Effects of oral and transdermal oestrogen replacement therapy on plasma levels of insulin-like growth factors and IGF binding proteins 1 and 3: a cross-over study

S. I. Helle*, I. H. Omsjø†, S. C. Cwyfan Hughes‡, L. Botta§, G. Hüls∥, J. M. P. Holly† and P. E. Lønning*

* Department of Oncology, Haukeland University Hospital, Bergen, Norway; † Laboratory for Osteoporosis, Oslo, Norway; ‡ Department of Medicine, Bristol Royal Infirmary, Bristol, UK and ∥ Bioanalytics & Pharmacokinetics, Ciba Geigy Limited, Basle, Switzerland

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Summary

OBJECTIVE Conflicting results have been reported on the effects of oral and transdermal oestrogen replacement therapy on IGF-I, while little information exists regarding the effects on IGFBP-1 and -3. In this study we evaluated the effects of oral and transdermal oestrogens on these parameters in post-menopausal women in a randomized cross-over study.

PATIENTS A group of 14 post-menopausal women were randomized to receive progestin-opposed oestrogen replacement therapy administered orally (Tri-sekvens Novo: 17β-oestradiol 2 mg daily on days 1–22 and 1 mg daily on days 23–28, norethisterone 1 mg days 13–22) or transdermally (Estracomb CIBA: oestradiol 50/22 g/24 h on days 1–28, norethisterone 250/22 g/24 h on days 15–28) for 6 months after which they were crossed over to the alternative treatment option. Fast- ing blood samples were obtained before treatment, and after 3, 6, 9 and 12 months on treatment.

MEASUREMENTS IGF-I, IGF-II, IGFBP-1, IGFBP-3, oestradiol and norethisterone were analysed by radioimmunoassays. In addition, IGFBPs were evaluated with Western ligand blots (WLB) in a subgroup of 12 patients.

RESULTS Plasma levels of oestradiol were not significantly different during oral and transdermal treatment. Norethisterone levels were below the detection limit in all situations in 8 patients, while 6 patients had detectable levels in one or several samples during treatment. Oral treatment caused a significant decrease (16%, P < 0.005) in IGF-I and a non-significant decrease in IGFBP-3. A similar effect was observed when samples containing detectable levels of norethisterone were excluded from the analysis. No significant effect on IGFBP-1 was observed when all samples were included in the analysis. However, when samples with detectable norethisterone were excluded IGFBP-1 increased by 46% (P < 0.01) during oral therapy. Contrary, transdermal treatment with oestrogens did not influence any of the parameters measured. None of the treatments had any effect on plasma IGF-II levels or IGFBP profile evaluated by WLB.

CONCLUSIONS Treatment with oral hormone replacement therapy significantly suppresses plasma IGF-I levels and increases plasma IGFBP-1 while transdermal treatment had no influence. This may be due to the route of administration, as plasma oestradiol levels showed little difference between the groups. The effect of oral oestrogens on IGFBP-1 seems to be attenuated by progestins.

Oestrogen replacement therapy has a well defined role in the treatment of menopausal complaints and osteoporosis in post-menopausal women (Grady et al., 1992). Both oral and transdermal hormone replacement therapies are available, but it is currently unknown whether one administration form has advantages compared to the other. A major difference is that oral treatment causes a higher exposure of oestrogens to the liver (Longcope et al., 1980) with alterations in synthesis of proteins like SHBG, ceruloplasmin and antithrombin, while transdermal oestrogens have little or no effect on hepatic protein synthesis (Holst et al., 1983; Lignieres et al., 1986).

Previous studies have shown unopposed oestrogens given by the oral route to decrease plasma levels of IGF-I despite enhancing basal and growth hormone releasing hormone-stimulated GH secretion (Dawson-Hughes et al., 1986; Duursma et al., 1984; Fröhlander & von Schoultz, 1988; Wiedemann et al., 1976). This may be attributed to a direct inhibitory effect of high concentrations of oestrogens on hepatic IGF-I production. Conflicting results have been
published regarding the effects of transdermal oestrogens with progestins on the IGF-system. Some groups have reported increased levels of plasma IGF-I (Slowinska-Szrednicka et al., 1992; Weissberger et al., 1991), while others have found transdermal oestrogens not to influence IGF-I levels (Bellantoni et al., 1991; Dall’Aglio et al., 1994). It is not known whether the different influence of transdermal and oral oestrogens on plasma IGF-I may be due to the route of administration or may be attributed to an influence of progestins included in the transdermal regimens.

