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Age Dependence of Blood Fibrinolytic Components and the Effects of Low-Dose Oral Contraceptives on Coagulation and Fibrinolysis in Teenagers

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Key words

Fibrinolysis – Tissue-type plasminogen activator – Tissue-type plasminogen activator inhibitor – Oral contraceptives

Summary

Basal t-PA antigen concentration, PAI-I activity and fibrinolytic capacity was studied in plasma from 20 healthy teenager girls (age 15.9 ± 1.3 years) and two groups of older healthy volunteers, consisting of 17 women (age 32 ± 8 years, group 1) and 35 men (age 34 ± 8 years, group 2). Basal t-PA antigen concentrations in plasma were found to be highly age-dependent with higher values with increasing age. The teenager girls had significantly lower values compared with the two groups of elderly volunteers. PAI-I levels were significantly higher in plasma from the teenager girls and the fibrinolytic capacity after 15 min of venous occlusion was significantly lower.

In this study we also determined the effect of low-dose oral contraceptives (OC) on coagulation and fibrinolysis in the teenager group. Each teenager served as her own control with samples drawn before and on OC after 4 months of use. The coagulation parameters, factor VIII activity, AT III, Protein C and platelet counts were all within reference values before and on OC. The fibrinolytic activity in plasma after venous occlusion (15 min) increased significantly when the teenagers had used OC for 4 months. This phenomenon was explained by significantly decreased PAI levels and also by significantly increased t-PA antigen release from the vessel wall after venous occlusion.

Introduction

Disturbances in the fibrinolytic system are correlated with development of venous thrombosis (1). It has recently been shown that this might be due to several different mechanisms, either a decreased synthesis of vessel wall tissue plasminogen activator (t-PA) or a defective release of t-PA from the vessel wall. Defective fibrinolysis can also be due to increased concentration of tissue plasminogen activator inhibitor (PAI-I) in plasma (2, 3). Oral contraception (OC) usage has been reported to be associated with an increased risk of thromboembolic events (4) especially for women over the age of 35 and those who smoke (5). A correlation was found between estrogen doses and thromboembolism in OC users (6). However, in an epidemiologic study, high dose of d-norgestrel tended to diminish thromboembolic complications (7). The effect of both high- and low-dose oral contraceptives on the fibrinolytic system is not clear. It has been claimed that patients developing thrombosis during use of oral contraceptives already have a defective fibrinolytic system (8). On the other hand increased fibrinolytic activity in plasma have been reported in women using oral contraceptives low in estrogen (9).

During the last years new and improved methods for t-PA and PAI-I measurements in plasma have been developed. The aim of the present investigation was twofold. Firstly, using these new methods, we studied basal fibrinolytic activity, basal t-PA antigen concentration and PAI-I in plasma from teenagers, to examine whether these parameters were age-dependent. Secondly the fibrinolytic components were evaluated after the influence of low dose oral contraceptives. We also studied the possible variations in some coagulation factors.

Subjects, Materials, and Methods

Twenty healthy teenager girls, of whom 17 came back, who called the department of gynecology to receive oral contraceptives participated in the study. All had a regular menstrual cycle (28–33 days). The age was 15.9 ± 1.3 (mean ± SD). Range 14–18 years. None was taking any drug. Three teenagers smoked 4–5 cigarettes per day.

All teenagers were investigated in the middle of the menstrual cycle (12–18 days after the first day of the last bleeding). Samples were drawn before and on low-dose oral contraceptives after 4 months of use. The OC contained 0.75 mg Lynestrenol and 37.5 µg ethinylestradiol, and were taken for 22 days per cycle commencing on day 7.

Plasma values from 52 apparently healthy volunteers served as reference values for the fibrinolytic parameters. The volunteers were divided in two groups. Group 1 consisted of 17 women (age 32 ± 8 years, mean ± SD) and group 2 of 35 men (age 34 ± 8 years; mean ± SD). Samples from the women were drawn in the middle of the menstrual cycle. They did not take any OC. Venous occlusion test was performed in 13 women and 23 men.

Sampling for Measurements of Coagulation and Fibrinolysis and Venous Occlusion

The teenagers and the healthy volunteers were fasting at least 8 hrs before sampling and were resting in supine position from 10 min before until the end of the test. All samples were drawn between 8–9 a.m. Blood samples were first drawn from an antecubital vein without a torniquet (for all coagulation and fibrinolysis determinations). Venous occlusion was produced by applying a sphygomanometer cuff to the upper arm and leaving it inflated for 15 min to a level half-way between systolic and diastolic blood pressure. Blood samples for fibrinolytic activity and t-PA antigen were drawn from the antecubital vein immediately before deflating the cuff. All blood samples were collected in plastic tubes containing 0.13 M trisodium-citrate and centrifuged immediately. The plasma was kept at −70° C until analyzed.

