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The Participation of Plasma Thromboplastin Antecedent (Factor XI) in Contact-Activated Fibrinolysis[1] (40839)

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Normal human plasma contains a precursor globulin, plasminogen, that can be converted to an active fibrinolytic (proteolytic) enzyme, plasmin (1). Since plasmin exerts a broad spectrum of proteolytic actions upon many proteins such as fibrinogen, fibrin, and antihemophilic factor (Factor VIII), activation of plasminogen to plasmin may have significant biologic consequences.

Plasmin may form in human plasma through many pathways. Streptokinase, urokinase, and tissue activators are potent activators of plasminogen, but are not normally present in the circulating blood. Under certain conditions, contact of plasma with foreign surfaces may lead to the generation of plasmin without the participation of exogeneous agents. The precise steps and components involved in this contact-activated generation of plasmin, however, have not yet been fully elucidated (2).

The contact-activated formation of plasmin requires the presence of at least four plasma proteins, Hageman factor (Factor XII), prekallikrein, high-molecular-weight kininogen, and plasminogen, since impaired fibrinolysis is found in plasmas deficient in any of these agents (3–5). The participation of additional factors in this pathway, however, has been debated; in particular, the role of plasma thromboplastin antecedent (PTA, Factor XI) is controversial (6–8). In order to evaluate the participation of PTA, we have examined contact-mediated activation of plasminogen in severely PTA-deficient plasmas. This paper will report impaired contact-activated plasmin generation in 11 PTA-deficient plasmas and its correction upon reconstitution with purified PTA. To our knowledge, this is the first demonstration that contact-mediated activation of plasminogen is impaired in PTA-deficient plasmas.

Materials and methods. Citrated plasmas from normal individuals and from patients with hereditary PTA deficiency and a standard pool of 24 normal male plasmas were prepared as described earlier (9). Venipuncture in normal subjects and patients was performed after informed consent for the procedure was obtained in accordance with the principles of the Declaration of Helsinki. A prekallikrein-deficient plasma was purchased from George King BioMedical, Inc., Overland Park, Kansas.

Human PTA, purified as reported (9), was free of any detectable plasmin, plasminogen, or prekallikrein.

Rabbit antiserum against PTA and prekallikrein were prepared as described earlier (9, 10). Antiplasminogen serum was raised in a New Zealand albino rabbit against human plasminogen purified by successive lysine–agarose and Sephadex G150 column chromatography.

Barbital-saline buffer, pH 7.4, contained 2.76 g of barbital, 7.3 g of sodium chloride, and 2.06 g of sodium barbital per liter.

PTA activity was measured by a clot-promoting assay (9), one unit of PTA activity being arbitrarily defined as that amount present in 1 ml of a standard pool of normal plasmas. Eleven PTA-deficient plasmas were used in this study; the titers of PTA activity were less than 0.01 units/ml in 7, 0.02 ~ 0.04 units/ml in 3, and 0.08 units/ml in 1 plasma.

Kaolin-induced plasmin generation was studied by a modification of a reported method (11). The 0.12-ml test plasma was shaken with 0.04 ml kaolin suspension in barbital–saline buffer (Fisher Scientific

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Co., Fairlawn, N. J., 3 mg/ml) at 37°C in a 12 × 75-mm polystyrene tube. At intervals, 2.3 ml 0.01 *M* Na acetate buffer (pH 4.7) was added to the tube and the tube was further incubated at 37°C for 60 min. The tube was then centrifuged at 1400 *g* for 5 min. The resultant euglobulin–kaolin precipitate was resuspended in 0.12 ml barbital–saline buffer containing 25% glycerol, 0.02 *M* lysine, and 0.001 *M* EDTA. The 0.05-ml aliquots were tested for plasmin activity by addition to 1 ml 0.6 m*M* *H*-D-Val–Leu–Lys–P-nitroaniline (S-2251, Kabi Diagnostica, Stockholm, Sweden) at 37°C for 10 min. The reaction was stopped by addition of 0.1 ml glacial acetic acid, the mixture was centrifuged, and the optical density of the supernatant solution was measured at 405 nm. The hydrolysis of S-2251 proceeded linearly under these conditions. The results were expressed as μmoles *P*-nitroanilide released per 10 min per 0.05-ml sample. The amidolytic activity generated in the kaolin–euglobulin precipitate was due to plasmin activity, since it was specifically inactivated by antiplasminogen serum, but not by anti-HF, anti-PTA, or anti-kallikrein serum (Table I). The amidolytic assay may detect not only free plasmin, but also plasmin-α_2 macroglobulin complexes. The contribution of plasmin-α_2 macroglobulin complexes to the amidolytic activity in the kaolin–euglobulin precipitates, however, appears to be minimal, since no significant amount of α_2-macroglobulin is present in the euglobulin fraction (12).

