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PARADOXICAL INCREASE IN HUMAN FACTOR VIII AFTER INFUSION OF PORCINE FACTOR VIII CONCENTRATE IN A PATIENT WITH ACQUIRED VARIANT VON WILLEBRAND'S DISEASE.

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A patient with acquired variant von Willebrand's disease was given an infusion of 2000 units of high purity porcine factor VIII (Hyate). Quantitative factor VIII parameters were assessed following infusion and human factor VIII multimers were analysed by radioimmunoelectrophoresis and autoradiography. We have previously described the patient to have acquired von Willebrand's disease due to a circulating inhibitor to the factor VIII complex (B.J. Haematol., 54,233,1983). Prior to infusion plasma from the patient contained factor VIIIC, RRCo, and vWFAg at less than 10 u/dl. Plasma factor VIII multimers showed an abnormal pattern with no high molecular weight bands present despite a normal triplet structure in the low molecular weight forms. After the infusion of porcine factor VIII concentrate a large increase in the levels of plasma VIIIC was detected with a disappearance half-life of 3.5 hours. A specific non-crossreacting immunoradiometric assay (IRMA) showed that plasma levels of porcine vWFAg did not rise significantly after the infusion. Despite this, human vWFAg levels were notably elevated at 1 hour (40 u/dl by Laurell) and 2 hours (30 u/dl by IRMA) post infusion. Similarly, ristocetin induced platelet aggregation and plasma RRCo levels showed significant elevations 2 hours after the infusion. Factor VIII multimers assessed on plasma samples taken over a similar time period revealed the transient appearance of a normal compliment of human factor VIII multimeric forms 2 hours after the infusion of porcine factor VIII conceptrate. This study indicates that the abnormal pattern of factor VIII multimeric bands present in inhibitor-related variant acquired von Willebrand's disease can be transiently normalised by infused porcine factor VIII concentrate. Whether this represents antibody displacement or de novo synthesis is yet to be determined.

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NEW VARIANT FORMS OF von WILLEBRAND'S DISEASE

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Two unusual variants of von Willebrand's Disease (vWD) have been observed; one resembling Type Ic vWD and one an unclassified variant form of the disease. Both are associated with a history of mild bleeding and with a prolonged bleeding time.

Two related patients (father and daughter) presented with reduced vWF activity (RiCoF) and vWF:Ag. Multimer analysis showed the presence of all molecular weight multimers but with a lack of triplet structure. This was confirmed in 3% agarose gels and resembled the pattern of multimers previously described as Type Ic vWD. Both patients responded well to DDAVP.

In the second case the patient had a lowered vWF activity (RiCoF) but normal vWF:Ag, analogous to a Type IIa vWD pattern. Mutimer analysis however demonstrated the presence of all MW multimers with a normal triplet structure. This defect was not detected with two monoclonal antibodies that recognise the GPIb binding site on vWF and is thought to represent a minor abnormality in the vWF molecule in this patient.

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INDUCED ANTIBODIES TO VON WILLEBRAND FACTOR (VWF).

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Plasma from 11 patients have been investigated for the presence of antibodies to VWF using an immunoblotting system. Eight patients had severe haemophilia A and 3 had severe VW's disease (VWD). One haemophiliac had a high titer of neutralizing activity against VIII:C, and two had previously shown neutralizing activity against VIII:C. Neutralizing activity against ristocetin cofactor was demonstrated in only two of the VWD-patients. None of the VWD-patients did show antiactivity to VIII:C. All patients except 2 haemophiliacs had been transfused with factor concentrate within the last three weeks before blood sampling. Four different concentrates had been used.

The following immunoblotting system was used: Highly purified VWF was applied to agarose gels, and electrophoresis performed. The proteins were blotted to nitrocellulose paper and the paper cut into strips. The strips were incubated with diluted plasma or the IgG-fraction of plasma (obtained by precipitation with ammoniumsulphate) from one or the other of the patients. Thereafter, the strips were treated with a second antibody (goat-anti human IgG) labeled with peroxidase before development with α-chloronaphtol. As a negativ control, normal plasma was used. As a positive control, a peroxidase labeled goat-antihuman VWF was used giving the known multimeric pattern of VWF-antigen.

Antibodies to VWF were found in 4 haemophiliacs and in all 3 VWD-patients. The pattern of multimeric bands obtained with the antibodies showed differences in different patients. The in vivo t 1/2 of VIII:C concentrate did not seem to be affected by these antibodies to VWF.

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VON WILLEBRAND FACTOR mRNA IS SEVERELY REDUCED IN PIGS WITH HOMOZYGOUS VON WILLEBRAND DISEASE.

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Porcine von Willebrand disease (vWD), an autosomal recessive disorder, is similar to some of the severe forms of vWD in humans and is characterized by a prolonged bleeding time and very low or undetectable amounts of von Willebrand factor (vWF) antigen and activity in plasma, platelets and endothelial cells. The molecular events that control the lack of expression of vWF in the vWD pigs is not known and could be at the transcriptional or post-transcriptional level. Lungs from normal and two homozygous vWD pigs were extracted immediately after harvesting of the animals and placed on dry ice. Tissues were homogenized in 6 M guanidinium thiocyanate and RNA isolated by centrifugation through cesium chloride. Total RNA was analyzed by Northern hybridization including denaturation in glyoxal, electrophoresis in 1.0 % agarose-2.2 M formaldehyde gels and transfer onto nitrocellulose. Messenger RNA was detected with a nick-translated human vWF cDNA probe or a human actin control probe. The vWF probe, cloned from a human lung library, was 2,280 bp in length and spanned nucleotides 960 to 3,240 of the human cDNA. These human probes were considered valid to detect levels of porcine vWF and actin mRNA because they hybridized with restriction enzyme digested genomic DNA from normal and vWD pig leucocytes under conditions of high stringency. The size of the vWF mRNA in the normal pigs after Northern hybridization was approximately 9.0 kb, similar to that of human vWF mRNA, and was easily detectable at the lowest concentration of RNA blotted (5 ug). In contrast, vWF mRNA from vWD pigs was at the lower limit of detection even at 10 ug of total RNA blotted. Nevertheless, although at extremely low levels, vWF mRNA from vWD pigs appeared to be the same size as the normal mRNA. These results agree with observations on the relationship of vWF secreted from 24 hr. cultures of endothelial cells from the pulmonary artery of normal and vWD pigs where the vWF levels were 0.90 and 0.06 U/10^8 cells, respectively. Therefore, it appears that the very low expression of vWF in the vWD pigs is due to a lack of transcription of the vWF gene. At this time, however, turnover of unstable transcripts in the vWD pigs can not be ruled out.