Consumption of fibrinolytic proteins in menstrual fluid from women with normal menstrual blood loss

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SUMMARY Fibrinolytic proteins in menstrual fluid collected from women with normal menstrual blood loss (<80 ml) were found to be completely exhausted. High levels of tissue plasminogen activator, fibrin(ogen) degradation products, and plasmin-inhibitor complexes were present, but little fibrinolytically active plasmin remained. No difference was observed between days 1 and 2 of menstruation.

The fluidity of menstrual discharge has intrigued investigators for many years,^{1,2} and the absence of clottable fibrinogen has been attributed to high fibrinolytic activity. The presence of plasminogen activator in endometrial tissue³ and the possibility that an increased level of this enzyme is an aetiological factor in menorrhagia have led to the use of synthetic antifibrinolytic agents to reduce excessive menstrual blood loss.⁴

Since these reports the molecular control of the fibrinolytic enzyme system has been explored in detail.^{5,6} Plasmin, the major fibrinolytic protease, is generated from the inactive proenzyme plasminogen by the action of the enzyme plasminogen activator, present only in trace amounts in peripheral blood.^{7,8} The action of plasmin on fibrin(ogen) is regulated by the inhibitors fast acting α_2 -antiplasmin and slow acting α_2 -macroglobulin.^{9,10} Fibrin, the primary substrate of plasmin, also plays an important regulatory role: both plasminogen activator and plasminogen bind to fibrin, bringing the proteins into catalytic contact and causing the generation of plasmin activity. In addition, fibrin bound plasmin is protected from α_2 -antiplasmin inhibition, localising the proteolytic action of plasmin to the immediate vicinity of the fibrin clot.^{5,6}

The recent exploration of the fibrinolytic enzyme system has been facilitated by methodological advances, notably the preparation of antibodies to fibrinolytic proteins and the development of tripeptide chromogenic substrates for functional assays. We have used these techniques to re-examine fibrinolytic proteins in menstrual fluid collected from women with normal menstrual blood loss (less

than 80 ml)¹¹ to see if further information can be obtained concerning the process of menstrual bleeding.

Subjects and methods

SUBJECTS

Menstrual fluid was collected from 12 women using Tassaway vaginal cups (containing no anticoagulant) inserted for 2 h during the first and second days of menstruation. Day one was taken to extend for 24 h from the onset of menstrual flow. The women (aged 25 to 46 years) all had regular menstrual cycles with an average cycle length of 28 days (range 23 to 33 days). Pelvic examination revealed no abnormality. None of the women had used either oral or intrauterine contraception or received hormone treatment for at least four months before the study. Coitus was prohibited for 24 h before sampling. The women collected all their soiled sanitary devices during two successive menstruations and menstrual blood loss was estimated using the alkaline haematin dilution method.¹²

METHODS

The menstrual fluid collected was centrifuged at 1800 g for 30 min at 10°C; the supernatant was removed and stored at –20°C until analysis.

When all the samples of menstrual fluid supernatant had been collected the assays were performed in single batches. Plasminogen, α_2 -antiplasmin, α_2 -macroglobulin, fibrin(ogen) related antigens, IgG, and serum albumin were each assayed by the radial immunodiffusion technique¹³; the results were related to calibration curves constructed from pooled peripheral plasma of known plasma factor

concentrations (Hoechst standard plasma). A correction was made for the presence of 1/10th volume anticoagulant in peripheral plasma samples.

Fast acting antiplasmin (α_2 -antiplasmin) activity was assayed¹⁴ using the Coatest antiplasmin kit (KabiVitrum, Stockholm) adapted to the Gilford 3800 reaction rate analyser (Gilford Inc, Ohio, USA). Enzymatically active plasmin was assayed by adding 5 μ l of menstrual fluid supernatant to 700 μ l (1 mmol/l) of the plasmin specific chromogenic substrate D-valyl-leucyl-lysine-p-nitroanilide (KabiVitrum, Stockholm) and measuring the reaction at 405 nm at 37°C over a 30 s interval. Concentrations were determined by reference to a standard curve constructed from dilutions of plasmin.

Plasminogen activator activity was measured in euglobulin fractions.¹⁵ Duplicate volumes (25 μ l) of each euglobulin fraction were incubated on fibrin plates¹⁶ and the lysis areas related to concentrations by reference to a standard curve constructed from serial dilutions of urokinase (CTA units). Similar assays were also carried out using fibrin-agar plates¹⁷ containing rabbit IgG fraction raised against human uterine tissue plasminogen activator. Proteolytic activity remaining after inhibition of the plasminogen activator represents the fibrinolytic activity of free plasmin. All antibodies used in this study were prepared by our research group.

Results

All the women had a normal menstrual blood loss of less than 80 ml (median 35 ml, range 15–75 ml).¹¹ There were no significant differences (Student's *t* test) for any factor when samples collected on days 1 and 2 were compared; therefore the following results are presented as the mean values for two samples (days one and two) from the 12 women. In no instance was the presence of clots observed in the vaginal cups.

