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EFFECT OF ELASTASE INDUCED CLEAVAGE OF VON WILLEBRAND FACTOR (vWf) ON ITS STRUCTURE AND FUNCTION

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A proteolytic product of vWf termed fast migrating protein (vWf:FMP) has been identified using crossed immunoelectrophoresis (CIE) in normal serum and in the plasma from patients with disseminated intravascular coagulation (DIC). A fragment of vWf antigen (vWf:Ag) migrating to a similar position on CIE as vWf:FMP results from digestion of vWf:Ag with polymorphonuclear cells (PMNC). Since parallels exist between the conditions for generation of vWf:FMP by PMNC and elastase release by these cells the effect of purified elastase from porcine pancrease on vWf was investigated.

VWf was purified from plasma using polyethylene glycol, ammonium sulphate and zinc acetate precipitation, high speed centrifugation and elution from column of Sepharose 4B-CL. A fraction rich in vWf was radiolabelled with 125-Iodine to spike the purified preparation of vWf in order to increase the sensitivity of the protein detecting systems.

A mixture of radiolabelled and non-labelled purified vWf was incubated with elastase at concentrations ranging from 2.5 to 40 U/ml for periods of 0-48 hours. Modifications of the structure were assessed by SDS-agarose multimeric analysis, SDS-polyacrylamide electrophoresis and CIE. Alterations of function were quantitated by antigen levels, ristocetin (RCof) and botrocetin (BCoF) cofactor assays and a binding assay to fixed washed platelets in the presence of ristocetin or botrocetin.

These investigations show, 1. all but the highest molecular weight multimers of vWf are present when elastase has cleaved vWf such that no intact 240K subunit is present. 2. an intact 240K subunit is not essential for RCoF and BCoF activity or for ristocetin or botrocetin induced binding to platelets.

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FACTOR VIII/VON WILLEBRAND FACTOR COMPLEX: ONLY THE 440 000 SUBUNIT OF ENDOTHELIAL CELL-DERIVED VON WILLEBRAND FACTOR FORMS A COMPLEX WITH PURIFIED PLASMA FACTOR VIIIC. B. Pötzsch (1), U. Delvos (1), E. Anders (1), N. Heimburger (2) and G. Müller-Berghaus (1). Clinical Research Unit for Blood Coagulation and Thrombosis of the Max-Planck-Gesellschaft, D-6300 Giessen (1) and Behringwerke Research Laboratories, D-3550 Marburg (2), West-Germany.

Von Willebrand Factor (vWF) circulates in plasma as a series of multimers with molecular weight ranging from $M_r = 0.44 \times 10^6$ up to more than 20×10^6. Besides the mediation of platelet adhesion to exposed subendothelium, the protein plays an important role in the stabilization and the transport of Factor VIIIC (FVIIIC). In the present study the interaction between FVIIIC and vWF was studied by recombination experiments. vWF was isolated from cultured human umbilical vein endothelial cells by immunoprecipitation. This source of vWF ascertained, that it was free of FVIIIC as indicated by the absence of FVIIIC activity as well as FVIIIC antigen. FVIIIC was prepared by immunoabsorption from human plasma yielding an activity of 1600 U/mg. SDS-PAGE analysis showed two main bands at $M_r = 0.28 \times 10^6$ and 0.18×10^6, respectively. vWF-multimers were separated by SDS agarose gel electrophoresis and were electrophoretically transferred onto nitrocellulose sheets. After extensive washing, the sheets were incubated for 12 h with 20 U/ml FVIIIC in PBS, pH 7.4, containing 2.5 mM calcium chloride. Subsequently, associated FVIIIC was detected by autoradiography with a 125-I-labelled monoclonal mouse anti-(human FVIIIC) antibody. The results of recombination experiments exclusively showed prominent staining of the $M_r = 0.44 \times 10^6$ vWF band in the autoradiography. However, proteolytically degraded FVIIIC with partly retained procoagulant activity did not show a positive stain. The results indicate that an intact FVIIIC molecule and the smallest multimer of vWF are required for the formation of a stable FVIII/vWF complex.

