1095

Neutrophil Elastase, Thrombin and Plasmin in Septic Shock: Influence on Prognosis. R. Seitz, M. Wolf, R. Egbring, K. Havemann. Div. of Internal Medicine, University of D-3550 Marburg, F.R.G.

The prognosis of septicaemia depends on the occurrence of disseminated disturbances of the microcirculation impairing organ function and haemorrhagic complications due to consumption of coagulation factors. The intravasal appearance of three potentially involved proteinases in active form can be detected by immunologic determination of their complexes with inhibitors: thrombin-antithrombin III (TAT), according to PELZER et al. (Thrombos. Haemostas. 54:24,1985); plasmin- antiplasmin (PAP), IdIE, antiserum donated by KARGES; human neutrophil elastase (HNE)- antitrypsin, ELISA, Merck, Darmstadt.

In 47 patients with septic shock (19 survived, group A; 28 lethal, group B) the PAP levels were moderately elevated throughout the course without vaviations related to the outcome. TAT was initially strongly increased in both groups (18.8±6.3 ng/ml/16.5±7.2), and decreased towards the end of the course in both groups (2.7±0.5/3.7±0.8). Though the intial HNE levels were higher in group A (2458±348ng/ml) than in group B (1291±295, p=0.017), they decreased, in group A more rapidly and were at the end almost significantly lower than in group B (315±54/652±161, p=0,059). The decrease of TAT as well as HNE was associated with substitution of antithrombin III concentate (ATIII) and fresh frozen plasma (ffp) given with the aim of normalization of haemostasis and replacement of inhibitors. Factor XIII, a substrate of both thrombin and HNE, was initially equally low about 50% of normal in both groups, but increased only in group A (69.1±7.1/49.6±5.5, p=0.045) towards the end.

Conclusions: Both TAT and HNE decreased after initial elevation under substitution of ATIII and ffp. A rapid decrease seems to be a favourable sign which is accompanied by rising levels of F XIII, while sustained elevation of HNE points to a poor prognosis.

1096

LIMITED CLEAVAGE OF HMW-KININOGEN BY PLASMIN ENHANCES RELEASE OF KININ BY PLASMA KALLIKREIN: CLEAVAGE BY LEUKOCYTE ELASTASE DOES NOT RELEASE KININ, BUT INACTIVATES ITS COAGULANT PROPERTIES. J. Kleniewski and V.H. Donaldson. Children's Hospital Research Foundation, University of Cincinnati, Departments of Pediatrics and Medicine, Cincinnati, OH, U.S.A.

After limited digestion of purified human HMW-kininogen by plasmin, the kininogen molecule consists of two disulfide-linked chains in which the bradykinin sequence resides in the "light chain" portion. Kinin was released from this molecule by plasma kallikrein at a two- to three-fold more rapid rate than from uncleaved HMW-kininogen. Similarly, when normal human plasma or prekallikrein deficient plasma was treated with sufficient streptokinase to activate plasminogen the subsequent rate of release of kinin by kallikrein was enhanced. The digestion of HMW-kininogen by plasmin as well as kinin release was inhibited by epsilon aminocaproic acid at a concentration of 10^{-3} M, suggesting that one or more lysine residues was critical to the plasmin-HMW-kininogen interaction. Leukocyte elastase cleaved HMW-kininogen into low molecular weight fragments without releasing kinin but plasma kallikrein could then release kinin from a low molecular weight component of elastase-digested HMW-kininogen ($\leq$ 50 kd mol. wt.). Elastase destroyed the coagulant properties of the kininogen.

When HMW-kininogen was converted to two-chain, disulfide-linked molecules, either by plasmin or kallikrein, the quantity of antigen detected in an ELISA with polyclonal antibody to human light chain antigens was significantly increased. Expression of antigen detectable with a monoclonal antibody to an epitope located close to the disulfide interchange in the light chain was not increased by prior limited digestion with these enzymes.

It is possible that minimal activation of plasminogen in vivo may facilitate kinin release by kallikrein. In addition, in quantifying antigenic properties of HMW-kininogen in plasma, care should be taken to block in vitro activation of plasminogen or prekallikrein. Since leukocyte elastase can be released during clotting of whole blood, it might then serve as a regulator of coagulation through its inactivation of coagulant properties of HMW-kininogen.

1097

IDENTIFICATION OF A NEUTROPHIL ELASTASE CLEAVAGE SITE ON THE Aα - CHAIN OF PRIMATE FIBRINOGEN. J. Weitz (1), S. Landman (2), and S. Birken (2). McMaster University, Hamilton, Ontario, Canada (1) and Columbia University, New York, N.Y., U.S.A. (2).

