Thromb Haemost
[HEADQ 69(6) 528-1155 (1993) June 30, 1993]
ISSN [illegible] 6245

27

Thrombosis and Haemostasis

Journal of the International Society on Thrombosis and Haemostasis

Abstracts

XIVth Congress of the International Society on Thrombosis and Haemostasis
New York, New York, USA
July 4 – 9, 1993

Schattauer

28

INHIBITORS OF THE FIBRINOLYTIC SYSTEM AND RELATED PROTEINS IN PATHOLOGICAL THROMBI. Linda A. Robbie, Nuala A. Booth, Alison M. Croll and Bruce Bennett. Departments of Medicine & Therapeutics and Molecular & Cell Biology, University of Aberdeen, Scotland UK.

The activity of the fibrinolytic system is regulated by plasminogen activator inhibitor (PAI-1) and α_2-antiplasmin (α_2-AP). We have previously shown that both platelet PAI-1 and plasma α_2-AP are important determinants of clot lysis *in vitro*. This study examines the concentrations of these inhibitors and related proteins in human pathological thrombi obtained at vascular surgery. PAI-1, t-PA and t-PA-PAI-1 complex were detected in thrombus extracts by specific ELISAs, while α_2-AP and vitronectin were measured by immuno-electrophoretic methods. In addition, immunostaining by the APAAP technique was performed. PAI-1 and α_2-AP were present at high concentrations, 158.7-8260.1 ng/g and 2.2-48.3 µg/g respectively. The t-PA concentrations were 0.9-58.8 ng/g, with only small amounts of t-PA-PAI-1, 0.07-0.13 pmol/g. Plasminogen concentrations were in the range 15.6-126.7 µg/g. Vitronectin was detected at around 4.2 mg/g. APAAP staining confirmed the presence of abundant PAI-1 (++) and α_2-AP (+++), with relatively little t-PA (+). The high concentration of PAI-1, up to 40 times greater than that in circulating blood, presumably reflects recruitment of platelets during thrombus formation and may also be due to local synthesis by the cells of the vessel wall. The presence of vitronectin at such high concentrations might well stabilise the PAI-1 present. The α_2-AP present in the thrombi can be accounted for by its high plasma concentration, together with its capacity to be cross-linked to fibrin. The low concentrations of t-PA, free and in complex with PAI-1, are consistent with the persistence of these pathological thrombi. Both PAI-1 and α_2-AP may contribute significantly to the resistance of thrombi to lysis *in vivo*.

29

CLONAL VARIATION OF SECRETION OF PLASMINOGEN ACTIVATORS AND THEIR INHIBITOR (PAI-1) AND THE METASTATIC POTENTIAL IN HUMAN FIBROSARCOMA (HT1080) CLONES

Eizo Matsuda[1], Hiroyuki Tsuchiya[2], Shin-ichi Katsuta[2], Hiroki Abe[2], Peter Hufnagl[1], and Bernd R Binder[1]. [1]Lab. Clin. Exp. Physiology, Univ. Vienna, Austria; [2]Dept. Orthopedics, Kanazawa Univ. Med. Sch. Japan, [3]Dept. Med. Kumamoto Univ. Med. Sch. Japan.

We could show recently that in subclones of HT-1080 obtained by consecutive in vivo passage through lung metastases, the metastatic potential correlated directly with their type 1 plasminogen activator inhibitor (PAI-1) and tissue factor (TF) expression and indirectly with their urinary-type plasminogen activator (uPA) expression. It was the aim of this study to investigate whether the metastatic potential of subclones of HT-1080 correlates also to their fibrinolytic potential in vitro. We selected subclones by the limited dilution method and subsequent screening for levels of tissue-type plasminogen activator (tPA) antigen, uPA antigen and PAI-1 antigen using enzyme linked immunosorbent assays (ELISAs). 4 stable clones with different fibrinolytic potential could be obtained (1-3B : PAI-1 98.2ng/10^5cells, uPA 26.6ng/10^5cells, TF 4.36U/10^5cells ; 1-3C : PAI-1 24.6ng/10^5cells, uPA 5.80ng/10^5cells, TF 4.32U/10^5cells ; 23-4B' : PAI-1 535ng/10^5cells, uPA 34.9ng/10^5cells, TF 13.8U/10^5cells ; 26-6 : PAI-1 985ng/10^5cells, uPA 5.52ng/10^5cells, TF 10.0U/10^5cells) ; there was no significant difference in the tPA antigen levels. TF activity was evaluated by measurement of prothrombin complex formation and chromogenic substrate conversion. The differences in antigen levels were also seen at the mRNA level. When these 4 clones were tested, no difference in their adhesiveness to human umbilical vein endothelial cells and their invasive potential through matrigel could be observed. However, there was a highly significant difference in their metastatic potentials as evaluated in nude mice (1-3B 7.3(0-25) ; 1-3C 16(2-46) ; 23-4B' 177(113-286) ; 26-6 206(38-373)). Our data indicate that also upon in vitro selection according to PAI-1 expression, a highly significant correlation between PAI-1 and TF expression on the one hand and formation of metastasis in nude mice on the other hand could be obtained.

30

PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 mRNA EXPRESSION IS INCREASED BY TRIGLYCERIDE RICH LIPOPROTEINS IN HUMAN HEPATOMA CELLS. Luciana Mussoni, Luigi Sironi, Livia Prati, Damiano Baldassarre, Marina Camera and Elena Tremoli. Institute of Pharmacological Sciences and E. Grossi Paoletti Center, University of Milan, Milan, Italy.

Previous studies have shown that plasminogen activator inhibitor type 1 (PAI-1) levels are elevated in plasma of patients with hypertriglyceridemia. In the present study we have evaluated the effects of very low density lipoproteins (VLDL) isolated from plasma of normal donors on PAI-1 synthesis by HepG2 cells. Cells ($4x10^6$) incubated in serum free medium for 14-16 hours released 11.2 $\pm$2.4 SE ng/ml (n=8) PAI-1 antigen determined by ELISA. Levels of PAI-1 antigen in supernatants of HepG2 cells incubated with 100 µg protein/ml VLDL were increased by two-fold (22.8$\pm$5.3 SE ng/ml) (n=9). The increases in PAI-1 synthesis observed in the presence of VLDL were accompanied by concomitant changes in PA-1 mRNA levels as determined by Northern blot analysis. In particular, 100 µg/ml VLDL increased three-fold the cellular levels of the 2.2 Kb PAI-1 mRNA species ($p<0.001$, n=7), whereas levels of the 3.2 Kb PAI-1 mRNA remained essentially unchanged or even decreased. Therefore, 2.2/3.2 Kb PAI-1 mRNA ratio increased in VLDL treated samples. Cycloheximide (25 µg/ml) changed the ratio between the two PAI-1 mRNAs in the favour of the 3.2 Kb PAI-1 mRNA species in VLDL treated cells. Time-course experiments showed that the effects of VLDL on the 2.2 Kb PAI-1 mRNA were evident after 9 hour incubation of the lipoproteins with cells. When VLDL were incubated with HepG2 in the presence of a concentration of insulin (135 nM) known to increase PAI-1 synthesis in this cell type, additive effects of VLDL on PAI-1 mRNA expression and on PAI-1 antigen release were observed. The effect of triglyceride rich lipoproteins on PAI-1 synthesis by HepG2 may be of importance in determining the increased levels of circulating PAI-1 described in hypertriglyceridemic patients.