Less Effect of Intranasal Than Oral Hormone Therapy on Factors Associated With Venous Thrombosis Risk in Healthy Postmenopausal Women

Majoie Hemelaar, Jan Rosing, Peter Kenemans, M. Christella L.G.D. Thomassen, Didi D.M. Braat, Marius J. van der Mooren

Objective—To compare the effects of intranasal and oral administration of 17β-estradiol (E2) and norethisterone(acetate) [NET(A)] in healthy postmenopausal women on activated protein C (APC) resistance and other hemostatic parameters associated with venous thrombosis.

Methods and Results—In this 2-center, randomized, double-blind, 1-year trial, 90 postmenopausal women (56.6±4.7 years of age) received daily either an intranasal spray with 175 μg/275 μg E2/NET (n=47) or 1 mg/0.5 mg oral E2/NETA (n=43). Normalized APC sensitivity ratios (nAPCsr) were determined with a thrombin generation-based APC resistance test. After 1 year, the increase in nAPCsr was smaller in the intranasal than in the oral group: 11% (95% CI, 1% to 22%) versus 53% (95% CI, 37% to 72%). Overall, the decrease in antithrombin and increase in prothrombin fragment 1+2 (F1+2) were smaller and the decrease in free protein S larger in the intranasal compared with the oral group after 1 year. In both groups, the decreases in protein C and prothrombin, and the increase in D-dimer were similar.

Conclusion—Compared with oral E2/NETA therapy, intranasal administration of E2/NET had less effect on APC resistance and on a number of other parameters associated with venous thrombosis. This observation suggests the possibility of a lower venous thrombosis risk for intranasal E2/NET compared with oral therapy. (Arterioscler Thromb Vasc Biol. 2006;26:1660-1666.)

Key Words: APC resistance ■ hormone therapy ■ intranasal ■ menopause ■ norethisterone ■ venous thrombosis

Use of oral postmenopausal hormone therapy (HT) is associated with a 2- to 4-fold increased risk of venous thromboembolism (VTE),1–3 with the highest risk during the first year of treatment. Although literature regarding possible risk differences between oral and transdermal is conflicting,1–3–5 the largest case-control study performed so far3 suggested that women on HT with transdermal estradiol (E2) may not be exposed to an increased risk of VTE (odds ratio [OR], 0.9; 95% CI, 0.5 to 1.6).

In 1993, Dahlbäck et al6 discovered that activated protein C (APC) hardly prolonged the clotting of plasma in members of families with multiple thromboembolic events. This defect, called APC resistance, which was attributed to a mutation in the factor V (FV) gene7 (FV Leiden), appeared to be a major risk factor of venous thrombosis.8 Later, it was shown that APC resistance in the absence of FV Leiden is also associated with an increased VTE risk.9 APC resistance in individuals without FV Leiden is particularly observed during use of combined oral contraceptives (OCs),10,11 during pregnancy,11 and while using oral HT.11–15 Compared with oral HT, HT with transdermal E2 has a much smaller effect on APC resistance.14,15

Important other risk factors for the development of VTE are deficiencies in protein C, protein S, and antithrombin and elevated plasma levels of factor VIII (FVIII) and prothrombin.16 Also, elevated levels of prothrombin fragment 1+2 (F1+2) and D-dimer, both reflecting ongoing coagulation, have been observed in individuals with an increased VTE risk.17–21

Oral HT induces changes in the plasma levels of coagulation factors that are indicative of the existence of a prothrombotic condition.22 Although it has been shown that HT with transdermal E2 has less effect on hemostatic parameters than oral HT,14,23,24 limited data are available for other forms of nonoral HT.

An intranasal spray has shown to be effective in climacteric symptom relief and a well-tolerated alternative route for E2 administration (Aerodiol; Servier)25 with an attractive theoretical advantage of less stimulation of the breast and endometrium,26 leading to less breakthrough bleeding and

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mastalgia. In addition to the E2-only spray, an intranasal spray for continuous combined 17β-E2 plus norethisterone (E2/NET) administration has been developed. Because the hepatic metabolism is largely bypassed, it is plausible that, like HT with transdermal E2, intranasal administration may have limited effect on VTE risk.

Because no data are available on the VTE risk during intranasal HT, we investigated the effect of the intranasal E2/NET spray on APC resistance and on the plasma levels of proteins that are associated with an increased risk of VTE. We compared the effects of the intranasal spray with those of low-dose continuous combined oral E2/NET acetate (NETA) therapy. This was the objective of a substudy among participants in 2 Dutch centers, in a large international, randomized, double-blind, double-dummy study, with endometrial safety as primary end point.

