LOCALIZATION OF A FACTOR VIII BINDING DOMAIN ON THE N-TERMINAL PORTION (FRAGMENT SpII) OF VON WILLEBRAND FACTOR (VWF) Y. Takahashi (1), J.P. Girna (1), M. Kalafats (1), K. Sewerin (2), L.O. Anderson (2) and D. Meyer (1), INSERM U. 143, Hôpital Bicêtre, Paris, France (1) and Kabi Vitrum Haematology, Stockholm, Sweden (2).

A new domain has been identified on the von Willebrand Factor (VWF) subunit. VWF binds to platelet glycoproteins GP Ib/IIIa and GPIb/IX, as to collagen and corresponding domains have been isolated. VWF also binds to Factor VIII (F.VIII). We show here that the corresponding domain is located in the N-terminal portion of the F.VIII subunit (residues 1 to 1,365). For this purpose, VWF was used for its ability to bind the purified VWF degradation fragments obtained by digestion with Saureus V-8 protease; a dimeric N-terminal fragment of 320 kD (SpII) and a dimeric C-terminal fragment of 260 kD (SpI). The C-terminal fragment of F.VIII was partially purified by cryoprecipitate by immunoadsorption of F.VIII/VWF onto a monoclonal antibody (MAb) to F.VIII coupled to Sepharose, followed by elution using 0.25 M CaCl2. The F.VIII preparation contained 106 U/ml VIIIC and less than 0.001 U/ml VWF.Ag. The binding assay was performed using polyethylene tubes coated with 2 ug/ml of purified VWF, SpII or SpI. Coated albumin, fibrinogen or fibronectin were used as controls. Purified V.WIII (0.1 to 2 U/ml VIIIC) was incubated in the coated tubes for 1 hr at 37°C. Following washing, bound F.VIII was estimated in situ by one-stage clotting and chromogenic assays. Immunoradiometric assay with 125-I-MAb to SpII or SpI demonstrated that the amount of coated protein remained constant throughout the experiments. F.VIII bound in a dose-dependent manner to coated VWF and SpII but not to SpI. Binding was specific for F.VIII as demonstrated by inhibition experiments. Bound F.VIII could be removed with 0.25 M CaCl2 and its coagulant activity inhibited by a MAb to polyclonal collagen antibodies or with an antibody against VWF VIIIIC. Binding of F.VIII to coated VWF or SpII was also inhibited in a dose-dependent way by VWF or SpII. In contrast, addition of SpII had no effect upon the binding of F.VIII to coated VWF. SpI could not be competitively inhibited by any MAb to F.VIII or VIIIC.

Among 28 MAbs which bound SpII and had anti-VIIIIC activity, 12 inhibited binding of F.VIII where the others had no effect. Among the latter, 3 MAbs blocked binding of VWF or VPII to GP Ib and 6 MAbs inhibited binding of VWF or SpII to collagen. Ten MAbs to SpII had no inhibitory effect upon binding of F.VIII. These results indicate that a F.VIII binding domain of VWF is located in the N-terminal portion of VWF (residues 1 to 1,365) and that it is distinct from the GP Ib and collagen binding domains. The 12 MAbs to SpII which block binding of F.VIII to VWF of SpII should allow the precise localization of this new domain.

IDENTIFICATION OF A SECOND COLLAGEN-BINDING DOMAIN IN HUMAN VON WILLEBRAND FACTOR (VWF) F. S. Parisi (1), K. Milv (1), P. W. Hostel (1), J. M. McPherson (2), T. S. Zimmerman (1) and Z. M. Huganir (1), Research Institute of Scripps Clinic, La Jolla, CA, U.S.A. (1) and Collagen Corporation, Palo Alto, CA, U.S.A. (2).

We have recently reported (Journal of Biological Chemistry 261: 1310-1315, 1986) that von Willebrand factor (VWF) possesses a collagen-binding domain localized in a reduced and alkylated tryptic fragment of apparent 52/48 kDa molecular weight, extending between residues Val (449) and Lys (728) of the constituent subunit. This proteolytic fragment of VWF also contains a glycoprotein Ib-binding domain and a heparin-binding domain. We have now identified a second collagen-binding domain in the Staphylococcus aureus V8 protease-generated fragment I that extends from residue Gly (911) to Glu (1365). The two binding domains exhibit different interaction with collagens of different origin. The reduced and alkylated 52/48 kDa tryptic fragment was a potent inhibitor of VWF binding to equine collagen type I, but had no effect on the binding of VWF to bovine collagen type I and III. In contrast, a purified fraction containing the unreduced 52/48 kDa domain inhibited VWF binding to equine collagen type I and III, but was less effective with equine collagen type I. Direct binding studies using 125-I-labeled fragment I demonstrated that the association constant was 5 to 10 times greater with the bovine collagens than with the equine collagen. The Staphylococcus aureus V8 protease-generated fragment II, with 52 kDa from residue (1) to Glu (1365) and contains both collagen-binding domains, was the most potent inhibitor of VWF binding to all types of collagen tested. The VWF fragment II has at least two collagen-binding domains. Native conformation appears to be necessary for binding of the 52/48 kDa domain to bovine collagen type I and III, but not to the equine collagen type I tested. The two domains appear to function concurrently in mediating VWF binding to collagen.

