A comparative study of the effect of continuous combined conjugated equine estrogen plus medroxyprogesterone acetate and tibolone on blood coagulability

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BACKGROUND: Hormone therapy (HT) after the menopause is associated with increased risk of venous thromboembolism (VTE). Tibolone has pharmacodynamic properties different from other hormone preparations. We compared the effect of a combined HT and tibolone on the inhibition of haemostasis. METHODS: Thirty-eight post-menopausal women were randomly assigned to 1.25 or 2.5 mg per day of tibolone or oral continuous combined conjugated equine estrogen plus medroxyprogesterone acetate (CEE/MPA). Inhibitors of haemostasis were measured at baseline and after 12 months. RESULTS: Results from the two groups of women receiving tibolone were not significantly different and, to improve the power of the study, the two groups were merged. Higher concentration at baseline and after 12 months. CONCLUSIONS: Tibolone induces fewer pharmacological alterations on blood coagulability than CEE/MPA and has a potentially favourable effect on APC-R. This may translate into a corresponding low risk of VTE, as also indicated from the existing clinical data.

Key words: coagulation/conjugated equine estrogen/medroxyprogesterone acetate/tibolone/venous thromboembolism

Introduction

Hormone therapy (HT) is associated with an increased risk for venous thromboembolism (VTE), as recently reviewed (Lowe, 2004). The risk for VTE induced by HT is dependent on the composition of the HT used. Users of estrogen-only preparations have lower risk of VTE than women receiving combined estrogen–progestin preparations (Daly et al., 1996; Perez et al., 1997; Varas-Lorenzo et al., 1998; Rossouw et al., 2002; Douketis et al., 2005), and also the route of administration seems to be of importance. Women treated with transdermal HT may have lower risk of VTE than women receiving orally administered HT, as demonstrated in a number of clinical studies (Daly et al., 1996; Perez et al., 1997; Scarabin et al., 2003). Genetic and pharmacological factors may contribute to the precipitation of VTE among HT users. We have previously shown that the pharmacological alterations induced by HT on the haemostatic system may be of particular interest (Bladhjerg et al., 2002; Sidelmann et al., 2003) because HT changes the inhibitory potential of coagulation as well as fibrinolysis significantly, as also shown by others (Hoibraaten et al., 2001; Salobir et al., 2002). Consequently, HT may increase the risk of VTE in thrombosis-prone individuals. Tibolone is a synthetic steroid compound with estrogen, progestogen and weak androgen activity. Tibolone has specific effects on different tissues due to tissue-selective metabolism, enzyme regulation and/or receptor binding and activation. It is, therefore, referred to as a selective tissue estrogenic activity regulator. As well as relieving vasomotor symptoms, tibolone has positive effects on sexual well-being and mood and improves vaginal atrophy and urogenital symptoms. Prevention of bone loss with tibolone is comparable with that seen with estrogen alone and combined HT. Tibolone rarely causes endometrial proliferation, and no additional progestogen is required. Phase 2 and 3 studies have indicated that tibolone may not
induce increased risk of VTE (Peverill, 2003), but only few clinical trials have addressed the VTE risk of tibolone (Daly et al., 1996; Jackson, 2001). On the other hand, a number of studies have focused on the effect of tibolone on some of the inhibitors of the haemostatic system (Walker et al., 1985; Cortes-Prieto, 1987; Parkin et al., 1987; van Wersch et al., 1994; Bjarnason et al., 1997; Winkler et al., 2000; Norris et al., 2002; Koh et al., 2003, 2005; Osmanagaoglu et al., 2005), and it has been suggested that tibolone has a favourable effect on fibrinolysis (van Wersch et al., 2005), whereas other studies indicate that tibolone has less effect on the inhibitors of coagulation than observed with other HT preparations (Winkler et al., 2000; Norris et al., 2002; Koh et al., 2003, 2005), whereas other studies indicate that tibolone has less effect on the inhibitors of coagulation than observed with other HT preparations (Winkler et al., 2000; Koh et al., 2003). The present study, however, is the first to make a thorough comparison of the effect of continuous combined conjugated equine estrogen plus medroxyprogesterone acetate (CEE/MPA) and tibolone on the inhibitory system of both coagulation and fibrinolysis.

