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CHARACTERIZATION OF DISORDERS OF PLATELET-VESSEL WALL INTERACTION IN AN AGGREGOMETER INCORPORATING BLOOD FLOW PAST AN ENDOTHELIAL CELL MONOLAYER. E.F. Grabowski and K. McKenny. Division of Pediatric Hematology/Oncology, Cornell University Medical Center

Epi-fluorescence videomicroscopy permits real-time imaging of platelet (plt) adhesion-aggregation to a defined microinjury site of an endothelial cell monolayer (ECM) exposed to flowing blood. The fluorescent label is the TAB murine monoclonal antibody (courtesy of Dr. R.P. McEver) directed against human plt GP IIB, together with a fluorescein-conjugated goat F(ab')2 against murine immunoglobulin. The combination assures specificity for plt membranes, yet leaves plt function intact. Bovine aortic ECM, grown on rectangular cover glasses, comprise one wall of a flow chamber mounted on a vertical microscope stage. A 6-0 sterile suture, drawn across the ECM in a direction transverse to flow, creates microinjuries of width 70 ± 15 μm (mean ± SD). Plt deposition is virtually absent upon intact and confluent regions of the ECM. On microinjury sites and at a shear rate of 270 sec^{-1}, however, computer-enhanced images show plt adherence, aggregation, and embolization. Pretreatment of the ECM with 1.0 mM FC lysine acetylsalicylate, further, leads to a three-fold increase in aggregate length. ECM products inhibitable by aspirin, therefore, modulate adhesion-aggregation in disease and normal states under physiologic flow conditions. The Table shows that percent coverage of the injury area, and mean aggregate length readily discriminate normal, post-aspirin, and von Willebrand's (vWD's) bloods. Aggregate length is reduced in vWD's blood to a greater degree ($p<0.01$) than by oral aspirin, while the latter is associated with a paradoxic increase ($p<0.01$) in single plt adhesion.

BLOOD	# RUNS	# DONORS	PERCENT COVERAGE*	AGGREGATE LENGTH*, μm
N1	10	5	24 ± 6.7	32 ± 3.2
N1 after 300 mg PO ASA	7	4	41 ± 8.1	29 ± 3.3
vWD's Disease	4	3	25 ± 2.8	22 ± 2.2
Hermansky-Pudlak Syndrome	2	1	12 ± 3.0	22 ± 8.1

*Mean ± SE: N= # Runs.

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AGGREGATES OF RED BLOOD CELLS, AND AGGREGATES OF PLATELETS UNDER ZERO GRAVITY: EXPERIMENT ON NASA SPACE SHUTTLE "DISCOVERY" STS 51-C, JANUARY 1985. L. Dintenfass, Department of Medicine, University of Sydney, Australia 2006.

The aim of experiment "ARC" on the space shuttle "Discovery STS 51-C, was to define effect of zero gravity on kinetics and morphology of aggregation of red cells in blood obtained from patients suffering from ischaemic heart disease, colon cancer, insulin-dependent diabetes, hyperlipidaemia, IgG and IgM paraproteins. Space-rated automated slit-capillary photo-viscometer contained a motorized infusion pump capable of handling eight different blood samples. Two cameras and a microscope allowed micro and macrophotography, and total of 500 photographs was obtained in space; and equivalent number on the ground, in the Kennedy Space Center, where a duplicate ground photo-viscometer was present. Identical blood samples have been used in the ground experiments. The slit had a gap of 12.5 microns (micrometers). Blood was anticoagulated with EDTA and adjusted to haematocrit of 0.30 using native plasma. Samples were kept at 5°C prior to the experiment, and at 25°C during experiment; duration of experiment was 9½ hours. The same computer program was used in both instruments. Photography was carried out at set intervals up to six minutes from the moment of stasis. There was a drastic difference between aggregation on the ground and at zero gravity. Blood from patients was greatly sludged on the ground, but normal rouleaux were formed under zero gravity. Also, aggregates under zero g were much smaller. However, red cell shape was not changed. Blood samples from normal donors, which showed normal rouleaux on the ground, exhibited random swarm pattern under zero gravity. Platelets, which tended to aggregate on the ground, and tended to accummulate at the slit entrance, remained monodisperse under zero gravity and no pseudopodia have been noted; under zero g platelet moved through the slit. Subject to future confirmation, it is suggested that zero gravity affects cell-to-cell interaction, and probably causes a modification of the cell membrane. If this is true, a new vista opens in the studies of immunology and oncology under zero gravity.

