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COMPARISON OF ENZYMATIC PROPERTIES OF HUMAN PLASMA FVIIa AND HUMAN RECOMBINANT FVIIa. T. Lund-Hansen and L.C. Petersen. Novo Research Institute, Bagsvaerd, Denmark.

Human plasma FVIIa (pFVIIa) and human recombinant FVIIa (rFVIIa) were both purified by immune adsorption chromatography using a calcium dependent monoclonal antibody. The FVII obtained is highly purified and contains only trace contaminants as revealed by SDS-PAGE and reverse phase HPLC chromatography. FVII was fully activated during the purification procedure. A FVIIa activity assay has been developed in microplates using human FX as a substrate and methoxycarbonyl-D-cyclohexyl-alanyl-glycyl-arginine-pNA as a chromogenic substrate for the FXa generated. The assay was linear at FVIIa concentrations between 0.5 and 10 nM. The concentration of the chromogenic substrate was 0.5 mM. A pH optimum at about 8 was found. An apparent $K_m = 0.2 \mu\text{M}$ for FX was found for both pFVIIa and rFVIIa. The results suggest that the kinetics of human FX activation by pFVIIa and rFVIIa are identical. The FVIIa activity was found to be calcium dependent with maximal activity at about 0.25 mM, while the activities at 1 and 2 mM were 20% and 3%, respectively. When rabbit brain extract is used, the well-known dramatic enhancement effect of thromboplastin could be demonstrated with both FVII preparations. Also this reaction is calcium-dependent; however, the profile of the curve is distinctly different. Poly-D-lysine (MW 160,000) was found to enhance the FVIIa activity in a concentration dependent manner. Maximum stimulation (five-fold) was obtained at a concentration of about 10 mg/l.

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COMPARISON OF THE EFFECT OF FACTOR VII PREPARED FROM HUMAN PLASMA (pVIIa) AND RECOMBINANT VIIa (rVIIa) IN VITRO AND IN RABBITS. U. Hedner, T. Lund-Hansen and D. Winther. Novo Research Institute, Bagsvaerd, Denmark.

Purified human FVIIa has been shown to induce haemostasis in pat. with haemoph. A compl. with antibodies against VIII:C (Hedner & Kisiel 1983). The in vitro effect of addition of pVIIa or rVIIa was studied by adding 0, 44, or 180 u pVIIa/rVIIa/ml to haemoph. A or haemoph. B plasma. The APTT shortened from 61 s (mean of 5 determin.) without any VIIa added to 38 s after addition of 44 u pVIIa to haemoph. A plasma, and to 33 s after addition of 180 u/ml. In haemoph. B plasma the corresponding APTTs were 64 s, 35 s (44 u pVIIa/ml VIIa) and 31 s (180 u pVIIa/ml). Similar results were obtained when rVIIa was added to the same plasmas. In haemoph. A plasma APTT was shortened from 66 s to 52 s (50 u rVIIa/ml) and to 41 s (159 u rVIIa/ml); and in haemoph. B plasma 71 s, 44 s, and 37 s. Also in plasma from pat. with acquired antibodies against VIII:C a shortening of APTT was found both of pVIIa (60 s, 37 s, and 32 s) and rVIIa (65 s, 38 s, and 33 s). In normal plasma the APTT only shortened a few seconds after addition of the same amounts of pVIIa/rVIIa per ml (30 s, 27 s, 25 s). The doses required to normalize the APTT in vitro exceed substantially the doses used in vivo so far. The pVIIa and rVIIa were therefore also given i.v. to rabbits. Plasma samples were drawn before inj., 15 min, 2 hrs, 4 hrs, 8 hrs, and 24 hrs after and platelets; APTT, fib.gen, ATIII, $\alpha_2\text{M}$, $\alpha_2\text{AP}$, ethanol gel. test, FVII, Hb, Hkr were followed. Doses of 500 u/kg b.w., 1000 or 5000 u/kg b.w. were given. No signs of a gen. activ. of the coag. were observed. The FVII level in plasma rose adequately. In conclusion higher doses of VIIa than used clin. so far may be needed to achieve full haemost. effect in haemoph. pat. or pat. with acq. antibodies against VIII:C. Such doses also seem to be safe. rVIIa was as active as pVIIa and as safe in rabbits.

