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INCORPORATION OF PLASMINOGEN ACTIVATOR INHIBITOR INTO FIBRIN, AN ALTERNATIVE REGULATORY PATHWAY OF FIBRINOLYSIS. H. Murayama and N. U. Bang, Indiana University School of Medicine, Dept. of Medicine, Lilly Laboratory for Clinical Research, Eli Lilly and Company, Indianapolis, IN, U.S.A.

A plasminogen activator inhibitor (PAI-1) M_r 50 kd is normally found in plasma at low concentrations. Plasma levels increase sharply upon stimulation of endothelial cells with endotoxin or monokines and activated platelets secrete significant quantities of PAI-1. It is possible that high levels of PAI-1 may be achieved at the local sites of intravascular thrombi. Semi-purified PAI-1 was therefore prepared from human platelets to study its affinity for fibrin (F). Approximately 50% PAI-1 adsorbed to F monomer immobilized on sepharose and desorbed under conditions of acidic pH and high ionic strength suggesting hydrogen bonding as the mode of interaction. Wells of 96-well microtiter plates were each coated with 50 µg [^{125}I] plasminogen (P)-free fibrinogen and clotted with thrombin in the presence and absence of different concentrations of PAI-1. After extensive washing of the wells, they were incubated with 5 mU of tissue plasminogen activator (t-PA) and 5 mU of P for 6 h. Appropriate calibration curves utilizing different concentrations of t-PA and different concentrations of PAI-1 added to the supernatant rather than to F established that 8-15% of 21-166 mU PAI-1 incorporated into crosslinked (XL) F or noncrosslinked (NXL) F. Incorporated PAI strikingly inhibited fibrinolysis (FL). Percent inhibition of FL of XL or NXLF (Mean±S.D., N=5) plotted in the presence of 166, 83, 42 and 21 mU of PAI were: 83±3.3, 59.5±1.8, 29.7±5.2 and 15.2±6.14 for XLF and 78±5.3, 31±8.1, 14.5±10.5 and 0 for NXL F. As demonstrated by radioautography on SDS PAGE PAI-1 incorporated into F readily formed complexes with [^{125}I] urokinase (u-PA). In these experiments, no evidence for crosslinking of PAI-1 into F has been obtained to date. In experiments utilizing agarose immobilized proteins, it was evident that not only F but also fibrinogen binds PAI-1; PAI-1 associated with F as well as fibrinogen is capable of forming complexes with [^{125}I] u-PA. In contrast, fibronectin, collagen, gelatin and albumin did not bind PAI-1. Thus, PAI-1 in analogy with alpha-2 plasmin inhibitor may modulate physiological fibrinolysis through incorporation into fibrin.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF PLASMINOGEN ACTIVATOR INHIBITOR (PAI) IN TISSUE. B. Risberg(1), G.K Hansson(2), E. Eriksson(1) and B. Wiman(3). Dept. of Surgery I (1) and Clinical Chemistry (2), Sahlgrenska Sjukhuset, Göteborg and Dept. of Clinical Chemistry (3), Karolinska sjukhuset, Stockholm, Sweden.

The origin of tissue plasminogen activator inhibitor (PAI) has not been fully elucidated. Platelets are rich in PAI and endothelial cells (EC) in culture produce the inhibitor (PAI 1), which seems to be a major secretory protein. Another inhibitor (PAI 2) has been demonstrated in the placenta. In the present study we localized PAI 1 in various human tissues using a polyclonal antibody against human PAI 1 and fluorescence technique. Tissue sections were incubated with a polyclonal rabbit-anti-human PAI antibody in various dilutions followed by incubation with biotinylated goat-anti-rabbit IgG and FITC-labelled Avidin. Positive identification using this technique was made in endothelium of liver sinusoids and in hepatocytes. Most vessels in systemic and pulmonary circulation showed positive fluorescence in the endothelial layer. No quantitative evaluation was possible with this technique. Synthesis of PAI in liver could provide an explanation for the efficient inactivation of tissue plasminogen activator (t-PA) during liver passage. Localization of PAI in vascular tissue corroborated studies from EC cultures.