Determination of plasminogen in plasma was performed by activating plasminogen to plasmin by streptokinase (the gift of Hoechst–Roussel Pharmaceuticals, Inc., Somerville, N.J.) and measuring plasmin activity upon S-2251 (13). A standard pool of normal plasmas was arbitrarily said to contain 100% plasminogen.

The effect of the addition of purified PTA to PTA-deficient plasma upon plasmin generation was studied by adding 0.012 ml of purified PTA (10 units/ml, sp act 150 units/mg protein) or barbital–saline buffer to 0.108 ml plasma and by measuring kaolin-induced plasmin generation as above.

Results. Kaolin-induced plasmin generation in normal, prekallikrein-deficient, and PTA-deficient plasmas. When normal plasma was exposed to kaolin for varying periods of time and was then diluted in an acidic buffer, plasmin activity was generated in the euglobulin fraction (Fig. 1). The ordinate showed plasmin activity as measured upon S-2251 and the abscissa the incubation time of plasma with kaolin before dilution and acidification. Plasmin activity was found after 1-min exposure to kaolin, reached its maximal level after 5-min exposure, and gradually decreased with time.

TABLE I. EFFECT OF VARIOUS ANTISERUMS UPON THE AMIDOLYTIC ACTIVITY OF THE KAOLIN–EUGLOBULIN PRECIPITATE ON S-2251

Addition	Amidolytic activity (%)
Buffer	100
Normal rabbit serum[a]	94
Anti-plasminogen serum[a]	14
Anti-HF serum[a]	100
Anti-PTA serum[a]	98
Antikallikrein serum[a]	116

[a] Crude immunoglobulin fraction that had been depleted of plasminogen by a lysine–agarose column. The 0.05 ml kaolin–euglobulin suspension was incubated with 0.15 ml of either buffer, normal rabbit serum, or antiserum for 10 min at 0°C. The 0.065-ml aliquots were then tested for the amidolytic activity on S-2251 as described under Methods and Materials.

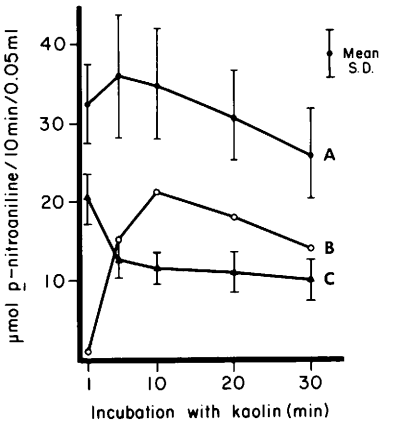

FIG. 1. Time course of the generation of plasmin activity in normal, prekallikrein-deficient, and PTA-deficient plasmas. A, B, and C indicate the mean ± SD plasmin activity in 11 normal, 1 prekallikrein-deficient, and 11 PTA-deficient plasmas, respectively.

As previously described (14, 15), no plasmin was activated in a prekallikrein-deficient plasma after a brief (1-min) exposure of plasma to kaolin, but significant amounts of plasmin were formed with prolonged exposure. Under the same conditions, the time course of the generation of plasmin activity in 11 PTA-deficient plasmas was different from that of both normal and prekallikrein-deficient plasmas. Maximum activity was reached after 1-min exposure. The titer of plasmin activity in PTA-deficient plasmas was significantly lower than that in normal plasmas at all incubation times with kaolin ($P < 0.01$).

The reduced plasmin generation in PTA-deficient plasmas was not due to the presence of excessive concentration of plasmin inhibitors, since a mixture of equal amounts of PTA-deficient plasma and normal plasma developed almost normal amounts of plasmin. Impaired plasmin generation was also not due to deficiency of HF, plasma prekallikrein, or high-molecular-weight kininogen, since PTA-deficient plasmas were shown to contain normal titers of these agents (10, 16). The level of plasminogen was normal in PTA-deficient plasmas when measured by streptokinase activation (mean ± SD, 102 ± 12%).

These data suggest that decreased plasmin formation was probably due to the deficiency of PTA.

Effect of addition of purified PTA to PTA-deficient plasma upon kaolin-induced plasmin generation. When purified PTA was added to nine PTA-deficient plasmas at a final concentration of 1 units/ml, the impaired plasmin generation in these plasmas was completely corrected (Fig. 2). The addition of PTA to normal plasmas did not significantly change the amount of plasmin activity developed.

These data further confirm that reduced activation of plasminogen in PTA-deficient plasmas is due to the absence of PTA, and not due to the presence of excessive concentrations of inhibitors nor to deficiencies of other components involved.

