The activity levels of the "new" coagulation inhibitor, Hirudin (HIR), have been determined with chromogenic substrate assays in patients with pneumonia (n=8), disseminated intravascular coagulation (DIC) (n=8) and various liver diseases (n=19). For comparison antithrombin III (AT) and Protein C (PC) were also measured. In cases with DIC low levels (<50%) for HC II, AT and PC were found, while EPI showed a much greater variation (60-190%). Persistent low values herald 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.

**Conclusion.** In cirrhosis, subnormal HC II values suggest reduced synthesis. High EPI values in cirrhosis suggest extrahepatic synthesis. The mechanisms for reduced HC II in DIC might be increased consumption and reduced synthesis, i.e. liberation of dermatan sulfate from injured intima with increased consumption.

Changes in HC II levels are similar, whereas EPI seems to have different production and metabolism.

**EXTRINSIC PATHWAY INHIBITOR (EPI): A SENSITIVE CHROMOGENIC SUBSTRATE ASSAY DEMONSTRATES THE RELEASE OF EPI TO THE BLOOD AFTER INJECTION OF HEPARIN.**

Control of the extrinsic pathway has been ascribed to negative feedback or 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 1986, 68, 66). In the chromogenic substrate 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 U of heated plasma was incubated with TP (1/100 dil) and amounts of F VII and F X similar to those found in normal plasma, EPI was not released. Higher amount of F X, the activation to F Xa dominated over inhibition of TP-F VIIa.

**Hirudin (EIR), a polypeptide of 65 amino acids, is the most potent natural anticoagulant available.** 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 HIR 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 experimental model of venous thrombosis. HV-2 was expressed in yeast, extracted from culture supernatant and purified by HPLC. Pure HV-2 had an esculin MU-ase activity and a specific activity of 13000 MU/mg. Male water rats (225-300 g) were anesthetized with pentobarbital, at 0.125 g/kg i.p. An i.v. infusion of normal saline at a rate of 0.1 ml/kg/min for 1 hour was given. Followed at t (5 min) by 25 mg/kg tissue factor (Thromboplastin C, Biak) i.v. and, 10 later, 0.5 U/kg heparin. Platelet aggregation, clotting, clot remodelling, coagulation and thrombus formation.

**COAGULATION INHIBITION: GENERAL**

**INHIBITOR OF THE FACTOR VIIA-TISSUE FACTOR COMPLEX IS REDUCED IN PATIENTS WITH DISEMINATED INTRAVASCULAR COAGULATION BUT NOT IN PATIENTS WITH SEVERE HEPATOCellular DISEASE.**

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 3H-factor IX and is sensitive to ~10% 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 of this inhibitor and have measured its activity in various disease states. This suggests that (a) the inhibitor is consumed in 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 30-80%.

**CONCLUSION.** 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 experimental model of venous thrombosis. HV-2 was expressed in yeast, extracted from culture supernatant and purified by HPLC. Pure HV-2 had an esculin MU-ase activity and a specific activity of 13000 MU/mg. Male water rats (225-300 g) were anesthetized with pentobarbital, at 0.125 g/kg i.p. An i.v. infusion of normal saline at a rate of 0.1 ml/kg/min for 1 hour was given. Followed at t (5 min) by 25 mg/kg tissue factor (Thromboplastin C, Biak) i.v. and, 10 later, 0.5 U/kg heparin. Platelet aggregation, clotting, clot remodelling, coagulation and thrombus formation.

**RECOMBINANT HIRUDIN INHIBITS EXPERIMENTAL VENOUS THROMBOSIS INDUCED IN INJECTION OF TISSUE FACTOR AND STAGE.**

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