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LOCALIZATION OF A FACTOR VIII BINDING DOMAIN ON THE N-TERMINAL PORTION (FRAGMENT SpIII) OF VON WILLEBRAND FACTOR. Y. Takahashi (1), J.P. Girma (1), M. Kalafatis (1), K. Sewerin (2), L.O. Andersson (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 GPIb and GPIIb/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-terminal portion of the vWF subunit (residues 1 to 1,365). For this purpose, F.VIII was tested for its ability to bind to purified vWF degradation fragments obtained by digestion with S.aureus V-8 protease, ie a dimeric N-terminal fragment of 320 kd (SpIII) and a dimeric C-terminal fragment of 220 kd (SpII). Human F.VIII was purified from cryoprecipitate by immunoadsorption of F.VIII/vWF onto a monoclonal antibody (MAb) to vWF coupled to Sepharose, followed by elution using 0.25 M $CaCl_2$. The F.VIII preparation contained 100 U/ml VIII:C and less than 0.001 U/ml vWFAg. The binding assay was performed using polystyrene tubes coated with 2 ug/ml of purified vWF, SpIII or SpII. Coated albumin, fibrinogen or fibronectin were used as controls. Purified F.VIII (0.1 to 2 U/ml VIII:C) 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 ^{125}I-MAbs to SpII or SpIII 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 SpII. Binding was specific for F.VIII as demonstrated by inhibition experiments. Bound F.VIII could be removed with 0.25 M $CaCl_2$ and its coagulant activity inhibited by a MAb or an oligoclonal (homologous) antibody neutralising VIII:C. 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 SpII had no effect upon the binding. Binding of F.VIII to SpIII was confirmed using MAbs to vWF. Among 28 MAbs which bound SpIII and had no anti VIII:C activity, 12 inhibited binding of F.VIII whereas the others had no effect. Among the latter, 3 MAbs blocked binding of vWF or SpIII to GPIb and 6 MAbs inhibited binding of vWF or SpIII 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 GPIb and collagen binding 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.

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IDENTIFICATION OF A SECOND COLLAGEN-BINDING DOMAIN IN HUMAN VON WILLEBRAND FACTOR. F.I. Pareti (1), K. Niiya (1), P.J. Kostel (1), J.M. McPherson (2), T.S. Zimmerman (1) and Z.M. Ruggeri (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: 15310-15315, 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 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 ^{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 III, which extends from residue Ser (1) to Glu (1365) and contains both collagen-binding domains, was the most potent inhibitor of vWF binding to all types of collagen tested. Thus, vWF 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.

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EFFECTIVENESS AND TOLERABILITY OF CV-3988, A SELECTIVE PAF ANTAGONIST, AFTER INTRAVENOUS ADMINISTRATION TO MAN. J. Arnout (1), A. Van Hecken (2), I. Delepeleire (2), Y. Miyamoto (3), I. Holmes (4), P. Deschepper (2) and J. Vermylen (1). Centre for Thrombosis and Vascular Research (1), Laboratory of Pharmacology (2), University of Leuven, Belgium, New Product Planning and Development Division, Takeda Chemical Ind., Osaka, Japan (3), and G.H. Besselaar Association AG, Zollikon, Switzerland (4).

Platelet activating factor (PAF) is a naturally occurring phospholipid with a wide spectrum of biological activities. Although PAF has been ascribed a potential role in various conditions including inflammation, asthma, glomerulonephritis and thrombosis, its precise function in physiologic/pathophysiologic 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 (RS)-2-methoxy-3-(octadecylcarbomoyloxy)propyl 2-(3-thiazolio)-ethylphosphate (CV-3988, Takeda Chem. Ind), a selective PAF antagonist with structural analogies with PAF, after intravenous infusion in man 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 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. 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, pulse or respiratory rate. However, small but clinically insignificant changes in plasma haemoglobin and serum haptoglobin were seen at the end and four hours after the end of the infusion, indicating a slight haemolysis 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.

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EFFECT OF Ca2+ and Mg2+ ON PLATELET ACTIVATING FACTOR (PAF) INDUCED AGGREGATION AND SPECIFIC BINDING TO HUMAN PLATELETS. C. M. Chesney(1,2) and D. D. Pifer(2). Departments of Medicine and Pathology, University of Tennessee (1) and Baptist Memorial Hospital (2), Memphis, Tennessee, U.S.A.

Gel filtered human platelets (GFP) collected in Tyrode's buffer containing 0.5 mM Ca+2, 1mM Mg+2, and 0.35% albumin exhibit high affinity binding of 3H-PAF with a Kd of 0.109 ± 0.029 nM (mean ± SD; n=13) and 267 ± 70 sites per platelet. When fibrinogen (1.67 mg/ml final concentration) is added to these GFP 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 GFP is prepared without added Ca+2 or Mg+2 in the presence of 3mM EDTA, platelets do not aggregate in response to PAF. However the number of specific binding sites remains unchanged (387 per platelet) with some decrease in affinity of binding (Kd = 0.214nM). In the presence of 1mM Mg+2 there is no significant difference in binding kinetics over a range of Ca+2 concentrations (0-2mM). 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 GFP in the presence of 1mM Mg+2 and 1mM EGTA 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, GFP under these conditions does not exhibit platelet aggregation with PAF in doses up to 80 nM.

From these data it appears that external Ca+2 is not necessary for specific binding of 3H-PAF to its high affinity receptor. However, calcium does appear to be necessary for second wave aggregation with PAF. While Mg+2 appears to enhance 3H-PAF binding to platelets Mg+2 cannot substitute for Ca+2 in PAF induced platelet aggregation. Although verapamil appears to competitively inhibit binding of PAF to GFP it is not clear whether the inhibition is due to competition at or near the actual PAF receptor or at a site involving the calcium channel.