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"NEW" COAGULATION INHIBITORS LEVELS IN PNEUMONIA, DISSEMINATED INTRAVASCULAR COAGULATION AND LIVER DISEASES. T.R.Andersson, H.Bell, P.M.Sandset, O.R.Ødegaard and L-M. Aamodt. Haematological Research Laboratory and Medical Department, Aker Hospital, Oslo 5, Norway.

The activity levels of the "new" coagulation inhibitors, heparin cofactor II (HC II) and extrinsic pathway inhibitor (EPI), have been determined with chromogenic substrates assays, in patients with pneumonia (n=8), disseminated intravascular coagulation (DIC) (n=8) and various liver diseases (n=19). For comparison antithrombin (AT) and Protein C (PC) were also measured. In cases with DIC low values (<50%) for HC II, AT and PC were found, while EPI showed a much greater variation (60-190%). Persistent low values heralds a poor prognosis. In survivors is rapidly normalized. In pneumonia, initially low levels (except HC II), were normalized on day 7. HC II may be an acute phase reactant.

	Pneumonia		Liver cirrhosis	
	Day 1	Day 7	mean (SD)	range
AT	72 ± 11	100 ± 8	45 ± 17	32-70
PC	73 ± 12	100 ± 10	51 ± 26	28-100
HC II	104 ± 24	143 ± 11	41 ± 27	14- 90
EPI	90 ± 28	110 ± 22	122 ± 44	53-182

Conclusion. In cirrhosis, subnormal HC II values suggests reduced synthesis. High EPI values in cirrhosis suggests extrahepatic synthesis. The mechanisms for reduced HC II in DIC, might besides consumption and reduced synthesis, be the liberation of dermatan sulfate from injured intima with increased consumption. Changes in HC II, AT and PC are similar, whereas EPI seems to have different production and metabolism.

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EXTRINSIC PATHWAY INHIBITOR (EPI): A SENSITIVE CHROMOGENIC SUBSTRATE ASSAY DEMONSTRATES THE RELEASE OF EPI TO THE BLOOD AFTER INJECTION OF HEPARIN. U. Abildgaard, P.M. Sandset, M. Pettersen and M. Hultin. Haematological Research Laboratory, Aker Hospital, Oslo 5, Norway.

Control of the extrinsic pathway has been ascribed to negative feedback or to plasma inhibitors. Sanders et al recently showed that the inhibitor in plasma cooperates with F X in the inhibition of the tissue thromboplastin (TP)-F VIIa complex (Blood 1985, 66, 204). Our chromogenic substrate (CS) assay for the plasma inhibitors (Dahl PE et al, Thromb Haemost 1982, 48, 253) was manipulated and the results confirm the cooperative effect of F Xa and EPI. When 1 ul of heated plasma was incubated with TP (1/100 dil) and amounts of F VII and F X similar to those in 1 ul of normal plasma, progressive inhibition of TP-F VII was observed. Using higher amount of F X, the activation to F Xa dominated over inhibition of TP-F VIIa. With optimal amounts (0.0005 U F VII, 0.0012 U of F X) in a volume of 250 ul, a 50% inhibition of TP-F VIIa developed in 10 minutes. Remaining TP-F VIIa was determined by adding 0.02 U of F X and, after 10 minutes, the CS S-2222. The A405 value is inversely related to EPI activity of test plasma or plasma fraction. Prior to assay, functional F II, F VII, F IX and F X in test plasma must be abolished, either by $BaSo_4$ adsorption or by heating to 56° for 15 minutes. $BaSo_4$ removes about 35% of EPI, heating about 15%. Antithrombin and heparin effects in the assay are prevented by specific antibodies and polybrene. EPI activity is reduced by phosphlipase C, suggesting lipoprotein nature. EPI is not released by venous occlusion, but following injection of heparin, EPI increases to 150-250% of base values. High EPI values are found in plasma from patients on heparin. We suggest that this increase in EPI contributes to the antithrombotic effect of heparin. Gel filtration separates plasma EPI into 3 distinct peaks. The macromolecular peak accounts for more than half the activity in normal plasma. The activity of this peak increases markedly following heparin injection.

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INHIBITOR OF THE FACTOR VII_A-TISSUE FACTOR COMPLEX IS REDUCED IN PATIENTS WITH DISSEMINATED INTRAVASCULAR COAGULATION BUT NOT IN PATIENTS WITH SEVERE HEPATOCELLULAR DISEASE. M.S. Bajaj, S.V. Rana, R.B. Wysolmerski, and S.P. Bajaj. St. Louis University School of Medicine, St. Louis, MO USA.

