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The Effect of Low-Dose Estroprogestinic Preparations on Prothrombin Complex Factors: No Significant Increase after an 8-Month Trial*

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Summary. The behavior of the prothrombin complex factors in 16 healthy women during low-dose estroprogestinic treatment (laevonorgestrel 0.15 mg and ethynilestradiol 0.30 mg) at basal conditions and during 8 months of therapy has been investigated. We found a statistically significant decrease of the PTT (Partial Thromboplastin Time). The prothrombin time, on the other hand, became slightly decreased, but not to a statistically significant extent. Among the prothrombin time derived tests for evaluating the prothrombin complex only the PP test (Prothrombin Proconvertin test) was significantly shortened. Of the coagulation factors (factors II, VII and X) only a modest, but not statistically significant, increase in Factor VII and Factor X was noted. We conclude that, during the 8 month observation period, prothrombin complex factors are not altered substantially.

Key words: Oral contraception – Prothrombin complex – Estroprogestinics – Hypercoagulability

The oral estroprogestinic drugs have been used for many years to inhibit ovulation for the purpose of contraception as well as in the treatment of disorders of the menstrual cycle. The side effects of these drugs have been studied by many authors. In particular, there have been many studies on the modifications of the coagulation system induced by these drugs [6, 9, 11, 13, 16, 19, 22, 24]. Many authors have shown evidence of a correlation between the use of oral contraceptives and the incidence of thromboembolic episodes [2, 3, 4, 8, 10, 23, 26, 27, 28]. This correlation seems to be due to the estrogen content of the estroprogestinic preparations. Changes in the coagulation process also seem to be correlated to the estroprogestinic content [14, 17, 27, 28]. Thus, at present the tendency is to use low-dose estroprogestin preparations in which the ethynilestradiol content has been reduced to 0.03 mg [20, 25]. It has, in fact, been demonstrated that the use of low dose preparations greatly reduces the incidence of

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thromboembolic phenomena [21] and that the reduced morbidity concerns almost exclusively venous thromboembolism [5].

The use of low dose preparations, however, does not appear to affect significantly the incidence of arterial thrombotic complications (cerebral, coronary, etc.). In order to characterize the causes of thromboembolic manifestations in subjects on estroprogestinic therapy, studies have been made of the principal parameters of coagulation and of the vessel wall. The interpretations of these studies have been very controversal. Some studies showed evidence of pathologic alterations of the vessel walls which could represent an important factor in the pathogenesis of thrombosis. Such alterations were attributed to the action of steroids and, in particular, that of estrogen. In recent years, however, this hypothesis has been abandoned because it has not been possible to demonstrate a significant correlation between the type of lesion produced and the use of estroprogestins, since such damage to vessel walls was also present in conditions other than that with oral contraceptives [15].

Most authors agree that a possible state of hypercoagulability may ensue from the use of these drugs. The changes in clotting parameters seem to be related to the dose of estroprogestin in the preparation used. Some of these alterations are dosedependent (i. e. dependent on the steroid content of the drug); others are secondary to the individual reactivity to the hormone therapy [21, 30]. The main alterations reported involve the coagulative parameters (global tests, levels of clotting factors, evaluation of the intrinsic and extrinsic pathways of coagulation), the inhibitors of coagulation (AT III) and the hematologic parameters (blood viscosity, etc.) [1, 2, 4].

The scope of our study was to evaluate the behavior of the prothrombin complex factors in 16 healthy women on low dose estroprogestinic therapy (Laevonorgestrel 0.15 mg, Ethynilestradiol 0.03 mg) in basal conditions and during 8 months of therapy. These factors have been reported to be elevated during pregnancy or during estroprogestinic therapy [1, 2, 3, 9, 14].

Material and Methods

16 women between 20 and 35 years old were studied. All subjects had negative histories of coagulation disorders, hepatic disease, episodes of thrombosis and previous pregnancy. All women took estroprogestinic contraceptives (Laevonorgestrel 0.15 mg, Ethynilestradiol 0.030 mg as supplied by Wyeth Lab. USA – Ovranet and by Schering AG Berlin Germany-Egogyn 30). The various tests were performed on blood samples taken before breakfast between 8 and 10 a. m.

