

Physiological activation of plasminogen in full term newborn infants

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SUMMARY Plasminogen in full term newborn infants has been measured by functional and immunological assays. Plasminogen functional activity and antigen concentration in newborn infants were about 44% and 48% of adult values respectively. Physiological activation kinetics of plasminogen in undiluted plasma at pH 7.4 and 37°C using various concentrations of streptokinase and urokinase showed no significant difference in the rate of plasmin generation between plasminogen from newborn infants and adult plasminogen.

These findings dispute the suggested existence of functional anomalies in plasminogen from full term newborn infants.

Plasminogen is a protein zymogen in plasma which is converted to plasmin by activators released from various sources in the body. In vivo the plasminogen-plasmin system is a major part of the fibrinolytic mechanism. Functional abnormalities of several coagulation proteins, especially fibrinogen¹⁻³ and prothrombin,^{4,5} have been described in full term newborn infants. Estelles *et al*⁶ studied plasminogen amino acids in full term newborn infants and found, besides small qualitative anomalies, a decrease in the number of amino acid residues per mole of protein. These abnormalities coupled with a decrease in plasminogen levels suggest the possible existence of dysfunctional plasminogen in full term newborn infants.

We have investigated plasminogen functional activity in the plasma of full term newborn infants under physiological conditions at pH 7.4 and 37°C to evaluate the possible existence of a functional anomaly in plasminogen.

Material and methods

Blood samples from normal adults were collected from antecubital veins and those from full term newborn babies, born spontaneously with no complications during or after labour, from the umbilical veins immediately after birth. The blood was anticoagulated with 3.8% sodium citrate (1/9 vol/vol) and centrifuged at 2000 g for 20 min to obtain the

plasma. Plastic containers containing Trasylol (100 Kallikrein inactivating units per millilitre of blood) were used. Plasma was stored at -40°C until use.

REAGENTS

The following reagents were used: chromogenic substrate S-2251 (Kabi Diagnostica); plasmin solution (Kabi Diagnostica); M-partigen single radial immune diffusion plates for plasminogen antigen assay (Behringwerke A G); streptokinase (Kabi-Vitrum); urokinase (Leo Laboratories); and Tris/NaCl buffer, 0.05 mol/l, pH 7.4.

ASSAY FOR PLASMINOGEN ACTIVITY

Plasma plasminogen was assayed by a chromogenic substrate method. Plasma was diluted in Tris/NaCl buffer, pH 7.4, (50 µl plasma in 2000 µl Tris/NaCl buffer). One hundred microlitres of pooled adult or test plasma was activated by streptokinase (10 000 units/ml). The last part of the assay was performed after Friberg *et al*.⁷ The results were calculated from a standard curve prepared from dilutions of pooled normal human plasma.

ASSAY FOR PLASMINOGEN CONCENTRATION

Plasminogen antigen concentration was measured by the single radial immune diffusion technique; M-partigen immune diffusion plates (Behringwerke A G) were used. The results were calculated from a standard concentration curve of pooled normal human plasma dilutions.

ASSAY OF ANTIPLASMIN IN PLASMA

A chromogenic substrate method was used for the

antiplasmin assay.⁷ Diluted plasma is incubated at 37°C with plasmin (0.3 Cu/ml). The plasmin activity inhibited within 20 s is proportional to the amount of antiplasmin activity. The remaining plasmin catalyses the splitting of P-nitroaniline from the substrate (S-2251). The rate at which the P-nitroaniline is released is measured photometrically at 405 nm. The concentration of antiplasmin is calculated from a standard curve prepared from normal human plasma dilutions.

ACTIVATION KINETICS OF PLASMINOGEN IN PLASMA

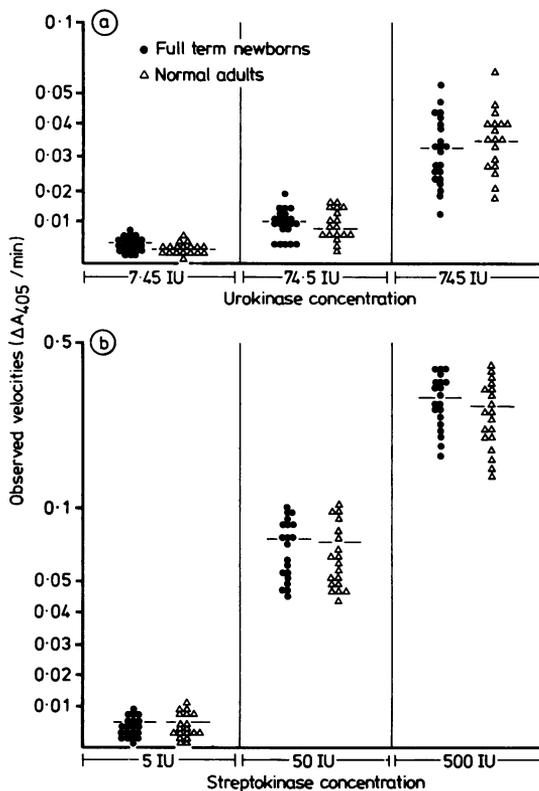
A modification of the method described by Wohl *et al*⁸ was used. Plasminogen in undiluted plasma was activated by various concentrations of activators. Two hundred and fifty microlitres of plasma was blanked against an identical sample to which 50 µl of chromogenic substrate S-2251/buffer solution had been added; 50 µl of activator was added to start the reaction. The reaction occurred at pH 7.4 and 37°C. The activation rate, measured as change in absorbance at 405 nm per minute ($\Delta A_{405}/\text{min}$), was calculated by following the activation curve for 7 min if the activator concentration was low and for 4 min if the activator concentration was high. Various concentrations of streptokinase and urokinase were used.

