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Efficacy and effects of transdermal hormone therapy in postmenopausal women

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Summary

The authors aimed to evaluate the influence of transdermal hormone therapy (HT) in the blood coagulation parameters. Fifty-eight postmenopausal women received: G1-transdermal estradiol (E2) (50 mg), G2-E2+oral micronized progesterone (100 mg/day), and G3-E2+oral medroxyprogesterone acetate (2.5 mg/day) for six months. Statistical relevance was seen mainly in G1 and G2. G1 showed a decrease in prothrombin time (PT), fibrinogen (FG), and thrombin time (TT) at six months ($p < 0.05$) compared to baseline values. G2 showed an increase of PT ($p < 0.05$) and a reduction of activated partial thromboplastin time (aPTT), AT III at three months ($p < 0.01$). AT III showed an increase after six months ($p < 0.05$), as well as F1+2 ($p < 0.05$). G3 exhibited a decrease of PT after three and six months of transdermal HT. Data suggests that E2 alone or E2+OMP are correlated with some variations of anticoagulant and procoagulant factors. E2+MPA avoids any major activation of blood coagulation in patients who receive transdermal HT.

Key words: Transdermal hormone therapy; Postmenopausal women; 17-beta-estradiol; Oral micronized progesterone; Medroxyprogesterone acetate.

Introduction

Postmenopausal women suffer from climacteric symptoms, which are associated with hypoestrogenism [1, 2]. These symptoms may include undesirable hot flashes, breast tenderness migraines [3], and sleep disturbance [4]. The decrease in estrogen level is also associated with loss of bone mass [5] and adverse effects on lipid metabolism [6]. Hormone therapy (HT), which consists of estrogen and progestogen administration [7], is the most effective treatment to counteract menopause-related symptoms [4, 8, 9]. However, estrogen plus progestin therapy, but not estrogen therapy, increase the risk of breast cancer and this risk may be greater when initiated close to menopause [10]. National Institutes of Health launched the Women's Health Initiative (WHI) trials to evaluate menopausal HT. Patients used standard-dose oral conjugated equine estrogens (CEE) with and without standard-dose continuous medroxyprogesterone acetate (MPA), which revealed not only an increased risk of breast cancer, but also cardiovascular disease, stroke, and thromboembolic events [10, 11]. Against this background, exogenous estrogen administration has been shown to affect the levels and activity of certain blood coagulation factors, such as antithrombin III (AT III) that is a potent coagulation inhibitor, and may increase the risk of thrombosis if not present in adequate levels [12].

Current clinical studies have demonstrated the risks of use of HT for postmenopausal women, mainly oral HT [13, 14]. Nevertheless, some recent epidemiological data suggest that transdermal estrogen therapy use does not expose women to an excess risk of a first venous thromboembolism (VTE) [15, 16]. Some evidence showed that transdermal estrogen therapy has little effect on blood homeostasis [15] and the route of administration seems to influence the risk factors. Transdermal estrogens are widely used in Europe, but the impact of this route of administration on the risk of recurrent VTE has not been elucidated [2]. Therefore, due to lack of consistent results about the risks associated with different types of progestins and the transdermal route administration, the present authors proposed to evaluate the effects of transdermal estrogen therapy, isolated or combined, with two types of progestogens - micronized progesterone or MPA. In addition, they aimed to evaluate the some coagulation and anticoagulation blood parameters, such as prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen (FG), AT III, prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin III complex (TAT) that affect blood homeostasis.

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Table 1. — *Reference values of coagulation tests.*

Coagulation test	Normal values
Prothrombin time	0.85 to 1.20
Activated partial thromboplastin time	0.80 to 1.25
Fibrinogen	2 to 4 g/L
Thrombin time	≤ 1.20
Fragement 1+2	< 1.1 nmol/L
Antithrombin III	70 to 120%
Thrombin-antithrombin complex	2.0 to 6.5 µg/mL

Materials and Methods

This study was approved by the Research Ethics Committee of the Federal University of São Paulo UNIFESP/EPM under protocol number 0333/02, and all participants previously agreed with and signed an informed consent form. Samples were selected between 2004 and 2005 from Climacteric Section, Department of Obstetrics and Gynecology - Federal University of São Paulo. Clinical data were collected from patient's charts. All patients were submitted to a medical interview, general and gynecological physical exams. Women included in the current study were at ages 45–65 years and body mass index (BMI) < 32 kg/m^2, and had not consumed soy and derivatives during the last 180 days prior the study. All women placed in group 1 of the study underwent a hysterectomy procedure and had follicle stimulating hormone (FSH) levels of up to 35 mU/ml. Patients with any kind of heart attack, coronary heart disease or stroke, estrogen-dependent neoplasia, abnormal vaginal bleeding, anticoagulant use and prior history of VTE were excluded from the study. The authors also excluded patients using medication that could interfere with blood clotting.