Materials and methods

The study population consisted of 38 healthy women 45–65 years old. All women had intact uterus and they were post-menopausal, defined as (i) amenorrheic for at least 1 year and (ii) amenorrheic for at least 6 months with serum estradiol (E2) ≤20 pg ml\(^{-1}\) and FSH ≥40 IU 1–1. In cases of previous HT use, appropriate washout periods required before drawing blood for E2 and FSH determinations were as follows: 4 weeks for transdermal HT or local estrogen; 8 weeks for phytoestrogens, tibolone, intrauterine or oral progestational and oral estrogen/progestogen therapy; 6 months for progestogen implants or injections or estrogen/progestogen implants or injections. Inclusion criteria were menopause for less than 15 years, atrophic or inactive endometrium at the screening biopsy and body mass index (BMI) ≥18 and ≤32 kg m\(^{-2}\).

The women were randomized in a 1:1:2 ratio to oral tibolone 1.25 mg per day (n = 10), tibolone 2.5 mg per day (n = 10) or oral CEE plus MPA (0.625 mg + 2.5 mg per day) (n = 18) for a 12-month treatment period.

Blood sampling

Citrate-stabilized venous blood samples were drawn at baseline and after at least 12 h of fasting and non-smoking. Minimal stasis was applied, and the samples were drawn in evacuated Hemogard 9NC tubes from Becton Dickinson, Plymouth, UK. The samples were centrifuged at 2000 g for 20 min at room temperature. Plasma was collected and stored at −65°C. Prior to analyses, the plasma samples were thawed at 37°C, stored at room temperature and analysed within 30 min.

Blood analysis

Activated protein C resistance ratio (APC-R) was determined with the Coatest APC Resistance kit from Chromogenix, Mölndal, Sweden. The assay was performed with the ACL 7000 from International Laboratories, Milan, Italy. This equipment was also used for determination of the activities of antithrombin and protein C, employing the coamatic antithrombin and the coamatic protein C kits, both from Chromogenix. The protein concentration of total protein S was determined with an enzyme-linked immunosorbent assay (ELISA), employing antibodies from DAKO, Glostrup, Denmark. The protein concentration of tissue factor pathway inhibitor (TFPI) was determined with the Aserachrom TFPI kit from Diagnostica Stago, Asnières-sur-Seine, France. The protein concentration of plasminogen activator inhibitor 1 (PAI-1) was determined with the TintElize PAI-1 kit from Biopool, Umeå, Sweden, whereas thrombin activatable fibrinolysis inhibitor (TAFI) was determined by an ELISA employing antibodies from Affinity Biologicals, Ancaster, ON, Canada.

The assay procedures were calibrated against World Health Organization International Biological Standards and reference materials when available. Antithrombin was calibrated against International Standard 93/768, protein C against International Standard 86/622, protein S against International Standard 93/590 and PAI-1 was calibrated against International Standard 92/654. All standards were provided by National Institute for Biological Standards and Controls, Potters Bar, UK. The TFPI assay was calibrated against standards provided by the manufacturer of the kit, and the TAFI assay was calibrated against an in-house collected pool obtained from 30 healthy persons not receiving HT.

Statistics

Non-parametric statistical methods were used because of non-Gaussian distribution of results. All statistical evaluations were performed with the SigmaStat program from Systat Software Inc. Richmond, CA, USA. A P-value of <0.05 was considered as statistically significant.

Within group comparisons

Comparison between the results obtained at baseline and those obtained after 12 months of treatment was performed with Wilcoxon’s signed rank test.

Between group comparisons

For each quantity, the Kruskall–Wallis one way analysis of variance on ranks was used to compare the results obtained in the treatment groups at baseline. Further comparison between the tibolone group and the CEE/MPA group was performed both for the baseline results and for the results obtained after 12 months of treatment with the Mann–Whitney rank sum test.