Results

The Table shows the results of plasminogen and antiplasmin assays in full term newborn infants and adults. Plasminogen functional activity in newborn infants was 44% of the adult value and plasminogen antigen concentration was 48% of the adult value.

Mean antiplasmin activity was 103% ± 29% in adult plasma and 67% ± 25.9% in plasma from newborn infants.

There were no significant differences in the velocities of plasminogen activation for plasminogens from adults and from newborn infants using two different activators and various concentrations.



Physiological plasminogen activation in whole plasma. The ordinate represents the observed initial velocity measured as change in absorbance at 405 nm per minute ($\Delta A_{405}/\text{min}$). The abscissa represents the concentration of urokinase (a) and streptokinase (b).

Discussion

The Figure shows that at low plasminogen concentration—that is, streptokinase 5 IU and urokinase 7.45 IU—only a small portion of plasmin generated is active. As the activator concentration increases, the observed velocity increases exponentially. The exponential plasmin generation means

Results of plasminogen and antiplasmin assays in full term newborn infants (FTN) and normal adults (NA)

	Plasminogen		Antiplasmin			
	Functional assay		Immunological assay		Functional assay	
	FTN	NA	FTN	NA	FTN	NA
Mean	37.92%	86.73%	45.57%	94.6%	67%	103%
SD	10.1%	21.8%	11.45%	19.7%	25.9%	29%
n	26	19	28	20	25	19
Significance*	p < 0.001		p < 0.001		p < 0.001	

*Student's t test.

that the data points for each activator will fall on a straight line on a log-log plot (Figure).

The increase in plasmin activity with activator concentration reflects a fall in the α_2 -plasmin inhibitor concentration. Two facts should be mentioned at this point. Firstly, the observed fibrinolytic activity is that of free plasmin since Harpel *et al*⁹ have shown that in the presence of α_2 -plasmin inhibitor α_2 -macroglobulin does not bind plasmin to any appreciable extent. Secondly, there is no appreciable competition between P-nitroaniline and α_2 -plasmin inhibitor under the conditions of the experiment.⁸ Wiman *et al*¹⁰ showed that P-nitroaniline competes appreciably with α_2 -plasmin inhibitor only at high substrate concentration and low α_2 -antiplasmin concentration. At physiological concentrations of α_2 -plasmin inhibitor such competition becomes minimal, as shown by the exponential rise of plasmin activity with increase in activator concentration while the tripeptide substrate concentration is kept constant.

Wohl *et al*⁸ showed that large changes in plasminogen concentration will produce small or insignificant changes in the observable velocities. This consideration verifies that an observed variation in kinetic behaviour represents a substantial departure from normal of the plasminogen species. Urokinase did not activate plasminogen in plasma as effectively as streptokinase (Figure). The extremely slow increase in the observable velocities indicates that the urokinase inhibitor effect is substantial. Moroi *et al*¹¹ showed a pronounced inhibition of urokinase by α_2 -plasmin inhibitor. This could explain the peculiar urokinase results in plasma.

In studies of the fibrinolytic system of newborn infants, plasminogen levels were found to be low in plasma from full term newborn infants and in pre-term newborn infants¹⁴⁻¹⁶. These results are in agreement with our own, where functional and immunological plasminogen assay results in plasma from full term newborn infants were low.

In studies of adult patients with inherited molecular abnormalities of plasminogen Aoki *et al*¹⁷ found that the abnormality was a depressed level of plasminogen activity, although plasminogen antigen concentration was normal. Wohl *et al*⁸ described two plasminogen variants, Chicago I and Chicago II, that showed impaired activator binding properties, low functional plasminogen levels, and subnormal plasmin generation rate.

The possible existence of dysfunctional plasminogen in full term newborn infants was suggested by Estelles *et al*,⁶ who found a decrease in the number of amino acid residues per mole of protein and different N-terminal amino acids. Their plasminogen activation kinetics were based on the

measurement of plasminogen light chains, which may reflect enzymatic activity,¹⁸ following streptokinase activation. The number of moles of light chain per mole of plasminogen was measured using a reduced sodium dodecyl sulphate gel electrophoresis technique.

In the present study activation kinetics were carried out on undiluted plasma under physiological conditions of pH 7.4 and 37°C. The rate of plasmin generation was measured directly by a tripeptide substrate method. Various concentrations of the clinical preparations of streptokinase and urokinase were used.

Our results confirm that plasminogen in full term newborn infants shows no significant departure from the functional behaviour of normal adult plasminogen. Our experience disputes the existence of functional anomalies in full term newborn plasminogen and confirms the decrease in plasminogen level in newborn babies as measured by functional and immunological assays.

This work was supported by West Dorset Health District. The authors thank Professor P Flute for critically reviewing the paper, Dr P Johnston and Dr R Purvis for their help, the staff of Somerleigh Court Maternity Unit, and Mrs Angela Pickett for typing the manuscript.

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