

Supplementary Abstracts

Thrombin

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BINDING OF THROMBIN BY PLASMA INHIBITORS. J.A. Penner and H.I. Hassouna. Dept of Medicine, College of Human Medicine, Michigan State University, East Lansing, Michigan, U.S.A.

Our study was undertaken with the purpose of clarifying the role of plasma inhibitors other than antithrombin III (ATIII) in binding thrombin in the presence and absence of heparin. In the clotting system, several antiproteases are involved in neutralizing active factors. The capacity to destroy thrombin is attributed to ATIII whose concentration in plasma is ten times less than α_1 antitrypsin (α_1A). If heparin is added to plasma, ATIII becomes an immediate inhibitor of thrombin. Several studies have indicated that a modest depression in ATIII activity leads to thrombosis, inferring that other plasma antiproteases play a minor role. Experimental approach was as follows: Defibrinated platelet poor citrated human plasma was depleted of ATIII and α_1A by means of monospecific insolubilized antibodies. Purified thrombin, labelled by Chloramine T incorporation of ^{125}I in NaOH, had a specific activity of 1.3×10^5 CPM/ μg . Total antiprotease thrombin binding capacity of defibrinated plasma was estimated by incubating 4×10^{-3} μM thrombin with plasma (100 μl) for 2 hours and 24 hours in the absence of heparin and for 5 minutes in the presence of heparin (20 units). Free from bound *thrombin was separated by gel filtration on Sephacryl S200 and chromatograms monitored on gamma analyzer. Following the same protocol, ATIII deficient plasma was used to evaluate thrombin binding capacity not attributable to ATIII, and role of ATIII in the absence of α_1A was assessed in α_1A depleted plasma. As a result, ATIII binding capacity was found to be 62 μg *thrombin/100 μl of normal and α_1A deficient plasma, in the presence and absence of heparin, eluting at a volume corresponding to 9×10^4 daltons. Binding capacity of other plasma inhibitors in ATIII deficient plasma amounted to 24 μg *thrombin in the absence of heparin and 7 μg in its presence. This confirms the role of ATIII as the major anti-thrombin, heparin cofactor. In its absence inactivation of thrombin is reduced by 61%.

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THROMBIN GENERATION STIMULATED BY OXIDATION PRODUCTS OF LINOLENIC AND ARACHIDONIC ACIDS. T.W. Barrowcliffe, E. Gray, J.M.C. Gutteridge and P.J. Kerry. National Institute for Biological Standards and Control, London NW3 6RB, U.K.

Autooxidation of unsaturated fatty acids yields mixtures of products which have potent biological activities in several systems. Such products have been shown to cause platelet aggregation and to stimulate thrombin generation in platelet-free plasma. The thrombin generating activity of products obtained by air or O_2 oxidation of linolenic and arachidonic acids was studied by standard in vitro clotting methods. Products were separated from the starting material by a variety of techniques, the most successful being column chromatography on phenoxymethyl lipidex, a modified LH-Sephadex.

Clotting studies showed that thrombin generating activity proceeded via the intrinsic system, requiring Factors VIII, IX and X but not Factor XI. Studies with lipoprotein-free plasma indicated that part of the activity of the oxidation product was a 'surfactant' effect, involving release of coagulant-active phospholipid from plasma lipoproteins. Such an effect was also seen to a lesser extent with the unoxidized fatty acids. However, addition of the products to a phospholipid preparation potentiated its thrombin generating activity, indicating the release of phospholipid is not the only mechanism. The products showed marked inhibition of antithrombin III, which would result in increased amounts of thrombin being generated in plasma.

Fractionation of the products showed that the highest activity was in the most polar fractions, and the activity was inhibited by horseradish peroxidase, suggesting that peroxide groups are involved.

These studies indicate that lipid peroxides produced by non-enzymic means have potent effects on the plasma coagulation system, and suggest the possibility that the endoperoxides produced enzymically by platelets could also be involved in plasma coagulation.

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IDENTIFICATION OF THROMBIN IN HUMAN SERUM BY ISOTACHOPHORESIS. D. Del Principe, G. Bianchini, C. D'Arcangelo, G. Mancuso, M. Persiani, A. Menichelli. I Department of Pediatrics, School of Medicine, University of Rome; CNR Centre Respiratory Viruses, Rome, Italy.

We investigated the possibility of detection of thrombin in blood by analytical isotachophoresis. This technique is a relatively new electrophoretic method for separation of different ions. At the equilibrium the ions move with the same speed and are spaced into consecutive zones between the leading and terminating electrolytes according to their mobilities. The instrument used for analytical isotachophoresis was equipped with an UV and thermal detectors. The leading was MES, Ammediol, hydroxypropylmethyl cellulose 0.25%, pH 9.2; the terminating was EACA, Ammediol, adjusted with Ba(OH) $_2$ to pH 10.8. Carrier ampholytes, pH 3.5-8 range were employed as spacers.

Free thrombin was not detected by analytical isotachophoresis in 30 normal sera, while it was demonstrated in 2 children with laboratory and clinical evidence of DIC.

Our data show that the detection of free thrombin by isotachophoresis is due to an in vivo decrease of the serum neutralizing capacity following either an enzyme excess or an inhibitor loss.

The isotachophoresis allows the assay of micro-quantities of sample (2 μl) in short time (15-20 min) and may reveal itself useful in clinical diagnosis.