Changes in the plasma levels of proteins C and S in young women on low-dose oestrogen oral contraceptives

A. GRANATA - G. A. SOBBRIO - F. D'ARRIGO - M. BARILLARI
P. DE LUCA - M. EGITTO - D. GRANESE (*) - C. PULLÈ (*) - F. TRIMARCHI

Summary: The physiological importance of proteins C and S as natural anticoagulants is demonstrated by the increased risk of thromboembolic disease among subjects with hereditary deficiency of both proteins. In the present study the effects were evaluated of low-dose oestrogen oral contraceptives (OC) on the plasma levels of immunological protein S, as free (PS-f), and in reversible complex with C4b-binding protein as well as functional protein C (PC) in a homogeneous group of 20 young healthy women. The participants were randomly given either gestodene (75 μg) or desogestrel (150 μg) in combination with ethinyl oestradiol (30μg). Blood samples were taken prior to the initiation of the treatment and at the end of the sixth 21-day treatment cycle. The mean concentration of both free and bound PS fell significantly, the decrease still being within the reference range. Conversely, the plasma values for PC rose to a statistically significant extent. There were no significant differences between the two OCs. Hypothetically, the changes in PS-f (active fraction) might be conducive to a procoagulant state, which the increased PC may compensate. The reverse effect of two OCs on the activity of the protein C-protein S anticoagulant system might suggest a different regulation of their synthesis.

Key words: Protein C; Protein S; Desogestrel; Gestodene; Oral Contraceptives.

INTRODUCTION

Oral contraceptive (OC) use has been reported to be linked with an increased risk of thromboembolic events (1). Among the mechanisms possibly responsible for enhanced morbidity, impairment of haemostatic balance has been implicated.

Changes in a number of factors of fibrinolytic and coagulation systems have been reported (review Beller). It has been suggested that the extent of the thromboembolic risk may vary with the type and dose of both estrogen and progestogen (3). So far, little attention has been paid to the influence of OCs on the status of protein C (PC) and S (PS) system, which, together with Antithrombin III, represent the most important natural inhibitors of coagulation.

The protein S is a vitamin k-dependent plasma protein, which serves as a cofactor for the anticoagulant (inactivation of Factors Va and VIIIa) and profibrinolytic activity (via its interaction with Type I plasminogen activator inhibitor) of ano-

Institute of Clinica Medica I,
University of Messina,
School of Medicine, Messina (Italy)
(*) Institute of Gynecology,
University of Messina,
School of Medicine, Messina (Italy)

ther vitamin K-dependent, activated protein C [4]. The PS circulates in plasma as a free protein and in reversible complex with C4b-binding protein, a regulatory protein of the complement system [4].

Only free PS (fPS) is active functionally [5]. Recurrent venous thromboembolic disease has been reported in patients with acquired [6, 7] or congenital deficiency in either PC [8] or PS [9]. Therefore, a longitudinal study was carried out to assess and compare the effects of two currently most used progestogens, 19-nortestosterone derivative gestodene (GD) and desogestrel (DG) as in monophasic combination with 30 μg ethinyl estradiol (EE), on PS and PC.

MATERIALS AND METHODS

Subjects

Twenty apparently healthy, non smoking women were recruited for the study. None of them had taken any hormonal medication or any drug known to interfere with xitamin K synthesis in at least the preceding six months. None of the 20 participants had a personal or familial history of thromboembolic disease or underlying conditions which contraindicated the use of OCs. After informed consent, all the subjects were randomly given either 150 μg desogestrel (DG) - group A (n = 10) - or 75 μg gestodene (GD) - group B (n = 10) in association with 30 μg ethinyl oestradiol (EE) for a 6-cycle treatment. The two groups were fully comparable both for age (22.00±4.22 vs 25.80±4.76; mean ± SD for group A and B, respectively) and for Quetelet's Index (23.10±3.46 vs 22.76±3.59; mean ± SD) [10].

Plasma collection and timing

Blood sampling was performed prior to OC treatment in the follicular phase (days 1 to 4) and at 6 months' treatment. The samples were withdrawn with minimum of stasis in the morning (between 8.30-10.30) in the supine position after a brief rest (20 min) and overnight fasting. Discarding the first 2 ml, venous blood (9.5 ml) was collected in plastic tubes containing 0.11 M sodium citrate (0.5 ml). The tubes were mixed and immediately transferred in crushed ice. The samples were centrifuged as soon as possible in a refrigerated centrifuge at 4 °C for 30 min at 2000×g. Citrated plasmas were stored at −70 °C until assayed.

Assays

PC activity was measured by an amidolytic method (Berichrom-Protein C, Boehringwerke AG, Marburg, West Germany) on an automatic Hitachi 705 analyzer (Naka Works, Katsuda, Japan). The reference interval was 60-140%.

PS (free and bound) was determined with an enzyme-linked immunosorbent assay (Elisa Protein S, Boehringer, Mannheim, FRG). The reference interval for total (PS (t PS) was 70-140%. Free PS (f PS) was separated from the PS complexed with C4b-bp according to Comp [5]. The reference interval for f PS was 21-84%.

Reference interval for PC and PS are mean ±2 SD from normal (n = 15) pooled plasma.

Statistical analysis

The significance of the differences between corresponding changes of PC, t PS and f PS prior to and on OC use was tested by Student's paired *t* test and further verified by Wilcoxon's non parametric rank sum test. The other significances were tested by means of *t* test for group means.

RESULTS

The data are presented as mean ±SD. The results are shown in Table 1.

