1026

A RAPID SCREENING METHOD TO INCREASE EFFICIENCY IN ASSAYING PLASMA LEVELS OF FACTOR VIII INHIBITORS. K.S. White[†], F.A. Dombrose[¶] and P.M. Blatt[§]. Bowman-Gray School of Medicine[†], Winston Salem, N.C. and Departments of Pathology[¶] and Medicine[§], Thrombosis and Hemostasis Center, University of North Carolina, Chapel Hill, N.C. 27514, USA

The plasma level of naturally acquired inhibitors to clotting Factor VIII activity (F.VIII:C) was assessed by the Bethesda Inhibitor Assay (BIA). At inhibitor concentrations outside the BIA working range (25% to 75% residual F.VIII:C), patient plasmas had to be diluted. However, when a rough estimate of the inhibitor concentration was unavailable, the choice of dilution was arbitrary and testing became tedious and inefficient. In an effort to improve the BIA, a screening test was devised to predict the appropriate plasma dilution. It is based on the partial thromboplastin time (PTT) of the incubated sample and is performed just prior to preparing dilutions for the F.VIII:C assay. Test plasmas were diluted serially in multiples of two, from 1:2 to 1:1024, and then incubated according to the BIA. After 90 min, samples were removed for PTT determinations to screen for the appropriate dilution to use in the F.VIII:C assay. The first two test dilutions to produce PTT values 21 sec (± 4 sec, 95% confidence interval) greater than the control resulted in a residual F.VIII:C for the BIA within the 25% to 75% acceptable range. When the log ratio of patient-to-control PTT determinations (specific PTT) for a given dilution was plotted as a function of log residual F.VIII:C at that test dilution, in 81 Bethesda assays a linear relationship was observed ($p > .25$; with $r^2 = .81$). The screening test was 89% effective in predicting an appropriate dilution (range 0 to 1020 Bethesda units/ml). The accuracy can be enhanced further if, based on the first of three dilution screen tests in which the specific PTT >21 sec, the F.VIII:C assay is performed using the middle dilution value first. Depending on whether that dilution yields an acceptable residual F.VIII:C, either the higher or lower dilution is chosen in the second F.VIII:C assay.

1027

KINETICS OF FACTOR VIII INHIBITORS IN THREE NON-HEMOPHILIAC PATIENTS. S. L. McClellan, C. V. Hussey, J. E. Fobian and A. V. Pisciotta. The Departments of Pathology and Medicine, The Medical College of Wisconsin and Milwaukee County Medical Complex, Milwaukee, Wisconsin, U.S.A.

Factor VIII (VIII) inhibitors were isolated from the gamma globulin fraction of the plasmas of three non-hemophilic patients. One of these patients is thought to have acquired the inhibitor secondary to Hodgkin's disease while the other two were apparently drug-related. These patients presented without significant prior replacement therapy and showed elevated levels of von Willebrand factor ($VIII_{VW-R}$) as measured by Ristocetin aggregation of fixed washed platelets and elevated levels of antigenic VIII ($VIII_{AGN}$) using a CELIA technique. The procoagulant VIII ($VIII_{AHF}$) activities, however, ranged from 12-20% of normal activity (one-stage method) and the inhibitors were present in levels of 6-50 Bethesda Units/ml of plasma.

The kinetics of the interaction between these inhibitors and VIII were studied to further characterize the inhibitors. In vitro inactivation of VIII by the inhibitors demonstrated a two-step kinetic model similar to that proposed by Biggs. In vivo neutralization of transfused VIII appeared to follow the same model. The high levels of $VIII_{AGN}$ and $VIII_{VW-R}$ in the presence of low levels of $VIII_{AHF}$ suggest binding of the inhibitor to the procoagulant moiety. Further, two-step inactivation of the procoagulant activity suggests binding of more than one molecule of inhibitor near rather than at the active procoagulant site which results in increasing stearic inhibition of the active site. This is in contrast to simple direct binding of a single molecule of inhibitor as described with bimolecular second order kinetics.

Finally, the presence of high levels of $VIII_{AGN}$ with markedly attenuated $VIII_{AHF}$ activities implies relatively slow clearance of the inhibitor-VIII complex.

1028

ACQUIRED F VIII INHIBITOR IN AN IDENTICAL SUBHEMOPHILIC TWIN. J.W. ten Cate, A. Sturk, C. Breederveld. Univ.Hosp."Wilhelmina Gasthuis" Div. Hemostasis, Amsterdam, The Netherlands.

An identical (94,3% certainty) twin developed a factor VIII inhibitor, almost simultaneously, which may imply a genetic predisposition. Both patients were high responders (18 an 22 Bethesda U). The initial FVIII;C plasma levels gradually decreased from 0.24 U to 0.08 U. Upon transfusion of 2500 U human FVIII concentrate 75% was inactivated immediately and the remaining factor VIII;C activity had a $T_{\frac{1}{2}}$ of 2 h. In order to investigate the $T_{\frac{1}{2}}$ of their own FVIII, plasmaphoresis was performed in one of them and 500 ml plasma was cryoprecipitated as the first step for factor VIII purification. Surprisingly, only 10% of the FVIII;C present in plasma was recovered in the cryoprecipitate. FVIII was purified by Sepharose 6B chromatography. Inhibitor activity eluted in later fractions seperate from FVIII. The void volume fractions were pooled and were negative for FVIII inhibitor activity. Purified FVIII was radiolabeled (^{125}I-lactoperoxidase technique). CIE followed by autoradiogrphy and PAGE of the ^{125}IFVIII, gave normal results. 12 u Ci ^{125}I FVIII was injected i.v. and revealed a biphasic normal half life: $T_{\frac{1}{2}}$ of the first phase was 2.0 h, $T_{\frac{1}{2}}$ of the 2nd phase 19.2 h. This may imply that this patient developed antibodies selectively directed against FVIII neoantigens present in the FVIII concentrates ($T_{\frac{1}{2}}$ 2 h!). However, i.v. administration of DDAVP resulted in a decreasing FVIII inhibitor titer presumably due to complex formation of released endogenous FVIII with the inhibitor. It is therefore concluded that ^{125}I VIII formes complexes with the inhibitor and that these complexes are slowly cleared from the circulation.

1029

DELETERIOUS EFFECT OF ASPIRIN IN THE ASYMPTOMATIC PATIENT WITH SPONTANEOUS FACTOR VIII INHIBITOR. M.E. Klein, S.A. Akman, and P.H. Wiernik. Division of Hematology, University of Maryland School of Medicine, and Baltimore Cancer Research Program, N.C.I., Baltimore, U.S.A.

A patient with spontaneous acquired circulating inhibitor to factor VIII and factor IX is reported. This 80 year old previously well black female developed spontaneous bleeding in the right forearm after ingestion of 650 mg of aspirin the day of the bleed. Acquired anticoagulants were identified by factor assay. Factor VIII and factor IX activities were 1.7% and 60% of normal. Dilution analysis revealed a factor VIII antibody titer of 1: 100. Diagnostic evaluations for collagen vascular disease, rheumatoid arthritis, inflammatory bowel disease, and paraproteinemias were negative. Treatment was instituted on day 1 with prednisone (80 mg per day) and azothiaprine (150 mg per day). Hemorrhage ceased within two days of therapy. After one week of therapy, factor VIII level had risen to 8%. On day 16 azothiaprine therapy was discontinued and prednisone was tapered. There was no further spontaneous bleeding. Aspirin therapy may result in clinically significant hemorrhage in otherwise asymptomatic patients with spontaneous factor VIII inhibitors.