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ASPIRIN ACETYLATES FIBRINOGEN AND ENHANCES FIBRINOLYSIS IN VIVO. FIBRINOLYTIC EFFECT IS INDEPENDENT OF CHANGES IN PLASMINOGEN ACTIVATOR LEVELS. Thorir D. Bjornsson, M.D., and Henry Berger, Jr., Ph.D. Division of Clinical Pharmacology, Department of Medicine, Thomas Jefferson University, Philadelphia, PA, and Department of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC, U.S.A.

In addition to its antiplatelet effect, aspirin has been reported to have fibrinolytic and hypoprothrombinemic effects. The objective of this study was to investigate possible mechanisms underlying the enhanced fibrinolysis observed after aspirin. Five healthy subjects received 650 mg of aspirin ql2hr for five days. Blood samples were collected before aspirin (control) and immediately before (0 hr) and two hours after (2 hr) the last dose for determinations of clot lysis time, time course of thrombin-induced fibrin aggregation, tissue plasminogen activator (tPA), intrinsic pathway fibrinolytic activity (IPFA), plasminogen, fibrinogen, aspirin and salicylic acid, and the coagulation tests activated partial thromboplastin time, thrombin time and prothrombin time. Clot lysis time was shorter after aspirin, control: 9.1±12.4 min (mean±s.d.), 0 hr: 4.6±4.0 min, 2 hr: 5.7±6.2 min (p:0.04), and the fibrin aggregation curves showed increased turbidity (expressed as AUC over 10 min), control: 72.7±17.8 mm•min, 0 hr: 94.6±1.6 mm•min, 2 hr: 112.8±45.1 mm•min (p:0.02). Control values of tPA (0.11±0.04 IU/ml), IPFA (2.20±0.59 IU/ml), plasminogen (10.9±1.0 mg/dl), fibrinogen (288±37 mg/dl), and the coagulation tests were not differnet from those after aspirin. Plasma aspirin concentrations were below detection limits at 0 hr and averaged 1.63±0.97 µg/ml at 2 hr. In vitro studies using fibrinogen-free plasma and added acetylated fibrinogen showed an inverse relationship between the extent of acetylation and clot lysis time. Studies using ^{14}C-acetyl-labeled aspirin and fibrinogen showed that fibrinogen is acetylated to form ε-N-acetyl-lysine on both D and E domains of the molecule (50.6±2.0 and 49.4±2.0%, respectively) and on α, β, and γ chains of the molecule (34.4±1.6, 30.7±3.4 and 34.9±4.7%, respectively), with preferential acetylation on the E domain. On the average, 2.88±1.49 ε-N-acetyl-lysyl residues were formed on each fibrinogen molecule. These results suggest that N-acetylation of lysyl residues of fibrinogen is responsible for the increased susceptibility of fibrin clots to lysis after aspirin.

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ROLE OF DISULFIDE BONDS NEAR THE CALCIUM BINDING SITES IN FIBRINOGEN. R. Procyk and B. Blomback. Plasma Proteins Coagulation Laboratory, New York Blood Center, N.Y., U.S.A. and Karolinska Institutet, Stockholm, Sweden.

Incubation of fibrinogen with 0.5 mM dithiothreitol in the presence of 20 mM calcium chloride cleaved disulfide bonds located at: the N-terminal end of the Aα-chain (either Aα28-Aα28 or Aα45-γ23), the C-terminal end of the Aα-chain (Aα442-Aα472) and the N-terminal end of the γ-chain (either of the symmetrical γ8, γ9 disulfides or the Aα45-γ23 disulfide bond). In the absence of calcium ions two additional disulfides, γ326-γ339, and one in the N-terminal end of the γ-chain were reduced.

Plasmin digestion of the reduced fibrinogens in buffers containing calcium chloride produced fragments D and E, except that smaller fragments of D were generated from the fibrinogen in which the γ326-γ339 disulfide bonds were reduced and alkylated. In these samples calcium did not protect the C-terminal end of the γ-chain from extensive digestion.