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THROMBOLYSIS: NEW THERAPEUTIC STRATEGIES

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PLASMA-CLOT LYSIS INDUCED BY MONOCLONAL ANTIBODY AGAINST α_2 -PLASMIN INHIBITOR. Y. Sakata (1), J. Mimuro (1), M. Matsuda (1) and Y. Koike (2). Institute of Hematology, Jichi Medical School, Tochigi-Ken (1), Central Research Laboratories, Teijin Limited, Tokyo (2), Japan.

A Monoclonal antibody (MCA) against α_2 -plasmin inhibitor (α_2 -PI) designated as JTP-1 inhibited antiplasmin activity and complex formation of α_2 -PI with plasmin. By using this MCA we tried to observe plasma-clot lysis (CL) in vitro and to estimate the level of total fibrinolytic capacity in plasma. As reported previously (Blood 55: 483, 1980) spontaneous CL is a striking feature of the plasma derived from a patient with α_2 -PI deficiency. We showed that this abnormally increased fibrinolysis was solely due to the deficiency of α_2 -PI. However, the contribution of plasminogen activator (PA) and its inhibitor to this specific patient's plasma-CL has been under discussion. Therefore, to test whether similar CL can be found in normal plasma without an addition of PA, plasma clots were made after incubation of plasma from normal volunteers containing ^{125}I fibrinogen with various concentration of JTP-1, and fibrinolysis was measured by counting the soluble radioactivity. The addition of JTP-1 to plasma led to a dose-dependent enhancement of the soluble ^{125}I fragment-release from the clot. However, JTP-1 had no effect on α_2 -PI-deficient plasma-CL. Other anti- α_2 -PI MCAs whose epitopes were not involved in the reactive site of α_2 -PI had no effect on CL and rabbit anti-mouse immunoglobulin IgG neutralized this JTP-1-inducing CL completely. Immunodepletion of tissue PA (tPA) or plasminogen from plasma decreased the rate of CL but that of prourokinase did not. To determine the role of PA inhibitor (PAI) released from platelets (plts) in the regulation of CL in vitro, plasma clots were made from plts poor plasma (PPP) and plts rich plasma (PRP), and CL was observed in the presence of JTP-1. There was almost no difference of the lysis time between PPP clot and PRP clot, although plasma clot from pregnant woman was lysed slowly. These results strongly suggest that endogenous t-PA in plasma is still functionally active after blood collection and CL is mainly prevented by α_2 -PI in vitro.

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EFFECT OF RECOMBINANT TISSUE-TYPE PLASMINOGEN ACTIVATOR (rt-PA) ON THE PREVENTION OF INTRAABDOMINAL ADHESION FORMATION. M. Mohler, S. Hollenbach, T. Nguyen, V. Reger* and A. Hotchkiss. Genentech Inc., South San Francisco, CA, USA and *Virginia Mason Hospital, Seattle WA, USA

The formation of intraperitoneal adhesions, the pathological adherence of organs and tissue surfaces, is the leading cause of postoperative intestinal obstruction following abdominal surgery, as well as the major contributing factor to infertility after reconstructive tubal surgery. At present, there is no generally accepted therapy for the prevention of adhesion formation. Since fibrin has been shown to be the physiological basis of adhesion formation, the current study was undertaken to determine if an application of exogenous rt-PA to the site of peritoneal injury would prevent adhesion formation. New Zealand white rabbits (3 kg) were anesthetized and midline laparotomies performed. A nine cm² area of the peritoneal wall was surgically removed and resutured in place, creating an ischemic patch of peritoneal tissue. A proximal area of the cecum (75cm²) was abraded with dry gauze until punctate bleeding occurred. Approximately 2.5 grams of an ointment containing 0, 0.16, 0.31, or 0.63 mg rt-PA was applied to the ischemic peritoneal tissue and the abraded cecum. After seven days the rabbits were euthanized and the adhesions scored. In the rabbits which received placebo ointment (n=6) the cecum was adhered to the entire area of the ischemic patch. In the rabbits which received the ointment containing 0.63 mg of rt-PA, five had no adhesions and one rabbit had a very minor adhesion. The high dose of rt-PA was equally effective in preventing reformation of adhesions after surgical lysis. No evidence of systemic fibrinogen degradation or abnormal wound healing were evident in this model.

In summary, these studies demonstrate that intraperitoneal administration of rt-PA is effective in preventing initial adhesion formation as well as the reformation of adhesions after surgical trauma or injury.