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THE EFFECTS OF THE PAI DERIVED FROM HUMAN PLATELETS ON TWO DIFFERENT TYPES OF t-PA. T. Yasukouchi (1), T. Fujie (2), S. Sakurama (2), M. Satoh (1), M. Ikeo (2) and S. Nakagawa (2). Department of Medicine,Higashi Nippon Gakuen University School of Dentistry, Ishikari-gun,Hokkaido, 061-02, Japan (1) and The second Department of Medicine, Hokkaido Unversity School of Medicine, North 14, West 5, Sapporo, 001, Japan (2).

We studied on the effect of PAI derived from human platelets on two different kinds of t-PAs; one was purchased from BioPool, Sweden, (human uterus or melanoma cell derived one-chain t-PA: one-chain u-mt-PA) and another was kindly supplied from Sumitomo Pharm. Co., Osaka, (recombinant two-chain t-PA: two-chain rt-PA). As the PAI-rich solution derived from platelets. We used the platelets extract, which was prepared as follows: The concentrated platelet-rich plasma (Red Cross Japan) was washed with phosphate buffer containing 0.4 % Triton X-100. The inhibitory activities of the PAI were measured by the method of parabolic rate assay and were calcurated from the activities remained in the mixtures of PAI and the t-PAs. The reactions of the PAI and the t-PAs were also analysed by the method of fibrin autography. The PAI suppressed the activity of two-chain rt-PA completely within 5 minutes, but could not suppressed that of one-chain u-mt-PA completely within such a short incubation time. These facts also recognized on fibrin autography. The molecular weights of both free t-PAs were recognized at 60 kDa and that of the complex of the PAI and two-chain rt-PA was recognized at 110 kDa, respectively. The fibrinolytic zone of the complex of PAI and one-chain u-mt-PA, however, could not be recognized. This indicates two possibilities; one is too small amounts of complex forming to detect and another is that SDS can not reveal the t-PA activity from the complex of PAI and one-chain u-mt-PA on fibrin autography. It remained unclear that the difference of the reactions of these t-PAs to PAI had been induced from whether the difference between one- and two-chain, or that between rt-PA and u-mt-PA. If the former is reasonable, it can be said that the molecular form of t-PA has an important significance in physiological fibrinolytic mechanism.

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EFFECT OF DEXTRAN ON PLASMINOGEN ACTIVATOR INHIBITOR (PAI). T. Saldeen (1), R. Moalli (2), F.M. Hasan (2), A. Carvalho (2), M. Eriksson (1). Department of Forensic Medicine, University of Uppsala, Uppsala, Sweden (1) and Brown University, Providence, USA (2).

Adult respiratory distress syndrome (ARDS) is often associated with impaired fibrinolysis and appearance of microemboli in the lung. Increased production of PAI, associated with acute lung injury, may contribute to this process. In patients at risk of developing microembolic complications, administration of dextran reduced the incidence of ARDS. Accordingly, we studied the effect of dextran administration on plasma PAI levels in a rabbit model of endotoxin-induced lung injury and in patients. E coli endotoxin (500 µg/kg bw, infused over 5 min) was administrered to three groups of anesthetized, ventilated rabbits; 10 rabbits were pretreated and maintained with a 10% dextran-40 infusion (20 ml/kg bw), 6 received a 5% albumin solution (20 ml/kg bw) and 8 control rabbits received saline. Plasma PAI levels were measured by chromogenic assay. By 180 minutes after endotoxin infusion, PAI levels increased from 28 ± 6 to 155 ± 7 U/ml in the saline group, to 152 ± 10 U/ml in the albumin group and to 85 ± 8 U/ml in the dextran group.
24 surgical patients were given 500 ml Dextran 70 (Macrodex) and PAI and t-PA were determined before, during and after dextran. PAI levels decreased 6 ± 8 U/ml during dextran infusion and 11 ± 11 U/ml (26 per cent) after dextran, and was significantly decreased after correction for hemodilution. t-PA antigen levels were not changed due to dextran. Dextran thus has an effect on PAI levels which is not due to release of t-PA. Dextran by decreasing PAI levels may have unique salutary effects on fibrinolysis which may be important in the prevention of thrombotic disorders.