PLATELET ACTIVATING FACTOR

EFFECTIVENESS AND TOLERABILITY OF CV-3988, A SELECTIVE PAF ANTAGONIST, AFTER INTRAVENOUS ADMINISTRATION TO MAN. J. Arnot (1), A. Van Heijen (2), I. Bellenpeleire (2), Y. Miyamoto (3), I. Holm (4), H. Okajima (4) (Laboratoire d'Immunochimie et d'Immunopathologie, Centre for Vascular Research (1), Laboratory of Pharmacology (2), University of Leuven, Belgium, New Product Planning and Development, Pfizer (3), and Department of Pathology, University of Tokyo (4)).

Platelet activating factor (PAF) is a naturally occurring phospholipid with a wide spectrum of biological activities. Although PAF has been described a potential role in various conditions including inflammation, asthma, thrombosis and thrombosis, its precise function in physiologic/pathophysiologic process remains unclear. The introduction of selective PAF receptor antagonists could represent a useful tool to extend our knowledge of the role of this mediator in health and disease.

We have investigated the efficacy and tolerability of (5S,8R)-2-[3-[10-ethynyl-2-[(10S,11R)-8-oxo-10-oxa­-7-aza­-5-phosphinanylidene]ethyl]phosphinyl]-1-propyl] (CV-3988, Takeda Chern Ind) as a selective PAF antagonist with structural analogies with PAF, after intravenous infusion in men in a double-blind, placebo-controlled study. The compound, dosed from 750 to 2,000 ug/kg, significantly reduced platelet sensitivity to PAF. The threshold aggregating concentration (TAC) of PAF was defined as the minimal concentration causing an irreversible aggregation with a maximal amplitude at least 50% of the difference in light transmission between platelet rich plasma and platelet-poor plasma. It increased in a dose-dependent manner reaching 3.6 times the basal TAC (p<0.0005) at the end and 2.60 times the basal TAC (p<0.0005) 4 hours after infusion of the highest dose of CV-3988. CV-3988 returned to the basal value within 24 hours after the end of the infusion. CV-3988 did not cause major side effects nor changes in blood pressure, body temperature, respiration rate, and no clinically significant changes in plasma hemoglobin and serum haptoglobin were seen at the end and four hours after the end of the infusion, indicating that light hemolysis probably by high local concentrations at the infusion site.

Our results indicate that, when adequate infusion volumes and infusion rates are used, CV-3988 can be safely administered to man and should be useful in elucidating the role of PAF in health and disease.

EFFECT OF Ca2+ and Mg2+ ON PLATELET ACTIVATING FACTOR (PAF) INDUCED AGGREGATION AND SPECIFIC BINDING TO HUMAN PLATELETS, C. M. Cheung(1,2) and D. R. Pifer(2). Departments of Medicine and Pathology, Washington University School of Medicine, St. Louis, Missouri, U.S.A.

Gel filtered human platelets (GPP) collected in Tyrode's buffer containing 0.5% CaCl2, 1mM MgCl2, and 0.35% albumin exhibit high affinity binding of 3H-PAF with a KD of 0.10 - 0.20 nM (mean = 0.17 nM) and Bmax = 70 sites per platelet. When fibrinogen (1.67 uM/ml total concentration) is added to these GPP preparations biphasic aggregation is observed with PAF (4 nM). Normal aggregation is also observed with other platelet agonists including ADP, epinephrine, collagen, arachidonic acid, A23187 and thrombin. If PAF is prepared without added Ca2+ or Mg2+ in the presence of 3M EDTA, platelets do not aggregate in response to PAF. However, the number of specific binding sites remains unchanged (357 sites per platelet) with some decrease in affinity of binding (Kd = 0.21 nM). In the presence of 1mM Mg2+ there is no significant difference in binding kinetics over a range of Ca2+ concentrations (0-200). On the other hand, the calcium channel blocker verapamil (5-10uM) exhibits competitive inhibition of 3H-PAF as analyzed by Lineweaver-Burk plots. Specific binding of 3H-PAF to GPP in the presence of 1mM Mg2+ and 1mM EDTA shows Kd of 0.166NM but with increase in specific binding sites to 665. Despite increase in number of sites and no change in binding affinity, PAF under these conditions does not exhibit platelet aggregation with PAF in doses up to 80 nM. From these data it appears that external Ca2+ is not necessary for specific binding of 3H-PAF to its high affinity PAF receptor. However, calcium does appear to be necessary for second wave aggregation with PAF. While Mg2+ appears to enhance 3H-PAF binding to platelets Mg2+ cannot substitute for Ca2+ in PAF induced platelet aggregation. Although verapamil appears to competitively inhibit binding of PAF to GPP it makes it unclear whether the inhibition is due to competition at or near the actual PAF receptor or at sites involving the calcium channel.