Results

Initially, we compared the results obtained in the group of women receiving 1.25 mg per day of tibolone with the results from the group receiving 2.5 mg per day. No significant differences were obtained either at baseline or after 12 months of treatment (data not shown). Thus, in order to improve the power of the study, the two groups were merged, and the results of the combined tibolone group were compared with the results obtained in the CEE/MPA group.

The women in the CEE/MPA group and the tibolone group were comparable with respect to age (median age 55.4 versus 54.0 years, respectively, P = 0.39), height (median height 1.68 versus 1.66 m, respectively, P = 0.27), weight (median weight 66.5 kg in both groups, P = 0.60), BMI (median BMI 24.0 versus 25.1 kg m\(^{-2}\), respectively, P = 0.76) and time since menopause (44 versus 37 months, respectively, P = 0.99) (Figure 1).

The within-group comparisons showed that treatment for 12 months with CEE/MPA reduced the concentration of antithrombin from 1.02 to 0.95 IU ml\(^{-1}\), P = 0.002, the concentration of total protein S was reduced from 1.17 to 0.99.
1.00 IU ml\(^{-1}\), \(P < 0.001\) (Figure 2). TFPI was reduced from 81.6 to 67.8 ng/ml, \(P < 0.001\), and PAI-1 antigen decreased from 18.5 to 15.6 ng ml\(^{-1}\), \(P = 0.048\) (Figure 2B). APC-R, protein C and TAFI were unaffected by the treatment with CEE/MPA (Figure 2A and B).

Treatment for 12 months with tibolone reduced the concentration of protein C from 1.13 to 1.09 IU ml\(^{-1}\), \(P = 0.004\) (Figure 2A), and the concentration of PAI-1 decreased from 16.3 to 11.2 ng ml\(^{-1}\), \(P = 0.006\) (Figure 2B). Tibolone induced a very pronounced increase in the APC-R from 3.85 at baseline to 4.20 after 12 months of treatment, \(P < 0.001\) (Figure 2A). Antithrombin, protein S, TFPI and TAFI were unaffected by the treatment with tibolone (Figure 2A and B).

The between-group comparisons performed at baseline revealed no significant differences in any of the biochemical quantities investigated (Figure 2A and B).

The between-group comparisons performed after 12 months of treatment showed that the APC-R was significantly higher in the tibolone group than in the CEE/MPA group (4.20 versus 3.65), \(P = 0.04\). The concentration of protein S was significantly lower in the CEE/MPA group (1.00 IU ml\(^{-1}\)) than in the tibolone group (1.16 IU ml\(^{-1}\)), \(P = 0.005\) (Figure 2A), and also the concentration of TFPI was lower in the CEE/MPA group (67.8 ng ml\(^{-1}\)) than in the tibolone group (79.9 ng ml\(^{-1}\)), \(P = 0.03\) (Figure 2B). The other quantities investigated showed no statistical significant differences after 12 months of treatment (Figure 2A and B).

**Discussion**

The effects of HT on the haemostatic system were carefully addressed at The Writing Group for the 3rd European Conference on Sex Steroids and Cardiovascular Diseases (2003). Here, it was concluded that since no single test or algorithm is currently able to detect a future thrombosis, haemostatic variables may continue to be used as relevant pharmacological risk markers for the effects of HTs on coagulation. It was furthermore recommended to use the type and dose of the preparations which cause the least change or no change in haemostatic risk markers. After oral administration, tibolone is rapidly absorbed and converted by the liver into two estrogenic metabolites (3α- and 3β-OH-tibolone), which bind to the estrogen receptor, and a third metabolite (δ4-isomer), which demonstrates binding to the progesterone and androgen receptors. Thus, the pharmacokinetic of tibolone is different from that of CEE/MPA, and it is therefore of interest to study the pharmacodynamics of the two preparations and how it might translate into different clinical profiles.