Manuscript describes the effects of estrogens and estrogen-progestin on the coagulation system in menopausal women. Due to the necessity to perform HT with isolated estradiol in hysterectomized women, this study was not a double-blind study, and all other data did not differ. In a prospective longitudinal study, 58 subjects were assigned to three different groups and treated for six months: 1) G1 isolated estrogens (E2) (hysterectomized women, transdermal 17-beta-estradiol 50 mcg every three days), 2) G2 (E2 plus 100 mg/day of oral micronized progesterone – OMP – continuously), and G3 (E2 plus 2.5 mg/day of oral medroxyprogesterone acetate – MPA – continuously).

Blood coagulation was evaluated at baseline (T0), at three months (T3), and at six months (T6) after HT treatment in order to set up the parameters of coagulation. Blood samples were collected from each patient between 8 and 9 AM; an overnight fast was not necessary. With regards to coagulation measurements, 20 mL of venous blood was collected in non-evacuated tubes containing 3.2% of trisodium citrate and conducted to laboratory hematology of São Paulo Hospital. Platelet-poor plasma was obtained by two centrifugation steps at 2,500 g for 15 minutes at 25ºC. Aliquots were transferred to plastic tubes and stored at -20ºC for further analysis.

PT, aPTT, and TT were measured on fresh citrated plasma by standard routine methods, described previously by Proctor and Rapaport [17]. All results were expressed as the ratio of test sample/normal plasma pool.

Plasma AT III activity and FG were measured by a functional method previously described by the present group [18], which consisted in the coagulation method (the results expressed in g/l) and practical method (amidolytic procedure) using a chromogenic substrate Tos-Gly-Pro-Arg-pNA (the results were expressed in percentage), respectively [12]. The analysis of F1+2 was determined according to the standard method Clauss in previously frozen plasma, and TAT was performed following the protocol established by Bonduki *et al.* [18]. All reference values of parameters are described in Table 1.

Statistical tests were performed using GraphPad Prism 3.00. Continuous data were described as mean and standard deviation (SD). All data were normally distributed, difference across groups was analyzed by ANOVA followed by Tukey-Kramer test. Statistical significance was established as $p < 0.05$.

Results

To investigate the effect of transdermal HT, the subjects received \ transdermal estrogen therapy isolated or combined with OMP or MPA and were followed up during six months. This study comprised 20 women in G1 group with 53.7 ± 3.6 years of age (mean ± S.D.) and the BMI range was 26.8 ± 3.3, 20 in G2 group with 54.5 ± 4.8 years of age and BMI range was 27.1 ± 2.4, and 18 in G3 group with 54.7 ± 4.0 and BMI range was 27.6 ± 1.9. The results obtained in the analysis of the coagulant and anticoagulant variables of the three groups of this study are shown in Table 2.

Levels of pro-coagulant PT showed statistically signifi-

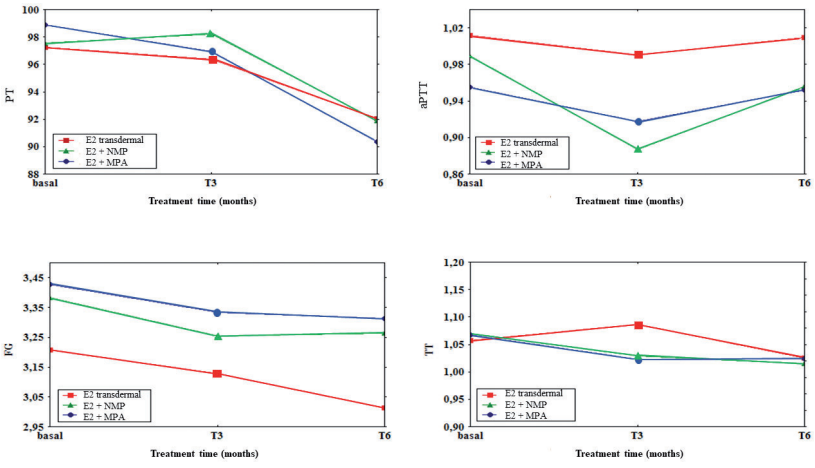

Figure 1. — Comparisons of prothrombin time, activated partial thromboplastin time, fibrinogen, and thrombin time concentrations before treatment initiation with baseline (T0), at three months (T3), and at six months (T6) of HT, which consist in transdermal 17-beta-estradiol (E2 transdermal), transdermal 17-beta-estradiol plus natural micronized progesterone (E2+OMP), and transdermal 17-beta-estradiol plus medroxyprogesterone acetate (E2+MPA).

