Modification of serum IGF-I, IGFBPs and SHBG levels by different HRT regimens

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Abstract

During the menopause, levels of SHBG, IGF-I and IGFBPs are significantly modified by the use of different HRT regimens. Objective: The aim of this study is to evaluate the influence of three different HRT regimens on serum levels of SHBG, IGF-I, IGFBP-1 and IGFBP-3 in postmenopausal women. Methods: 41 postmenopausal women requesting HRT were enrolled in the study. Subjects were divided in three groups according to the therapy assigned; Group A: estradiol 2 mg/day + cyproterone acetate 1 mg/day in a cyclic sequential regimen; Group B: estradiol hemihydrate 2 mg/day plus norethisterone acetate (NETA) 1 mg/day in a continuous combined regimen; Group C: estradiol hemihydrate 1 mg/day plus NETA 0.5 mg/day in a continuous combined regimen. Blood samples were drawn before the start of hormonal treatment and after 6 months of HRT. Levels of SHBG, IGF-I, IGFBP-1 and IGFBP-3 in the serum were measured by means of a specific immunoassay. Results: In group A, a significant increase of SHBG, no change of IGFBPs and a significant decrease of IGF-I were observed; in group B and in group C, no significant variations for any of the parameters were recorded. Conclusions: The association of cyproterone acetate to oral estradiol determines a significant reduction of IGF-I levels and an increase of SHBG; nevertheless, it does not seem to influence the serum levels of the IGF-I binding proteins. The treatment with oral continuous combined estrogens plus androgenic progestins, at low doses, produces minor, not significant, changes in the circulating levels of IGF-I, SHBG and IGFBPs.

Keywords: IGF-I; SHBG; IGFBPs

1. Introduction

Insulin-like Growth Factor I (IGF-I) is a basic polypeptide structurally related to pro-insulin. Liver is the major source of IGF-I in the blood. The production of IGF-I in the liver and in the other tissues is stimulated by Growth Hormone
(GH) and modulated by nutritional conditions and insulin levels. IGF-I is virtually ubiquitous in the body, partly because it is transferred to target organs through blood circulation and partly due to its peripheral production [1]. Circulating IGF-I is bound to specific binding proteins (IGFBPs), which modulate its biologic effect at the tissue level [2–5].

Although insulin-like effects may derive from the weak binding capacity of IGF-I to the insulin receptor, its predominant role is to promote the cellular growth of several normal and cancerous tissues by interacting with specific receptors [1,6]. This is consistent with the observation that high serum IGF-I levels increase the risk of breast, lung [7], colon [8] and prostate cancer [9].

The breast is an important target of IGF-I activity; in vitro studies indicate that IGF-I, and to a lesser extent IGF-II, are potent mitogens for several breast cancer cell lines, particularly if estrogen-dependant [10]. Recent studies have underlined the importance of IGF-I in the regulation of breast cancer growth also in humans and IGF-I is now considered to be a good “surrogate marker” of breast cancer risk.

Sex Hormone Binding Globulin (SHBG) is a protein of hepatic derivation, which binds to sex hormones reducing their bio-availability. Although its role in the pathogenesis of breast cancer has not yet been defined, several studies have shown, in breast cancer patients, higher free-androgen and estrogen serum levels associated with a reduction of SHBG levels [11].

During the menopause, serum levels of SHBG, IGF-I and IGFBPs are significantly modified by the use of HRT; the effects of HRT on these proteins are different according to the route of administration of estrogens (oral, transdermal), or to the type and the dose of progestin association.

Because of the potential role of the IGF-I system and SHBG in the pathogenesis of breast cancer, it has been hypothesised that metabolic differences among the various HRT regimens could have some clinical impact, mainly when long-term treatments are prescribed.

The aim of this study is to evaluate the influence of three different HRT regimens on serum levels of SHBG, IGF-I, IGFBP-1 and IGFBP-3 in post-menopausal women.

2. Materials and methods

2.1. Subjects

Forty-one postmenopausal women requiring HRT due to symptoms related to estrogen deprivation and followed up at the Menopause Clinic of our Department were enrolled in the study. Subjects were divided in three groups.

Group A: 16 women, aged 48–56 years (median = 52.5; range: 48–56), who underwent spontaneous menopause 6 years to 6 months before enrolment (m ± S.D. = 21.9 ± 21.1 months), received estradiol 2 mg/day for 21 days a month + norethisterone acetate 1 mg/day from the 12th to the 21st day of treatment in a cyclic sequential regimen (Climen®–Schering).