More than 75% of plasma IGF-I and IGF-II circulate in a 150-kDa ternary complex with IGFBP-3 and an acid labile subunit, while the remaining peptides are found in a 50-kDa complex with IGFBP-1 to -6 (Jones & Clemmons, 1995). Thus, to evaluate the effects on IGF-I bioavailability, alterations in the concentrations of IGFBPs need to be taken into account. One study reported transdermal oestrogens do not influence plasma levels of IGFBP-1 and IGFBP-3 (Dall’Aglio et al., 1994) but, except for preliminary results (Stock et al., 1993), to our knowledge no study has addressed the effect of oral oestrogens on IGFBPs.

The aim of this study was to evaluate the influence of oestrogens given by the oral and transdermal routes on plasma levels of IGF-I, IGF-II, IGFBP-1 and IGFBP-3 measured by RIA and IGFBP profile evaluated by Western blotting in post-menopausal women. To minimize the confounding factor of inter-individual variation, each patient was evaluated on both treatment regimens in a cross-over design. Plasma norethisterone was measured, in addition to oestradiol, in order to determine whether persisting progestins might influence the results.

Patients and methods

Subjects

Post-menopausal women who were to receive hormone replacement therapy due to clinical symptoms related to oestrogen deprivation were included in the study. Each patient gave her written informed consent. The study was approved by the regional ethical committee. A total of 17 patients were randomized. Two patients had insufficient cycle control on transdermal treatment and one patient did not take part after inclusion. Thus, only the 14 patients who completed the study on both treatments were included for analysis. Median age, weight and height were 49.5 years (range 43–63), 68 kg (range 44–85) and 167 cm (range 156–177.5) respectively. All patients had gonadotrophin levels in the post-menopausal range. None of the patients suffered from diabetes mellitus, liver or renal diseases. Any other hormone therapy was terminated at least 6 weeks before inclusion in the study. Six patients smoked 10–20 cigarettes daily, the other patients were non-smokers.

Design

Patients were randomized to receive cyclical therapy with oestradiol and norethisterone orally (Trisekvens Novo: 17β-oestradiol 2 mg days 1–22 and 1 mg days 23–28, norethisterone 1 mg days 13–22) or by the transdermal route (Estracomb CIBA: oestradiol 50 μg/24 h days 1–28, norethisterone 250 μg/24 h days 15–28).

Patients were treated with the assigned regimen for 6 months after which they were switched to the other treatment regimen without any wash-out period. Total time of the investigation period was 12 months. Fasting blood samples were obtained before treatment and after 3, 6, 9 and 12 months on treatment. According to protocol, samples were to be drawn on the first half of the cycle when patients were treated with oestradiol only in both regimens.

Blood was collected in heparinized vials. Plasma was separated by centrifugation and stored at −20°C until time of analysis.

Assays

Human recombinant IGF-I peptide was purchased from GroPep Pty Ltd (Adelaide, Australia). Human recombinant IGF-II and IGFBP-1 were provided by Kabi-Pharmacia (Stockholm, Sweden). Human recombinant non-glycosylated IGFBP-3 was a gift from Dr C. Maack, Celtrix (Santa Clara, CA, USA). IGF-I, -II, IGFBP-1 and -3 were iodinated using the chloramine-T method. Labelled peptide was separated from non-incorporated 125I by AcA 202 columns (BioSepra, Villeneuve, France) using 1 × 40 cm columns for IGF-I and -II and 1 × 10 cm columns for IGFBP-1 and -3. EDTA was obtained from Sigma (Poole, Dorset, UK).

Plasma levels of IGF-I (Holly et al., 1988) and IGF-II (Hopkins et al., 1994) were measured by RIA following acid–acetone extraction. Potential residual IGFBP binding sites were blocked by adding 25 ng IGF-I in each tube in the IGF-II assay. Serum levels of IGFBP-1 (Holly et al., 1988) and IGFBP-3 (Cwyfan-Hughes et al., 1993) were directly measured by RIA.

Plasma oestradiol was measured by RIA as previously recommended (Dowsett et al., 1987; Lønning et al., 1995). Norethisterone levels were measured by a specific RIA developed and performed by the Bioanalytics and Pharmacokinetics Department of CIBA Basle. The sensitivity limit in the assay is 50 ng/l.