Materials and Methods

Participants

Healthy postmenopausal women 40 to 75 years of age were recruited from gynecological outpatient clinics of 2 centers in the Netherlands and through advertisements in local newspapers. Specific indications for HT use were not required, but women were willing to participate in the study because of some kind of climacteric symptoms. All women were nonhysterectomized and had their last menstrual period ≥2 years before inclusion and had serum levels of E2 < 30 pg/mL and of follicle-stimulating hormone > 30 mIU/mL. All participants had a normal cervical smear and mammography within 12 months before inclusion. A normal transvaginal ultrasound and an endometrial biopsy without hyperplasia or polyps were required, as well as blood tests (lipids, liver enzymes, kidney function, glucose and thyroid-stimulating hormone) without any clinically relevant abnormalities. At screening, all participants had plasma levels of total cholesterol ≤ 8.0 mmol/L and triglycerides ≤ 3.0 mmol/L. Exclusion criteria were a body mass index > 32 kg/m², any contraindication for use of estrogen or progestogen, any ear-nose-throat disease that might interfere with intranasal drug administration, and concomitant use of any treatment for menopausal symptoms, chronic treatment liable to interfere with the coagulation profile, treatment liable to interfere with intranasal administration, enzyme inducers, and systemic vasoconstrictors. The present study was done in a subset of women who were not taking lipid-lowering drugs, and who had either never used HT or had a washout of previous HT of ≥ 6 weeks before the baseline visit.

All participants gave written informed consent before participation in the trial, which was conducted in accordance with the ethical principles stated in the Declaration of Helsinki, with Good Clinical Practice, and was approved by central and local institutional review boards.

Study Design

This study was performed in a subgroup of 90 women who were included in 2 centers in the Netherlands, as part of a large international, randomized, double-blind, double-dummy study with 2 parallel treatment arms including 954 women. Eligible women were randomized to either 1 intranasal spray containing a fixed dose of 175 μg E2 plus 275 μg NET (S21405; Servier) and 1 placebo capsule (intranasal E2/NET) or 1 capsule containing 1 mg E2 plus 0.5 mg NETA (Activate; Novo Nordisk) and 1 placebo spray (oral E2/NET) daily. Study medication was manufactured, packaged, and labeled by the Institut de Recherches Internationales Servier. Placebo and active treatments were identical in appearance and smell. Centralized computerized subject randomization was done by an Interactive Voice Response System in blocks of 12 (6 active spray and 6 active capsules) per center. Treatment was administered for 52 weeks.

Throughout the whole study period, all participants, clinical investigators, and laboratory personnel were blinded for the study medication. Unblinding was done after all data were collected in the database.

Hemostatic Parameters

For assessment of hemostatic parameters, venous blood samples were taken at baseline and in weeks 12 and 52. After fasting and refraining from smoking for ≥ 10 hours and no alcohol intake for ≥ 24 hours, blood samples were taken between 8 and 10 AM. After 20 minutes of rest, blood was collected into precooled tubes (Becton Dickinson) containing sodium citrate, theophylline, adenosine, and dipiridamole for FVIII activity or 0.129 mol/L sodium citrate (for protein C, free protein S, antithrombin, F1+2, and D-dimer) or into tubes at room temperature containing 0.129 mol/L sodium citrate (for normalized APC sensitivity ratios [nAPCsr], prothrombin, and the test for the FV Leiden mutation). After blood collection, precooled tubes were immediately placed on ice. Within 1 hour after collection, plasma was separated by centrifugation at 2000g for 30 minutes at 4°C (for blood collected into precooled tubes) or at 20°C (for blood collected into tubes at room temperature). Plasma was divided into aliquots, snap-frozen, and stored at −80°C until analysis. All samples of a given subject were assayed within a single run.

nAPCsr were determined with the endogenous thrombin potential (ETP)–based APC resistance test, in which the effect of APC on the time integral of thrombin generation (ETP) is quantified. A normal plasma pool consisting of 45 men and 27 women (all without OCs/HT/pregnancy) with a mean age of 40–49 years was used to normalize the APCsr. In this assay, women (without OCs/HT/ pregnancy) generally have a nAPCsr between 1 and 2, whereas the mean nAPCsr of men is < 1. Protein C activity was measured with the Coamatric protein C activity kit (Chromogenix), free protein S antigen with the automated latex ligand immunoassay, interleukin test free protein S (Instrumentation Laboratory), antithrombin with the Coamatric antithrombin test (Chromogenix), FVIII activity with the Coatest Factor VIII (Chromogenix), prothrombin with the ecarin activation test, and D-dimer with an ELISA (Enzygnost; Dade Behring), and D-dimer with an ELISA (TintElize d-dimer, Biopool). All women were tested for the FV Leiden mutation with a functional test that fully discriminates between homozygous, heterozygous, and noncarriers of the mutation.

