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ABSTRACT

Objective: Estetrol (E4) represents a novel estrogen of interest to relieve vasomotor symptoms. E4 activates the nuclear estrogen receptor α (ERα) but antagonizes the estradiol ERα-dependent membrane-initiated steroid signaling pathway. The distinct pharmacological properties of E4 could explain its low impact on hemostasis. This study aimed to assess the effect of E4 on coagulation in postmenopausal women, using the thrombin generation assay (TGA).

Methods: Data were collected from a multicenter, randomized, placebo-controlled, dose-finding study in postmenopausal women (NCT02834312). Oral E4 (2.5 mg, $n=42$; 5 mg, $n=29$; 10 mg, $n=34$; or 15 mg, $n=32$) or placebo ($n=31$) was administered daily for 12 weeks. Thrombograms and TGA parameters were extracted for each subject at baseline and after 12 weeks of treatment.

Results: After 12 weeks of treatment, all treatment groups showed a mean thrombogram (±95% confidence interval [CI] of the mean) within the reference ranges, that is, the 2.5th–97.5th percentile of all baseline thrombograms ($n=168$), as well as for TGA parameters.

Conclusions: The intake of E4 15 mg for 12 weeks led to significant but not clinically relevant changes compared to baseline as the mean values (±95% CI of the mean) remained within reference ranges, demonstrating a neutral profile of this estrogen on hemostasis.

Introduction

The risk of developing venous thromboembolism (VTE) is still a matter of concern for many women starting oral hormonal therapy (HT) [1]. The risk of VTE is increased especially with preparations containing oral conjugated equine estrogen (CEE) [2], is less pronounced with oral estradiol (E2) but is lowest with transdermal E2 preparations [2,3]. First ever users of HT had a twofold increased risk of pulmonary embolism within 4 months after starting therapy, compared with non-users [4].

The VTE risk has been explained, at least in part, by the hemostatic imbalance induced by HT which causes changes in plasma levels of almost all proteins involved in coagulation and fibrinolysis [5–8]. Although these changes might be considered relatively modest when measured separately, they have a supra-additive effect leading to a procoagulable state responsible for this increased risk of VTE [9]. These hormonal changes are also associated with activated protein C (APC) resistance which results from increases in FII, FVIII or FX levels and/or decreases in protein S and tissue factor pathway inhibitor levels [10–13].

The endogenous thrombin potential (ETP)-based APC resistance assay is one of the most sensitive assays toward APC resistance acquired following HT and has been linked to an increased risk of VTE in women using HT [11–16]. This technique relies on the thrombin generation assay (TGA) which allows to obtain a thrombogram, that is, a visual and quantitative representation of the amount of thrombin generated over time in a testing cupule [17]. On top of using this marker for the determination of APC resistance, the parameters of the thrombogram can also be exploited [18] to provide information on the prothrombotic tendency independently of the resistance toward exogenous APC [19–21]. As the use of HT and other known hypercoagulable states have been shown to enhance thrombin generation *in vitro* [5,22–25], the development of convenient, easily administrable HT with no or low impact on thrombin generation and the associated risk of thrombosis is of utmost importance.

Estetrol (E4) is the first native estrogen with selective tissue activity (NEST) and represents a novel estrogen of

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interest to relieve vasomotor symptoms [26–29] and genito-urinary syndrome of menopause [30]. Indeed, like other estrogens, E4 activates the nuclear estrogen receptor α (ERα), but in contrast to other estrogens, induces limited activity via ERα-dependent membrane-initiated steroid signaling in several tissues including the breast, and antagonizes this pathway in the presence of E2, thereby uniquely uncoupling nuclear and membrane activation.

E4 demonstrated a low impact on the liver with minimal effects on lipids, lipoproteins, sex hormone-binding globulin (SHBG) and several coagulation and fibrinolytic proteins [31].