Recently, inhibition of factor VIIa-tissue factor activity by a plasma component(s) which requires factor Xa has been described. In this communication, we have developed a specific radiometric assay (which utilizes ^{3}H-factor IX and is sensitive to <1% of plasma level) for this inhibitor and have measured its activity in various disease states. Strikingly, the levels of this inhibitor were found to be normal in patients with advanced chronic hepatocellular disease but low in patients with disseminated intravascular coagulation (DIC). When endotoxin was used to induce DIC in rabbits, the levels of this inhibitor fell by 30 to 90%. Human umbilical vein endothelial cells (HUVE), bovine pulmonary artery endothelial cells, and a human hepatoma cell line (HepG2) all synthesized and secreted this inhibitor whereas a promyelocytic cell line (HL-60) did not and a monocytic cell line (U937) appears to synthesize only small amounts. When ammonium sulfate fractionated human plasma, and serum-free conditioned media from both HUVE and HepG2 cells were electrophoresed on sodium dodecyl sulfate acrylamide gels, two activity peaks corresponding to Mr ≃45,000 and Mr ≃33,000 were eluted in each case. These observations suggest that (a) the inhibitor is consumed in DIC and that (b) endothelial cells (or other cells) synthesize sufficient amounts of this inhibitor in vivo to compensate for any decreased production by liver cells. Furthermore, the inhibitor levels were found to be normal in patients on chronic warfarin therapy suggesting that the inhibitor is not a vitamin K-dependent protein.

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RECOMBINANT HIRUDIN INHIBITS EXPERIMENTAL VENOUS THROMBOSIS INDUCED BY INJECTION OF TISSUE FACTOR AND STASIS. M. Freund (1), J.-P. Cazenave (1), M.-L. Wiesel (1), C. Roitsch (2), N. Riehl-Bellon (2), G. Loison (2), Y. Lemoine (2), S. Brown (2) and M. Courtney (2). INSERM U.311, Centre Régional de Transfusion Sanguine, Strabourg, France (1) and Transgène S.A., Strasbourg, France (2).

Hirudin (HIR), a polypeptide of 65 aminoacids, is the most potent natural inhibitor of coagulation by forming rapidly a very stable and specific non covalent 1:1 complex with α-thrombin, independent of antithrombin III. Although natural HIR has in vivo anticoagulant and antithrombotic properties, its limited availability for large scale purification has prevented further clinical testing and potential use; this can now be solved by recombinant DNA technology. We have previously reported the cloning and expression of a cDNA encoding one variant (called HV-2) of Hirudo medicinalis HIR (Proc. Natl. Acad. Sci. USA. 1986, 83, 1084-1088). The main factors responsible for venous thrombosis are stasis and thrombin generation secondary to tissue factor liberation from vascular cells and monocytes by injury, endotoxin, interleukin-1 or cachectin and the subsequent activation and circulation of activated clotting factors. We have studied the antithrombotic properties of recombinant HIR, HV-2, in a rat experiemental model of venous thrombosis. HV-2 was expressed in yeast, extracted from culture supernatant and purified by HPLC. Pure HV-2 had an isoleucine NH_2-terminus and a specific activity of 13000 ATU/mg. 30 male Wistar rats (225-300g) were anesthetized with pentobarbital. At time t (0 min) an i.v. (penis) injection of 0.4 ml of saline or HV-2 (2000 to 8000 ATU/kg) was given, followed at t (5min) by 25 mg/kg tissue factor (Thromboplastin C, Dade) i.v. ; 10 s later stasis of the exposed vena cava between 2 sutures 0.7 cm apart and at t (15 min) removal, blotting, fixation and weighing of the thrombus. Linear regression analysis showed a correlation (r=0.99) between the dose of HV-2 and thrombus weight and a calculated IC_{50} = 3000 ATU/kg. Total inhibition of thrombus formation was seen after injection of 6000 ATU/kg HV-2 and lasted up to 15 min of circulation, HV-2 being completely eliminated from blood in 60 min and accumulated in the kidneys as shown by gamma imaging with ^{131}I-HV-2. In conclusion, the recombinant HIR HV-2 is a potent immediate antithrombin which inhibits venous thrombosis induced by tissue factor and stasis.