

The fibrinolytic system in pre-eclampsia

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SUMMARY Plasma fibrinolytic activity and plasma inhibitory activity against urokinase and tissue activator were measured in primigravidae with moderate or severe pre-eclampsia and in gestation-matched primigravidae with uncomplicated pregnancy. The mean levels of fibrinolytic activity and inhibitory activity against urokinase and tissue activator did not differ significantly between the pre-eclampsia and uncomplicated pregnancy groups. The pattern of inhibitory fractions of plasminogen-depleted plasma from pre-eclamptic and uncomplicated primigravidae after gel filtration on Sephadex G-100 was similar.

The long-standing observation that widespread fibrin deposition is common in patients with severe pre-eclampsia and eclampsia has led to the suggestion that there is an abnormality in the mechanism for the deposition or removal of fibrin in toxæmia of pregnancy. Some have championed the view that fibrin persistence through failure of the fibrinolytic system might be aetiologically important in pre-eclampsia.

Studies on the fibrinolytic system in pre-eclampsia have provided conflicting results. While Bonnar and colleagues¹ reported that plasma fibrinolytic activity is lower in women with severe pre-eclampsia, others have observed no alteration in fibrinolytic activity in patients with pre-eclampsia or eclampsia.²⁻⁴ A diminished sensitivity to urokinase-induced fibrinolysis was found by Bonnar *et al.*¹ and Howie *et al.*³

We report a study comparing plasma fibrinolytic activity and, using a new technique, plasma inhibition of plasminogen activators in primigravidae with pre-eclampsia and those with uncomplicated pregnancy.

Material and methods

Plasma Blood was withdrawn from an antecubital vein and mixed with 1/9 volume of 3.8% sodium citrate. The plasma was separated by centrifugation at 2500 *g* for 10 min and used immediately or stored at -20°C until required.

Plasminogen-free plasma was prepared by mixing one part plasma with two parts of lysine-Sepharose (AB Pharmacia, Uppsala, Sweden). After 5 min the plasma was separated by centrifugation. Removal of

plasminogen was confirmed by double diffusion in agar gels against anti-plasminogen serum (Hoechst Pharmaceuticals, Hounslow, Middlesex, UK).

Urokinase was obtained from Leo Pharmaceuticals, Copenhagen, Denmark and dissolved to the required concentration in water containing 200 µg/ml bovine serum albumin (Sigma Chemical Co, Poole, Dorset, UK).

Tissue activator was prepared from pig heart by the technique of Rickli and Zaugg.⁵ Dilutions were made in bovine serum albumin.

Gel filtration was carried out at 8°C on 2.6 cm × 70 cm columns of Sephadex G-100 (AB Pharmacia) equilibrated with 0.1 M Tris/HCl buffer, pH 7.4; 10 ml plasma was applied and 3.0 ml fractions collected.

Plasma fibrinolytic activity was measured in blood maintained in ice water and carried out within 30 min of collection. Euglobulin precipitates were prepared by the method of Nilsson and Olow,⁶ resuspended to the original plasma volume in Tris/HCl buffer (pH 7.4) and reactivity measured on fibrin plates as described previously.⁷ The activity was expressed as units of a standard preparation of tissue activator.

Plasma inhibitory activity on urokinase or tissue activator was measured by incubating equal quantities of diluted plasminogen-free plasma with each activator for 10 min at 37°C before application of 30 µl aliquots to untreated fibrin plates. The percentage inhibition was determined for control values obtained with urokinase or tissue activator in the absence of plasma. The plasma dilutions used were 10% and 25% for urokinase and tissue activator respectively. The dilutions were made in 0.02 M Tris/HCl buffer, pH 7.4.