The haemoconcentration induced by the venous occlusion was not measured and the data were not corrected.

Assays

A pooled human plasma (from thirty healthy blood donors) was used as control in all assays.

Reference interval for FVIII:C, Antithrombin III and Protein C are mean ± 2 SD from the plasma values from 30 apparently healthy volunteers.

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Table 1 Age dependence of t-PA antigen and PAI-I in plasma

	No. of subjects	t-PA ag µg/l		No. of subjects	PAI-I U/ml		
Women (14–18 years)	20	2.8 ± 1.4		20	11 ± 6.1		
			p = 0.002			p = 0.002	
Women (22–43 years)	17	4.7 ± 1.7		17	6.2 ± 2.9		ns
			p = 0.02			ns	
Men (22–44 years)	35	6.7 ± 2.8		28	8.5 ± 4.9		

Results are mean ± SD.

FVIII:C (biologic activity) was assayed using a one-stage clotting assay with FVIII:C-deficient plasma as test base (10). The reference interval was 60–160%.

Antithrombin III (ATIII) was enzymatically assayed using a chromogenic substrate (S-2238) for thrombin as described in the manual for Antithrombin COATEST (Kabi-Vitrum, Sweden). The reference interval was 86–120%.

Protein C was assayed with an enzym-immunoassay method (Elisa Protein C, Boehringer Mannheim). The reference interval was 64–140%.

Platelet counts were calculated with a Coulter S+. Reference interval was 150–350 × 10^9/l.

Elisa for t-PA. Determination of t-PA antigen concentrations in plasma was performed with the "Imulyse 5" t-PA antigen kits (Biopool AB Umeå, Sweden) (11).

Tissue plasminogen activator inhibitor (PAI-I) in plasma was measured with Spectrolyse/pL kits from Biopool Sweden using polylysine as a stimulator (12).

The fibrinolytic activity in plasma was measured by means of the digestion of radiolabelled fibrin coated into plastic tubes as described by Moroz and Gilmore (13). The procedure was performed as follows: Human fibrinogen was purchased from Kabi, Sweden. The preparation was made plasminogen-free by absorption of plasminogen to lysine-sepharose (Pharmacia, Uppsala, Sweden) as described by the manufacturer. Fibrinogen was labelled with ^{125}Iodine by means of the chloramine-T method.

Iodidelabelled fibrinogen was diluted to working solution of about 200,000 cpm/ml in 0.015 M Na-phosphate buffer, pH 8.1. 100 µl of the fibrinogen working solution was added to 2.5 ml plastic tubes and allowed to rotate at a 45° C angle for 2.5 hrs after which 0.5 ml bovine albumin (10 g/l) was added and incubated for 20 min. This incubation was followed by four washes of the tubes by means of 0.015 M TRIS-NaCl, pH 7.4. 200 µl of thrombin (10 U/ml) was added and the tubes were incubated at 37° C for 10 min to transform fibrinogen into fibrin. Finally the tubes were washed twice in the TRIS-NaCl buffer. The fibrin coated tubes were counted in a gamma counter and tubes which deviated more than 10% in activity from the mean activity of the particular batch were discarded. The tubes were stored until use (maximal 3 weeks) in the refrigerator.

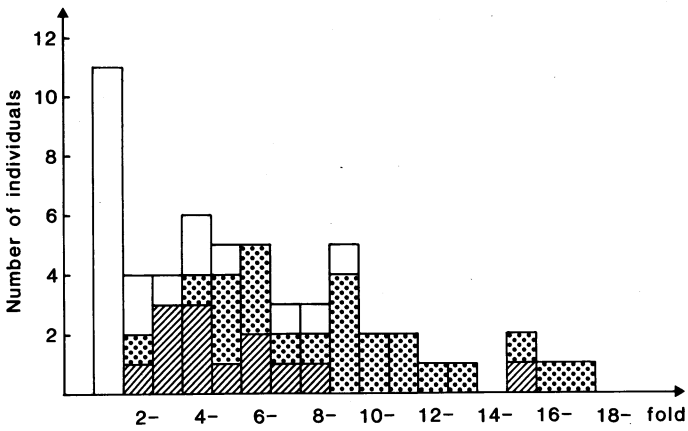

Fig. 1 Increase in plasma fibrinolytic activity after venous occlusion compared with preocclusion values. □ bars represent teenagers; ▨ bars represent the reference group constituted of elderly women; ▩ bars represent the reference group of men

The assay was run as follows: The material to be assayed (100 µl) and 100 µl TRIS-NaCl buffer were added to the tubes and incubated in triplicates at 37° C for 30 min. After this 1 ml of buffer was added and the fluid transferred to another tube and the activity in the fluid counted in a gamma counter. The results are expressed in percent of activity of 25 µg trypsin after correction for background activity i.e. the activity in the tubes incubated with buffer alone.