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Western ligand blotting

The IGFBP profile in the plasma was analysed by a modified version (Coulson et al., 1991) of the technique originally described by Hossenlopp et al. (1996). Briefly, samples were diluted with buffer (0.15 mol/l TRIS-HCl, pH 6.8, 6% SDS, 22% glycerol and 0.02% bromophenol blue) in a ratio of 1:15, boiled at 100°C for 5 minutes, cooled and immediately applied to a 12.5% SDS-polyacrylamide gel. Samples were electrophoresed with low molecular weight rainbow markers (Amer- sham, Aylesbury, UK) using a constant current (40 mA/gel) for 15 hours. The separated proteins were electroblotted onto Hybond-C Extra membrane (Amer sham, Aylesbury, UK) at 4°C for 4 hours at constant current (0.8A). After blocking with BSA (3%), the Hybond-C mem-brane was probed with 125I-labelled IGF-I (0.6 × 106 c.p.m./ml) for 2 hours at 21°C. Radiolabelled IGFBPs were visualized by autoradiography. The IGFBP pattern was compared with the profile of a normal serum pool (NSP), and samples from each patient were analysed in the same run for comparison.

Statistics

In a previous study we found plasma levels of IGF-I and IGFBP-1 to be well fitted to a log normal distribution (Lenning et al., 1995). Unpublished data from our group suggest plasma levels of IGF-II may be described by a similar distribution while IGFBP-3 is normally distributed. Thus, parameters are given as their geometrical mean value with 95% confidence intervals of the mean except IGFBP-3 where arithmetical values are given. Values obtained before treatment and during oral and transdermal treatment were compared using the Friedman’s test (non-parametrical analysis of variance).

Results

Because no differences were seen between values obtained after 3 and 6 months on treatment with either of the regimens, the mean values obtained on each treatment modality were used in the statistical analysis (Fig. 1). The influence of the different treatment modalities on the IGFs and their binding proteins was similar whether the treatment was given as first or second line therapy. Accordingly, results obtained in all patients were analysed together independent of the sequence of administration of the two treatment modalities. As no difference between smokers and non-smokers was observed (data not shown), all patients were handled as a single group.

Plasma oestradiol increased from a mean pretreatment level of 34 pmol/l to 272 and 178 pmol/l during oral and transdermal treatment respectively. No significant difference in plasma oestradiol was found between the administration routes.

Norethisterone levels above the detection limit (50 ng/l) were observed in 6 patients in one or more samples during treatment (14 samples in all) while it was not detectable on any occasion in 8 patients. In some patients a low level of norethisterone during treatment with transdermal drug was probably due to a ‘hangover’ effect of the hormone into the follicular phase while in other cases it could be due to sample collection during the combined treatment phase. Thus, data were analysed including the total number of samples as well as after excluding all samples in which detectable levels of norethisterone were found. Only IGFBP-1 levels differed between the groups and data for the total number of samples are given below.

Plasma IGF-I decreased to 84% (76–93) of control values on treatment with oral oestrogens. On the contrary, transdermal treatment did not influence plasma IGF-I (Table 1). There was a significant difference between plasma levels of IGF-I in the 3 situations (P < 0.005). Similar findings were observed when norethisterone containing samples were excluded with values corresponding to 83% (76–93) and 100% (82–119) of pretreatment levels during oral and transdermal treatment respectively.

IGFBP-1 increased to 118% (73–192) of control values on oral treatment, all samples included, while no alterations were noted on transdermal therapy (97% of control values). However, when samples containing residual norethisterone were excluded, IGFBP-1 increased to 146% (98–218) while a slight decrease (89% of control values) was observed during transdermal treatment (Fig. 2). The difference between IGFBP-1 values obtained in the 3 situations was statistically significant (P < 0.01). No differences in plasma levels of IGF-II and IGFBP-3 were observed, although there was a slight decrease in IGFBP-3 on oral therapy (to 95% of control values with all samples included) which was not statistically significant.

Plasma from 12 patients were available for analysis of IGFBP profile by Western ligand blotting. No major alterations

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Fig. 1 Plasma levels of IGF-I after 3 and 6 months on treatment with oral and transdermal hormone replacement therapy expressed as percentage of pretreatment values. Error bar indicates upper 95% confidence interval of the mean.
were observed during either treatment, and the bands corresponding to IGFBP-3 were comparable to those seen in the normal plasma pool in all test situations. Immunoblots for IGFBP-3 in one of the patients did not reveal any alteration in fragmentation pattern during therapy. Although data are limited, these results suggest that hormone replacement therapy does not influence IGFBP-3 protease activity in normal women.

Discussion

Values of IGF-I, IGF-II, IGFBP-1 and IGFBP-3 were in the same range as previously reported by us (Frost et al., 1996).