To further delineate the minimal effective dose of E4 for the treatment of postmenopausal symptoms, the E4Relief Phase 2 trial was subsequently designed and revealed that E4 at a dose of 15 mg daily is the minimum effective daily dose for the treatment of vasomotor symptoms [32,33]. This study also allowed to confirm the low impact of E4 on hemostasis and supports the hypothesis that E4 may be associated with a lower risk of thrombosis, like its use in contraception [34]. Nevertheless, while some coagulation factors such as protein S and tissue factor pathway inhibitor were slightly impacted by rising doses of E4, the synergistic effect of these changes on hemostasis could not be fully captured by the singular measurements proposed in these initial hemostasis investigations [35,36]. Therefore, as TGA allows to assess the coagulation process in its entirety and as it has been shown to be sensitive to the synergistic hemostatic alterations induced by HT [17], we present here the impact of multiple ranging doses of E4 on TGA.

Materials and methods

Trial design

This was a multicenter, randomized, double-blind, placebo-controlled, phase 2, dose-finding trial in hysterecto-mized and non-hysterectomized postmenopausal women (ClinicalTrials.gov NCT02834312, EudraCT 2015-004018-44). The trial was approved by the independent ethics committees of participating centers and conducted in accordance with the ethical principles established by the Declaration of Helsinki and the International Conference on Harmonization – Good Clinical Practice (ICH E6 [R2]) guidelines. All participants gave written informed consent and had the right to withdraw at any time.

Trial population and treatments

Enrolled (n = 260) were postmenopausal women (amenorrhea for at least 12 consecutive months with follicle stimulating hormone [FSH] > 40 IU/l; or amenorrhea for at least 6 months with FSH >40 IU/l and E2 < 20 pg/ml; or at least 6 weeks after surgical bilateral oophorectomy with FSH >40 IU/l), aged 40–65 years with a body mass index (BMI) of 18.0–35.0 kg/m². Women with an intact uterus were eligible if transvaginal ultrasound showed a bilayer endometrial thickness ≤5 mm. More detailed selection criteria have been described previously, as well as potential washout conditions [32]. Eligible

participants (n = 257) were randomly allocated (1:1:1:1:1) to daily oral treatment with E4 2.5 mg, 5 mg, 10 mg or 15 mg (SEQENS VLG CHEM, France), or placebo, for 12 weeks. E4 and placebo were manufactured by Haupt Pharma Munster GmbH, Germany and packaged and supplied by Almac, UK. All non-hysterectomized women received 10 mg dydrogesterone (Dufaston®, Abbott Italy and Mylan Italy, supplied by Almac, UK and BGP Products, Hoofddorp, The Netherlands) once daily for 14 days, after completion of E4 or placebo treatment.

Included in the thrombin generation analysis were all participants with a baseline assessment and a post-dose assessment at week 12, with treatment compliance ≥80% and no changes in concomitant medication during trial treatment that could affect any of the hemostatic parameters (TGA set population n = 168; see Supplementary Figure 1).

Measurements and outcome parameters

Trial visits were scheduled for screening (visit 1), randomization (visit 2, baseline), after 4 weeks of treatment (visit 3), after 12 weeks of treatment (visit 4 [W12], end of treatment) and after dydrogesterone treatment in non-hysterectomized women only (visit 5, follow-up). Blood samples for the assessment of hemostasis and metabolic parameters were taken at baseline and end of treatment (W12). Samples were analyzed by central laboratories (BARC, Belgium and InterLab GmbH/ Synlab, Germany), and samples for the normalized APC sensitivity ratio were analyzed by QUALIblood, Belgium. Outcomes on several hemostasis, metabolic and bone parameters have been reported previously [36].

The TGA was performed on a Calibrated Automated Thrombogram (Diagnostica Stago, Asnières-sur-Seine, France) using STG-ThromboScreen® (Diagnostica Stago) as a triggering reagent. A complete description of the method is reported in detail elsewhere [18]. Thrombogram parameters were integrated using the Thrombinoscope software (Thrombinoscope bv, version 5.0) and the following TGA parameters were extracted from the thrombin generation curve: the lag time, corresponding to the start of thrombin generation (expressed in minutes); the peak height, corresponding to the maximal concentration of thrombin generated (expressed in nanomoles); the time to peak, corresponding to the time to reach the peak (expressed in minutes); the ETP, corresponding to the area under the curve (expressed in nanomolar minutes); and the mean velocity rate index (mVRI), corresponding to the maximal rate of thrombin generation (expressed in nanomoles per minute). Supplementary Figure 2 provides a representation of a thrombogram with the corresponding TGA parameters.

