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The selective estrogen receptor modulator DT56a (Femarelle) does not affect platelet reactivity in normal or thrombophilic postmenopausal women

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Abstract

Objective: The purpose of this study was to assess the effect of DT56a (Femarelle), a selective estrogen receptor modulator, on platelet function in normal and thrombophilic women being treated for severe menopausal symptoms.

Methods: The Platelet Function Analyzer-100 (PFA-100) was used to asses platelet reactivity at baseline and after 8 weeks of treatment with Femarelle (644 mg/d in divided doses) in 25 symptomatic postmenopausal women with normal clotting times and seven symptomatic women with shortened clotting times (<61 s). The PFA-100 measure of closure time is considered equal to clotting time in assessing clotting function and platelet adhesion, aggregation, and blood coagulation factors. Closure times were measured after 3 and 8 weeks in all participants and at 1 year in the women with shortened clotting times. The nonparametric Wilcoxon signed rank test was used to assess the changes between baseline and each of the three subsequent measurements.

Results: Pretreatment study of all seven women with shortened closure times confirmed abnormalities associated with thrombophilia: four women were heterozygous for the factor V Leiden gene mutation, one was heterozygous for the prothrombin gene mutation, one was found to have protein S deficiency, and one had increased anti-cardiolipin antibodies. All participants reported improved symptoms during the treatment period. No significant change in closure times was found in the normally clotting participants after 3 or 8 weeks of Femarelle therapy ($P > 0.26$). No significant change in closure time was seen in the seven thrombophilic women after 3 or 8 weeks or 1 year of Femarelle treatment ($P > 0.26$). The regression curve for measures over time was not significant ($P = 0.26$).

Conclusions: Femarelle, whose active ingredient is DT56a, did not adversely affect platelet reactivity as measured by PFA closure times in symptomatic thrombophilic postmenopausal women or normal controls. Femarelle, a novel selective estrogen receptor modulator that inhibits menopausal symptoms without thrombogenicity, may offer a new clinical choice for therapy of symptomatic postmenopausal women.

Key Words: Hormone therapy – DT56a – Femarelle – Clotting – Thrombophilia – Menopause.

It is well established in the literature that postmenopausal women using hormone therapy (HT) are at an increased relative risk of venous thromboembolism (VTE).[1-3] The factor V Leiden mutation is the most common inherited risk factor for VTE. Factor V Leiden is an autosomal dominant condition producing a factor V variant, which hinders degradation by the endogenous anticoagulant activated protein C.[4] The frequency of inherited factor V Leiden and other risk factors for VTE in the general population is estimated at 5% to 10%. This population has a 5- to 21-fold greater risk of developing spontaneous VTE.[5] Although isolated factor V Leiden is associated with a relatively mild hypercoagulable state, the risk of thrombosis is greatly magnified when other prothrombotic disorders are also present. These additional risk factors may be hereditary or acquired, including pregnancy, acquired antiphospholipid antibodies, prolonged immobility, and surgical operation. Studies have shown that the presence of the factor V Leiden mutation in women using HT increases the risk of thrombosis 15-fold.[6] Women taking HT develop acquired activated protein C resistance,[7] which provides a plausible explanation for the greatly increased thrombotic risk among women taking estrogen who are carriers of a prothrombotic mutation. As general screening for prothrombotic abnormalities has not yet been recommended by the

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professional societies,[8] essentially every woman taking HT is at an increased risk for a thrombotic event.

The role of sex steroid hormones on platelet function has been investigated for years, with conflicting results. Previous studies have demonstrated that the presence of estrogen receptors on platelets is subject to nongenomic regulation by estrogens.[9] However, the effects of administered estrogen on platelet function and activation are unclear. Some studies have shown that estrogen inhibits platelet function, whereas others have shown that estrogen augments the activation of platelets induced by physiologic agonists.[9,10]

The Platelet Function Analyzer-100 (PFA-100) is an instrument designed to evaluate platelet function in whole blood under conditions mimicking physiologic shear. The instrument measures the time (referred to as closure time [CT]) it takes for platelets in whole blood to block the flow through a perforated membrane coated with collagen and epinephrine (CEPI) or collagen and adenosine diphosphate (CADP). CT is a combined measure of platelet adhesion, aggregation, and the blood coagulation factors.