Statistical Methods

The significance of the differences between corresponding changes of t-PA antigen and PAI-I before and on OC use was tested by means of t-test for paired observations, and further verified by a Wilcoxon non parametric rank sum test. The other significances were tested by means of t-test for group means. The probability level for statistical significance was chosen to be at least $p < 0.05$.

Results

Fibrinolytic Components in Plasma from Women and Men of Different Age

Both reference groups had higher mean t-PA antigen concentrations in plasma before venous occlusion than the teenagers had (Table 1). There was also a significant difference between women and men in the same age group.

Mean PAI-I level in plasma from the teenagers were significantly higher compared with the reference group consisting of elderly women but did not differ from the mean PAI-I level from the reference group of men (Table 1).

The fibrinolytic pattern in plasma studied after venous occlusion was regarded as a measure of the fibrinolytic capacity i.e. fold increase compared with preocclusion values. The twenty teenagers had a 2.8-fold mean increase (range 0.7–9.0-fold) in fibrinolytic activity in plasma after 15 minutes of venous occlusion (Fig. 1). Women [31 ± 5 years (mean ± SD)] had a 5.3-fold mean increase (range 2-15-fold) and the reference group consisting of men [34 ± 8 years (mean ± SD)] had a 8.8-fold mean increase (range 2-17-fold). The teenagers had significantly ($p < 0.05$) lower fibrinolytic capacity than the older women which had significantly lower capacity than men.

Results of Some Coagulation Parameters in Plasma from Teenagers before and on OC Mediation

All teenagers had values within the reference interval for platelet counts, FVIII:C activity, ATIII concentrations and Protein C antigen concentrations before and after 4 months on oral contraceptives (Table 2). 8/17 individuals had decreased levels of ATIII after use of oral contraceptives compared with pretreatment values.

Fibrinolytic Components in Plasma from Teenagers before and on OC Medication

After 4 month medication on OC the fibrinolytic activity in plasma after venous occlusion was significantly increased

Table 2 Effects of low dose oral contraceptives on some coagulation components

	Before OC	On OC
AT III (%)	108 ± 17	103 ± 22
Protein C (%)	113 ± 18	117 ± 16
FVIII:C (%)	121 ± 26	110 ± 23
Platelets ($\times 10^9$/l)	257 ± 61	275 ± 63

Results are mean ± SD.

Table 3 Fibrinolytic components in plasma from teenagers before and on OC medication after 4 months

	Before OC	On OC	
Fibrinolytic act %			
before v.o.	4.42 ± 0.99	5.0 ± 2.5	n.s.
after v.o.	11.39 ± 8.8	18.5 ± 10.5	p = 0.03
t-PA antigen µg/l			
before v.o.	2.8 ± 1.44	1.96 ± 1.2	n.s.
after v.o.	15.9 ± 6.4	24.0 ± 12.6	p = 0.016
PAI U/ml	11.55 ± 6.1	5.5 ± 5.7	p <0.01

Venous occlusion = v.o.
Results are mean ± SD for the whole group.

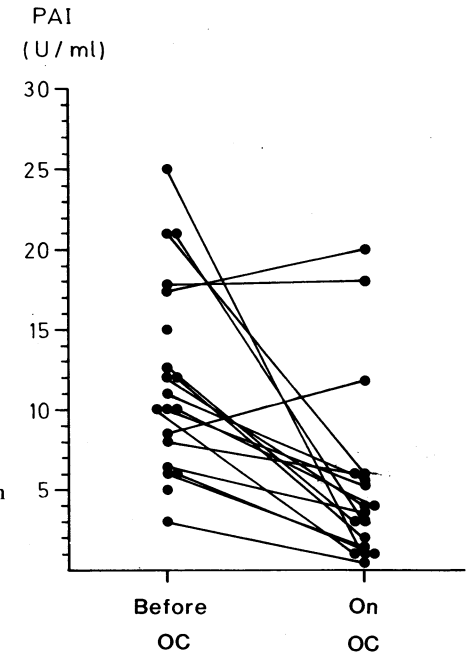

Fig. 3 PAI-I levels in plasma before venous occlusion before and on oral contraceptives. The PAI-I levels were significantly decreased (p <0.001, t-test for paired observations) after 4 months on OC

(Table 3). The teenager girls now had a 4.5-fold mean increase of the fibrinolytic activity in plasma after venous occlusion. PAI-I mean level was significantly decreased. The mean level of t-PA antigen before venous occlusion did not differ from the level before OC use. 10/17 young women had t-PA antigen concentrations of 2 µg/l or less compared to 5/20 before OC use. However t-PA antigen mean level after venous occlusion was significantly increased.

