Monday, July 13, 1981

Poster Presentations

Fibrinogen – I

Degradation Products

11:00–12:30 h

Grand Ballroom Lobby Boards 201–209

0150

PURIFICATION OF VASOACTIVE PEPTIDES FROM HUMAN FIBRINOGEN DEGRADED BY HUMAN LEUCOCYTE ELASTASE. R. Wallin, M. Belew, K. Ohlsson and T. Saldeen, Institute of Forensic Medicine, University of Uppsala, Uppsala, Sweden.

The presence of leucocytes around extravascular fibrin deposits suggests that the leucocyte elastases might be partly responsible for the extravascular degradation of fibrin. Our previous studies have shown that the degradation of fibrin(ogen) by plasmin leads to the release of 2 small peptides which markedly increase vascular permeability and induce oedema e.g. in the lungs. The results of this investigation show that small peptides released from fibrinogen after degradation by leucocytes elastases also increase vascular permeability.

Human fibrinogen (Kabi, Grade L) was made plasminogen-free by affinity chromatography on Lysine-Sepharose 4B prior to use. The human leucocyte elastases were isolated from extracts of lysosome-like granules of human leukaemic myeloid cells by a combination of gel filtration, affinity chromatography and preparative agarose gel electrophoresis. The fibrinogen (0.5 %) and the leucocyte elastases (in a molar ratio of 100:1) were incubated together for 48 h at +37°C and at pH 8.5. The mixture was then cooled to +4°C to stop the lysis and ultrafiltrated on a DIAFLO PM 10 membrane until the retentate was approximately 10 % of the starting volume. The peptides in the diffusate accounted for about 20 % of the starting material as estimated from absorbance measurements at 280 nm. The diffusate was concentrated by lyophilization and fractionated by chromatography on a column of Bio-Gel P-6. At least 8 fractions were obtained of which only two showed a significant activity in their ability to increase vascular permeability in rat skin. The active peptides in these two fractions were further purified to homogeneity by column zone electrophoresis at various pHs and their amino acid compositions established.

0151

MECHANISMS OF COAGULOPATHY AFTER ENVENOMATION BY THE EASTERN DIAMONDBACK RATTLESNAKE. C.S. Kitchens and L.H.S. Van Mierop. Departments of Medicine, Pathology, and Pediatrics, University of Florida College of Medicine, Gainesville, Florida, USA.

All 34 patients seen at this hospital during the 1978-1980 period who were envenomated by poisonous snakes were studied in a prospective manner with respect to their hemostatic system. Blood was drawn on the patient's arrival to the emergency room and every 6 h thereafter. Blood was analysed for platelet count; routine coagulation tests; levels of factors II, VIII, IX, XII; clottable fibrinogen; fibrinogen antigen; fibrin degradation products (FDP); plasminogen (Pl); antithrombin III (AT III); α_2 plasmin inhibitor (API); and plasminogen activator (PA). Twelve of the 34 patients underwent a coagulopathy as described below. These patients included all 10 patients envenomated by the Eastern diamondback rattlesnake (Crotalus adamanteus) and 2 of 17 patients bitten by the pygmy rattlesnake (Sistrurus miliarius). Values of all the above coagulation tests in 15 pygmy rattlesnake and 7 moccasin (Agkistrodon piscivorus) victims were indistinguishable from normal. Patients undergoing coagulopathy rapidly developed noncoagulable blood as defined by a thrombin time (TT) >120 s; blood remained incoagulable for an average of 18 h. The nadir clottable fibrinogen (0 mg/dl), fibrinogen antigen (99 mg/dl), Pl (20% of normal), API (17% of normal), and maximal levels of FDP (1:4096) and PA (20 times normal) were all significantly ($p < 0.001$) altered when-compared with normal values. The platelet count and AT III levels were only midly decreased. Factors II, VIII, IX, and XII were normal. Because venom from the Eastern diamondback rattlesnake does not directly activate Pl, we conclude that the coagulopathy following envenomation by that reptile appears to be due to partial proteolysis of the fibrinogen with secondary activation of Pl by PA released from the endothelium. The resulting defibrination is distinguishable from disseminated intravascular coagulation.

0152

UNIQUE DEGRADATION OF FIBRINOGEN BY WESTERN DIAMONDBACK RATTLESNAKE VENOM PROTEASES. Bharat V. Pandya, Andrei Z. Budzynski, Ronald N. Rubin and Stephanie A. Olexa. Thrombosis Research Center and Department of Biochemistry, Temple University H.S.C., Philadelphia, PA.

The venom of western diamondback rattlesnake, Crotalus atrox, renders blood unclottable even after addition of thrombin. The phenomenon is due to a limited proteolytic degradation of fibrinogen. Purified fibrinogen (1 mg/ml) or human plasma incubated with crude venom (7.5 µg/ml) became unclottable. Fibrinogen in a purified system was affected at a faster rate than that in plasma suggesting that plasma protease inhibitors partially inhibited the proteolysis of fibrinogen. The degradation of plasma fibrinogen did not result from plasminogen activation since the venom did not activate purified human plasminogen as determined by an amidolytic assay and SDS polyacrylamide gel electrophoresis. The degradation of the fibrinogen molecule (Mr 340,000) proceeded steadily with a continuous cleavage of small peptides as demonstrated by SDS polyacrylamide gel electrophoresis. The bulk of the degraded fibrinogen was represented in gels as a single band with gradually increasing electrophoretic mobility as the incubation time progressed. The correlation of molecular weight and clottability demonstrated that the earliest unclottable derivative had Mr 285,000; its Aα and Bβ chains were degraded loosing peptides of Mr 20,000 and 6,500, respectively, but the γ chain appeared intact. The degradation pattern of fibrinogen in plasma was different than that of fibrinogen alone. An unclottable fibrinogen derivative isolated from plasma by precipitation with 2.2 M glycine had Mr 325,000; its Aα and γ chains appeared unaffected but the Bβ chain had lost a peptide of Mr 6,500. This product represents a novel unclottable derivative of fibrinogen with apparently intact Aα and γ chains, cleaved Bβ chain and high molecular weight. The cleavage of the Bβ chain would indicate that the intact Bβ chain may have an important role in the conversion of fibrinogen to fibrin clot.