Statistical analysis

Statistical analysis was performed using GraphPad version 9.3.1 (GraphPad Prism 9.3.1 for macOs, GraphPad Software, San Diego, CA, USA; www.graphpad.com). Descriptive statistics were used to analyze the data (n, mean, standard deviation, median, minimum–maximum range, 10th–90th percentile and 95% confidence interval [CI]). Changes from

baseline were assessed using ordinary one-way analysis of variance. A Dunnett's multiple comparisons test with a single pooled variance was then run to assess the difference between the E4 groups and the placebo group. Reference ranges for thrombin generation and associated parameters are reported as the 2.5th–97.5th percentile of the baseline of the entire cohort, in accordance with the definition of the reference intervals as reported in the Clinical & Laboratory Standards Institute (CLSI) EP-28-A3C [37]. Within a particular group, a comparison of the data at baseline and after 12 weeks was performed using a paired t-test. All statistical tests were evaluated with a level of significance of 0.05.

Results

Study population

The trial was conducted in 35 centers in Europe (Belgium, Czech Republic, Great Britain, Ireland and Poland). In total, 260 women were randomized, of which 257 women received treatment. A total of 168 participants were included in the analysis of TGA parameters (E4 2.5 mg, n = 42; E4 5 mg, n = 29; E4 10 mg, n = 34; E4 15 mg, n = 32; placebo, n = 31)

(Supplemental Figure 1). Baseline characteristics in each of the five groups are summarized in Table 1. Mean age ranged between 53.0 and 54.8 years, mean BMI ranged between 25.1 and 26.7 kg/m^2 and mean time since menopause ranged between 3.5 and 5.2 years among the different treatment groups. Treatment groups were comparable for all characteristics assessed.

Thrombograms and TGA parameters

Reference range and absolute values at baseline and after 12 weeks of treatment

Analyses of thrombograms and resulting TGA parameters were performed on data from the 168 subjects in the TGA set population.

The baseline thrombogram (n = 168, entire cohort) and those after 12 weeks of treatment with either E4 2.5 mg, 5 mg, 10 mg or 15 mg, or placebo are shown in Figure 1. The mean baseline thrombogram and its 2.5th–97.5th percentile represents the reference interval. The reference interval was 2.1–4.3 min for the lag time, 4.3–8.8 min for the time to peak, 146.1–398.5 nM for the peak height, 1013–1893 nM*min for the ETP and 32.5–201 nM/min for the mVRI. After 12 weeks of

Table 1. Baseline characteristics by treatment group of all participants who received at least one dose of oral estetrol (E4) or placebo.

