LOCALIZATION OF A FACTOR VIII BINDING DOMAIN ON THE N-TERMINAL PORTION (FRAGMENT SpII) OF VON WILLEBRAND FACTOR (VWF) was investigated. Purified VWF binds to platelet glycoprotein Ibb/IIIa (GPIbb/IIIa) as well 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 on the N-terminus of the F.VIII subunit (residues 1 to 1,365). For this purpose, F.VIII was used for its ability to bind to a purified vWF degradation fragments obtained by digestion with S.aureus V-8 protease, in a dimeric N-terminal fragment of 320 kd (SpIII) and a dimeric C-terminal fragment of F.VIII was obtained from cryoprecipitate by immunoadsorption of F.VIII/vWF onto a monoclonal antibody (MAb) to vWF coupled to Sepharose, followed by elution using 0.2 M CaCl2. The F.VIII preparation contained 100 U/ml VWF and less than 0.001 U/ml vWF-Ag. The binding assay was performed using polystyrene tubes coated with 2 µg/ml of purified vWF, SpIII or SpI. Coated albumin, fibrinogen or fibronectin were used as controls. Purified F.VIII (0.1 to 2 U/ml) was incubated in the coated tubes for 1 h at 37°C. Following washing, bound F.VIII was estimated in situ by one-stage clotting and chromogenic assays. Immunoradiometric assay with 125I-labeled MAb to vWF demonstrated that the amount of coated protein remained constant throughout the experiments. F.VIII bound in a dose-dependent manner to coated vWF and SpIII but not to SpI. Binding 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 glycoprotein Ibb/IIIa and a polyclonal antibody (MAb) to GPIbb/IIIa. Binding of F.VIII to coated vWF or SpIII was also inhibited in a dose-dependent way by vWF or SpIII. In contrast, addition of SpI had no effect upon the binding of F.VIII to GPIbb/IIIa. This indicates that GPIbb/IIIa is located on the N-terminal portion of VWF (residues 1 to 1,365) and that it is distinct from the GPIbb and GPIbbIIIa domains. The 12 MAbs to SpIII which block binding of F.VIII to vWF or SpIII should allow the precise localization of this new domain.

IDENTIFICATION OF A SECOND COLLAGEN-BINDING DOMAIN IN HUMAN VON WILLEBRAND FACTOR (VWF) was performed. F.VIII possesses a collagen-binding domain localized in a reduced and alkylated tryptic fragment of apparent 52/48 kDa molecular weight which is 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 VR P3a gene-encoded 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 all types of collagen, as did anti-52/48 kDa monoclonal antibodies. Some of these antibodies, however, were more effective in inhibiting binding to equine collagen. On the other hand, fragment I markedly inhibited the binding of vWF to bovine collagen type I and III, but was less effective with equine collagen type I. Direct binding studies using 125I-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 VR P3a gene-encoded fragment I contains one potential N-linked glycosylation site (Gly, 911) to Glu (1365) and contains both collagen-binding domains, was the most potent inhibitor of vWF binding to all types of collagen tested. The VR P3a gene-encoded fragment I is located on the N-terminal portion of the VWF molecule. 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.

EFFECTIVENESS AND TOLERABILITY OF CV-3988, A SELECTIVE PAF ANTAGONIST, AFTER INTRAVENOUS ADMINISTRATION TO MAN. J. Arnut (1), A. Van Hecken (2), I. Delpeeleher (2), Y. Miyamoto (3), I. Holm (4), S. K Anaheim (5), C. Vercelline (6), M. Hennings (7), D. J. Turpie (8), Departments of Pharmacology and Clinical Research, AstraZeneca Research (1), Laboratory of Pharmacology (2), University of Leuven, Belgium, New Product Planning and Development, Stenvisie, Chemical Ind. (3), Japan (4), and C.H. Bennelse, Association AG, Pollution, Switzerland (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, glomerulonephritis and thrombosis, its precise role in physiology/pathophysiological processes 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 CV-3988, a 2-[2-[(3α-tetradecyloxy)lonylopropyl]-3-(4-ethyl)-3]-phenyl-4-ethylphosphonic acid (CV-3988, Takeda Chem. Ind), a selective PAF antagonist with structural analogies with PAF, after intravenous infusion in men in a double-blind, placebo-controlled study. The compound, in doses from 750 to 2,000 µg/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 of at least 50% of the difference in light transmission between platelet rich plasma and platelet poor plasma. It increased by a dose-dependent manner reaching 3.6 times the basal TAC (p < 0.0005) at the end and 2.6 times the basal TAC (p < 0.0005) 4 hours after infusion of the high dose. The TAC of PAF 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, heart rate, body temperature, or weight. No clinically significant changes in plasma haemoglobin 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.