A basal sample was taken prior to initiation of treatment; the follow-up samples were taken every 60 days for 8 months. These were then compared to the base sample. These same women before the initiation of treatment were considered as normal control subjects (basal samples).

Blood was taken in the fasting state, with a no. 19 needle and a plastic syringe. Sodium citrate was used in a 1:10 proportion as anticoagulant. The Prothrombin Time (PT) was measured by the classic method using tissue thromboplastin supplied by General Diagnostic, Morris Plains, USA. The Partial Thromboplastin Time (PTT) was carried out on citrated plasma, using activated partial thromboplastin (Cephaloplastin, Dade Laboratories, Miami, USA). The Prothrombin Proconvertin Test (PP test) was carried out on citrated plasma diluted 1:10 with Michaelis buffer. 0.20 ml of Simplastin A (General Diagnostic) was incubated at 37 °C for 10–15 min and then 0.10 ml of the diluted plasma was added. Thrombotest and Normotest were performed on citrated plasma, mixing 0.25 ml of reagent with 0.05 and 0.025 ml of plasma respectively. Factors II, VII, X activities were evaluated according to previously described methods [12].

The mean, the standard deviation, the variance and the standard error were calculated for

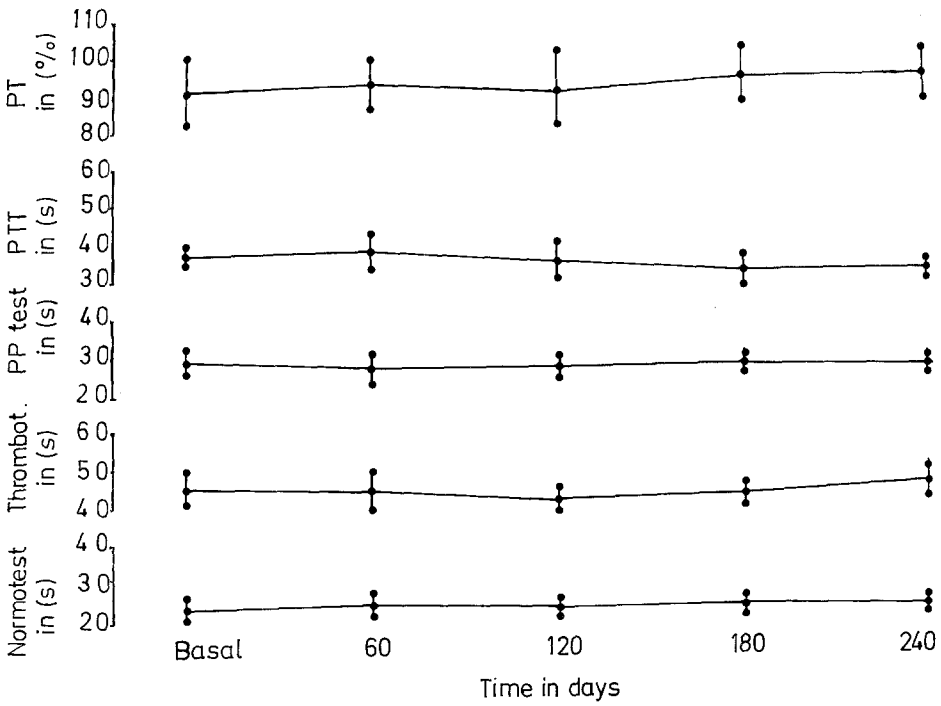

Fig. 1: Overall behavior of PT, PTT, PP test, Thrombotest and Normotest during estroprogestinic therapy. The PP test showed statistically significant changes at every control. On the contrary, the PTT showed significant changes at 180 and 240 days. All other changes were not significant

each parameter. The comparison of averages for each parameter at the basal state with the various samples taken afterward was executed utilizing the Fisher test.