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LARGE SCALE PREPARATION OF VON WILLEBRAND FACTOR BY AFFINITY CHROMATOGRAPHY. **J. Newman and D. Farb. Meloy Laboratories-Rorer Group, Springfield, Va., U.S.A.**

Treatment of bleeding in von Willebrand's disease usually consists of the infusion of cryoprecipitate or plasma. DDAVP is effective in some patients. Commercial concentrates for the treatment of Hemophilia A are ineffective as a source of von Willebrand Factor (vWF) replacement in von Willibrand's disease presumedly because of the absence of higher molecular weight forms of vWF protein. A vWF concentrate obtained during the course of preparation of an affinity purified Factor VIII may provide an alternative therapeutic agent without impacting the available Factor VIII supplies. The process used for the preparation of a highly purified Factor VIII concentrate (Monoclate™, Armour Pharmaceutical Co.) from cryoprecipitate includes an affinity chromatography step which separates vWF/Factor VIII complex from other proteins in cryoprecipitate using an anti-vWF monoclonal antibody (C.A. Fulcher & T.S. Zimmerman, 1982). Factor VIII is then dissociated from the vWF remaining on the column and is eluted immediately by 3M sodium thiocyanante (NaSCN) as a step in the regeneration of the column. Unless the NaSCN is rapidly removed from the elutriant, the vWF activity as measured by platelet agglutination is destroyed. We have taken the NaSCN eluate and processed it immediately over an in-line G-10 or G-25 Sephadex column which removes the NaSCN while the vWF is eluted with a buffered isotonic solution. Alternatively, the vWF has been precipitated from the NaSCN by ammonium sulfate or polyethylene glycol. VWF prepared by any of these methods retains platelet agglutinating activity and has a distribution of vWF multimers similar to those of vWF in normal plasma. The potency of vWF prepared from cryoprecipitate by this process is 20 units/ml and the specific activity is 20 units/mg. In several fractionations of kilogram amounts of cryoprecipitate, vWF was isolated and subjected to lyophilization and heating at 68° for 30 hours without loss of bioactivity. Drug development in progress suggest that the combined effect of affinity chromatography and exposure to NaSCN may negate the need for a heating procedure to reduce the risk of viral transmittance.

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INCREASED ADHESIVE PROPERTIES OF THE CARBOHYDRATE-MODIFIED VON WILLEBRAND FACTOR (CHO-VWF): CRITICAL ROLE OF THE MULTIMERIC STRUCTURE AND CORRELATION WITH PLATELET AGGREGATION - A.B. Federici, (1) C. De Romeuf, (2), P.G. De Groot (3), P.M. Mannucci (1), B. Samor, (2) R. Lombardi, (1), P. D'Alessio (1,3) C. Mazurier (2), and J.J. Sixma (3). (1) A. Bianchi Bonomi Hemophilia Thrombosis Center, University of Milano, (2) Centre Regional de Transfusion Sanguigne, Lille, (3) Department of Hematology, Utrecht

We have reexplored the role of the carbohydrate moiety (CHO) on the von Willebrand Factor (vWF) structure and function by critically evaluating its different purification steps and modifications in CHO content by specific enzymes. Structural and functional assays have been evaluated separately in each laboratory (Milano and Lille) and jointly in Utrecht during several organized experiments. Under our conditions, the CHOVWFs obtained were characterized by less than 5% of sialic acid "(Neu)asevWF" and about 45% of D-Galactose "(Neu-Gal)ase-vWF" remaining, by increased electrophoretic mobility without any significant losses of the high molecular weight multimers and by their capacity to induce spontaneous aggregation in normal platelet rich plasma (PRP). Platelet adhesion to these different CHO-vWFs was tested in the flat chamber devised by Sakariassen in the presence of different subendothelial matrices and data expressed as the percentage of the surface covered by platelets. The blood reconstituted with different plasma samples showed the following percentual values of surface coverage (mean ± SD):

- Normal plasma = 15 ± 3.8
- Severe vWd plasma = 4 ± 1.9
- SvWd pl + Native vWF = 14 ± 2.8
- SvWd pl + (Neu) ase-vWF = 23 ± 3.5
- SvWd pl + (Neu-Gal) ase-vWF = 19 ± 2.9

This significantly increased adhesion to the subendothelium of the CHO-vWFs corresponded to the spontaneous aggregation present in normal PRP but it disappeared when the multimeric structure was damaged by in vitro proteolysis and/or by storage conditions (changes in temperature and freezing and thawing). From these results we may conclude that removal of terminal sugars enhances not only platelet-vWF interactions, but also platelet adhesion to the subendothelium.

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