Monday, March 4, 1991
4:00PM–5:30PM, Room 367, West Concourse
Fibrinolysis and Platelets

4:00

THE EFFECTS OF A FREE FATTY ACID PREPARATION OF OMEGA-3 POLYUNSATURATED FATTY ACIDS ON PLATELET FUNCTION ALONE AND IN COMBINATION WITH ASPIRIN AND LOW MOLECULAR WEIGHT HEPARIN.

Gregory Mishkel, John Gill, John Cairns, Mike Buchanan, Bruce Holub, Robin Roberts, Jack Hirsh. McMaster University, Hamilton, Ontario, Canada.

Prior to performing a trial using Omega-3 Polyunsaturated Fatty Acids (PUFA) and/or low Molecular Weight Heparin (LMWH) in the prevention of PTCA restenosis, we examined the effects of PUFA, aspirin (ASA), and LMWH on bleeding time (BT) and other measures of platelet function. Ten subjects consumed 7.4 g/d of PUFA administered as a free fatty acid (FFA) for 21 days. ASA (325 mg) was administered on day 7 and also on day 21 in combination with 30 mg sc of LMWH. Significant platelet membrane incorporation of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids was seen at 48 hrs ($p<.01$) and continued to rise to day 21 ($p<.001$) along with an associated fall ($p<.001$) in platelet arachidonate. PUFA had no inhibitory effect on 1) BT (baseline 3.8 ± 0.4 min vs. day 21 3.9 min ± 4.0 min), 2) platelet aggregation (in response to ADP and collagen) and 3) platelet adhesion to a cultured endothelial cell monolayer. BT prolongation occurred with PUFA+ASA (1.9X baseline, $p<.001$) and PUFA+ASA+LMWH (2.2X baseline, $p<.001$), but there was no evidence to suggest that PUFA or LMWH enhanced the effect of ASA on BT. The mean BT with the combination of PUFA+ASA+LMWH was 8.1 ± 0.9 min.

Despite the demonstration of early incorporation of EPA and DHA into platelet membranes using a FFA preparation of PUFA, we were unable to demonstrate an inhibitory effect on platelet function. Furthermore PUFA combined with ASA and LMWH does not prolong bleeding times beyond that seen with ASA alone.

4:15

NEGATIVE FEEDBACK OF FIBRINOLYSIS MEDIATED BY ENDOTHELIAL CELLS IN RESPONSE TO PLATELETS

Satoshi Fujii, William E. Hopkins, Burton E. Sobel, Washington University, St. Louis, MO

Locally increased activity of plasminogen activator inhibitor type-1 (PAI-1) can attenuate fibrinolysis and may retard recanalization, potentiate early reocclusion, or both after pharmacologic coronary thrombolysis. To determine whether elaboration of PAI-1 by vascular endothelial cell is influenced by products of activated platelets, we incubated human umbilical vein endothelial cells with human platelet lysates (from 0.5 to 8.0 x 10^4 platelets/mm^3 media; equivalent to < 10% of platelets in blood). Although lysates did not augment synthesis of t-PA or total protein, they increased synthesis and release of PAI-1 protein (ELISA) into conditioned media by 3.3 ± 0.4 (SD) fold (n = 7) in 24 hr and into matrix (2.8 ± 0.4 fold in 6 hr, n = 5). PAI-1 mRNA was increased as well, by 2.1 - 2.3 fold in 4 hr (n = 2; Northern blots). The increased synthesis of PAI-1 was confirmed by immunoprecipitation of ^{35}S-PAI-1 after metabolic labeling of the cells. Secreted PAI-1 was functionally active and bound exogenous t-PA (1,000 ng/ml, n = 4). Antibody to transforming growth factor-β (TGF-β) reduced simulation by platelet lysates by 69.0 ± 8.0% (n = 5) and effects of lysates were simulated by TGF-β (0.25 ng/ml, n = 4), known to be present in platelet α-granules and to be released with activation. Other platelet constituents (TGF-α, platelet derived growth factor, histamine, serotonin, and norepinephrine) had no effect. Thus, activation of platelets accompanying thrombosis may attenuate fibrinolysis locally through release of TGF-β with consequently increased endothelial cell elaboration of PAI-1.

