0287

10:00 h

CIRCULATING HUMAN PLATELET MEMBRANE MICROPARTICLES.

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"Platelet dust" providing coagulant activity has been postulated to exist in human plasma. We studied human subjects to identify platelet membrane microparticles (MP) by immunologic methods in cell-free plasma. Blood was drawn into a syringe containing ACD-PGE_1 and centrifuged at 30,000 g·min to obtain plasma with no identifiable platelets. The plasma concentration of platelet factor 4 was normal (9.4 ng/ml, n = 14), demonstrating no in vitro platelet activation. MP were isolated from diluted plasma by centrifugation at 7 x 10^5 g·min, the pellet was washed once, and solubilized in Triton X-100. Antibody from rabbits immunized with whole platelets did not cross-react with red cells or white cells. Using tandem crossed immunoelectrophoresis with MP and whole platelets, platelet antigens were identified in the MP. A specific antibody to platelet membrane glycoprotein II-III was prepared by adsorption of the anti-whole platelet antibody with cryoprecipitate and thrombasthenic platelets and used to quantify MP by rocket immunoelectrophoresis. Normal plasma MP concentration was 4.37 $\pm$ .71 (SE) µg/ml (n = 20). MP concentration was greater in serum (65.2 $\pm$ 13.2 µg/ml, $p < .001$) demonstrating platelet microparticle formation during coagulation. In a patient with thrombocytosis (1.76 x 10^6 platelets/µl) plasma MP were normal (7.5 µg/ml) but serum MP were increased (185 µg/ml). Plasma MP were assayed in 11 normal subjects before and after aspirin (640 mg/day x 7 days) and MP decreased in 9 (mean values: 4.45 µg/ml pre- and 1.92 µg/ml post-aspirin, paired t test: $p < .02$). Therefore platelet membrane fragmentation occurs during normal circulation and is inhibited by aspirin.

0288

10:15 h

THE ENDOGENOUS LECTIN OF HUMAN PLATELETS IS AN α-GRANULE COMPONENT.

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Thrombin-activated platelets express a hemagglutination activity (enhanced lectin activity) not expressed by non-activated platelets and this agglutinin mediates the direct platelet-platelet interactions which cause the aggregation of thrombin-activated platelets. The enhanced agglutinin activity of thrombin-activated platelets is composed of two components: a plasma membrane-bound component and a soluble component. The expression of both forms of the agglutinin activity is dependent upon secretion. The purpose of this study is to characterize the platelet-bound and platelet-free (soluble) agglutinin activities of activated washed platelets from patients with the bleeding disorders, congenital afibrinogenemia, Glanzmann's thrombasthenia, gray platelet syndrome, and Hermansky-Pudlak syndrome. Thrombin and A23187 activated afibrinogenemic, Hermansky-Pudlak and thrombasthenic platelets had normal platelet-bound hemagglutination activity. Gray platelets activated by the same agents had deficient platelet-bound hemagglutination activity. In contrast, thrombin-activated afibrinogenemic, gray and thrombasthenic platelets lacked platelet-free hemagglutination activity. Only thrombin-activated Hermansky-Pudlak platelets had a normal level of platelet-free hemagglutination activity. On the basis of these results and the distinguishing characteristics of the defective platelets, it is concluded that the α-granules are the origin of the enhanced hemagglutination activity. Furthermore, it is suggested that the insufficiency of the platelet-bound agglutinin may be the cause of the inability of gray platelets to aggregate normally in response to thrombin.

0289

10:30 h

PLATELET FACTOR 4 BINDING PROTEINS.

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Platelet factor 4 (PF-4) is a platelet alpha granule specific protein with a high affinity for heparin, and a progressively decreasing affinity for other glycosaminoglycans. PF-4 is reportedly bound to a proteoglycan (PG) carrier within the platelet and can be dissociated by either high salts or by the presence of heparin. In an effort to isolate and characterize this PG, we synthesized PF-4 resin by carbodiimide coupling human PF-4 to diaminobutyl-Sepharose. Platelet lysate, which had been previously passed over heparin-Sepharose, was applied to the PF-4 resin. PG was determined by the carbazole reaction. All of the carbazole reactive material applied to such columns bound, and was subsequently eluted with 1.0 M NaCl. Gel filtration of this fraction on Sepharose 6B resolved 3 distinct components, the largest of which was exclusively carbazole positive. The product was found to contain: 13% protein; 35% hexuronic acid; 17% neutral sugar; 35% amino sugar, identified as galactosamine. This PG is similar to the chondroitin sulfates. PG antisera, when tested against sucrose gradient fractionated platelet homogenates, localize this material in the alpha granule. This PG does not bind β-thromboglobulin proteins. PG-PF-4 complexes, as judged by filtration data, can be readily dissociated by heparins. This PG is not an exclusive PF-4 binding protein. A 70,000 dalton protein has also been isolated from the platelet lysate. Upon reapplication to PF-4 resins, it quantitatively binds and is dissociated only by molar salts. This 70,000 dalton is not albumin and appears to be released upon platelet activation. It is unresolved as to the ability of this 70,000 protein to compete with PG for PF-4 binding affinity. The concentration (yield) of the 70,000 protein is greater than that of the PG. Studies are currently underway to further characterize this new protein and determine its effect on PF-4 release, quantitation by RIA and plasma half-life.

0290

10:45 h

HUMAN HIGH MOLECULAR WEIGHT KININOGEN - A SECRETED PLATELET COAGULANT PROTEIN.

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Platelets have been shown to contain a number of secretable coagulant proteins, which participate as substrates or cofactors in plasma coagulation reactions. Since we have previously demonstrated that high molecular weight kininogen (HMWK) is immunochemically present in platelet extracts, we posited that HMWK is secreted during activation of platelets. Fresh normal platelets were washed by a combination of albumin-gradient and gel-filtration procedures. In 11 experiments the supernates of freeze-thaw lysates of normal human platelets contained a mean of 5.7 Units (range 3.16 to 8.14) of HMWK coagulant activity/3 x 10^{11} platelets. This coagulant activity was neutralized by a goat antikininogen antibody. Using a ^{125}I-HMWK tracer in PRP, the supernate of washed activated platelets contained 0.082% radioactivity as the starting PRP, suggesting that 14% of the total HMWK coagulant activity could be accounted for by plasma contamination. In four experiments, ionophore A23187 (15µM) induced a net secretion of 39% of the total platelet HMWK (range 16 to 49%). Platelet HMWK secretion by A23187 was concentration dependent (1 to 15 µM). At A23187 (15µM) platelets released 75% ^{14}C-5HT (range 61 to 99%) and 81% low affinity platelet Factor 4 (range 60 to 99%). Ninety-five percent of A23187-induced secretion of HMWK could be blocked by platelet pretreatment with metabolic inhibitors. LDH determinations indicated that only 5% (range 0 to 10%) of total secreted platelet HMWK could be attributed to lysis. Collagen and PGH_2 also caused secretion of platelet HMWK coagulant activity. This study indicates that human platelets contain functional HMWK which may be secreted locally to modulate the reactions of the contact phase of plasma proteolysis.

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