Different effects of low-dose transdermal and oral oestrogen therapy on procarboxypeptidase U, an inhibitor of fibrinolysis, in healthy postmenopausal women: A randomised, placebo-controlled study

Dear Sir,

The effect that oestrogen therapy exerts on markers of coagulation and fibrinolysis in postmenopausal women depends on the route of administration (oral or transdermal) (1–3) and whether or not a progestogen is added (1–5). Recently, Scarabin et al. found that transdermal oestrogen therapy, in contrast to oral therapy, is not associated with an elevated risk of venous thromboembolism (6). This indicates that different forms of hormone therapy not only differentially affect the balance of markers of coagulation and fibrinolysis but also have different effects on the risk of venous thromboembolism. In addition, the early increased risk of coronary heart disease observed in postmenopausal women using oral hormone therapy (7, 8) has been thought to be related to procoagulatory changes.

Procarboxypeptidase U (proCPU, EC 3.4.17.20), also known as thrombin-activatable fibrinolysis inhibitor (TAFI), is considered to be a link between coagulation and fibrinolysis (9–11). During coagulation and fibrinolysis the active enzyme, carboxypeptidase U (CPU) is generated from proCPU (12). CPU exerts its antifibrinolytic effect by removing C-terminal lysine residues from partially degraded fibrin (11), thereby abrogating the enhanced plasminogen activation and preventing an acceleration of fibrinolysis. Elevated levels of proCPU have been found to be a mild risk factor for venous thrombosis (13) and were observed in patients with angina pectoris (14).

Data on the effect of oral hormone therapy on proCPU levels are scarce and inconclusive, whereas no data are available on the effect of transdermal treatment. In the present study, we intended to elucidate the role of the route of oestrogen administration and the effect of the addition of the progestogen gestodene to oral oestradiol on proCPU levels.

The design of this study was published previously (2, 3, 15–18). Briefly, in this randomised, placebo-controlled, double-blind, double-dummy, multicentre study, 152 healthy hysterectomised postmenopausal women aged 45 to 65 years received daily either placebo (n=49), or transdermal 17β-oestradiol (E_2) 50 µg (Climara®, tE_2 group, n=33), or oral E_2 1 mg (oE_2 group, n=37), or oral E_2 1 mg combined with gestodene 25 µg (oE_2+G group, n=33) for thirteen 28-day treatment cycles, followed by four cycles of placebo for each group. The investigation conformed to the principles outlined in the Declaration of Helsinki. Institutional Review Boards of all participating centres approved the protocol. Written informed consent was obtained from each participant before entry into the study.

Venous plasma samples were collected at baseline and in cycles 4, 13 and 17 and stored at –80°C until analysis. Samples of 146 participants were available for analysis. Plasma proCPU concentrations were determined by converting the zymogen to its active form and subsequently measuring the carboxypeptidase activity with a colorimetric assay (5, 19, 20). The mean intra-assay coefficient of variation was 3.7%.

Statistical analysis was performed using the Statistical Package for the Social Sciences PC + 9.0 (SPSS Inc., Chicago, Illinois). ProCPU concentrations are given as mean ± standard deviation (SD) and the individual percentage changes from baseline as mean with 95% confidence interval (CI). Standard parametric tests were performed. Analyses of covariance (ANCOVA) for repeated measurements, with the baseline concentrations of proCPU and serum oestradiol as constant covariates, were used for comparisons among and between the groups.

At baseline, no significant differences were found between the groups in either proCPU levels or demographic characteristics (age, body mass index (BMI), blood pressures, number of smokers, serum cholesterol, FSH and oestradiol levels). The women had an age of 54.6 ± 4.5 years (mean ± SD) and a BMI of 25.6 ± 2.9 kg/m^2 (mean ± SD).