Parameter	Statistic	Placebo (n = 31)	E4 2.5 mg (n = 42)	E4 5 mg (n = 29)	E4 10 mg (n = 34)	E4 15 mg (n = 32)	p-Value
Age (years)	Mean (SD)	54.00 (4.70)	54.17 (4.56)	52.97 (4.32)	54.09 (4.35)	54.78 (3.92)	0.6121[a]
	Median (minimum, maximum)	54 (45, 63)	54 (43, 64)	53 (46, 61)	53 (45, 64)	54 (47, 62)	
Body mass index (BMI) (kg/m^2)	Mean (SD)	26.36 (3.79)	25.60 (3.69)	25.07 (3.95)	26.65 (3.72)	26.38 (4.37)	0.4653[a]
	Median (minimum, maximum)	25.70 (19.90,33.60)	25.50 (19.90,34.20)	24.40 (18.90,34.30)	26.85 (19.80,35.00)	26.80 (18.30,34.50)	
Smoking		7 (23%)	5 (12%)	3 (10%)	8 (24%)	2 (6%)	0.1914[b]
Number of pregnancies	Mean (SD)	2.13 (0.99)	2.17 (1.31)	2.31 (1.26)	2.76 (1.16)	2.06 (1.56)	
	Median (minimum, maximum)	2 (0, 4)	2 (0, 5)	2 (0, 5)	3 (0, 5)	2 (0, 8)	0.0512[c]
Time since menopause (years)	Mean (SD)	5.20 (4.77)	5.21 (5.29)	3.46 (2.36)	4.61 (4.31)	4.17 (3.72)	0.5930[c]
	Median	4 (1, 21)	4 (1, 28)	3 (1, 10)	3 (1, 22)	3 (1, 12)	
FSH (IU/ml)	Mean (SD)	94.50 (32.14)	88.16 (29.75)	86.57 (28.11)	85.81 (21.86)	97.31 (26.43)	
	Median (minimum, maximum)	81.00 (43.00,180.20)	77.60 (40.70,168.00)	84.90 (44.00,154.70)	87.90 (43.70,143.80)	95.70 (49.90,159.90)	0.3315[c]
E2 (pg/ml)	n[d]	13	24	13	17	17	
	Mean (SD)	36.26 (27.27)	36.56 (28.65)	29.69 (21.04)	36.72 (39.13)	20.49 (9.64)	
	Median (minimum, maximum)	22.40 (11.80,95.40)	21.85 (11.80,99.10)	19.10 (11.80,69.70)	23.60 (11.80,172.50)	17.00 (11.80,43.30)	
Hysterectomized	All, n (%)	3 (19%)	7 (28%)	2 (15%)	4 (20%)	7 (37%)	0.5921[b]
	Complete hysterectomy	1 (33%)	3 (43%)	0	2 (50%)	4 (57%)	
	Partial hysterectomy	2 (67%)	1 (14%)	1 (50%)	0	2 (29%)	
	Other hysterectomy	0	3 (43%)	1 (50%)	2 (50%)	1 (14%)	
Selected medical history	Diabetes mellitus	0	0	1	0	1	
	Hyperlipidemia	11	6	3	7	5	
	Hypertension	6	6	3	6	4	
	Thyroid disorders	6	10	3	3	6	
Concomitant medication	All, n (%)	15 (48%)	14 (33%)	9 (31%)	16 (47%)	12 (38%)	0.4882[b]
	Antidiabetics	0	0	1	0	1	
	Cholesterol lowering drugs	7	8	3	9	5	
	Antihypertensive drugs	9	5	3	8	5	
	Thyroid therapy	6	7	3	2	5	

FSH, follicle stimulating hormone; SD, standard deviation.
[a]One-way analysis of variance; [b]Chi-square test; [c]Kruskal-Wallis test; [d]Estradiol (E2) was only measured for participants who had their last menstruation within the last 6 and 12 months.
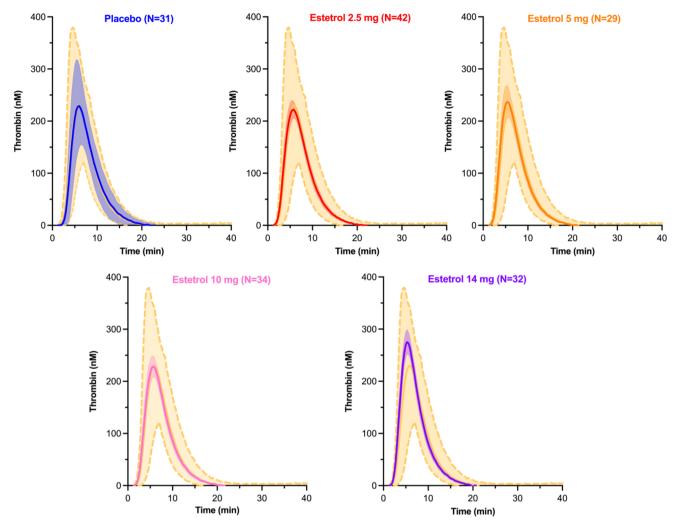
Figure 1. Mean (2.5th–97.5th percentile) thrombogram of the entire baseline cohort (*n* = 168) and mean thrombograms (95% confidence interval [CI] of the mean) after 12 weeks of treatment. Mean thrombogram of the entire baseline cohort is represented by the yellow line and 2.5th–97.5th percentiles, indicating reference ranges, are represented by yellow dotted lines. Women treated with placebo or E4 2.5 mg, 5.0 mg, 10 mg and 15 mg are represented in blue, red, orange, pink and purple, respectively. None of the mean thrombograms were outside the reference range established at baseline, demonstrating the low impact of estetrol (E4) on the entire coagulation process.