Results

The results are summarized in Figure 1 and in Tables 1, 2, 3 and 4. The mean PT in the basal control was 90.94% ± 8.76 and similar values were observed after 60, 120, 180 and 240 days of therapy. The mean basal PTT value was 37.02 s ± 2.30, 33.44 ± 3.97 and 33.81 ± 2.89 respectively after 180 and 240 days of therapy. These differences were statistically significant. The mean PP test in basal conditions was 29.57 s ± 2.45. In the following samples it was 27.41 s ± 3.49, 27.89 s ± 1.82, 27.97 s ± 1.63 and 27.68 s ± 1.46 respectively after 60, 120, 180, 240 days of therapy. None of these values was significantly different from the basal value. Mean Thrombotest value in basal conditions was 45.51 s ± 3.58; mean basal Normotest value was 24.43 s ± 2.18; Factor II was 122.75 ± 18.45 in basal conditions, Factor VII Activity was 95.13 ± 17.28 and mean Factor X Activity was 109.47 ± 23.82: none of these values varied significantly after 60, 120, 180 or 240 days of therapy.

Table 1. Averages and standard deviations of clotting parameters investigated during the observation period

Parameters		Basal	60 days	120 days	180 days	240 days
PT	mean	90.94	92.69	91.62	94.80	95.88
(%)	s.d.	8.76	7.16	9.74	6.71	6.25
PTT	mean	37.02	37.69	36.55	33.44	33.81
(s)	s.d.	2.30	4.56	4.85	3.97	2.89
PP Test	mean	29.57	27.41	27.89	27.97	27.68
(s)	s.d.	2.45	3.49	1.82	1.63	1.46
Thromobotest	mean	45.51	44.94	43.31	43.72	46.39
(s)	s.d.	3.58	4.57	2.69	2.61	3.74
Normotest	mean	24.43	26.02	24.77	24.91	24.20
(s)	s.d.	2.18	2.70	2.03	2.06	1.96
Factor II	mean	122.75	125.23	118.25	121.56	112.07
Activity (%)	s.d.	18.45	31.15	18.89	28.74	26.64
Factor VII	mean	95.13	100.90	102.27	97.20	109.50
Activity (%)	s.d.	17.28	15.80	14.36	19.90	27.12
Factor X	mean	109.47	114.67	109.62	107.00	122.28
Activity (%)	s.d.	23.82	31.64	21.94	31.96	30.08

Table 2. Statistical analysis of Prothrombin Time (PT) and Partial Thromboplastin Time (PTT) values at 60, 120, 180 and 240 days as compared to basal values. Asterisk indicates statistical significance

Parameters	number of cases	time	mean ± s.d.	p
PT	16	0	90.94 ± 8.76	0.546
(%)	16	60	92.69 ± 7.16	
	16	0	90.94 ± 8.76	0.671
	16	120	91.62 ± 9.74	
	16	0	90.94 ± 8.76	0.118
	16	180	94.80 ± 6.71	
	16	0	90.94 ± 8.76	0.087
	14	240	95.88 ± 6.25	
PTT	16	0	37.02 ± 2.30	0.606
(sec.)	16	60	37.69 ± 4.56	
	16	0	37.02 ± 2.30	0.680
	16	120	36.55 ± 4.85	
	16	0	37.02 ± 2.30	0.003*
	16	180	33.44 ± 3.97	
	16	0	37.02 ± 2.30	0.002*
	14	240	33.81 ± 2.89	

Table 3. Statistical analysis of prothrombin time derivative tests (PP test, Thrombotest, Normotest) values at 60, 120, 180, 240 days as compared to basal values. Asterisk indicates statistical significance

Parameters	number of cases	time	mean ± s.d.	*p*
Prothrombin Proconverting Test (PP Test) (s)	16	0	29.57 ± 2.45	0.048*
	16	60	27.41 ± 3.49	
	16	0	29.57 ± 2.45	0.033*
	16	120	27.89 ± 1.82	
	16	0	29.57 ± 2.45	0.027*
	16	180	27.97 ± 1.63	
	16	0	29.57 ± 2.45	0.016*
	14	240	27.68 ± 1.46	
Thrombotest (s)	16	0	45.51 ± 3.58	0.671
	16	60	44.94 ± 4.57	
	16	0	45.51 ± 3.58	0.056
	16	120	43.31 ± 2.69	
	16	0	45.51 ± 3.58	0.113
	16	180	43.72 ± 2.61	
	16	0	45.51 ± 3.58	0.522
	14	240	46.39 ± 3.74	
Normotest (s)	16	0	24.43 ± 2.18	0.074
	16	60	26.02 ± 2.70	
	16	0	24.43 ± 2.18	0.637
	16	120	24.77 ± 2.03	
	16	0	24.43 ± 2.18	0.537
	16	180	24.91 ± 2.06	
	16	0	24.43 ± 2.18	0.688
	14	240	24.20 ± 1.96	