Serum levels of E2 and of follicle-stimulating hormone were measured with an electrochemiluminescence immunoassay (Roche Diagnostics), and serum sex hormone binding globulin levels were measured using an immunoradiometric assay (Orion Diagnostica).

Statistical Analyses

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 11.5 for Windows (SPSS Inc.). Values of the hemostatic parameters are given as mean ± SD or as median (25th to 75th percentile) if skewed. Percentage changes from baseline are given as mean (95% CI) or as geometric mean (95% CI) if the changes had a skewed distribution and for D-dimer as median (25th to 75th percentile) because changes remained skewed after log transformation.

We used standard parametric tests. If variables were skewed, analyses were done after log transformation. Within-group changes over time were tested using ANOVA for repeated measurements. For between-group comparisons, we used analyses of covariance (ANCOVA) for repeated measurements, with the baseline value of the variable under consideration as covariate. Nonparametric tests were used for comparison between groups of smoking at baseline (χ² test) and percentage changes in d-dimer (Mann–Whitney U test).

Only data from women, of whom data were available at baseline and ≥ 1 other time point, were used for analyses; for ANCOVA for repeated measurements, the last observation carried forward procedure for the missing values was applied. Sample size calculation for this cardiovascular substudy was based on changes in nAPCsr. To find a 35% difference in change in nAPCsr between the groups with an SD in percentage change of
TABLE 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Intranasal</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>47</td>
<td>43</td>
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<tr>
<td>Age, y</td>
<td>57.5±5.2</td>
<td>55.9±3.9</td>
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<tr>
<td>Amenorrhoea, months</td>
<td>74 (51–123)</td>
<td>73 (45–106)</td>
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<tr>
<td>Weight, kg</td>
<td>68.4±9.2</td>
<td>68.5±9.3</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<td>25.2±3.6</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
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<tr>
<td>Systolic, mm Hg</td>
<td>126±17</td>
<td>121±17</td>
</tr>
<tr>
<td>Diastolic, mm Hg</td>
<td>80±10</td>
<td>76±11</td>
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<tr>
<td>Smoking, n (%)</td>
<td>12 (27.7%)</td>
<td>11 (25.6%)</td>
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<tr>
<td>Previous HT use, n (%)</td>
<td>14 (32.6%)</td>
<td>12 (27.7%)</td>
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<tr>
<td>Washout from HT, weeks</td>
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<td>15.7 (13.7–20.7)</td>
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<tr>
<td>Serum cholesterol, mmol/L</td>
<td>6.1±1.1</td>
<td>6.1±0.8</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.15 (0.92–1.54)</td>
<td>0.99 (0.76–1.48)</td>
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<tr>
<td>Serum estradiol, pmol/L</td>
<td>62 (43–83)</td>
<td>56 (44–91)</td>
</tr>
<tr>
<td>Serum FSH, IU/L</td>
<td>83±32</td>
<td>82±25</td>
</tr>
</tbody>
</table>

Values are given as mean± SD, as median (25th–75th percentile), or as No. (n).

Previous HT use indicates use of hormone therapy in year before inclusion in the study; FSH, follicle-stimulating hormone; intranasal, spray containing 175 μg 17β-E₂ and 275 μg NET; oral, capsule containing 1 mg E₂ and 0.5 mg NETA.

Results

Participants
Between September 2001 and June 2002, 125 women were screened in the 2 participating centers, of whom 94 women were randomized. Because 4 women either had no washout from their previous HT (n=3) or used lipid-lowering drugs (n=1), 90 women were eligible for the current substudy. The last patients completed the study in May 2003. No differences in baseline demographic characteristics were found between the 2 groups (Table 1).

Two women in the intranasal group discontinued the study versus 10 women in the oral group (P<0.01; Figure 1). Premature study discontinuation was mainly related to the occurrence of an adverse event. No women stopped because of coronary or cerebrovascular events. In the intranasal group, 1 woman discontinued in week 11 because of the occurrence of deep venous thrombosis which, in retrospect, was already present before study entry, and another woman discontinued because of vaginal candidiasis. One woman in the intranasal group was excluded from analyses after week 12 because she started preventive anticoagulant therapy because of a family history of cerebrovascular disease. In the oral group, 1 woman discontinued in week 36 because of clinical symptoms of venous thrombosis, which, however, could not be confirmed ultrasonographically. This woman was 1 of the 4 women who carried the FV Leiden mutation. Furthermore, 1 woman in the oral group stopped because of the detection of breast cancer, which, in retrospect, was already present at the mammography before study entry. Other reasons for discontinuation in the oral group were vaginal bleeding, fatigue, lack of efficacy, withdrawal of consent, nasal symptoms, arthralgia, abdominal symptoms, and headache.

