Skin contamination by oestradiol gel—a remarkable source of error in plasma oestradiol measurements during percutaneous hormone replacement therapy

Tarja Vihtamäki a,*, Tiina Luukkaala b, Risto Tuimala a

a Department of Obstetrics and Gynecology, Tampere University Hospital, P. O. Box 2000, 33521 Tampere, Finland
b Tampere School of Public Health, University of Tampere, 33521 Tampere, Finland

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Abstract

Objectives: To study the consequence of skin contamination by oestradiol gel on circulating plasma oestradiol levels. Methods: We studied ten healthy, hysterectomized postmenopausal women who had used percutaneous oestradiol gel for at least 2 years. After wash-out period percutaneous dose of 1.5 mg 17β-oestradiol was administered once a day in the evening. The gel was applied with a bare or gloved hand to an arm or thigh according to the schedule. Blood samples for assay of plasma oestradiol concentrations were collected from both cubital veins 12 h after gel administration, at baseline and every time after using the gel, for 2 weeks. Results: Plasma oestradiol concentrations were significantly higher in the gel-contaminated samples: in the cubital vein of the gel-applying arm and in the cubital vein of the forearm on which the gel had been spread. Conclusions: Skin contamination by topical 17β-oestradiol can distort plasma oestradiol measurements by giving much higher oestradiol concentrations than in reality there are in the systemic circulation. This has an important meaning when tailoring individual oestrogen therapy.

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1. Introduction

Plasma oestradiol (E2) measurements are necessary when tailoring hormone replacement therapy (HRT) individually for early findings have shown that the beneficial effects of HRT are not completely contributed with low plasma E2 levels [1–3]. Similarly, in a recently published prospective study, women with no bone response to oestrogen therapy had significantly lower plasma E2 levels and higher follicle-stimulating hormone levels compared with HRT responders [4].

Use of transdermal E2 therapy is increasing, since it provides well-known advantages compared with oral administration [5–7]. During topical therapy, however, plasma E2 concentrations vary inter-individually, this being at least partly a result of differences in absorption of the drug and its metabolism [8]. When using 17β-oestradiol gel the material is applied to the skin by hand and E2 is absorbed both from the gel-treated skin area and from the gel-spreading hand. If the samples for E2 assays are taken from the cubital vein of
the forearm on which the gel had been spread or from the cubital vein of the gel-applying arm (E2 contaminated samples), plasma E2 levels may be high and are not reflecting the levels in systemic circulation. We studied the influence of 17β-oestradiol gel skin contamination on circulating plasma E2 levels.

2. Materials and methods

Ten healthy, hysterectomized, postmenopausal women, aged 52–58 years and weighting 55–80 kg were included in the study. The women had been on percutaneous 17β-oestradiol gel for at least 2 years. They had relatively constant intra-individual E2 levels and were accustomed to spreading the gel. There was a wash-out period of 2 weeks before the start of the study. A percutaneous dose of 1.5 mg 17β-oestradiol (Estrogel R, Leiras, Finland) was administered once a day in the evening. The gel was applied with a

| Table 1 |
| Study protocol |
| (1) Wash-out period of 2 weeks |
| (2) Percutaneous 17β-oestradiol gel was applied to a thigh with a disposable glove on the right hand, for 2 weeks |
| (3) Percutaneous 17β-oestradiol gel was applied to the left arm with a disposable glove on the right hand, for 2 weeks |
| (4) Percutaneous 17β-oestradiol gel was applied to a thigh with a bare right hand, for 2 weeks |
| (5) Percutaneous 17β-oestradiol gel was applied to the left arm with a bare right hand, for 2 weeks |

Blood samples were taken from both cubital veins at baseline and every time after using the 17β-oestradiol gel, for 2 weeks.

bare or gloved hand (disposable plastic glove) to an arm or thigh (area: as large as possible) according to the schedule outlined in Table 1. Each subject went through all five steps indicated in Table 1. Blood samples for assay of plasma E2 concentrations were collected from both cubital veins 12 h after gel administration, at baseline and every time after using the gel, for 2 weeks. Oestradiol concentrations were

![Fig. 1. Plasma oestradiol concentrations in left (open circle) and right (open triangle) cubital vein [1] at baseline, after spreading 17β-oestradiol gel [2] to a thigh with a disposable glove on the right hand, [3] to left arm with a disposable glove on the right hand, [4] to a thigh with a bare right hand, and [5] to the left arm with a bare right hand. Cross-lines are representing medians.](image)
The data were assessed for normality of distribution before analysis and natural logarithmic transformation was undertaken. Despite this, normality was not observed and therefore, non-parametric tests were applied. To assess the differences in plasma E2 concentrations between the right and left cubital vein, Wilcoxon’s test was used. Friedman’s test was applied to assess the differences between E2 gel-contaminated samples and between uncontaminated samples. The data were analyzed using spss for windows version 9.0 statistical software.

