suggesting ever lower aortic production or lower sensitivity to the inhibitory effect of PGI₂-like material in treated animals. In addition a lower inhibition of platelet aggregation occurred after perfusion of PRP from HC animals through aortas of N rabbits, indicating a decreased platelet sensitivity to the inhibitory effect of PGI₂-like material released.

It appears that in experimental HC rabbits, platelet aggregation and their sensitivity to the antiaggregatory effect of PGI₂ are significantly affected. In our experimental conditions, production of PGI₂-like material in the aorta is not reduced, but the overall outcome of platelet-vessel wall interaction is a reduced inhibition of platelet aggregatory response.

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09:00 h

Ca²⁺ AFFECTS PLATELET-SUBENDOTHELIUM INTERACTION INDEPENDENT OF F VIII-VWF. K.S. Sakariassen, J.J. Sixma and P.A. Bolhuis. Department of Haematology, University Hospital Utrecht, The Netherlands.

The effects of Ca²⁺ in the presence or absence of F VIII-VWF on blood platelet adherence to subendothelium of human arteries were investigated in an annular perfusion chamber according to Baumgartner, using a non-pulsatile perfusion system. The perfusates consisted of citrated blood or reconstituted fluids with washed red blood cells, ⁵¹Cr-labelled platelets, and the appropriate plasma replacements. F VIII-VWF was purified from cryoprecipitate by Sepharose CL-4B chromatography and radio-labelled with ¹²⁵I by the lactoperoxidase technique. Platelet adherence was measured by registration of ⁵¹Cr count or by morphometry. Free Ca²⁺ was assayed with a calcium electrode, with a minimum detection limit of 10 µM. A close correlation was observed between the Ca²⁺ concentration and the haematocrit in normal blood anticoagulated with 1/10 vol 110 mM Na₃-citrate. The plasma concentration of citrate and Ca²⁺ had mean values of 18 mM and 65 µM respectively. Perfusion for 7 min at a vessel wall shear rate of 1000 s⁻¹ with this blood, produced a monolayer of adherent platelets and only occasionally small platelet aggregates on the subendothelium, but no thrombi > 5 µm in height. At plasma citrate and Ca²⁺ concentrations of respectively 40 mM and 25 µM the platelet spreading on the subendothelium was impaired. At a citrate concentration of 160 mM and a Ca²⁺ concentration below the detection limit, the initial contact between platelets and subendothelium was abolished. In perfusion fluids in which plasma was substituted by a human albumin solution (HAS), the adherence at Ca²⁺ concentrations above 10 µM was higher in the presence of F VIII-VWF than in its absence. However, at a Ca²⁺ concentration below 10 µM induced by addition of citrate (160 mM in HAS), the adherence with and without F VIII-VWF was abolished. The binding of ¹²⁵I F VIII-VWF to the subendothelium was unchanged at levels below 10 µM Ca²⁺.

These data indicate that the various steps of platelet interaction with subendothelium require specific Ca²⁺ concentrations. The effect of F VIII-VWF is independent of this.

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09:45 h

EFFECT OF TICLOPIDINE ON PLATELET DEPOSITION IN GORE-TEX AND AUTOLOGOUS VEIN GRAFTS. K. P. Murphy, M. K. Dewanjee, V. Fuster, P. Didisheim and M. P. Kaye. Mayo Clinic and Foundation, Rochester, MN 55905 USA

To quantitatively study platelet deposition on arterial prostheses and the effect of platelet inhibitory therapy with ticlopidine, 14 dogs underwent bilateral femoral artery excision and replacement with one polytetrafluoroethylene (Gore-Tex) and one autologous vein graft. Five dogs received 60 mg/kg/day of ticlopidine orally on four consecutive days prior to surgery. Nine untreated dogs served as controls. Collagen-induced platelet aggregometry studies were performed at the end of day 4. Autologous indium-111-labeled platelets were injected 24 hours prior to surgery to serve as a platelet marker. All animals received heparin (1 mg/kg I.V.) 5 minutes prior to excision of the arterial segment. Grafts were removed 1 hour following resumption of blood flow and gently flushed with 30 cc normal saline. Radioactivity per unit weight of Gore-Tex, vein and blood was determined with a gamma counter. The relative radioactivity with respect to the internal reference standard, blood, is tabulated below.

	Gore-Tex Blood	Vein Blood	Gore-Tex Vein
Control	19.70±5.40	3.02±1.77	11.20±4.11
Oral ticlopidine	1.23±1.58	0.97±0.66	1.19±0.14

Ticlopidine treated dogs showed a 15-fold reduction in platelet deposition on Gore-Tex grafts and a 3-fold reduction on venous grafts. Platelet deposition was reduced significantly in Gore-Tex grafts (P<0.001) to a level comparable to that in autologous veins. Platelet inhibitory effect was also observed in collagen-induced platelet aggregometry.

According to this "in vivo" data, ticlopidine appears to be a promising platelet inhibiting agent.