Table 2. — *Hormonal and metabolic features of the women studied. Values distribution of treatment months in mean and SD.*

Variable	**Min-max***	**G1/E2** n=20	**G2/E2 + OMP** n=20	**G3/E2 + MPA** n=18
Prothrombin time	51 - 100			
T0 (basal)		97.3 ± 4.6	97.6 ± 4.4	98.9 ± 3.0
T3		96.4 ± 7.1	98.2 ± 8.1	96.9 ± 4.7[c]
T6	92.1 ± 6.4[a]	91.9 ± 11.3[b]	90.3 ± 9.8[d]	
Activated partial thromboplastin time	0.71 – 1.29			
T0 (basal)		1.01 ± 0.10	0.99 ± 0.10	0.96 ± 0.12
T3		0.99 ± 0.13	0.89 ± 0.09[e,f]	0.92 ± 0.09
T6		1.01 ± 0.11	0.96 ± 0.11	0.95 ± 0.09
Thrombin time	0.89 – 1.95			
T0 (basal)		1.06± 0.06	1.07 ± 0.07	1.07± 0.05
T3		1.09± 0.02	1.03± 0.09	1.02 ± 0.07
T6		1.03± 0.04 [g]	1.02± 0.07	1.02 ± 0.05
Fibrinogen	2.21 – 5.3			
T0 (basal)		3.21 ± 0.72	3.38 ± 0.48	3.43 ± 0.34
T3		3.13 ± 0.54	3.26 ± 0.47	3.33 ± 0.42
T6		3.01 ± 0.52[a]	3.26 ± 0.57	3.32 ± 0.50
Antithrombin III	67 - 138			
T0 (basal)		110.4 ± 11.7	114.1 ± 10.7	110.8 ± 13.3
T3		104.8 ± 17.4	102.4 ± 9.0[e]	102.6 ± 18.5
T6		107.8 ± 7.5	105.5 ± 8.1[b]	106.2 ± 8.7
Fragement 1+2	0.29 - 9			
T0 (basal)		0.66 ± 0.26	0.66 ± 0.20	0.67 ± 0.18
T3		0.74 ± 0.42	0.80 ± 0.28	0.73 ± 0.30
T6		0.88 ± 0.56	1.54 ± 0.89[a]	0.84 ± 0.35
Thrombin-antithrombin complex	1 – 10.5			
T0 (basal)		2.74 ± 1.37	2.75 ± 1.63	2.48 ± 1.06
T3		2.82 ± 1.35	2.86 ± 2.26	2.82 ± 2.02
T6		3.28 ± 2.31	2.98 ± 2.30	3.49 ± 2.6

**Reference values for all groups. SD = standard deviation; OMP = oral micronized progesterone; MPA = medroxyprogesterone acetate. [a]p < 0.05 compared to G1/T0; [b]p < 0.05 compared to G2/T0; [c]p < 0.01 compared to G3/T0; [d]p < 0.001 compared to G3/T0; [e]p < 0.01 compared to G2/T0; [f]p < 0.01 compared to G1/T3; [g]p < 0.05 compared to G1/T3.*

cant differences at T6 in the three studied groups, but the only G3 group was relevant at T3. The authors observed only significant variation in the anticoagulant aPTT values in G2 at three months and a greater reduction in G2 *vs.* G1 group at three months.

TT revealed a reduction in G1 at T6; significant differences is shown in the comparisons between the values at T6 and T3 (Figure 1).

After three months of HT application, the authors observed a greater decrease of AT III levels in G2 at T3 and a significant increased at T6 compared to baseline values.