To provide insight into the haemostatic risk profile of tibolone, we have performed a thorough evaluation of the
effect of tibolone on the inhibitory potential of haemostasis and compared this effect with the effect induced by CEE/MPA, the HT formulation used in the Women’s Health Initiative study (Rossouw et al., 2002). We demonstrate that the effect of tibolone on the inhibitory potential of coagulation is independent of the dosage used, i.e. 1.25 versus 2.5 mg per day, confirming the study of Bjarnason et al. (1997), and the results in the two groups of women treated with tibolone were combined to improve the power of the study. We have previously shown that various oral combined HT regimens alter the inhibitory mechanisms of coagulation significantly (Bladbjerg et al., 2002, 2003; Siedlmann et al., 2003), and the specific effect of CEE/MPA on inhibition of haemostasis has been addressed in a number of studies (Nozaki et al., 1999; Lobo et al., 2001; Koh et al., 2003; Osmanagaoglu et al., 2005; Sumino et al., 2005). In accordance with these studies, we presently demonstrate that treatment with CEE/MPA decreases the concentration of antithrombin, protein S and PAI-1, whereas the concentration of protein C is unaffected by the treatment. In addition, we demonstrate that CEE/MPA, as other combined oral HT preparations (Hoibraaten et al., 2001; Bladbjerg et al., 2002, 2003), reduces the concentration of TFPI significantly, but is without effect on the APC-R.

The concentration of TAFI is unaffected by both CEE/MPA and tibolone, as shown for other HT preparations (Bladbjerg et al., 2003), and tibolone causes a decrease in the concentration of PAI-1 comparable with that induced by CEE/MPA, as previously demonstrated (van Wersch et al., 1994; Bjarnason et al., 1997; Winkler et al., 2000; Norris et al.,

![Figure 2.](image)

**Figure 2.** (A) Results of the analysis of plasma taken from post-menopausal women before (baseline) or after 12 months of treatment with CEE/MPA (black dots) or tibolone (white dots). (B) TFPI, tissue factor pathway inhibitor; PAI-1, plasminogen activator inhibitor 1; TAFI, thrombin activatable fibrinolysis inhibitor.
2002; Koh et al., 2003, 2005). In contrast to other studies (Winkler et al., 2000; Osmanagaoglu et al., 2005), we observe a small, but significant, decrease in the concentration of protein C. However, the effect of tibolone on the inhibition of coagulation is much less pronounced than that of CEE/MPA because tibolone does not affect the concentration of antithrombin, protein S and TFPI—proteins representing the three major inhibitory pathways of coagulation. The lacking effect of tibolone on TFPI may be of particular importance, as all other combined HT preparations studied so far reduce the concentration of TFPI significantly (Hoibraaten et al., 2001; Bladbjerg et al., 2002, 2003), and reduced concentration of TFPI is associated with increased risk of thrombosis (Amini-Nekoo et al., 2001; Dahm et al., 2003; Hoke et al., 2005). The analysis of APC-R provides insight into the effect of APC on the coagulation system. Some HT preparations have the capacity to reduce APC-R (Sidelmann et al., 2003), which is coherent with an increased resistance towards the inhibitory effect of APC. This condition is associated with an increased risk of thrombosis (Rosen and Sturk, 1997). However, tibolone shows, also in this respect, a unique pharmacological quality, as we demonstrate a very significant increase in APC-R in the women treated with tibolone, confirming the study of Winkler et al. (2000). Taken together, the present study shows that tibolone induces fewer pharmacological alterations of the inhibitory potential of haemostasis than those observed with CEE/MPA and other conventional combined HT therapy, i.e. no effect on antithrombin, protein S and TFPI and a potentially favourable effect on APC-R.

We recognize that the present study is limited by the rather low number of women included and the lack of a control group of placebo-treated women, and a large randomized placebo-controlled study would have been the optimal design. Despite these limitations, the present study, however, confirms that the modest pharmacological effect of tibolone on the haemostatic system translates into a corresponding low clinical risk of VTE, as reported in the few clinical studies performed so far (Daly et al., 1996; Jackson, 2001).

References
Hormones and blood coagulation in post-menopausal women


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