Fig. 2 shows all t-PA antigen values before and after venous occlusion before and on OC. The t-PA antigen concentration was significantly increased. In 3 girls the t-PA antigen concentrations increased more than 30 times after occlusion.

PAI-I values were significantly decreased after 4 months on OC (Fig. 3).

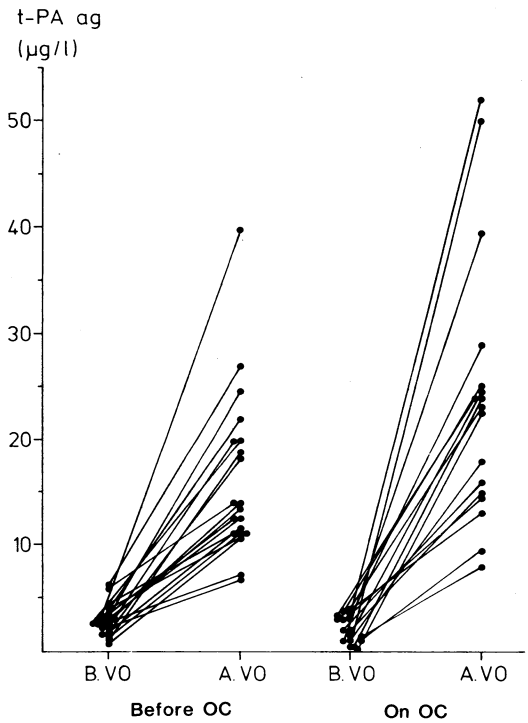

Fig. 2 t-PA antigen concentrations in plasma from 20 teenagers before and after venous occlusion before OC use and in 17/20 on OC. The t-PA release on OC was significantly increased (p <0.05, t-test for paired observations)

Discussion

Basal t-PA antigen concentrations in plasma were found to be highly age dependent with higher values with increasing age. These results confirm those recently reported by Rånby et al. (11) concerning two groups of adults. In accordance with these results, teenagers seem to have lower concentrations than adults. PAI-I levels were higher in the group of teenagers. It is known that PAI-I activity in bloodplasma of healthy individuals show a diurnal fluctuation (14) and increase with age (15, 16, 17). The fibrinolytic capacity was also lower in the group of young women. These results indicate the necessity of age- and sex matched controls when one is evaluating the different components of fibrinolysis in plasma from patients. It has been speculated that the increasing t-PA antigen levels at higher age may reflect the biological aging of the vascular endothelial cells (11). The mechanism behind the phenomenon is however still unexplained.

In this study the mean values for Antithrombin III and Protein C were unaltered after four month on OC. However, the teenager group showed a larger SD in Antithrombin III values compared to the reference group. This could reflect an age variation.

The fibrinolytic activity in plasma before venous occlusion was surprisingly not significantly increased after OC use. A decrease of circulating basal t-PA antigen was effected by OC. This was also shown recently by Gevers Leuven et al. (18). This decrease of t-PA ag, however not significant, may help to explain the not occurring increase in fibrinolytic activity but is most probably not the whole truth.

One must consider that partly different methods were used when measuring the fibrinolytic activity in plasma (19). However, in this study we also investigated the fibrinolytic capacity and found it enhanced after OC use. There was also an enhanced concentration of t-PA antigen after venous occlusion. Perhaps oral contraceptive agents may induce enhanced synthesis of t-PA, which then can be released at a proper stimulus. This might be due to a stimulating effect of the gestagen component since it has

previously been shown that addition of d-norgestrel to culture medium stimulated the t-PA antigen release from incubated human veins in vitro (20).

The reduction of circulating PAI-I certainly also contributed to the enhanced fibrinolytic capacity in blood plasma. Low dose OC reduced PAI-I levels in this and Gevers Leuvens study. Jespersen also found lower PAI-I levels in a group of women on OC compared with a group of non users (21).

The regulating mechanisms of synthesis and release of PAI-I are still quite unknown. Endotoxin, Il-I and thrombin can induce PAI-I activity in plasma and conditioned medium from endothelial cells (22, 23, 24). Longterm oral administration of Stanozolol decreases PAI-I activity in blood plasma and activated human Protein C pursuit a direct inhibitory effect on PAI-I (25, 26). It may be possible that also the hormones in OC affect the production and/or release of PAI-I from the endothelial cells. The decrease of PAI-I levels after OC use could, at least to a part reflect the steroid influence on the hepatocytes, since these cells might also contribute to plasmatic PAI-I levels (27).

Teenagers are a significant group of OC users. The observed decrease in venous thromboembolism with low dose OC is compatible with the unaltered values of Antithrombin III and Protein C and the profibrinolytic changes noted in our young subjects.

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