Discussion. The pathways leading to the activation of plasminogen by surface contact have been extensively studied for the past 20 years. Still, the precise steps involved are not completely known. Colman (17) showed that plasma kallikrein can directly convert plasminogen to plasmin, suggesting a major role of this agent. It is well recognized, however, that prekallikrein-deficient plasma can develop almost normal amounts of fibrinolytic activity after prolonged exposure to kaolin (14, 15). Therefore, another mechanism must be present in plasma. A recent report from this laboratory demonstrated direct activation of plasminogen by a mixture of HF and kaolin or by HF fragments (18).

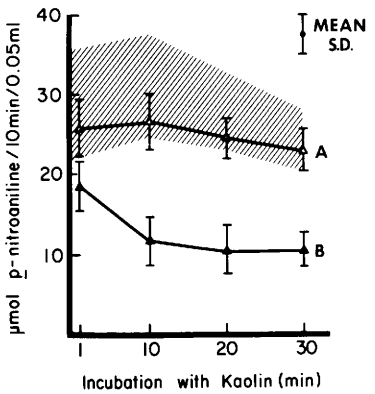

FIG. 2. Effect of the addition of purified PTA upon plasmin generation in PTA-deficient plasmas. Purified PTA (A) or buffer (B) was added to nine PTA-deficient plasmas and kaolin-induced plasmin generation was measured. The shaded area indicates the normal range (mean ± SD).

The data presented in this paper strongly indicate the participation of PTA in the contact-mediated activation of plasminogen in whole plasma. Impaired generation of plasmin in severely PTA-deficient plasmas was not due to deficiency of HF, prekallikrein, high-molecular-weight kininogen, or plasminogen, nor due to the presence of excessive concentration of inhibitors. Reconstitution of PTA-deficient plasmas with purified PTA corrected defective plasmin formation. Our results are in contrast to previous reports (11, 19) that the kaolin-induced clot lysis time was normal in PTA-deficient plasmas. Although the reason for the discrepancy is not clear, it is likely that fibrinolytic assays used in previous studies were not as sensitive and quantitative as the amidolytic assays used in the

present study. Alternatively, nonplasmin-dependent fibrinolytic activity may be partly responsible for clot lysis.

Our data are consistent with a recent report of Mandle and Kaplan (7) that a plasminogen proactivator activity present in γ-globulin fraction of a prekallikrein-deficient plasma is associated with PTA. Later, we demonstrated that the inactivation by anti-PTA serum of PTA in prekallikrein-deficient plasmas abolished the development of plasmin activity and the conversion of plasminogen to plasmin after prolonged exposure of prekallikrein-deficient plasmas to kaolin (20), suggesting a role of PTA in contact-activated plasmin generation under certain conditions. Still more recently, the direct activation of plasminogen by activated PTA was reported (21). These findings are in contrast to other studies. Both Venneröd and Laake (6) and Bouma and Griffin (8) were unable to show any HF-dependent plasminogen activator or proactivator in the γ-globulin fraction of prekallikrein-deficient plasmas. The latter investigators appeared to use HF fragments (MW 28,000) to activate the γ-globulin fraction containing PTA and it is pertinent to point out that HF fragments are only very weak activators of PTA (22), perhaps one reason for the difference in their results.

Thus, it appears that there is more than one pathway leading to the activation of plasminogen in plasma upon contact with foreign surfaces. Since HF and its substrate (prekallikrein, PTA, and plasminogen) activate reciprocally (23), it is difficult in whole plasma experiments to assess the relative contributions and the mode of actions of each component. But the present study provides evidence to support the hypothesis that PTA functions not only as a blood-clotting factor, but also as a component in the fibrinolytic system.

Summary. Exposure of human plasma to foreign surfaces, under certain conditions, may lead to the development of fibrinolytic activity. To assess the role of plasma thromboplastin antecedent (PTA, Factor XI) in contact-mediated activation of plasminogen, kaolin-induced plasmin generation was examined in 11 severely PTA-deficient plasmas. PTA-deficient plasmas generated significantly less plasmin activity, as measured upon a substrate of *H*-D-Valyl-L-leucyl-L-lysine-*P*-nitroanilide, than normal plasmas. Impaired plasmin generation was not due to the presence of excessive inhibitors, nor due to a deficiency of plasminogen, Hageman factor, prekallikrein, or high-molecular-weight kininogen. Reconstitution of PTA-deficient plasmas with purified PTA completely corrected impaired contact-activated plasmin generation. These results suggest a requirement of PTA in the contact-activated generation of plasmin in human plasma, and support a hypothesis that PTA functions not only as a blood-clotting factor, but also as a component in the fibrinolytic system.

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