Previous studies using the PFA-100 have correlated CTs to the presence of known coagulation defects, indicating use for this device in detecting and following abnormalities in primary hemostasis.[11] The CTs recorded by the PFA-100 have been shown to be a more objective measure of clotting times than the usual subjective observation method. PFA-100 precision testing revealed a coefficient of deviation of less than 10% within days and between days in a 5-day analyses.[12]

Femarelle is derived from a botanical source containing, as its main substance, DT56a, a selective estrogen receptor modulator (SERM) also derived from a botanical source. Several studies have shown data defining the safety profile and clinical efficacy of DT56a.[13-18] It has been shown to alleviate menopausal symptoms,[13] particularly relieving vasomotor symptoms, with no effect on the endometrium or the sex steroid blood level, with additional positive effects on all menopausal symptoms.[13]

The purpose of the current study was to use the PFA-100 to assess the effect of Femarelle on platelet function in whole blood in both normal and thrombophilic women.

METHODS

Participant selection

The patient population for the study of thrombophilic women was taken from a previously published case control study[19] in which symptomatic postmenopausal women were screened with the PFA-100 before receiving prescription estrogen therapy (ET). In that study, symptomatic women (n = 91) were recruited to evaluate the effect of either oral or transdermal ET on CT as measured by the PFA-100. Before the initiation of ET, a baseline CT (CEPI and CADP) was obtained from all participants who met the eligibility requirements. Baseline analysis of CADP-CT was used to classify participants into normal (>66 s), borderline (61-66 s), or short (<61 s) CADP-CT. Women with normal CADP-CTs (n = 71) or with borderline CADP-CTs (n = 13) were treated with either oral or transdermal estrogen. Women with a CADP-CT of less than 61 seconds (n = 7) were excluded from the study because of the contraindication for women with increased clotting potential to receive ET. It was recommended that no ET be prescribed, and they were offered further study of their clotting disorder. These seven women were included in the current study. This was a new study with a separate institutional review board approval. The women were aged 40 to 67 years and had no previous exogenous estrogen exposure. An additional 25 symptomatic postmenopausal women who were screened and were found to have normal CT (>66 s) served as a control group. All women were required to have a follicle-stimulating hormone level greater than 40 IU/L and a last menstrual period of more than 1 year previously. Women were excluded from this Femarelle study if they had a history of a bleeding disorder, breast cancer, or other malignancy. Additional exclusion criteria included diabetes, coronary artery disease, and liver disease or concurrent use of anticoagulants. Women did not take aspirin, nonsteroidal anti-inflammatory drugs, clopidogrel, ticlopidine, heparin, warfarin, or other anticoagulant medications during the course of the study. If the women were taking any of these substances, they were told to stop before beginning the study. The 25 women who became the controls for the study received treatment with 644 mg/day (two 322-mg capsules) of Femarelle for 8 weeks. The seven thrombophilic women underwent coagulation screening and received treatment with 644 mg/day (two 322-mg capsules) of Femarelle for 1 year. The CT of the seven thrombophilic women was measured at 3 and 8 weeks and 12 months. All women signed informed consent forms approved by the institutional review board that had approved this protocol.

PFA-100 testing

The PFA-100 is an instrument designed to evaluate platelet function in whole blood under physiologic conditions and flow rates. The PFA-100 measures the time required for whole blood drawn through a fine capillary to occlude a microscopic aperture cut in a membrane coated with CEPI or CADP. This time is referred to as CT and is recorded in seconds. The CT is actually a measure of clotting time. Blood samples for analysis of platelet function were collected into evacuated tubes (Vacutainer, Becton Dickinson) containing 3.8% citrate. A total of 0.8 mL of citrated whole blood was transferred into the reservoir of a disposable test cartridge. The anticoagulated blood was warmed