Inhibitory activity of fractions of plasma separated by gel filtration Aliquots of each fraction were incubated

Plasma fibrinolytic activity and inhibitory activity against urokinase (UK) and tissue activator (TA) in primigravidae with uncomplicated pregnancy and primigravidae with pre-eclampsia

	Normal primigravidae			Pre-eclamptic primigravidae		
	No	Mean	SD	No	Mean	SD
Plasma fibrinolytic activity (units)	12	1.24	1.23	12	0.77	0.66
Plasma inhibitory activity (% inhibition)	UK	8	61.9	9.0	8	51.9
	TA	8	69.2	9.7	8	66.5
					13.8	

with an equal volume of urokinase or tissue activator (final concentration 2.5 CTA U/ml) for 10 min at 37°C before application of 30 µl aliquots to untreated fibrin plates.

Subjects Primigravidae with established proteinuric pre-eclampsia as defined by Nelson⁸ and a control group of primigravidae with uncomplicated pregnancy were studied. The age range of the 12 pre-eclampsia women was 17 to 36 yr (mean 22.0 yr) and of the controls was 17 to 31 yr (mean 23.4 yr). The controls were matched with the pre-eclamptics for gestational age at time of sampling. The pre-eclamptic group was subdivided into the five with urinary protein of less than 2 g/l ("moderate") and the seven with over 2 g/l ("severe").

Results

Plasma fibrinolytic activity The mean fibrinolytic activity of euglobulin precipitates from the plasma of pre-eclamptic primigravidae was lower than that of primigravidae with uncomplicated pregnancy (Table), but the difference did not reach the 5% level of significance (unpaired *t* test). The women with pre-eclampsia graded as moderate had a mean

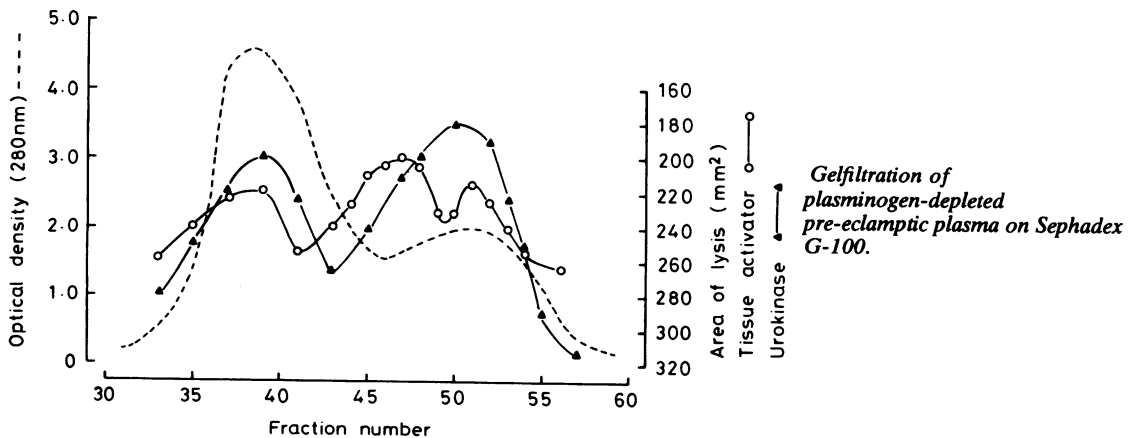
fibrinolytic activity of 0.64 units compared to 0.87 units for those graded as severe.

Plasma inhibition of urokinase and tissue activator The mean inhibitory activity of plasma against tissue activator was very similar in the control and pre-eclampsia groups (Table). The inhibitory activity against urokinase was higher in the primigravidae with pre-eclampsia, but the difference for the women with uncomplicated pregnancy did not reach statistical significance. The mean plasma inhibitory activity against tissue activator for the four women with "moderate" pre-eclampsia was 69.5% compared to 63.5% for those graded as "severe." The corresponding values for urokinase inhibition were 56.4% and 47.4%.

Inhibition of urokinase and tissue activator in fractions of plasma separated by gel filtration Gel filtered plasma of women with pre-eclampsia showed a pattern of elution of inhibitory fractions against urokinase and tissue activator similar to that noted previously in plasma from women with uncomplicated pregnancy.⁹ One example showing the three regions of inhibitory fractions against tissue activator and the two regions inhibitory to urokinase activity is shown in the Figure.

