PHARMACOKINETICS OF HUMAN FACTOR IX IN A DOG WITH SEVERE HEMOPHILIA B. E.A. Centy, A.B. Thompson and A.W. Forrey. Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada and Hematology, University of Washington and Puget Sound Blood Center, Seattle, WA, U.S.A.

In preparing a factor IX concentrate with a high yield and low hepatitis and chromoblastic risks, we have tested this material for survival in an in vivo system, the hemophilia dog. By following the disappearance of radiolabeled, isolated factor IX in addition to the classic clotting assays, data on protein survival and more accurate kinetic parameters were obtained.

Crude factor IX concentrate was prepared by batchwise adsorption-elution with DEAE-Sephadex using cryoprecipitate-poor human plasma. Isolated human factor IX was radiolabeled with 125I by chloramine-T without in vitro loss of clotting activity (Thompson, J Clin Invest, in press, 1977). A preparation containing less than 0.01% radioactivity of the activated partial thromboplastin time (0.22 μM) and lyophilization; clotting and radioactivity were not altered by these steps. Following infusion of the combined preparation into a dog with severe hemophilia B 0X baseline factor IX, 10 post infusion samples were taken over three hours. The radioactivity and factor IX clotting activity. These data were then analyzed by fitting to a two exponential expression using a Marquart non-linear least squares numerical procedure for a two compartment open model. The central volume was 35% of the animal's body weight; the total volume of distribution was 28% with a t 1/2 of 11.4 min. The t 1/2 elimination was 20 h; the slower phase of elimination (b, c) that affected the distribution had a t 1/2 of 40 h. Factor IX clotting activity from the crude concentrate closely paralleled radioactivity from the isolated factor IX throughout the 96 h; t 1/2 was slightly longer from the clotting activity data.


Venous thromboses have been reported following the clinical use of some factor IX preparations. This may be due to several causes as for instance traces of FVIII or thrombin formed during processing or presence of surface activation products involved in the early stages of the coagulation pathway. There is an urgent need for simple and reliable thrombogenicity tests. Activation by Ca²⁺ should preferentially be avoided, as Ca²⁺ by itself is capable of activating the prothrombin complex. All clotting methods are affected by the heparin content (4–5 units) and excess amount of sodium citrate present in the factor IX concentrates. We have therefore developed a colorimetric method using synthetic substrates. The assay is performed in the absence of presence of xonon proteolytic inhibitors in order to improve the specificity of the test system and identify the nature of the thrombogenicity. Using this method, various types of hot material can be classified in three groups according to the rate of cleavage of the substrates. Hot materials have been titrated with several inhibitors. The contaminating enzymes have also been isolated and characterized. The results clearly indicate the presence of two entirely different types of hot material. One group contains mainly FVIII and thrombin and can be neutralized by A1 III. The other group probably contains large amounts of surface products. The "thrombogenicity" of this group can be mimicked by kaolin activation of plasma and inhibited by Trasylol.