Addition of thrombin to partially reduced and alkylated fibrinogen prepared in the presence of calcium gave a clotting time similar to control unreduced fibrinogen. However, when the γ326-γ339 disulfide bonds and another γ-chain disulfide bond most likely in the N-terminal region were cleaved in reduced fibrinogen prepared in the absence of calcium, the thrombin clotting time was extremely prolonged. Apparently the disulfide bonded structure supported by γ326-γ339 was important both for binding of calcium and also for normal clotting.

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REDUCED TISSUE PLASMINOGEN ACTIVATOR RELEASE AND NORMAL PLASMINOGEN ACTIVATOR INHIBITOR LEVEL IN A FAMILY WITH RECURRENT VENOUS THROMBOSES
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We have studied the fibrinolytic system in one asymptomatic and six symptomatic members of a family with recurrent DVTs in three generations. Tissue plasminogen activator activity (TPA) and fast acting inhibitor of TPA (PAI) were determined using chromogenic substrate and TPA antigen with ELISA. Measurements were made at rest and after 10 and 20 minutes of venous occlusion (VO). 17 healthy subjects served as controls. The mean TPA in the seven family members was significantly lower than in controls at 10 and 20 min VO (p< 0.01).TPA was below the lowest value of controls (<1.7 U/ml) in five of the six patients with DVT at 10 min VO and remained below the range of controls in three at 20 min VO (<3.3 U/ml). The lowered TPA activity was associated with impaired release of TPA antigen (mean level at 10 min VO 12.4 ng/ml, controls 19.5 ng/ml, p<0.05; at 20 min VO 18.2 ng/ml, controls 43.2 ng/ml, p<0.01). Four patients with and one without DVT had TPA antigen below the lowest level of controls at 10 and/or 20 min VO. The level of PAI at rest was normal in all cases (from 0.6 to 1.7 U/ml, controls from 0 to 3.7 U/ml). In accordance with low release of TPA antigen PAI was consumed less during VO in patients than in controls (mean level at 20 min VO 0.9 U/ml, controls 0.4 U/ml, p<0.05). The levels of antithrombin III, protein C, protein S, plasminogen, fibrinogen, F V, F VIII:C, vWf:Ag, fibrinopeptide A and beta-thromboglobulin were normal . Circulating anticoagulant was not found. It is concluded that impaired release of TPA, independent of PAI, is associated with DVT in this family. The pattern of inheritance suggested autosomal dominant trait.

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A NEW AT III VARIANT WITH DEFECTIVE PROTEASE BINDING SITE.
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A decreased plasma antithrombin activity in presence heparin cofactor (hep-cof) or in absence of heparin (AT) was discovered in a 47 year-old patient presenting with recurrent venous thromboembolism. The immunoreactive material (AT III-IR) was normal. The same biological abnormalities were found in two relatives of the patient, leading to the diagnosis of hereditary qualitative AT III deficiency.

The propositus'AT III was coeluted with normal AT III from an heparin sepharose column. An additional step of ion-exchange chromatography on a Mono Q column using a FPLC system (Pharmacia, Bois d'Arcy, France) allowed the purification of a protein which was homogenous in SDS - 10 % polyacrylamide electrophoresis gel (PAGE). AT III purified from propositus'plasma, normal plasma and the plasma of a patient known to have an AT III variant with defective protease binding (1) were compared. The specific activities measured as hep-cof AT or factor Xa inhibition in absence of heparin (anti Xa) were respectively 6.4 and 4.8 U/mg for the propositus'AT III and 13.6 and 8.5 U/mg for the normal AT III (one unit is the activity of 1 ml of a plasma pool prepared from 30 normal subjects). The formation of protease inhibitor complexes was studied by incubating purified AT III with purified thrombin (in molar ratio 1:4, 1:2, 1:1) during 5 minutes at 37 °C and submitting the mixture to PAGE. The densitometric scan showed that in equimolar ratio the percentage of an AT III-thrombin complex (with 92 kΔ Mr) reaches 70 for normal AT III and respectively 30 and 23 for the propositus'AT III and the already described variant AT III Charleville (1). A 70 % proportion of free AT III (58 kΔ) remained for the propositus. As previously observed an unidentified 63 kΔ compound appeared for AT III Charleville. This results strongly suggest that in the patient described here, half th the AT III molecules are normal, the others having a defective protease binding site. We propose to call this new variant AT III Avranches, the town where the propositus was born.

(1) Thrombosis Research 1985, 39, 559-570.