Discussion

Our results show that the use of low-dose estroprogestinic preparations can shorten significantly the Partial Thromboplastin time in agreement with previous studies [7, 21, 23]. Such modification manifests itself only after a few months of therapy. In fact, it became statistically significant only in the 3nd set of samples. This could be related to the increase of Factor VII already noted by other authors [23], but the importance of such an increase in the pathogenesis of thromboembolism associated with the use of estroprogestinic preparations is doubtful [17]. In considering the prothrombin complex, it appears evident that the low-dose estroprogestin preparations are not able to modify significantly the prothrombin activity during the 8 months of treatment over which the patients were followed. In fact, a modest increase in the prothrombin activity was observed during the treatment, but the difference was not statistically significant. With respect to the other global tests for the evaluation of the prothrombin complex (PP test, Normotest, Thrombotest), only the PP test presents a statistically significant difference between basal values and samples taken after initiation of estroprogestin therapy. Such a difference was seen already in the 1st sample and persisted

Table 4. Statistical analysis of Factor II, Factor VII and Factor X levels at 60, 120, 180 and 240 days as compared to basal values

Parameters	number of cases	time	mean ± s.d.	*p*
Factor II	16	0	122.75 ± 18.45	0.658
Activity	16	60	125.23 ± 31.15	
in %	16	0	122.75 ± 18.45	0.507
	16	120	118.25 ± 18.89	
	16	0	122.75 ± 18.45	0.561
	16	180	121.56 ± 28.74	
	16	0	122.75 ± 18.45	0.205
	14	240	112.07 ± 26.64	
Factor VII	16	0	95.13 ± 17.28	0.344
Activity	16	60	100.90 ± 15.60	
in %	16	0	95.13 ± 17.28	0.159
	16	120	102.27 ± 14.36	
	16	0	95.13 ± 17.28	0.676
	16	180	97.20 ± 19.90	
	16	0	95.13 ± 17.28	0.103
	14	240	109.50 ± 27.12	
Factor X	16	0	109.47 ± 23.82	0.624
Activity	16	60	114.67 ± 31.64	
in %	16	0	109.47 ± 23.82	0.394
	16	120	109.62 ± 21.94	
	16	0	109.47 ± 23.82	0.683
	16	180	107.00 ± 31.96	
	16	0	109.47 ± 23.82	0.210
	14	240	122.28 ± 30.08	

during the entire study. The other test results did not change significantly. The evaluation of the activity of the individual factors shows a slight increase in activity for both Factor VII and Factor X which, nevertheles, was not statistically significant. Thus the alterations of the coagulation system observed during treatment with oral estroprogestins are not always univocal. This could be the result of various factors such as the variations in hormone dosage, the variations in the technique adopted for the various tests, or the variations in the diet and other features of the patients being examined.

In conclusion, our results suggest that the low-dose estroprogestin preparations are not capable of significantly influencing the activity of the individual factors of the prothrombin complex even if the estroprogestin content is sufficient to modify significantly some of the global clotting tests. The low-dose oral estroprogestin contraceptives, therefore, show a much lower effect on coagulation than the analogous preparations with higher estroprogestin content previously studied [21]. The low-dose preparations are, without doubt, to be preferred for women with positive histories of thromboembolic episodes or with »borderline« hemocoagulation parameters but with the need of an efficient contraception. Based on these results, we may state that no hypercoagulable state, as reflected by the evaluation of the prothrombin complex, appears in patients on low-dose estroprogestin therapy. The duration of treatment in our study appears to be sufficiently long (8 months) to cause any state of hypercoagulability.

In fact, it has been noted that the shift towards a hypercoagulable state with estroprogestinic therapy usually occurred by the $2^{nd}-3^{rd}$ month of treatment [7, 9, 18]. One may therefore reasonably assume that if no significant alterations occur after 8 months of therapy such alterations will not occur afterwards.

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