treatment, the mean (95% CI of the mean) thrombograms were all within the reference range.

Mean values of each TGA parameter for each subgroup at baseline and after 12 weeks of treatment are presented in Table 2. The evolution of the different TGA parameters from baseline to week 12 and their comparison with the reference range are provided in Figures 2 and 3.

Table 3 presents the number of individual values outside the reference range after 12 weeks of treatment. More than 85% of the values were within the reference ranges, independent of the treatment group.

Changes from baseline

Results after 12 weeks of treatment were statistically different from baseline for all TGA parameters for E4 15 mg, for the lag time and time to peak for E4 10 mg and for all parameters except ETP for E4 5 mg, and no changes were observed for E4 2.5 mg and placebo (Figures 2 and 3). Changes remain mostly within the reference range (Table 3 and Figure 4). The absolute change from baseline is statistically significant for all TGA

parameters. The multiple comparisons allowed to highlight that doses of 5 mg, 10 mg and 15 mg E4 showed statistically significant absolute change from baseline compared to placebo for all TGA parameters. The dose of 2.5 mg is not different from placebo.

Discussion

Hemostasis is a finely regulated physiological process and HT may induce changes in the levels of individual coagulation proteins which are not considered clinically relevant according to established normal ranges [37,38]. Nevertheless, it has been demonstrated that treatment with CEE or oral E2 may induce changes that, even if not considered clinically relevant when assessed individually (e.g. levels of prothrombin, FVII, FVIII, protein S), can be clinically relevant in global coagulation tests like the TGA [5,23]. Numerous publications reported an association between elevated TGA parameters and the risk of developing VTE [19,20,39]. Therefore, as conventional oral HT increases thrombin

Table 2. Thrombin generation parameters with different doses of estetrol (E4) (from 2.5 mg to 15 mg once daily) at baseline and after 12 weeks of treatment.