Analyses were based on 86 women (46 in the intranasal and 40 in the oral group) of whom values at baseline and at least at 1 other time point were available.

Hemostatic Parameters
No women in the intranasal group compared with 4 women in the oral group were heterozygous carriers of the FV Leiden mutation. Baseline levels of protein C were lower in the intranasal than in the oral group. At baseline, there were no other significant differences between the 2 groups (Table 2). FV Leiden carriers had higher baseline nAPCsr (range 3.03 to 6.97), which remained higher throughout the study period.

Figure 1. Trial profile. n=number of subjects. Intranasal indicates spray containing 175 μg 17β-E₂ and 275 μg NET; oral, capsule containing 1 mg E₂ and 0.5 mg NETA.

Figure 2. Box and whisker plots: boxes represent 25th and 75th percentile with the median; ends of whiskers indicate 10th and 90th percentile of nAPCsr. *P<0.05 vs baseline; §P<0.001 vs baseline. Intranasal indicates spray containing E₂/NET; oral, capsule containing E₂/NETA.
The increase in nAPCsr in the intranasal group was smaller (P<0.001) than in the oral group. After 52 weeks of treatment, the mean increase of the nAPCsr was 11.2% (95% CI, 1.0%, 22.3%) in the intranasal group and 53.8% (95% CI, 37.1%, 72.5%) in the oral group. Increases in nAPCsr were already apparent after 12 weeks of treatment: 15.5% (95% CI, 8.3%, 23.2%) in the intranasal and 51.3% in the oral group (95% CI, 39.0%, 64.7%) (Figure 2).

The decrease in protein C was similar (difference +0.5% [95% CI, −2.8%, 3.8%]) in both groups: −11.1% in the intranasal and −11.6% in the oral group in week 52. Free protein S was more decreased (difference −4.4% [95% CI, −7.8%, −0.8%]) in the intranasal group: −2.2% in week 52 than in the oral group (+2.2%). For antithrombin, the decrease was less pronounced (difference +4.5% [95% CI, 2.6%, 7.35%]) in the intranasal (−5.7%) than in the oral (10.2%) group (Figure 3).

No significant difference in change was seen between the groups in FVIII (difference +3.1% [95% CI, −3.9%, 10.2%]), although in week 12, a transient decrease (−3.7%) was observed in the intranasal group. In week 52, prothrombin was equally decreased (difference −0.4% [95% CI, −3.3, 2.5%]) in both groups: −5.2% in the intranasal and −4.8% in the oral group.
The increase in F1+2 in the oral group (19.0% in week 52) was larger (difference +13.2% [95% CI, −2.8%, 29.15%]) than the nonsignificant increase in the intranasal group (5.8%). After 52 weeks, the increase in d-dimer did not significantly differ between the groups, although in week 12, the increase was less pronounced in the intranasal group (29.8%) than in the oral group (50.7%; Figure 3).

Results did not change when analyses were performed after exclusion of the 4 carriers of the FV Leiden mutation or when the presence of FV Leiden mutation was added as an additional covariate in the ANCOVA (data not shown). Also, exclusion of outliers in the other parameters did not affect the results.

Discussion

At present, there are no reports on the risk of venous thrombosis associated with intranasal HT. The current study is the first to describe the effects of intranasal administration of combined estrogen plus progestogen therapy on hemostatic parameters that are associated with an increased risk of venous thrombosis.

Whereas use of oral postmenopausal HT is known to increase VTE risk,1,2,20 less is known about the risk of nonoral administration. So far, only 4 case-control studies1,3–5 have investigated the association between VTE and HT with nonoral (ie, transdermal) E2. Scarabin et al,3 the largest of these studies, investigating healthy women who had their first idiopathic venous thrombosis, found no increased risk (OR 0.9 [0.5 to 1.6]) among users of HT with transdermal E2 compared with the increased risk (OR, 3.5 [1.8 to 6.8]) among users of oral HT. The other 3 studies included very small numbers of cases (2, 5, and 7), and 2 of them1,4 found a nonsignificant increase in VTE during HT with transdermal E2, however, smaller than for oral HT.1

Intranasally administered E2 is rapidly absorbed and induces very steep and short peaks in serum E2 levels. Because of the lack of a first-pass liver effect, intranasally administered hormones may have less effect on plasma levels of coagulation factors than orally administered hormones. Hence, like has been suggested for HT with transdermal E2,3 HT with intranasal E2 may be associated with a lower risk of venous thrombosis. The dosages we compared have shown similar exposure to E2,30 and NET, the active hormone of NETA (unpublished data, 2001).

