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INTACT MICROTUBULES ARE NECESSARY FOR COMPLETE PROCESSING, STORAGE AND REGULATED SECRETION OF VON WILLEBRAND FACTOR BY ENDOTHELIAL CELLS. S. Sinha and D.D. Wagner. Hematology Unit, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA.

The importance of intact microtubules in the processing, storage and regulated secretion of von Willebrand factor (vWf) from Weibel-Palade bodies in endothelial cells was investigated. Human umbilical vein endothelial cells treated for one hour with colchicine (10^{-6}M) or nocodozole (10^{-6}M) lost their organized microtubular network. Stimulation of these cells with secretagogues (A23187, thrombin) produced only 30% release of vWf in comparison to control cells containing intact microtubules. The nocodazole treatment was reversible. One hour incubation in the absence of the drug was sufficient for microtubules to reform and to restore the full capacity of the cells to release vWf.

Long-term incubation (24 hours) of endothelial cells with microtubule destabilizing agents had a profound effect on vWf distribution. In control cells vWf was localized to organelles in the perinuclear region (i.e. endoplasmic reticulum and Golgi apparatus) and to Weibel-Palade bodies. In drug-treated cells vWf staining was dispersed throughout the cytoplasm and Weibel-Palade bodies were absent. The vWf synthesized in the absence of microtubules contained significantly less large multimers than that produced by control cells. This was not due to possible side effects of the drugs on the cells because the presence of lumicolchicine, an inactive analogue of colchicine, had no effect on vWf processing. Since Weibel-Palade bodies specifically contain the large multimers (Sporn et al, Cell 46:185-190, 1986), we hypothesize that the structural defect in vWf secreted by cells in the absence of microtubules is due to the lack of Weibel-Palade bodies in these cultures.

In summary, the intact microtubular cytoskeleton in the endothelial cells in culture, appeared to be crucial for normal release of Weibel-Palade bodies after stimulation with secretagogues, for reformation of new Weibel-Palade bodies and for the efficient intracellular multimerization of vWf dimeric molecules.

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ABNORMAL EXPRESSION OF VON WILLEBRAND FACTOR BY ENDOTHELIAL CELLS FROM A PATIENT WITH TYPE IIA VON WILLEBRAND'S DISEASE. Richard B. Levene (1), François M. Booyse (2), Juan Chediak (3), Theodore S.Zimmerman (4), David M. Livingston (1), and Dennis C. Lynch (1). Dana-Farber Cancer Institute, Boston, MA, U.S.A. (1); University of Alabama, Birmingham, AL, U.S.A. (2); Michael Reese Medical Center, Chicago, IL, U.S.A. (3); Scripps Clinic and Research Foundation, La Jolla, CA, U.S.A. (4).

Studies were conducted to characterize the biosynthesis of von Willebrand factor (vWf) by cultured endothelial cells (EC) derived from the umbilical vein of a patient with type IIA von Willebrand's disease. The patient's EC, compared with those from normal individuals, produced vWf which had decreased amounts of large multimers and an increase in rapidly migrating satellite species, features which are characteristic of plasma vWf from patients with type IIA von Willebrand's disease. The type IIA EC produced a full spectrum of vWf multimers in both cell lysates and post-culture medium, although the relative amounts of the larger species were decreased. The large multimers were degraded in conjunction with the appearance of rapidly migrating satellites which contained ≈170 kDa proteolytic fragments. Kinetic studies demonstrated that the ≈170 kDa species is not a primary translation product. Normal metabolically labeled vWf, incubated with either the patient's EC or medium conditioned by these cells, was not similarly degraded. These results demonstrated that this patient's clinical phenotype is due to abnormal proteolysis and not to a primary failure of subunit oligomerization. Moreover, the increased degradation is attributable to increased proteolytic sensitivity of an abnormal vWf molecule rather than to pathologically elevated levels of endogenous proteases. Experiments using monoclonal antibodies which recognize either N- or C-associated epitopes have localized the defect to the N-terminal portion of the vWf molecule, which is believed to be involved in the inter-dimer polymerization reaction. The type IIA EC also contained a single vWf mRNA species which comigrated with that from normal EC. However, the type IIA EC contained 8-10 fold more vWf mRNA than their normal counterparts. These results suggest that the functional defect in this patient is caused by a subtle mutation in the vWf coding sequence leading to increased proteolytic sensitivity of its protein product.