Human neutrophil elastase (HNE) cleaves the Aα21-22 bond of fibrinogen thus releasing the fibrinopeptide A (FPA)-containing fragment Aα1-21. Plasma Aα1-21 levels reflect in vivo HNE activity and peptide levels are increased in cigarette smokers and patients with chronic lung disease. To further explore the HNE-fibrinogen interaction, we set out to develop an animal model. The digestion of purified baboon and marmoset fibrinogen by human thrombin, HNE and extracts of baboon and marmoset neutrophils was monitored with a specific radioimmunoassay for human FPA. Thrombin produced quantitative release (2 mol/mol fibrinogen) of FPA. In contrast, HNE and the neutrophil extracts did not release FPA, but rather, produced quantitative release of a larger, FPA-containing fragment. Immunochemically, this fragment was clearly distinguishable from FPA in that in vitro thrombin treatment increased its immunoreactivity 1,000-fold (thrombin increasable FPA or TIFPA). TIFPA release by the neutrophil extracts was blocked by α_1-proteinase inhibitor, a specific HNE inhibitor (MeO-Suc-Ala$_2$-Pro-ValCH$_2$Cl) and an anti-HNE IgG, indicating that elastase was the responsible proteinase and that there was homology between the human and primate enzymes. The products of HNE and neutrophil extract proteolysis of the primate fibrinogens were then separated by high performance liquid chromatography and the TIFPA-containing fractions were subjected to amino acid sequence analysis. The FPA-containing fragments each consisted of 21 amino acids, had minor substitutions when compared with human Aα1-21 [Baboon: Aα(3) Ser - Thr; Marmoset Aα(1) Ala - Thr, Aα(3) Ser - Thr, Aα(11) Glu - Ala], and exhibited complete crossreactivity with the human peptide. Using the TIFPA assay, there was good recovery of primate or human Aα1-21 added to primate blood and the mean peptide level in 8 healthy marmosets was similar to that in man (0.5 nM and 0.4 nM, respectively). In conclusion, (1) the Aα21-22 bond of baboon and marmoset fibrinogen is a cleavage site for human and primate elastase, (2) baboon and marmoset Aα1-21 can be measured with the assay for the human peptide, and (3) the primate serves as a useful model for the study of elastase-fibrinogen interactions.

1098

CLEAVAGE PATHWAY AND SPECIFICITY OF LEUCOCYTE ELASTASE AS COMPARED TO PLASMIN DURING FIBRINOGEN DEGRADATION. L. Goretzki, E. Müller and A. Henschen. Max-Planck-Institute for Biochemistry, Martinsried/Munich, FRG.

Plasmin and leucocyte elastase are regarded as the two medically most important fibrin(ogen)-degrading proteolytic enzymes. There is, however, a considerable difference in information available about the cleavage specificities and fragmentation pathways of these two enzymes. Degradation by plasmin has been studied already for a long time in great detail so that now the time course of the degradation, the cleavage sites and the functional properties of many fragments are well known. In contrast, relatively little is known about the degradation by leucocyte elastase, except that the overall cleavage pattern resembles that obtained with plasmin.

In this investigation the leucocyte elastase-mediated degradation of fibrinogen has been examined by means of proteinchemical methods. Human fibrinogen was incubated with human enzyme material for various periods of time and at some different enzyme concentrations. The split products formed at the various stages were isolated in pure form by gel filtration followed by reversed-phase high-performance liquid chromatography. The fragments were identified by N-terminal amino acid sequence and amino acid composition. The course of the degradation was also monitored by sodium dodecylsulfate-polyacrylamide gel electrophoresis. All cleavage patterns were compared with the corresponding patterns from plasmic degradation. It could be confirmed that X-, D- and E-like fragments are formed also with elastase. However, several early elastolytic Aα-chain fragments are characteristically different from plasmic fragments. The previously identified N-terminal cleavage site in the Aα-chain, i.e. after position 21, was found to be the most important site in this region of fibrinogen. The very early degradation of the Aα-chain N-terminus by elastase is in strong contrast to the stability against plasmin. Several cleavage sites in N-terminal region of the Bβ-chain were observed, though the low amino acid specificity of elastase partly hampered the identification. The γ-chain N-terminus was found to be as highly stable towards elastase as towards plasmin. The results are expected to contribute to the understanding of the role of leucocyte elastase in pathophysiologic fibrino(geno)lysis.