Parameter	Baseline	Week 12	Change from baseline	
			Absolute difference	Relative difference (%)
ETP (nM*min)				
E4 2.5 mg	1456 ± 219	1452 ± 234	−4 ± 160	0.18 ± 10.85
E4 5.0 mg	1405 ± 208	1450 ± 239	45 ± 186[a]	3.77 ± 13.62
E4 10 mg	1387 ± 203	1428 ± 204	40 ± 143[a]	3.54 ± 11.18
E4 15 mg	1457 ± 214	1558 ± 290	101 ± 145[a]	6.70 ± 9.61
Placebo	1532 ± 224	1447 ± 219	−86 ± 156	−5.16 ± 10.51
Peak height (nM)				
E4 2.5 mg	257 ± 54	259 ± 64	3 ± 53	2.27 ± 21. 45
E4 5.0 mg	252 ± 65	273 ± 75	21 ± 54[a]	9.38 ± 22.07
E4 10 mg	243 ± 69[a]	261 ± 66	17 ± 63[a]	11.78 ± 30.40
E4 15 mg	259 ± 50	306 ± 63	47 ± 63[a]	19.37 ± 18.18
Placebo	293 ± 66	268 ± 69	−26 ± 71	−6.90 ± 25.13
Lag time (min)				
E4 2.5 mg	2.95 ± 0.41	2.80 ± 0.62	−0.15 ± 0.60	−4.28 ± 19.67
E4 5.0 mg	2.97 ± 0.49	2.75 ± 0.46	−0.22 ± 0.46[a]	−6.27 ± 16.38
E4 10 mg	3.17 ± 0.59	2.82 ± 0.50	−0.34 ± 0.48[a]	−9.71 ± 14.11
E4 15 mg	3.10 ± 0.63	2.72 ± 0.40	−0.38 ± 0.50[a]	−10.10 ± 15.91
Placebo	2.91 ± 0.35	3.05 ± 0.69	0.14 ± 0.57	4.66 ± 19.14
Time to peak (min)				
E4 2.5 mg	6.11 ± 0.86	5.94 ± 1.15	−0.17 ± 1.10	−2.00 ± 17.97
E4 5.0 mg	6.19 ± 1.07	5.77 ± 1.17	−0.42 ± 0.90 [a]	−6.14 ± 15.72
E4 10 mg	6.48 ± 1.33	5.87 ± 1.11	−0.61 ± 1.10[a]	−7.77 ± 16.52
E4 15 mg	6.27 ± 1.17	5.40 ± 0.80[a]	−0.87 ± 0.92[a]	−11.99 ± 15.93
Placebo	5.78 ± 0.84	6.12 ± 1.41	0.36 ± 1.30	6.96 ± 21.99
mVRI (nM/min)				
E4 2.5 mg	86.2 ± 33.2	90.3 ± 42.9	4.2 ± 36.4	9.44 ± 45.67
E4 5.0 mg	86.5 ± 40.0	102.1 ± 49.1	15.6 ± 35.7[a]	22.81 ± 44.35
E4 10 mg	83.5 ± 44.6[a]	95.1 ± 44.9	11.6 ± 44.4[a]	31.19 ± 65.17
E4 15 mg	88.3 ± 37.5	121.0 ± 47.2	32.7 ± 41.3[a]	46.18 ± 47.45
Placebo	110.7 ± 43.2	96.6 ± 43.0	−15.3 ± 45.6	−5.14 ± 46.46

Mean values ± standard deviations are reported. Absolute and relative changes from baseline for each treatment group are reported. Absolute changes from baseline were used for statistical evaluation of treatment effect on thrombin generation assay (TGA) parameters. ETP, endogenous thrombin potential; mVRI, mean velocity rate index.
[a]Different from placebo, the p value had been estimated using ordinary one-way analysis of variance. A Tukey's multiple comparisons test with a single pooled variance was run to assess the difference between the groups. $p < 0.05$ considered statistically significant.

generation [5,23,40,41], it is not surprising that this increase is also linked with the observed increased risk of thrombosis with oral HT containing CEE or E2 [42]. On the other hand, it has been demonstrated that E2, once administered by a transdermal route, has a lower impact on the hemostatic system [40,43] and is not associated with an increased risk of thrombosis [3].

In this study, we report for the first time TGA data with E4, the first NEST. The highest dose tested in this study, that is, E4 15 mg once daily, was shown to be effective for the relief of vasomotor symptoms [32]. Interestingly, we demonstrated that E4 does not clinically impact thrombin generation (Table 2). Importantly, the mean thrombograms remain within the normal range established according to CLSI standards (Figure 1) [37]. At the individual level, the impact of E4 on thrombin generation is very limited since only very few subjects have abnormal TGA parameters after 12 weeks of treatment compared to placebo (Table 3). Also, for some of these patients, the TGA results were already outside the

reference range at baseline (Figure 2 and 3). The most influenced parameter is the peak but it is only translated into very few subjects outside the reference range ($n = 4/32$ for E4 15 mg versus $n = 1/31$ for placebo after 12 weeks of treatment; Figure 4). Importantly, the high thrombin generation curves observed in the placebo group at baseline undoubtedly influenced the change from baseline in this group. This finally also influenced the difference between the E4 groups and the placebo group when comparing the changes from baseline. This observation is confirmed by the fact that when comparing only the time point after 12 weeks of treatment, none of the E4 doses showed statistically different ETP, peak height, lag time or mVRI results compared to the placebo group after 12 weeks of treatment. It is important to note that the same dose of E4 has been used in combination with drospirenone in contraception and the results also revealed that this estrogen has a low impact on thrombin generation compared to ethinylestradiol combined with either levonorgestrel or drospirenone [44].