Functional PC concentrations increased significantly in both groups as compared with the pretreatment levels. Conversely, a statistically significant decrease in PS was found. The decrease was observed in both free and bound form as compared with their respective baseline values. There were no significant differences at any time between groups for each variable measured.

DISCUSSION

These results show that the effect on PC and PS system of monophasic desogestrel are similar to those of monophasic gestodene. In the few earlier investigations, women taking OCs had unchanged [11, 12, 13, 14] or increased [15, 16, 17] PC levels. These discrepancies may be accounted for by methodologic differences as well as different oestrogen content or progestogen

Table 1. – *Plasma concentrations of protein C activity (PC), free protein S (fPS) and total protein S (t PS) during intake of a monophasic ethinyl estradiol/desogestrel combination (Group A, n=10) and a monophasic ethinyl estradiol/gestodene combination (Group B, n=10).*

	Baseline		After 6 months		Normal range
	Group A	Group B	Group A	Group B	
PC (%)	98.48± 9.64	104.79±31.52	73.96±12.07 [5]	83.14±18.28 [6]	60 - 140
t PS (%)	99.63±21.99	117.27±31.88	43.84± 4.04 [3]	44.66±14.17 [4]	60 - 140
f PS (%)	55.70± 9.84	68.80±19.11	117.48±21.29 [1]	135.60±28.66 [2]	21 - 84

[1] P=0.0036; [2] P=0.000061; [3] P=0.0079; [4] P=0.0083; [5] P=0.013; [6] =P0.00080.
The P values refer to the differences between baseline and treatment values for each group.

type. In the present study we found an increase in PC. The physiopathological significance of the increased PC levels is not clear. One may speculate that the changes in PC is an effect induced by oestrogen on the hepatic synthesis of K-vitamin-dependent factors.

Indeed, in women taking OCs the four vitamin-K-dependent factors (II, VII, IX, and X) have been reported as being elevated ([1]). That the PC increase occurs to counterbalance the rise in the above procoagulant factors, is another hypothesis ([14, 15]).

Previous studies have reported that OCs decrease total PS ([13, 14, 17, 18]). However, in these investigations there are no concordant results as to the effects on free protein S. In keeping with Malm ([17]) and Boerger ([18]) we found a significant fall in f PS. A recent publication provides further support for our results ([19]). Since the only free fraction of PS is functional as a cofactor for the anticoagulant effect of activated PC ([5]), the variations observed in f PS might result in being prone to thrombosis for OC users. Of interest is the fact that the PS and PC, though both are vitamin-K-dependent factors, showed an opposite pattern. This reverse effect induced by OC treatment would suggest that the two proteins have a different regulation for their synthesis. Furthermore, since the results for PC and PS (free and bound) in both groups parallelled, the progestogens in use, DG and GD, do not interfere with oestrogen-induced changes.

BIBLIOGRAPHY

1) Mammem E. F.: *Am. J. Obst. Gyn., 142,* 781, 1982.
2) Beller F. K., Ebert C.: *Obst. Gyn. Surv., 40,* 425, 1985.
3) Meade T. W.: *Am. J. Obst. Gyn., 142,* 758, 1982.
4) Clouse L. H., Comp P. C.: *N. Engl. J. Med., 314,* 1298, 1986.
5) Comp P. C., Doray D., Patton D., Esmon C. T.: *Blood, 67,* 504, 1986.
6) Pabinger I.: *Blut, 53,* 63, 1986.
7) Comp P. C., Viganò S., D'Angelo A., Thurneau G., Kaufman C., Esmon C. T.: *Blood, 66* (Suppl.) 348 a., 1985.
8) Griffin J. H., Evatts B., Zimmerman T. S., Kleiss A. J., Wideman C.: *Clin. Invest., 68,* 1370, 1982.
9) Schwarz H. P., Fischer M., Hopmeier P., Batard M. A., Griffin J. H.: *Blood, 311,* 1525, 1984.
10) Garrow J. S., Webster J.: *Int. J. Obes., 9,* 147, 1985.
11) Jespersen J.: " A study of the antithrombotic potential in women on oral contraceptives low in oestrogen ". In: *Jespersen J., Kluft C., Korsgaard O. (eds.). Clinical aspects of fibrinolysis and thrombosis, pp. 399-419* (Esbjerg: South Jutland University Press, 1983).
12) Cohen H., Mackie I. J., Walshe K., Gillmer M. D. G., Machin S. J.: *Br. J. Haematol., 69,* 259, 1988.

13) Gilabert J., Fernandez J. A., España F., Aznar J., Estelles A.: *Thromb. Res., 49,* 319, 1988.
14) Huisveld I. A., Hospers J. E. H., Meijers I. C. M., Starkenburg A. E., Erich W. B. M., Bouma B. N.: *Thromb. Res., 45,* 109, 1987.
15) Meade T. W., Stirling Y., Wilkes H., Mannucci P. M.: *Thromb. Haemost., 53,* 198, 1985.
16) Gonzalez R., Alberca E., Vicente V.: *Thromb. Res., 39,* 637, 1985.
17) Malm J., Laurell M., Dahlbäck B.: *Br. J. Haematol., 68,* 437, 1988.
18) Boerger L. M., Morris P. C., Thurnau G. R., Esmon C. T., Comp P. C.: *Blood, 69,* 692, 1987.
19) Melissari E., Kakkar V.V.: *Thromb. Res.: 49,* 489, 1988.

Address reprint request to:
A. GRANATA
Viale S. Martino, 13-366
28124 Messina (Italy)