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COMPARISON BETWEEN IN VITRO AND IN VIVO AGGREGATION OF PLATELETS **C. TAPPARELLI, P. GFELLER, S. SANJAR, J. MORLEY. Preclinical Research, Sandoz AG, Basel CH-4002, Switzerland.**

The extensive utilisation of in vitro tests of platelet aggregation presumes that corresponding effects occur in vivo. Platelet aggregometry in vitro and in vivo have been compared using a range of agonists in order to test this assumption.
PRP from citrated peripheral blood of man, rat and guinea-pig was exposed to ADP, adrenaline, serotonin, collagen, thrombin and PAF in a Born aggregometer to define the time course and amplitude of these responses. In rat and guinea-pig, these aggregatory stimuli have also been used to define the time course and amplitude of intrathoracic accumulation of 111 -Indium labelled platelets, using an automated monitoring system (AIMS 8000).
Concordance between these tests was evident for ADP, collagen, thrombin and PAF in both species; but, substantial discrepancy was observed between in vitro and in vivo responses to serotonin and adrenaline, since sustained aggregation followed injection of these agonists in the rat.
For these platelet stimuli, in vivo aggregometry in rat and guinea-pig may more faithfully reflect the behaviour of human platelets than in vitro studies.

	Human	Guinea-pig		Rat	
	in vitro	in vitro	in vivo	in vitro	in vivo
ADP	+	+	+	+	+
ADRE.	+	-	-	-	+
5-HT	+	-	-	-	+
COLL.	+	+	+	+	+
THROM.	+	(+)	(+)	+	+
PAF	+	+	+	-	-

+ response, - no response, ( ) weak response.

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INHIBITION OF HUMAN PLATELET AGGREGATION BY THE THROMBOXANE MIMETIC, U46619.
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Recent publications suggest that high concentrations (10-100μM) of the stable analogues of PGH_2, U46619 and U44069 stimulate adenylate cyclase (Avdonin et al., 1985) and cause an elevation in cAMP in intact platelets (Best et al., 1979). The effect of high concentrations of U46619 on responses to ADP [1.0-30μM] and vasopressin [1-10nM] was investigated in human platelet rich plasma preincubated with the thromboxane antagonist, GR32191, [10μM] (Lumley et al., this meeting) and aspirin [0.1mM]. Concentration-effect curves to ADP were not affected by preincubation with 0.1mM U46619 in the presence of GR32191 [10μM]. Under the same conditions, aggregation to vasopressin was inhibited. U46619 at 30μM had no effect on the vasopressin concentration-effect curve, but at 0.1mM, it resulted in an eight-fold rightwards shift and also reduced the maximum response by 30%. This was equivalent to the inhibition of vasopressin-induced aggregation achieved with prostacyclin at a concentration of 1nM in the same experiments. At 0.3mM, U46619 caused total inhibition of aggregation to vasopressin, equivalent to that seen with prostacyclin at 3nM. The failure of U44619 to inhibit ADP-induced aggregation may reflect inhibition of adenylate cyclase by ADP (Cusack et al, 1982). Aggregation induced by vasopressin is independent of inhibition of adenylate cyclase (Thomas et al., 1983) and under these conditions, U46619 may induce increases in cAMP which exert an inhibitory action on platelet function. These results indicate that experiments using U44619 as an aggregating agent should be interpreted with caution. The high concentrations of U46619 required to induce aggregation in the presence of thromboxane antagonists may also cause stimulation of adenylate cyclase, thereby making an additional contribution to the inhibition observed.

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