Until now, the effect of intranasal E2 on hemostatic parameters has been reported in only 1 study.25 Given with oral dydrogesterone in a sequentially combined fashion, 24 weeks of intranasal 300 μg E2 did not show significant changes from baseline in antithrombin or d-dimer.25

Increased resistance to APC is associated with an increased VTE risk.3,9 APC resistance was measured with the ETP-based APC resistance test.10 This is a functional assay that, in contrast to the commonly used activated partial thromboplastin time–based APC resistance test,6 is very sensitive for changes in the levels of sex steroid hormones.10–15

In this 1 year, randomized, double-blind study the increase in nAPCsr during intranasal E2/NET therapy (11%) was significantly less than the increase observed during oral E2/NETA therapy (54%). With respect to oral E2/NETA therapy, our observations are in agreement with placebo-controlled studies with various oral HT regimens12–15 including E2/NETA therapy.12 Literature on the effect of nonoral HT on APC resistance is only available for transdermal therapy. Compared with placebo and oral HT,14,15 HT with transdermal E2 caused a smaller increase14 or had no significant effect15 on the nAPCsr.

Our study further shows that, among the antithrombotic proteins, protein C was similarly decreased in both groups, and free protein S showed a larger decrease and antithrombin a smaller decrease in the intranasal E2/NET compared with the oral E2/NETA group. For the comparison between oral HT and HT with transdermal E2, previous studies generally reported no significant difference in changes in antithrombotic proteins.14,15,23 During oral HT plasma levels of anticoagulant proteins are significantly reduced,14,31–33 although in some studies, a transient decrease or no change of protein S was observed.15,31 During HT with transdermal E2, both decreases14,34 and no changes15,23,34 of anticoagulant proteins were reported.

In the current study, intranasal E2/NET and oral E2/NETA decreased the plasma level of prothrombin, increased d-dimer to a similar extent, and had no effect on the FVIII level. A significant increase of F1+2 was only observed during oral E2/NETA therapy. Our findings with oral E2/NETA are largely in line with previous randomized studies in which HT

![Figure 3. Percentage change from baseline of the plasma levels of hemostatic parameters in weeks 12 and 52. *Significant difference between the groups; intranasal, spray containing E2/NET; oral, capsule containing E2/NETA; F1+2, prothrombin fragment 1+2.](image-url)
using oral and transdermal E2 were compared \(^{14,23,35}\) and with placebo-controlled studies in which the effect of oral E2/NETA was investigated.\(^ {31,33}\) During transdermal E2, either unopposed,\(^ {14,24,35}\) or combined with transdermal\(^ {16}\) or oral progestogen,\(^ {23}\) slight increases or no effect on FVIII, prothrombin, F1+2, or n-dimer were found.

The significantly smaller increase in nAPCsr in the intranasal group might reflect a lower risk for the development of VTE during intranasal HT. This would be in line with the observations that HT using transdermal E2 induces a relatively small increase in the nAPCsr\(^ {14,15}\) and may not increase the risk of VTE.\(^ {3}\) Also in OC users, there appears to be a good correlation between the nAPCsr and the risk of venous thrombosis.\(^ {10,11}\) Women who use second-generation OCs are exposed to a lower thrombotic risk than third-generation pill users, and their plasma is less APC resistant than that of third-generation pill users.\(^ {10,11}\)

One of the strengths of our study is the randomized, double-blind study design and the 1 year duration, including the measurement of short-term effects after 3 months of therapy. The study also has limitations. We did not include an untreated or placebo control group. However, we compared the effects of the new intranasal spray with a well-studied reference product, the effects of which were found to be comparable with those observed in placebo-controlled studies. The study was not designed to detect differences in venous thrombotic risk or a possible interaction between FV and predisposing factors.

In conclusion, in this study, in healthy postmenopausal women, intranasal continuous combined E2/NETA therapy showed smaller changes in nAPCsr, antithrombin, and F1+2 than oral E2/NETA therapy. This might be indicative for a lower VTE risk during intranasal E2/NETA therapy when compared with oral therapy.

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Disclosures
None.

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