818

THE EFFECT OF LOW DOSE HEPARIN ON THE FEEDBACK ACTIVATION OF FACTOR VIII IN VIVO. P.P. Devilée (1), H.F.B.M. Fiolet (2), M.C.E. van Dam-Mieras (1), H.C. Hemker (1). Department of Biochemistry, University of Limburg (1), Department of Internal Medicine, University Hospital Maastricht, (2) Maastricht, The Netherlands.

Heparin is a well established drug in the treatment and prophylaxis of thrombosis. However, the in vitro methods for the control of heparin therapy (aPTT, anti-Xa, anti-II) often are not sufficiently sensitive to monitor low-dose heparin therapy. We used an assay that is based upon the in vivo activation of factor VIII at the site of skin punction: the factor VIII activity in blood samples taken from a capillary wound increases with time (Hurlet-Birk Jenssen et al. Path. Biol. 24, 6 (1976).

Assay: 50 µl blood samples are taken 30, 60 and 90 sec after capillary puncture. The samples are diluted with 950 µl, 10 mM sodium citrate, Mich. buffer and centrifugated (3 min, 4 °C). In the diluted samples the factor VIII activity is determined.

In healthy volunteers the increase in factor VIII activity in the successive samples is fairly constant (1.4 - 1.8 fold) in spite of distinct interindividual differences in the factor VIII level. In patients receiving continuous intravenous heparin (20000 IE/24 h) and in a hemophilia A patient there was no increase in factor VIII activity with the time. In individuals receiving 5000 IE heparin subcutaneously the increase in factor VIII activity was dependent upon the time interval between heparin injection and sampling. There was almost no influence of heparin on the 30 s samples but the 90 and 150 sec. samples showed distinctly prolonged clotting times at sampling points from 2 to 4 hours after heparin administration. It was not possible to detect the heparin effect by an automated anti-IIa assay or in a prothrombin time assay because the heparin levels were below the detection limit of the tests.

These results suggest that low-dose heparin therapy has a definite influence on the feedback activation of factor VIII even in concentrations that are not detectable with the usual heparin tests.

819

VON WILLEBRAND FACTOR PROTECTS FACTOR VIII FROM INACTIVATION BY ACTIVATED PROTEIN C AND PROTEIN S. Joost A. Koedam, Joost C.M. Meijers, Jan J. Sixma, and Bonno N. Bouma. Dept. of Haematology, University Hospital Utrecht, P.O.Box 16250, 3500 CG Utrecht, The Netherlands.

Activated protein C (APC) inactivated the cofactors factor V (FV) and factor VIII (FVIII). In the case of FV, this reaction and the respective roles of Ca^{2+}, phospholipids and protein S have been well documented. We investigated the role of protein S and von Willebrand factor (VWF) on the inactivation of FVIII.

Purified human factor VIII (3 units/ml) was incubated with protein C (0.2 µg/ml) in the presence of 8 µg/ml phospholipid, 5 mM $CaCl_2$, and 1 unit/ml hirudin. Factor VIII coagulant activity decreased with a pseudo first-order rate constant of 0.09 min^{-1}. The reaction rate increased linearly with the concentration of protein S in the incubation mixture.

^{125}I-FVIII was incubated under the same conditions. SDS-polyacrylamide gel electrophoresis showed cleavage products of Mr 43 and 22 kDa. High Mr bands (FVIII-heavy chain) ranging from Mr 108 to 208 kDa disappeared while the Mr 80 kDa FVIII-light chain remained unchanged. The degradation pattern was not changed by addition of protein S.

The FVIII-VWF complex was reconstituted by mixing the two components (± 2 units VWF/units FVIII) and lowering the calcium concentration to 2 mM. The inactivation of the FVIII-VWF complex by APC proceeded at a 15- to 20-fold slower rate as compared to the isolated FVIII, indicating a protection of FVIII by VWF. Protein S exhibited no cofactor activity on the inactivation of FVIII-VWF by APC. The protective effect of VWF was lost completely after activation of the FVIII-VWF complex with thrombin (0.05 units/ml).

When FVIII (0.1 units/ml) was added to plasma of a patient with severe von Willebrand's disease, 96% of its activity was lost in 20 min after the addition of APC. All of the FVIII activity was retained when haemophilic plasma was used. Mixing experiments showed that one unit of VWF unit FVIII is needed to fully protect FVIII against APC. These results may explain the observed lability of FVIII in von Willebrand's disease patients.