In cycle 13, proCPU was significantly decreased in the tE_2 and oE_2+G group compared to placebo ($P<0.001$), but not in the oE_2 group (Table). The mean percentage changes from baseline versus placebo were –8.6% [95% CI –12.1 to –5.1%] in the tE_2 group and –7.9% [95% CI –10.9 to –5.0%] in the oE_2+G group. The decreases were already apparent in cycle 4. Changes in the tE_2 and oE_2+G group differed significantly from the changes in the oE_2 group. After four cycles of wash-out, when compared to baseline and placebo, the decrease observed in the oE_2+G group persisted (–4.6% [95% CI –7.9 to –1.3%]).

In earlier randomised controlled trials and in the present study it was observed that neither unopposed oral 17β-oestradiol in a dosage of 1 or 2 mg daily (5) nor oral oestrogens combined with dydrogesterone (5), medroxyprogesterone acetate (20), or norethisterone acetate (21) changed proCPU levels. In contrast, decreased plasma proCPU levels were observed during transdermally administered oestradiol 50 µg daily and during oral oestradiol combined with the progestogens gestodene or trimegestone (5). Taken together, data from randomised controlled trials indicate that both the route of administration and the addition of different progestogens play a role in the effect of oestrogens on plasma proCPU. Consequently, this will have differential effects on the haemostatic balance and, therefore, may differentially affect clinical venous and arterial thromboembolic risk. However, results from the present study are puzzling, since

Correspondence to:
M.J. van der Mooren, M.D., PhD,
Department of Obstetrics & Gynaecology
VU University Medical Center
PO Box 7057, 1007 MB Amsterdam
The Netherlands
Tel.: +31 20 444 32 44, Fax: +31 20 444 44 22,
E-mail: mj.vandermooren@VUmc.nl

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Table: Plasma procarboxypeptidase U concentrations.

	N	Baseline	cycle 4	cycle 13	ANCOVA‡	ANCOVA‡‡	%Δ 0–13	N	cycle 17	ANCOVA#	ANCOVA##	%Δ 0–17
Placebo	47	619 ± 80	625 ± 72	633 ± 92	<0.001			40	624 ± 84	0.001		
tE_2	32	631 ± 100	601 ± 90	591 ± 97		<0.001	–8.6 (-12.1 to –5.1)**	29	642 ± 94		0.001	–0.6 (-4.1 to 2.9)
oE_2	37	659 ± 85	649 ± 99	654 ± 97		0.13	–3.2 (-6.5 to 0.2)	36	659 ± 93		0.27	–2.2 (-5.6 to 1.2)
oE_2+G	30	643 ± 115	594 ± 111	620 ± 102		<0.001	–7.9 (-10.9 to –5.0)**	29	637 ± 92		<0.001	–4.6 (-7.9 to –1.3)*

Concentrations (U/L) are given as mean ± SD.
‡ANCOVA with the baseline values of proCPU and oestradiol as constant covariate for among-group differences over the 13-cycle study period.
‡‡ANCOVA over the 13-cycle study period: treatment versus placebo. In addition, significant differences were found between the tE_2 and oE_2 group (P<0.001) and between the oE_2 and oE_2+G group (P<0.001).
%Δ, Mean (95% CI) of the individual percentage changes from baseline compared to placebo at cycle 13 and at cycle 17. *P<0.05 and ** P<0.001.
#ANCOVA with the baseline values of proCPU and oestradiol as constant covariate for among-group differences over the study period from cycle 13 to cycle 17.
##ANCOVA from cycle 13 to cycle 17 (after correction for baseline values): treatment versus placebo. In addition, significant differences were found between the tE_2 and oE_2 group (P<0.05) and between the oE_2 and oE_2+G group (P<0.05).
N = number of participants of whom data were analysed.

previous studies clearly showed that orally but not transdermally administered oestrogens increase fibrinolysis.

Since the proenzyme proCPU is mainly produced in the liver, the observed difference in effect of orally and transdermally administered unopposed oestrogen may be the consequence of avoiding the hepatic first-pass effects in the latter. Besides a decreased production, the observed reductions in plasma proCPU levels can be the result of an increase in the conversion of proCPU to active CPU as well. The activation of proCPU to CPU occurs during coagulation and fibrinolysis by plasmin, thrombin, and by a complex of thrombin with thrombomodulin (22). Therefore, effects of transdermal oestradiol and oral oestradiol combined with gestodene on these haemostatic factors may have contributed to our observations.