Table 1 compares the concentrations of albumin, IgG, and fibrinolytic proteins in menstrual fluid

supernatant with those found in pooled peripheral plasma. Adjustment was made to account for the presence of 1/10th volume trisodium citrate in the plasma pool, and an average packed cell volume of 0.42 for menstrual blood was assumed. Concentrations of albumin, IgG, α_2 -antiplasmin, α_2 -macroglobulin, plasmin(ogen), and fibrin(ogen) antigens in menstrual fluid supernatant were similar to or slightly higher than those in peripheral plasma. Plasminogen activator activity was much higher than in peripheral plasma, and fast acting α_2 -antiplasmin activity was zero in each sample (Table 2). Although high levels of enzymatically active plasmin were found using the chromogenic peptide assay, there was low fibrinolytic activity (17%) when assayed with inhibiting antibody against plasminogen activator, which suggests that little free plasmin is present in menstrual fluid (Table 2).

Discussion

This study has shown that menstrual fluid supernatant has features that are distinct from those of serum obtained from the clotting of peripheral venous blood. The very high concentrations (3.7 mg/ml) of fibrin(ogen) related antigen (normally <5 μ g/ml in serum), enzymatically active plasmin (normally undetectable), and high levels of plasminogen

Table 2 Activity levels for fibrinolytic proteins present in menstrual fluid supernatant and peripheral plasma

	Menstrual fluid supernatant (n = 24) (mean $\pm$ SD)	Pooled peripheral plasma (mean)
Plasmin activity (μ mol/l)	0.83 $\pm$ 0.97	0
Plasminogen activator (CTA units/ml)	1.04 (range 0–3.5)	0.15 (range 0–0.2)
Plasminogen activator + anti-PA antibody. (% inhibition)	83 $\pm$ 28	ND
α_2 -antiplasmin (%)	0	100 $\pm$ 20%

ND = not done.

Table 1 Protein concentrations in menstrual fluid supernatant and peripheral plasma

	Menstrual fluid supernatant (n = 24)		Pooled peripheral plasma	
	Mean $\pm$ SD (g/l)	Mean (μ mol/l)	Mean $\pm$ SD (g/l)	Mean (μ mol/l)
IgG	12.5 $\pm$ 2.3	84.5	12.0 $\pm$ 2.3	81.1
Albumin	43.6 $\pm$ 11.8	641	44 $\pm$ 8.8	647
Fibrin(ogen) antigen	3.71 $\pm$ 2.3	10.9	2.25 $\pm$ 0.54	6.62
Plasmin(ogen)	0.17 $\pm$ 0.05	1.88	0.15 $\pm$ 0.03	1.67
α_2 -antiplasmin	0.56 $\pm$ 0.12	0.81	0.70 $\pm$ 0.14	1.0
α_2 -macroglobulin	2.51 $\pm$ 0.37	3.49	2.65 $\pm$ 0.61	3.68

The plasma pool was prepared from blood drawn from 20 normal subjects. Concentrations were determined using a standard plasma from Hoechst Pharmaceuticals, Hounslow.

activator, together with undetectable levels of functionally active α_2 -antiplasmin are in striking contrast to normal serum.¹⁸ That albumin and IgG, the major plasma proteins, were present in menstrual fluid in concentrations similar to those found in peripheral venous blood indicates the absence of haemoconcentration or haemodilution phenomena that would have otherwise invalidated these comparisons.

Menstrual fluid was obtained from the vagina instead of the uterine cavity since this procedure is noninvasive and was the method of collection most acceptable by the women. In addition, the presence of a probe in the uterine cavity is thought to affect endometrial blood flow, at least in sheep.¹⁹

Our findings show an exhaustive activation of the fibrinolytic mechanism in normal menstrual discharge which is probably responsible for its fluid properties. This study did not indicate whether fibrinogen or fibrin is digested before or after clotting, though plasminogen activation is normally a fibrin dependent reaction.^{5*} The detection of substantial quantities ($0.83 \mu\text{mol/l}$) of enzymatically active plasmin that retained little fibrinolytic activity on fibrin plates is probably explained by the action of α_2 -macroglobulin. This inhibitor inactivates a wide range of proteases by an "entrapment" mechanism, which destroys fibrinolytic activity while leaving enzymatic activity still detectable using small synthetic substrates.¹⁰ The enzyme activity so detected may therefore represent the complex between plasmin and the slow reacting α_2 -macroglobulin.

As inactivation of all the fast acting α_2 -antiplasmin ($0.81 \mu\text{mol/l}$) in menstrual fluid represents $0.81 \mu\text{mol/l}$ plasmin(ogen) consumed (the reaction has a 1:1 stoichiometry) then the remaining plasmin ($1.88 - 0.81 = 1.07 \mu\text{mol/l}$) would react with α_2 -macroglobulin, giving rise to the $0.83 \mu\text{mol/l}$ activity detected by chromogenic assay. α_2 -macroglobulin represents a substantial reservoir of inhibitory activity since each mole can inhibit two moles of protease and it is present in a molar excess over plasmin. The discrepancy ($0.24 \mu\text{mol/l}$) between expected and measured figures may be due to the small contribution of α_1 -antitrypsin inactivating plasmin. The potent proteolytic activity of plasmin is thus confined to the uterine compartment and does not appear to extend to the vaginal tract.

These observations are especially important since they have been made on menstrual fluid collected from subjects with normal blood loss ($<80 \text{ ml}$)¹¹ assessed by objective procedures. That normal menstrual blood appears to be fibrinolytically exhausted strongly suggests that other factors within the endometrium are responsible for the heavy mens-

trual blood loss that is amenable to treatment with synthetic antifibrinolytic agents.^{4, 20}

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