F1+2 levels were most prominent in G2 that showed an increase in the sixth month of treatment compared to baseline. All groups reveled a tendency to increase of levels F1+2, but G1 and G3 groups did not show significant values. With regards to marker of thrombin generation, dur-

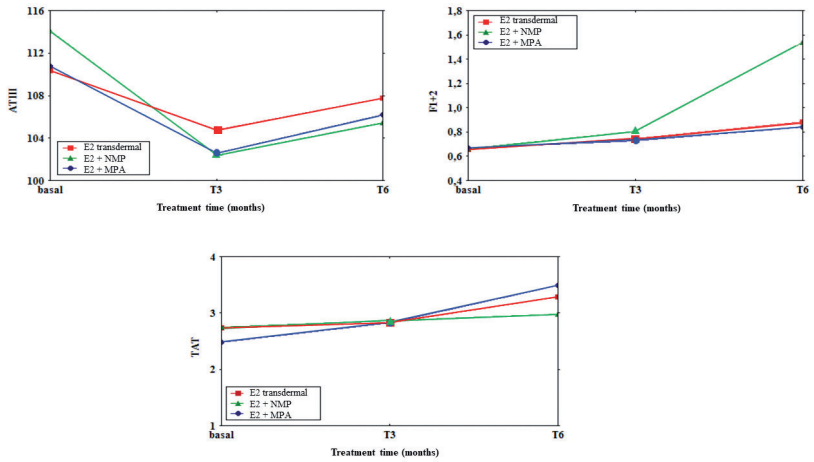

Figure 2. — Comparisons of antithrombin III, fragement 1+2 and thrombin-antithrombin complex concentrations before treatment initiation with baseline (T0), at three months (T3) and at six months (T6) of HT, which consist in transdermal 17-beta-estradiol (E2 transdermal), transdermal 17-beta-estradiol plus natural micronized progesterone (E2+ OMP), and transdermal 17-beta-estradiol plus medroxyprogesterone acetate (E2+ MPA).

ing the HT, clearly no significant change was observed in TAT complex levels (Figure 2).

Discussion

Despite a constant decrease in the use of postmenopausal HT over the last years, for many women, hormones are still prescribed, in order to alleviate climacteric symptoms [19]. Nonetheless, postmenopausal women are subject to a number of adverse changes in the coagulation and fibrinolytic systems [20]. Studies of coagulation systems in postmenopausal women have shown divergent results regarding hypercoagulable states and the risk of thromboembolic phenomena resulting from HT [21, 22]. Progestogens are needed in hormone replacement therapy to prevent endometrial hyperplasia or neoplasia when estrogen is administered. Synthetic progestins are used in usual HT, and these progestins have different affinities for the progesterone receptor and they may also activate non-progesterone receptors' steroid in different tissues. The most physiological way to apply HT is to give bioidentical hormones transdermally without a first-pass mechanism in the liver to avoid non-physiological changes and actions of the hormones [23]. Therefore the present authors decided to investigate the hypercoagulable state and action of blood coagulation factors on transdermal HT in postmenopausal women.

PT was evaluated to estimate prothrombin, a glycoprotein [24]. In the three studied groups (G1, G2, and G3) PT levels showed a decrease mainly at six months of HT. The association with MPA has shown significant reduction beginning at the three initial months of treatment. The shortening of PT observed in oral and transdermal HT could be related to the increase in factor VII [18]. Extrinsic coagulation pathway is measured by aPTT, which can indicate a thrombophilia state if in high levels [25]. Present data showed a trend towards a reduction in aPTT levels. These two coagulation factors have unsettled compositions and suffer some influences that may alter their values. Thus, they showed limited relevance in the studies that evaluate hormonal influence in the coagulation systems.

TT may indicate an abnormality in the conversion of soluble protein FG into fibrin [26]. The present authors did not observe greater changes in TT, which corroborates with other studies [18, 27]. In accordance with the TT results, the FG showed a discreet reduction in the same group (G1) after six months of HT, confirming the direct influence. Furthermore, this fact might indicate a beneficial effect of estroprogestative therapy in decreasing FG levels and blood viscosity after six months of treatment. This might reduce the possibility of occurrence of thromboembolic events. Data analyzed were more consistent when MPA associated with estradiol was used. A remarkable decrease in these values was found at six months of treatment. In another study, Postmenopausal Estrogen/Progestin Interventions (PEPI), which is the most important regarding the effects of HT related to FG in postmenopausal women, an increase in FG levels in relation to its basal levels was described. Independently of the type of hormonal treatment, there was a reduction in the levels of FG compared with placebo group [28]. Curiously, the present authors detected this small alteration only in the G1 group. Perhaps this effect may be protective against blood viscosity, once the low levels of fibrinogen might lead to blood viscosity reduction [29].