A direct comparison of the results obtained in this study cannot be made with HT containing oral CEE or E2 as this study did not include other active comparator arms. Nevertheless, the change from baseline reported in other studies using similar thrombin generation methods suggests that the changes induced by E4 15 mg is not different from that of transdermal estrogen [45]. Interestingly, this is in line with the data observed on APC resistance [36]. Indeed, the median relative change from baseline in the normalized APC sensitivity ratio with E4 15 mg was 42% after 12 weeks of treatment, which is very close to the 28% observed by Post et al. with transdermal E2 at a dose of 50 μg [43]. In any circumstances, the change from baseline is far lower than the ±100% normalized APC sensitivity ratio increase observed with the different oral preparations containing 17β-E2 as the estrogen [43,46,47]. This clearly delineates that the different pharmacological profile of oral E4 compared to oral CEE or oral E2 results in a lower impact on the liver.

There is currently scientific evidence that explains the differences between oral E2 and oral E4. Orally administered E2 is already partly metabolized to estrone (E1) in the intestines [48,49]. The ileum produces more E1 after incubation with E2 than any other tissue except for the placenta [50,51]. E1 is less potent than E2 on nuclear and membrane ERα receptors, but once given orally it can accumulate in liver cells, the major site of coagulation protein synthesis [52,53]. Plasma levels of E1 have also been reported to be higher in users of oral E2 than in users of transdermal E2. In fact, the plasma levels of E1 and E2 are equal in transdermal E2 users while they are fivefold higher in oral E2 users [54]. This also supports the possible link between E1 and increased impact on hemostasis with oral E2 [5,23]. The risk of a VTE event has been shown to correlate with E1 plasma levels. This risk has also been demonstrated to be 30-fold higher in women with the *CYP3A5*1* genotype, encoding a high expression of the cytochrome P450 enzyme responsible for E1 formation from E2, thus implying that the formation of E1 and E1 sulfate is suggestive of an increased risk of VTE [55–57]. This higher plasma E1 concentration has also been directly linked to higher thrombin generation potential [23]. This evidence-based
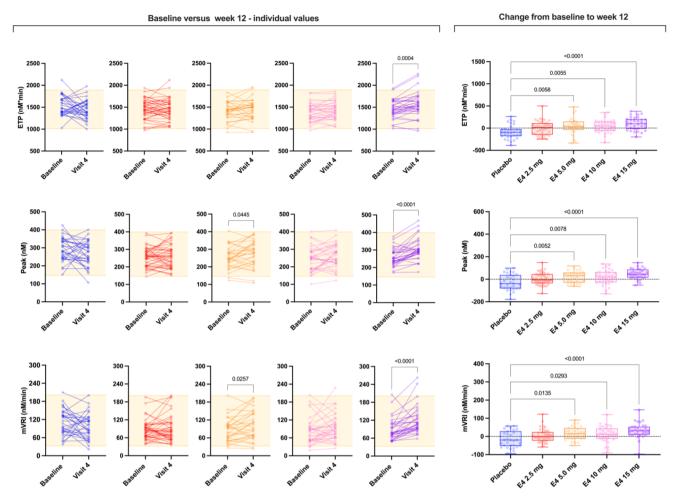
Figure 2. Spaghetti plot of individual data comparing the baseline endogenous thrombin potential (ETP), peak and mean velocity rate index (mVRI) and absolute changes from baseline versus week 12 (visit 4). Changes from baseline were assessed using ordinary one-way analysis of variance with a Dunnett's multiple comparison test. Only differences versus placebo are reported. E4, estetrol.
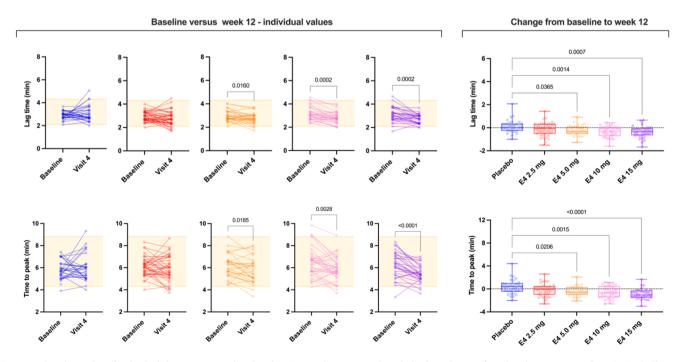