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PLATELET INTERACTION WITH ARTIFICIAL SURFACES UNDER DEFINED FLOW CONDITIONS: ROLE OF VON WILLEBRAND FACTOR. L. Badimon, J.J. Badimon, V. Turitto and V. Fuster. Division of Cardiology, The Mount Sinai Medical Center, N. Y.

Platelets deposit onto artificial surfaces to form mono or multilayers which under certain conditions lead to gross thrombus formation and eventual failure of graft material. Both hemodynamic and blood related factors determine the interaction of blood cells with prosthetic implants. The objective of this study has been to ascertain the extent to which vWF plays a role in platelet deposition onto artificial surfaces over a range of shear conditions. Polytetrafluoroethylene (PTFE) vascular graft segments were exposed to blood flow in a tubular perfusion chamber with well characterized rheological conditions. The perfusion chamber with test material was placed within an extracorporeal circuit (carotid artery-jugular vein) in normal swine and swine with inherited homozygous vWD. Platelet deposition was measured by labelling autologous platelets with ^{111}Indium and also by optical morphometry of epoxy embedded specimens. Electron microscopy was performed in selected samples. Studies were performed with heparinized blood (300u/Kg iv) for perfusion times of 1 to 10 minutes. Platelet deposition increased with wall shear rate and with exposure time in the presence of vWF (vWF: 89 ± 11%) but in its absence (vWF: < 3%) platelet deposition was significantly(*) inhibited at high ($1690s^{-1}$, shown below) but not at low ($212s^{-1}$) shear rate. Results are Platelets x $10^6/cm^2$ ±1SE.

		212 s^{-1}		1690 s^{-1}	
		3'	5'	3'	5'
Heparin	N	2.2±0.9	3±1	4±1.5	15±6
	vWD	2.5±0.7	6±2	4±2.2	6±3 (*)

Plasma and platelet vWF play an important role in platelet deposition and stabilization of thrombus growth on a synthetic biomaterial as we have previously observed on vascular surfaces.

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PLATELET DEPOSITION ON ARTIFICIAL SURFACES: EFFECT OF PROTEIN PRECOATING, ENDOTHELIAL CELL COVERAGE AND SHEAR RATE. A. Poot, A. Dekker, T. Beugeling, A. Bantjes and W.G. van Aken. Twente University of Technology, Enschede, The Netherlands.

In the present study the in vitro capillary perfusion system according to Cazenave was used. This system consists of a capillary tube connected to a syringe containing the perfusate which consisted of washed human platelets (^{111}In-labeled or native) and washed red cells in Ca^{2+}/Mg^{2+}-containing Tyrode-albumin buffer. Perfusate flow is controlled by a syringe pump. Polyethylene tubes (PE, 0.75 mm ID, 25 cm long) were precoated with purified human von Willebrand factor (vWF), fibrinogen (Fb), fibronectin (Fn), immunoglobulin G (IgG), albumin (HSA) or plasma. Compared with uncoated PE, platelet deposition increased after precoating with vWF, Fb or Fn, and decreased by preadsorbed IgG, HSA or plasma. Platelet deposition was positively correlated with shear rate only on surfaces precoated with vWF, Fb or Fn. Scanning electron microscopy showed platelet aggregates on IgG-coated PE, whereas on all other surfaces single adherent platelets were observed. Complete platelet spreading was only observed after precoating with Fn. In contrast with PE coated with vWF, Fb or Fn, platelet adhesion on uncoated PE did not increase further after 5 minutes of perfusion probably due to passivation of the surface by albumin present in the perfusate. This could be overcome by addition of Fb to the perfusate. Perfusates prepared in this way were used for studying the effect of human endothelial cell (HEC) coverage on platelet deposition. HEC were seeded at different densities in PE tubes precoated with partially purified Fn. Platelet deposition decreased with increasing HEC coverage, and was negligible at 60,000 HEC/cm^2. Our results indicate the applicability of this perfusion model for the in vitro testing of artificial surface thrombogenicity.