In contrast to oral postmenopausal hormone therapy, transdermally administered hormone therapy is not associated with an increased risk of venous thrombosis (6). The risk of venous thrombosis depends, in part, on the balance between markers of coagulation and fibrinolysis. Since plasma proCPU plays a role in this balance as well, differences in effect of the two routes of administration on proCPU levels as observed in our study may therefore contribute to the difference in venous thrombosis risk in users of oral and transdermal hormone therapy.

In elderly women, oral combined oestrogen plus progestogen therapy is possibly associated with a slightly early increased risk for coronary heart disease (CHD) (7, 8), whereas a non-significant increased risk of CHD was observed in elderly women using a high dose of transdermal oestradiol (23). Interestingly, recent data suggest a protective effect of oral unopposed oestrogen therapy in postmenopausal women aged 50–59 years (24). Contradictory results have been found in trials investigating the association of proCPU levels and arterial thrombotic events (14, 25). This might be the consequence of differences in study populations and the use of different assays (25). We want to stress that the reductions in proCPU in relatively young postmenopausal women as observed in our study should not be extrapolated to elderly women who are at increased CHD risk during oral combined oestrogen plus progestogen therapy.

In conclusion, transdermal unopposed oestradiol reduced plasma proCPU levels whereas oral unopposed oestradiol did not. Oral oestradiol combined with gestodene also lowered proCPU levels. A reduction in proCPU may contribute to increased fibrinolysis.

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Marinka S. Post[1], Judith R. Leurs[2], Marius J. van der Mooren[1], W. Marchien van Baal[1], Dirk F. Hendriks[2], Coen D.A. Stehouwer[3], Peter Kenemans[1]

Project 'Ageing Women' and the Institute for Cardiovascular Research-Vrije Universiteit (ICaR-VU), Departments of [1]Obstetrics & Gynaecology and [3]Internal Medicine, VU University Medical Center, Amsterdam, and [3]Internal Medicine, Maastricht University, Maastricht, The Netherlands; [2]Department of Medical Biochemistry, University of Antwerp, Wilrijk, Belgium

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Rebuttal: A problem of calibration in the paper 'Point of care monitoring of oral anticoagulant therapy in children'

Dear Sir

We read with interest the paper by Ignjatovic and colleagues (1) and were encouraged to learn about their efforts to improve the quality of their service to children and young people.

We were disturbed, however, at the way in which the study was carried out and concerned about its conclusions. In particular, the idea that the laboratory venous method of INR determination should be deemed the "gold standard" is an extraordinary assertion. The laboratory method described was an ACL 100 with Instrumentation Laboratory's ILTest PT Fibrinogen. The laboratory relied on the International Sensitivity Index (ISI) assigned to the reagent by the manufacturer. No attempt was made to determine a local system ISI. The authors used as justification a paper by Hobbs et al. (2) describing a study to compare INR measurements in hospital laboratories and primary care settings. Hobbs et al. used three hospitals and applied the term "gold standard" to the one which had taken the trouble to assign a system ISI to their combination of coagulometer and thromboplastin reagent. We do not believe that these authors were suggesting that any laboratory INR method can be a "gold standard"!

We feel that the only proper way to undertake studies of this sort must be to use manual prothrombin time testing with an International Reference Preparation (IRP) as the "gold standard", or, at least, to perform a system ISI calibration of the laboratory method to align it to the WHO system. Shiach et al. (3) described the ideal approach in a comparison study by performing system ISI for both laboratory and point of care testing. This should surely be the model for similar studies.

Steven Craig, David Leeming, Allan Sidebotham , Ken Stevenson
Anticoagulation and Haematology Research Unit, South Manchester University Hospitals Trust, Wythenshawe Hospital, Manchester, UK

Correspondence to:
Ken Stevenson
Anticoagulation and Haematology Research Unit
Wythenshawe Hospital
Manchester M23 9LT
UK
Tel.: +44 161 291 4747, Fax +44 291 4749
E-mail ken.stevenson@smuht.nwest.nhs.uk

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