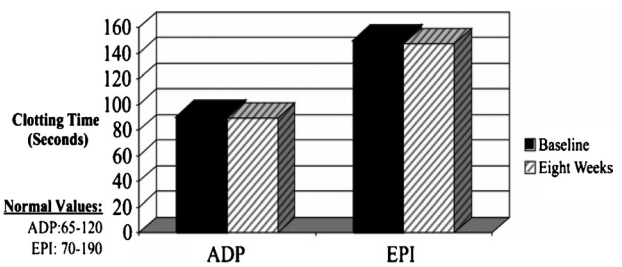

FIG. 1. Average clotting time in 25 women at baseline and after 8 weeks of treatment with Femarelle. ADP, membrane coated with adenosine diphosphate; EPI, membrane coated with epinephrine.

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TABLE 1. *Clotting times and genetic diagnosis of seven thrombophilic women treated with Femarelle for 1 year*

Participant identifier	Result of screen for hypercoagulable state	Baseline CADP-CT	CADP-CT at 3 wk	CADP-CT at 8 wk	CADP-CT at 1 y
1	Heterozygous prothrombin gene mutation	61	54	54	53
2	Heterozygous factor V Leiden mutation	58	55	55	55
3	Heterozygous factor V Leiden mutation	53	54	54	55
4	Heterozygous factor V Leiden mutation	55	59	59	60
5	Heterozygous factor V Leiden mutation	61	55	55	53
6	Protein S deficiency	60	54	54	54
7	Elevated anticardiolipin (IgG)	60	61	61	61

CADP-CT, collagen and adenosine diphosphate closure time.

to 37°C and drawn under vacuum through a 200-μm-diameter stainless steel capillary and a 150-μm-diameter aperture in a nitrocellulose membrane coated with CADP or CEPI. The time required to occlude the aperture is reported as the CT and is measured to a maximum of 300 seconds. The reference range is 62 to 120 seconds for CADP-CT and 70 to 190 seconds for CEPI-CT. Interassay coefficient of variation in CADP-CT and CEPI-CT is less than 10%.[12,20,21]

Data analysis

Nonparametric statistical tests were applied. Pairwise comparisons were performed between the baseline and each of the three subsequent measurements using the Wilcoxon signed rank test.

RESULTS

The 25 control symptomatic women with normal (CADP-CT >66 s) baseline CTs were treated with Femarelle for 8 weeks. No significant change in CT was found after treatment with Femarelle (CT at beginning vs end of trial, $P > 0.05$; Fig. 1).

The seven women with short baseline CT underwent a full thrombophilia investigation for a hypercoagulable state (Table 1). Four of the seven women were found to be heterozygous for the factor V Leiden gene mutation, one was found to be heterozygous for the prothrombin gene mutation, one was found to have a protein S deficiency, and one was found to have increased anticardiolipin antibodies.

After discussion of risks and benefits, all women chose to be treated for their symptoms, and this was carried out without any reported side effect.

CTs in the seven thrombophilic women after 3 weeks (56 ± 2.8 s), 8 weeks (56 ± 2.8 s), and 1 year (56 ± 3.3 s) of Femarelle were not significantly different from that at baseline (56 ± 2.6 s; $P > 0.05$; Tables 1 and 2).

TABLE 2. *Simple descriptive statistics for CTs (in seconds) for the seven thrombophilic women after 3 weeks (56.0 ± 2.8 s), 8 weeks (56 ± 2.8 s), and 1 year (55.9 ± 3.3 s) of Femarelle treatment*

	Baseline	Week 3	Week 8	1 y
Mean CADP-CT	58.3	56.0	56.0	55.9
Median	60.0	55.0	55.0	55.0
SD	3.1	2.8	2.8	3.3
Minimum	53.0	54.0	54.0	53.0
Maximum	61.0	61.0	61.0	61.0

There was no significant difference from baseline (58.3 ± 3.1 s; $P > 0.05$).
CADP-CT, collagen and adenosine diphosphate closure time.

Pairwise comparisons between baseline and each of the three subsequent measurements using the Wilcoxon signed rank test showed no significant differences between the groups for all tests ($P > 0.200$). Checking the regression of repeated measures from time zero overtime was nonsignificant ($P = 0.262$).