3. Results

At baseline, plasma E2 concentrations were at post-menopausal levels in all women. In samples from the right and left cubital vein there were not significant differences intra-individually or inter-individually. When the gel was applied with a disposable glove on the right hand to a thigh for 2 weeks there were not differences in E2 concentrations between the right (median 0.21 nmol/l) and left (median 0.17 nmol/l) cubital vein (\(P = 0.34\); Fig. 1). After applying the gel to the left arm with a disposable glove on the right hand, markedly higher E2 concentrations were measured in the left cubital vein (median 0.26 nmol/l) in all subjects (\(P = 0.03\)). In addition, higher E2 concentrations were found in right forearm vein samples (median 0.21 nmol/l) after applying the gel to a thigh with a bare right hand (\(P = 0.07\)). When the gel was measured by RIA (Sorin biomedica, S.p.A., Saluggia, Italy, intra-assay variation CV (%) at low, medium and high level of E2 is 4.2, 4.8, and 7.9, respectively) all samples from any one subject being assayed together. Informed consent was obtained from each woman, and the study was approved by the Ethical Committee of Tampere University Hospital.
Fig. 3. Contaminated samples. Plasma oestradiol concentrations in ten women treated with percutaneous oestradiol 1.5 mg/day. Oestradiol gel was spread [1] to a thigh with a bare right hand (the sample was taken from the right cubital vein), [2] to left arm with a bare right hand (the sample was taken from the left cubital vein), [3] to left arm with a bare right hand (the sample was taken from the right cubital vein), and [4] to left arm with a disposable glove on the right hand (the sample was taken from the left cubital vein).

applied to the left arm with a bare right hand there was not difference in E2 concentrations in the right (median 0.32 nmol/l) and left (median 0.34 nmol/l) cubital vein \( (P = 0.72; \text{Fig. 1}) \). When assaying E2 gel-contaminated samples together there were not significant differences \( (P = 0.52) \) neither were any differences found when assaying uncontaminated samples together \( (P = 0.52) \). No general agreement exists about the need to monitor plasma E2 levels routinely during HRT, but most clinicians agree that in cases with no clinical response to HRT or in cases with hyper-oestrogenic symptoms, plasma E2 measurements should be utilized. However, according to our earlier findings \([9]\) ca. 10% of women using the recommended topical 17β-oestradiol dosage show exceptionally high plasma E2 levels 12 h after administration, indicating a need to decrease the dosage.

4. Discussion

The possibility that skin contamination by 17β-oestradiol gel might distort the concentrations of E2 in plasma was supported in all subjects. Nevertheless the E2 levels attained varied inter-individually, as in previous studies \([9-11]\). Oestradiol concentrations 12 h after gel administration were significantly higher in the cubital vein of the gel-applying arm and also in the cubital vein of the forearm on which the gel had been spread.
differences in percutaneous absorption due to variations in oestrogen metabolism, skin thickness, retention time within the skin, vascularity of the adipose tissue, and the surface area of application [8,12,13]. The pharmaceutical formulation and drug content of different preparations also have an effect on the pharmacokinetics and bioavailability of a drug [14].

In the current study, E2 concentrations following 17β-oestradiol application with a bare hand on the opposite forearm were similar in both cubital veins but significantly higher compared with the levels in uncontaminated samples. This indicates that E2 penetrates the stratum corneum and diffuses through the epidermis, dermis and subcutaneous connective tissue into the systemic circulation over a period of 12 h. The tissue under the skin of the application area functions as a store. When spreading 17β-oestradiol gel over a large area of skin including the forearms, upper arms and shoulders, as many women do and as recommended with the preparation used, plasma E2 concentrations, if monitored, may vary greatly causing confusion when evaluating the situation as a whole.

The surface areas of application in published studies have varied from about 200–800 cm² or have not been specified. In this study, 17β-oestradiol was applied to as large a skin area as possible, since the area of application has been shown to be a significant and limiting parameter of steroid absorption when the surface area is too restricted [15]. With a larger surface area absorption is only dependent on the dose applied to the skin [16]. In contrast, Jarvinen et al. demonstrated that absorption of 17β-oestradiol applied to a small area of skin resulted in higher plasma E2 levels than when applied to an area as large as possible [17]. However, they used a different preparation whose pharmaceutical formulation as well as E2 content were different from those in the preparation used in the present study [17]. Various cutaneous applica-
tion sites had no influence on E2 plasma levels in our study nor in previous studies [11,18,19]. Blood samples for E2 assays were collected 12 h after gel application, and the 17β-oestradiol gel used has been shown to provide relatively stable plasma E2 concentrations [20–23]. Furthermore, 12 h after administration is a time generally used in determination of circulating drug concentrations. In addition, in practice, blood samples for E2 assay taken 12 h after administration are easy to collect, for most women spread the gel in the evenings. It would have been interesting to know if the differences in plasma E2 levels between contaminated and uncontaminated samples no longer exist 24 h after administration but as we took only 12 h samples it remains unknown.

There was one participant whose plasma E2 concentrations between contaminated and uncontaminated samples varied greatly. The woman did not use any other medication and her BMI did not differ from those of the other subjects, though weight, according to our observations, does not correlate with circulating E2 concentrations in topical therapy. In such cases, the gel may have been stored in the skin for longer period producing lower plasma E2 concentrations. In animal models, it has been suggested that the outer layers of the skin may act as an E2 reservoir for topically applied E2 [24].

5. Conclusion

This study shows that skin contamination by topical 17β-oestradiol can distort plasma E2 measurements by giving much higher E2 concentrations than in reality there are in the systemic circulation. This has an important meaning when tailoring individual oestrogen therapy. Contamination may also be one reason for the large individual variations in plasma E2 reported during topical therapy. When monitoring plasma E2 during HRT, samples for E2 assay should not be taken from a gel-contaminated forearm.

References

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