Figure 3. Spaghetti plot of individual data comparing baseline lag time and time to peak and absolute changes from baseline versus week 12 (visit 4). Changes from baseline were assessed using ordinary one-way analysis of variance with a Dunnett's multiple comparison test. Only differences versus placebo are reported. E4, estetrol.

information suggests that the higher hepatic exposure to E1 that follows oral E2 administration, which is absent with transdermal HT, leads to increased thrombin generation with oral HT use [23]. On the contrary, E4 is an end-stage product which means that it is not metabolized into active metabolites like E1, E2 or E3 [29]. In addition, unlike E2, E4 is not converted into hydroxylated metabolites, precursors of quinone estrogens that can react and damage the DNA, which has been linked to breast cancer development [58]. E4 is excreted in urine in the form of a D-ring monoglucuronide and is otherwise metabolically unaltered [59]. Considering also the antagonism of E4 for the membrane-initiated steroid signaling pathway, which has been demonstrated to be an important regulator of lipid content in the liver [60], there is a strong rationale to support the hypothesis that the weak effect of E4 on hemostasis is due to a combination of its specific pharmacodynamic and pharmacokinetic properties.

As estrogen response is highly tissue dependent, E4 represents the interesting combination of an estrogen with an efficient action on the uterus, bone and brain, which provides a rational for use for the relief of menopausal symptoms, while its limited effect on the liver and the breast may delineate a never-reached safety profile for an orally administered estrogen.

Conclusions

This study confirms the current evidence for the weak impact of E4 on hemostasis in postmenopausal women. After having demonstrated that E4 only minimally impacts APC resistance, the results presented in this manuscript reveal that its impact on thrombin generation, a global coagulation test sensitive to the changes induced by estrogenic compounds, are negligible and not clinically relevant. When comparing these results with the literature, it appears that oral E4 has a similar impact on thrombin generation to transdermal E2. Obviously, a direct comparison would be required to confirm this observation but the current knowledge about its pharmacological profile provide a rationale for this statement. More and more evidence is reinforcing the message that E4 has a neutral profile on hemostasis. Results from the phase 3 program will provide important additional information on the safety profile of E4 in menopause.

Table 3. Reference intervals (2.5th–97.5th percentile of entire baseline cohort) of thrombin generation parameters and out-of-range results after 12 weeks of treatment.

Treatment	Lag time (min)	Time to peak (min)	Peak (nM)	ETP (nM*min)	mVRI (nM/min)
Reference interval (2.5th–97.5th percentile of entire baseline cohort)					
Reference range (n = 168)	2.1–4.3	4.3–8.8	146–399	1013–1893	32.5–201.0
Out of reference range[a] after 12 weeks of treatment, n (%)					
Placebo (n = 31)	1 (3%)	1 (3%)	1 (3%)	1 (3%)	0 (0%)
E4 2.5 mg (n = 42)	5 (12%)	4 (10%)	0 (0%)	2 (5%)	1 (2%)
E4 5 mg (n = 29)	3 (10%)	2 (7%)	0 (0%)	2 (7%)	0 (0%)
E4 10 mg (n = 34)	4 (12%)	2 (6%)	1 (3%)	0 (0%)	1 (3%)
E4 15 mg (n = 32)	2 (6%)	3 (9%)	4 (13%)	3 (9%)	2 (6%)

E4, estetrol; ETP, endogenous thrombin potential; mVRI, mean velocity rate index.
[a]Either 2.5th or 97.5th percentile.

Figure 4. Comparison of thrombin generation assay (TGA) parameters after 12 weeks of treatment.

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