820

APSAC versus placebo: A Multicentre Study of Safety and Early Mortality in Acute Myocardial Infarction.
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A preliminary study was carried out to obtain data on mortality, benefit and the safety of anisoylated plasminogen streptokinase activator complex (APSAC). 90 patients from 7 centres, with symptoms of acute myocardial infarction of not more than 4 hours duration, were randomised to receive either 30U of APSAC as an intravenous injection over 4 to 5 minutes or placebo given by the same route. 45 patients received APSAC and 45 placebo, mean time to treatment was 3 hours 20 minutes and 3 hours, respectively.

Mortality at 30 days was 7 deaths in the placebo group and 1 death in the APSAC group (p=0.058), these were all related either directly or indirectly to the infarct: cardiogenic shock (2), ventricular asystole (2) further acute myocardial infarction (2), ventricular fibrillation (1) and pulmonary embolism (1). The patient who died after APSAC therapy had presented with an anterior infarction; 5 patients who died in the placebo group presented with anterior infarcts and 2 with inferior infarcts. Most deaths occurred in the group randomised 2 hours 30 minutes to 4 hours post-infarction.

Both systolic and diastolic blood pressure were generally similar in each group from 1 hour to 1 month after treatment. 35 per cent of patients in the placebo group experienced cardiovascular events (2 of whom died from cardiogenic shock) compared with 20 per cent of APSAC treated patients (1 of whom died from pulmonary embolism). Minor haemorrhagic adverse events were experienced by 22 per cent of patients on APSAC compared with 6.6 per cent on placebo. These events were uncomplicated and did not require blood transfusion. Haematuria was apparent in a greater number of patients in the APSAC group after 12 hours, but there was no difference between groups after 1 or 2 weeks.

The results of this study, although encouraging, should be interpreted with caution, mainly due to the small numbers of patients enrolled. There was however a trend towards increased survival in the APSAC treated group (97.8 per cent) compared with 84.4 per cent survival in the placebo group at 30 days post-infarction.

821

APSAC VERSUS PLACEBO: THE FIRST PLACEBO CONTROLLED CORONARY RECANALISATION STUDY WITH PRE AND POST TREATMENT CORONARY ARTERIOGRAPHY. A.D.Timmis (1), B.Griffin (1), J.C.P.Crick (1), J.S.Flax (2), E.Sowton (1). Guy's Hospital, St. Thomas's Street, London, UK (1) and Beecham Pharmaceuticals, Brentford, UK (2).

40 patients entered the first double-blind placebo controlled invasive arteriographic study of intravenous anisoylated plasminogen streptokinase activator complex (APSAC) for coronary recanalisation in acute myocardial infarction. Coronary arteriography was performed before and 90 minutes after a single intravenous injection of APSAC or matched placebo given over 2-5 minutes. Pre-treatment coronary arteriography was performed in all 40 patients 3.1 ± 1.2 hours after the onset of major symptoms of myocardial infarction. All coronary arteriograms were read blindly and scored according to the TIMI criteria by an independent cardiologist. Following randomisation occlusion of the infarct related coronary artery was demonstrable in 29 of the 40 cases at pre-treatment coronary arteriography. Patients were given either APSAC 30 units (n=16) or matched placebo (n=13), 3.3 ± 1.3 hours after the onset of symptoms. Repeat coronary arteriography was performed 90 minutes after administration of study drug. 9 of the 16 patients who received APSAC had demonstrable coronary recanalisation of the infarct related vessel compared with only one patient in the placebo group (P 0.05). A third full diagnostic coronary arteriogram was performed three days after treatment and this showed persistent patency of all the recanalised coronary arteries except one in the APSAC group as well as late recanalisation in a further 4 cases, 3 of whom had received APSAC.

Of the 11 patients who had patent infarct related arteries on initial arteriography, 4 received APSAC and 7 placebo. Patency was maintained in all these throughout the study period. There were haemorrhagic complications related to APSAC therapy. This data confirms the thrombolytic efficacy of APSAC in acute myocardial infarction, and indicates that the drug may be used safely by the intravenous route with a 56 per cent recanalisation rate at 90 minutes. Re-occlusion of the recanalised vessel was rare.