Discussion

This study was carried out to determine whether an alteration in the fibrinolytic system could be demonstrated in women with pre-eclampsia. The level of plasma plasminogen activator is reduced in the later months of normal pregnancy, but we have been unable to show a significant difference in plasma fibrinolytic activity, as assessed by the activity of euglobulin precipitates on fibrin plates, between primigravidae with pre-eclampsia and gestation-



matched primigravidae with uncomplicated pregnancy.

The euglobulin fraction of plasma does not include inhibitors of the fibrinolytic system except for C \bar{I} inactivator, and the activity of euglobulin precipitates on fibrin plates is primarily a measure of plasminogen activator. In a separate study we found that the mean levels of α_2 -antiplasmin, the major plasma inhibitor of plasmin, and the other plasma protease inhibitors (α_2 -macroglobulin, α_1 -antitrypsin, C \bar{I} inactivator and antithrombin III) were not significantly different between women with uncomplicated pregnancy and those with pre-eclampsia.¹⁰

The existence and characterisation of inhibitors of plasma activators distinct from the known protease inhibitors has not been established, and the measurement of plasma inhibition of activators has been unsatisfactory. The technique used in this study involves the removal of plasminogen from the test plasma to obviate the problem of plasmin formation on the addition of activator to plasma. Using this method we have been able to detect inhibition of both tissue activator and urokinase by plasma. The level of inhibition against both activators, however, did not differ between the women with uncomplicated pregnancy and those with pre-eclampsia, although the sample was small. The pattern of inhibition against tissue activator and urokinase seen in gel filtered plasminogen-free plasma from healthy, non-pregnant adults¹¹ and pregnant women⁹ was also seen in plasma from women with pre-eclampsia. The technique is not quantitative, but the peaks of inhibition appeared to be similar in magnitude in the plasma fractions from the normal and pre-eclamptic women.

Overall the results of this study provide no evidence to support the hypothesis that an abnormality in the fibrinolytic enzyme system is related to pre-eclampsia.

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References

- ¹ Bonnar J, McNicol GP, Douglas AS. Coagulation and fibrinolytic systems in pre-eclampsia and eclampsia. *Br Med J* 1971;ii:12-6.
- ² Birmingham Eclampsia Study Group. Intravascular coagulation and abnormal lung-scans in pre-eclampsia and eclampsia. *Lancet* 1971;ii:889-91.
- ³ Howie PW, Prentice CRM, McNicol GP. Coagulation, fibrinolysis and platelet function in pre-eclampsia, essential hypertension and placental insufficiency. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1971;78:992-1003.
- ⁴ Condie RG, Ogston D. Sequential studies on components of the haemostatic mechanism in pregnancy with particular reference to the development of pre-eclampsia. *Br J Obstet Gynaecol* 1976;83:938-42.
- ⁵ Rickli EE, Zaugg H. Isolation and purification of highly enriched tissue plasminogen activator from pig heart. *Thrombosis et Diathesis Haemorrhagica* 1970;23:64-76.
- ⁶ Nilsson IM, Olow B. Fibrinolysis induced by streptokinase in man. *Acta Chirurg Scand* 1962;123:247-66.
- ⁷ Beattie AG, Ogston D, Bennett NB, Douglas AS. Inhibitors of plasminogen activation in human blood. *Br J Haematol* 1976;32:135-43.
- ⁸ Nelson TR. A clinical study of pre-eclampsia. Part I. *Journal of Obstetrics and Gynaecology of the British Commonwealth* 1955;62:48-57.
- ⁹ Walker JE, Gow L, Campbell DM, Ogston D. The inhibition by plasma of urokinase and tissue activator-induced fibrinolysis in pregnancy and the puerperium. *Thromb Haemost* 1983;49:21-3.
- ¹⁰ Gow L, Campbell DM, Ogston D. Blood levels of proteinase inhibitors in pre-eclampsia. *Br J Obstet Gynaecol* 1983;90:950-2.
- ¹¹ Walker JE, Ogston D. The inhibition of tissue activator and urokinase by human plasma. *Thromb Haemost* 1982;47:265-8.

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