Previous studies have shown contrasting effects of oral and transdermal oestrogens on the GH-IGF axis. While oral oestrogens suppress plasma IGF-I levels despite increasing basal GH secretion (Dawson-Hughes et al., 1986; Duursma et al., 1984; Fröhlander & von Schoulz, 1988; Wiedemann et al., 1976), transdermal oestrogens have been found to increase or have no effects on plasma IGF-I with little influence or an increase in basal GH secretion (Copeland et al., 1984; Dall’Aglìo et al., 1994; Weissberger et al., 1991). Such a difference could be due to the administration form. Alternatively, it may be due to a confounding effect of progestins in the transdermal regimens as suggested by others (Bellantoni et al., 1991). With the exception of one small study (Weissberger et al., 1991) most studies evaluating the effects of oral oestrogens on the IGF-system used unopposed oestrogens (Dawson-Hughes et al., 1986; Duursma et al., 1984; Fröhlander & von Schoulz, 1988; Wiedemann et al., 1976), while studies on transdermal oestrogens evaluated combined oestrogen/progestin therapy (Bellantoni et al., 1991; Dall’Aglìo et al., 1994; Słowińska-Srzednicka et al., 1992; Weissberger et al., 1991).

Our study was designed to compare the effect of oral and transdermal oestrogen therapy on the IGF system. According to the protocol, all samples were obtained during the first 2 weeks on each treatment cycle (oestrogen administration only). However, in some samples (14/70) detectable levels of norethisterone were found. While high oral doses of progestins like medroxyprogesterone acetate 500 mg (Reed et al., 1992) and megestrol acetate 160 mg daily (Frost et al., 1996) are found to increase plasma IGF-I, the effect of norethisterone given in substitution doses on the IGF system is not known. In this study only alterations in plasma IGFBP-1 were affected by persisting norethisterone levels, while it had no effect on plasma IGF-I.

We found oral and transdermal oestrogen therapy to have different influences on plasma levels of IGF-I. The lack of effect of transdermal oestrogens on plasma levels of IGF-I, IGFBP-1 and IGFBP-3 is consistent with a previous report by Dall’Aglìo et al. (1994). While the observed decrease in plasma IGF-I levels during oral therapy is moderate (16%), our result is consistent with previous findings (Dawson-Hughes et al., 1986; Wiedemann et al., 1976) as well as oral treatment with partial oestrogen agonists like tamoxifen and droloxifene (Helle et al., 1996, Lønning et al., 1992). Studies by us and others have shown tamoxifen and droloxifene to cause a pronounced increase in fasting plasma levels of IGFBP-1 in breast cancer patients (Helle et al. 1996; Lønning et al., 1992). The effect of oral oestrogens on plasma IGFBP-1 has not been evaluated previously. Because tamoxifen and droloxifene have oestrogen-agonistic effects on the synthesis of other plasma proteins such as SHBG and TBG (Fex et al., 1981), we postulated that oral oestrogens (and, possibly, transdermal oestrogens) caused similar effects. No significant change in IGFBP-1 was observed.

Table 1

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<tr>
<th>Hormone values (all samples included) before and during treatment with oral and transdermal oestrogens. Values are given as mean with 95% confidence intervals</th>
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Fig. 2 Plasma levels of IGFBP-1 during treatment with oral and transdermal oestrogens expressed as percentage of pretreatment level in all samples (ALL) and after excluding norethisterone (NET) containing samples.
when all samples were included in the analysis. Excluding samples containing norethisterone, a significant increase in plasma IGFBP-1 (mean increase of 46%) was observed during oral treatment but no alteration was seen during transdermal therapy. This indicates that progestins may counteract the effects of oral oestrogens on IGFBP-1. It is noteworthy that treatment with high oral doses of megestrol acetate and medroxyprogesterone acetate has previously been shown to cause a decrease in plasma IGFBP-1 (Frost et al., 1996; Reed et al., 1992).

Similar to observations on tamoxifen and droloxifene (Lahti et al., 1994; Helle et al., 1996), immunoreactive IGFBP-3 levels were not altered on treatment with either oral or transdermal oestrogens. Western ligand blots did not suggest any increased or reduced protease activity for IGFBP-3 on any treatment form.

In summary, our study has shown oral hormone replacement therapy to increase plasma IGF-I while no alterations are observed during transdermal treatment in the same patients. A significant increase in plasma IGFBP-1 was observed when samples with persisting norethisterone were excluded, indicating that progestins may counteract the effect of oral oestrogens on IGFBP-1.

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References


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