DISCUSSION

Women taking exogenous HT are at an increased risk of VTE.[5,6] Since the publication of the Women's Health Initiative results showing a significant increase in thrombotic events among HT users, the use of HT has been dramatically altered.[3]

Identification of postmenopausal women at increased risk of VTE before the initiation of ET should decrease the morbidity associated with ET. However, generalized screening has not yet come into practice.

In their review of both the Heart and Estrogen/Progestin Replacement Study and the Estrogen Replacement and Atherosclerosis trial, two studies of women with preexisting coronary disease, Herrington et al[22] found a 16.7% incidence of the factor V Leiden mutation in women who had VTE as opposed to a 6.3% occurrence in controls without VTE. In women without the factor V Leiden mutation, the use of estrogen plus progestin (HT) significantly increased their VTE risk. The overall risk for this group was 3.7 (CI, 1.4-9.4); in women receiving HT with factor V Leiden mutation, the overall risk was 14.1 (CI, 2.7-72.4) when comparing them to women receiving placebo. The authors estimated that 376 women would have to be screened for factor V Leiden to avoid an HT-associated VTE during 5 years of treatment. They pointed out that genetic testing was too expensive at the time to consider such screening.

In the Estrogen and Thromboembolism Risk (ESTHER) study,[23] a multicenter case control study of 271 cases of women with idiopathic VTE and 610 controls, the authors concluded that when ET is administered transdermally, VTEs are not increased, but they did confirm that oral estrogen increased these events, with a relative risk (RR) of 4.2 (CI, 1.5-11.6).[23] The PFA-100 provides an excellent measure of platelet function in whole blood, and the cost and the swift response of this office machine could provide an excellent solution to reduce the occurrence of VTE in a heightened-risk group. In our previous study,[19] 8% of the study population (7/91 women) was found to have thrombophilia after their shortened CT was noted using the PFA-100. The incidence of thrombophilia in this

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cohort mirrors estimates of the incidence of thrombophilia in the general population.[5] This would seem to indicate that the screening for thrombophilia was effective and that using the PFA-100 for this type of screening before women were given HT might be cost-effective particularly in a high-risk population.[12] As demonstrated in the current study, the PFA-100 was a sensitive tool to identify women with underlying clotting abnormalities.

The seven women with shortened CT were not given any hormonal treatment. Because of the large amount of safety data for DT56a[13-18] and the unchanged CT of the 25 normal clotters, these 7 thrombophilic women were treated for their symptoms with Femarelle (644 mg/d) and showed no change in their CTs after 3 and 8 weeks and 1 year of treatment. These results are an important addition to accumulating data regarding the safety profile of Femarelle. It is particularly important that this SERM did not affect the clotting indices of women at high risk for thrombotic disease. In addition, Femarelle has been shown to be successful in decreasing menopausal symptoms.[13] Femarelle is the first SERM that was shown to decrease menopausal symptoms.[12] The two widely used SERMs, tamoxifen and raloxifene, do not decrease vasomotor symptoms, but they actually increase them. Moreover, these two SERMs were found to significantly increase thromboembolic events in repeated studies.[24] It is hoped that the current study on Femarelle will encourage larger studies that can lead to a nonestrogenic product that decreases flushing in women with a high-risk profile.

The main drawback of the current study is the small number of thrombophilic women tested, which limits the chance of a thrombotic event, although studies evaluating the incidence of VTE in women taking HT report the greatest absolute increase in the incidence of VTE shortly after initiation of therapy.[2] None of the seven thrombophilic women had a significant change in clotting time after 1 year of treatment with Femarelle, which points to the safety of this medication with regard to platelet function.

CONCLUSIONS

The PFA-100 is successful in detecting short clotting times and might serve as a faster, less expensive screening test for thrombophilia before women are given ET. Femarelle, a SERM with potential for vasomotor instability reversal, without thrombogenicity, may offer a new clinical choice for menopausal symptom therapy, even in women with clotting risks.

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