4:30

THE EFFECTS OF THROMBIN INHIBITION AND ANTIPLATELET MEMBRANE GLYCOPROTEIN IIb/IIIa ON PLATELET DEPOSITION IN AN *EX VIVO* ANGIOPLASTY MODEL.

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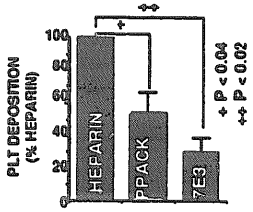
We examined the role of thrombin inhibition with $PhePro ArgCH_2Cl$ (PPACK) and inhibition of the platelet membrane receptor IIb/IIIa (GPIIb/IIIa) with monoclonal antibody 7E3 on platelet (plt) deposition at the site of balloon injury. Fresh rabbit aortas were mounted in a perfusion chamber, underwent balloon injury and were perfused with human blood at physiologic pressure and shear rates of 180-250 sec^{-1} for 30 minutes. Plt deposition was measured with ^{111}In-labeled plts. With heparin (2U/ml) 8.2 2.2x10^6 plt/cm^2 were deposited at the site of injury, compared to 0.7 0.2x10^6 on uninjured segments ($p<0.02$, n=7). These results were confirmed by scanning electron microscopy. PPACK was tested at a 10 M which totally inhibited thrombin induced plt aggregation. 7E3 was tested at 10 g/ml which totally inhibited plt aggregation. Plt deposition was reduced 47% by PPACK (n=4) and 70% by 7E3 (n=3) when compared to heparin.



Conclusions: At shear rates seen in non-stenotic coronary arteries: 1) PPACK and 7E3 reduce plt deposition more than heparin. 2) Inhibition of plt deposition by PPACK demonstrates the importance of thrombin in plt thrombus formation 3) 7E3 data suggests that~70% of plt deposition is GPIIb/IIIa dependent and ~30% is due to plt-subendothelial adhesion. 4) This *ex vivo* angioplasty model may be useful for testing anti-thrombotic and antiplatelet agents.

4:45

HIRUDIN, CONTRARY TO HEPARIN, DOES NOT INDUCE PLATELET ADHESION TO BLOOD CLOTS AND EXTRACELLULAR MATRIX IN VITRO.

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Platelet adhesion to fibrin and to extracellular matrix (ECM) participate in thrombolysis resistance or reocclusion, as well as post-angioplasty occlusion and restenosis. They can occur despite heparin (HEP) treatment. Recombinant hirudin (r-HIR) is a potent and more specific anti-thrombin agent than HEP. Since HEP can activate platelets, we tested the effects of HEP (up to 1 U/ml) and r-HIR, used at the same anti-thrombin activity, on platelet adhesion to preformed fibrin and to ECM.

^{111}Indium-labelled platelets were incubated in plasma with standardized fibrin clots for up to 45 min in the presence of HEP, r-HIR or saline (control), and adhesion to the clot was measured after clot washing.The same experiments were performed in the presence of ECM. HEP, but not r-HIR, induced a time and dose-dependent platelet adhesion to the clot and to ECM, in comparison to control. Aspirin (200 µg/ml) failed to prevent this HEP-induced platelet adhesion to clot and ECM.

	Platelet adhesion after 30 min (% of control, Mean ± SD, p vs control) to clot	to ECM
HEP (n=5)	149 ± 15 ($p < 0.002$)	193 ± 31 ($p < 0.001$)
r- HIR (n=8)	99.5 ± 15 (NS)	99 ± 7 (NS)
	$p < 0.0001$ vs HEP	$p < 0.001$ vs HEP

Conclusion : *in vitro*, HEP, at pharmacological concentrations, but not r-HIR, increases platelet adhesion to fibrin clot and to ECM. *In vivo*, this difference may favor the use of r-HIR over HEP in clinical situations associated with platelet adhesion to clot or ECM such as thrombolysis and angioplasty.