Others parameters of coagulation system indicate that oral estrogen seems to have a procoagulant effect, even in a short time, leading to a reduction of antithrombin activity [30]. This important coagulation inhibitor acts in the level of activated factor X and thrombin. These effects were confirmed with the use of high doses of conjugated equine estrogens (CEE) for three months; an increase of fibrinopeptide A and a decrease of AT III levels were observed [31]. However, these authors also observed that the dose of daily 0.625 mg CEE could also be procoagulant, but its effect was significantly lower than the highest dose, especially in the decrease of AT III activity. The present authors observed that only transdermal E2+OMP had pronounced reduction in the first three months, and after the third initial month a significant increase of AT III levels occurred. Enzelsberger *et al.* [27] reported that significant alterations in the levels of AT III after one year of oral treatment with 0.625 and 1.25 mg of conjugated estrogen or transdermal with 50 μg of 17-beta-estradiol per day combined with progestogens were not observed. Another study showed that treated women with transdermal 17-beta-estradiol (25 to 200 μg) did not exhibit alterations in the levels of AT III [32]. Several authors described that neither progestins nor steroids derivatives do not determine the alterations in the coagulation system, while lynestrenol can reduce AT III, most likely by the metabolic products which have estrogenic activity [33-35].

F1+2 and TAT are the most useful parameters for the diagnosis of thrombosis [18, 36]. Interestingly, the present authors also observed that postmenopausal women exhibited a greater increase of procoagulant factor F1+2 in the treatment with E2+OMP mainly after the third month of treatment. Combination of E2+OMP determined a negative impact in the coagulation system with a tendency of hypercoagulability state in comparison with E2+MPA. This data has shown a significant increase of 43% in the treatment with estrogen and 100 mg of micronized progesterone. Previous studies have demonstrated elevated levels of F1+2 after both oral and transdermal administration of estrogen therapy [29, 31, 37]. It was suggested that patients who showed high levels of F1+2 might be in a prothrombotic state [38, 39]. On the other hand, it may be explained by the fact that OMP was used orally. Biologic evidence supports a differential effect of oral *vs.* transdermal estrogen on hemostasis. Prothrombin F1+2 is a marker in vivo thrombin generation which is related to the risk of recurrent

VET [36, 40]. Nevertheless, reported data emphasized that transdermal estrogen therapy had no detrimental effect on coagulation especially prothrombin F1+2 plasma level [18, 40-42].

There is a hypothesis that the estroprogestative therapy (CEE associated with MPA) does not affect the coagulation system and the thrombosis may be a multifactorial phenomenon [43]. Genetics factors might partially explain the susceptibility of thrombosis in women after estrogen treatment. There are contradictions in the literature about the action of estrogenic isolate or associated in the coagulation system [44].

TAT complex levels reflect coagulation activity [7] and in the present study, the data suggest that the treatment with E2+MPA does not induce coagulatory activity, once the TAT complex did not significantly change. Callejon *et al.* [7] asserted that there is no increase of coagulation activity when the levels of TAT complex have not significant changed, suggesting that the decrease of protein C and antithrombin and a decrease of factor VII and factor X neither induce a state of hypercoagulability nor coagulation activation. Transdermal administration of postmenopausal estrogen therapy seems not to be associated with an increased risk of cardiovascular complications, especially stroke and VTE [44-46]. It is necessary to take into account that low doses of estrogen to postmenopausal women with risk factors for stroke should be administered, and the transdermal route is indicated for older women and for women with a high VET risk [46, 47]. Furthermore, the occurrence of VET, gallbladder disease, and stroke can be prevented by the use of transdermal route of estradiol administration. Optimized HT will allow us to treat symptomatic women for as long as required, taking into account that their individual risk/benefit ratio are favorable [47].

In the current study, the therapy with transdermal estradiol combined with natural progesterone might determinate procoagulant alterations, due to the increase of F1+2 and the substantial reduction of AT III during the treatment, as well as the partial and discreet increase in TAT complex. Initially, the E2+MPA combination could have an important procoagulant effect by AT III reduction. In addition, the present authors noted that in the transdermal estradiol (isolated) group, a significant reduction of FG values occurred, which would be beneficial. The present results suggest that during six months, the transdermal estrogen therapy promoted less effect in the coagulation parameters, and improvement of hormonal, hematological, and metabolical variables. Large prospective studies are needed to determine the role of transdermal estrogen therapy in postmenopausal women.

Conclusion

In conclusion, transdermal estrogen and estroprogestative therapies seem to promote the reduction of procoagulant factor PT. Transdermal estradiol combined with OMP is correlated with the reduction of anticoagulant factors aPTT and AT III. Estrogen alone might be associated with a reduction of FG and TT levels and exhibit a tendency to decrease in patients treated with progestogens. Treatment with transdermal estradiol combined with MPA avoids any major activation of blood coagulation in patients who receive this type of HT.

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