Group B: 15 women, aged 44–62 years (median = 54.56; range: 44–62), who underwent spontaneous menopause 11 years to 10 months before enrolment in the study (m ± S.D. = 59.8 ± 44.1 months) received estradiol hemihydrate 2 mg/day + norethisterone acetate (NETA) 1 mg/day in a continuous combined regimen (Kliogest®–Novo Nordisk).

Group C: 10 women, aged 49–61 years (median = 53.83; range: 49–61), who underwent spontaneous menopause 11 years to 6 months before study entry (m ± S.D. = 62.7 ± 51.9 months), received a halved dose as compared with group B: estradiol hemihydrate 1 mg/day + NETA 0.5 mg/day in a continuous combined regimen (Activelle®–Novo Nordisk).

None of the subjects had received any kind of hormonal treatment for at least 6 months since enrolment in the study.

Patients’ characteristics are reported in Table 1. No differences were recorded among the three groups with respect to age and body mass index (BMI).

Medical history, basic physical examination, routine serum analysis of all participants to the study showed no abnormalities; in particular, normal kidney and liver function was documented.
All the women were non-smokers and none was taking any other concomitant medication.

2.2. Study design

Before the start of hormonal treatment and after 6 months of HRT, blood samples were drawn at 09:00 h after overnight fasting. The blood was permitted to clot at room temperature, and the serum was separated and stored at −80 °C until assay.

Levels of SHBG, IGF-I, IGFBP-1 and IGFBP-3 were measured by means of a commercially available specific immunoassay. Serum IGF-I was measured in duplicated by radioimmunoassay after acid ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); at IGF-I concentration of 122.8 ng/ml, the lowest detectable dose of this assay was of 13.5 ng/ml, and the intra- and inter-assay coefficient of variation (CV) was 6.7 and 13.13% respectively. Serum SHBG was measured by time-resolved fluoroimmunoassay (Delfia SHBG, Wallac Oy, Turku, Finland); the intra- and inter-assay CV was 4.0 and 5.2%; 3.3 and 2.5%; 2.4 and 3.0% at SHBG concentration of 25.5, 63.8 and 139.0 nmol/ml, respectively.

Serum IGFBP-1 was measured by IRMA using a commercial kit (DSL, USA); the intra- and inter-assay CV was 5.2 and 6.0%; Serum IGFBP-3 was measured by RIA using a commercial kit (Media Gnost, Germany); the intra- and inter-assay CV was 5.5 and 7.0%.

2.3. Statistical analyses

Results are expressed as mean ± standard deviation (m ± S.D.). The statistical significance of the effects of HRT on IGF-I and SHBG serum levels for each group were assessed by the paired t-test. For values not depicting a Gaussian distribution, the Wilcoxon test was applied. A P value < 0.05 was considered as significant.

3. Results

Descriptive data obtained from the three groups of women are shown in Table 1.

In group A, the following variations in serum levels were registered by comparing the values at study entry and after 6 months of treatment: a significant decrease of IGF-I (m ± S.D. = −21.05 ± 12.2%; P = 0.0002); a significant increase of SHBG (from 58.8 ± 28.6 to 99.9 ± 52.5 nmol/ml; P = 0.026; Wilcoxon = 0.016); no change of IGFBPs.

In group B after 6 months of treatment no significant variations of IGF-I levels were observed as compared with basal values (from 125.5 ± 30.5 to 129.3 ± 34.6 ng/ml; P = 0.66). A case by case analysis reveals a high variability: IGF-I levels showed either an increase (range: 4.68–47.36%, median = 25.61%) in eight women or a decrease of similar entity in seven women (range: 8.10–36.57%, median = 19.26%). Similar results were observed for SHBG; overall, no difference was recorded between basal values (66.8 ± 23.3 nmol/ml) and after 6 months of therapy (81.8 ± 19.7 nmol/ml; P = 0.123; Wilcoxon = 0.124). In nine women an increase of SHBG levels was observed (range: 3.07–184.21%; median = 58.20%), whereas six women showed a slight reduction (range: −5.81 to −48.5%; median = −23.07%). Also in this group, serum IGFBP-1 and IGFBP-3 levels were not modified by hormonal treatment.

Table 1

<table>
<thead>
<tr>
<th>Patients number</th>
<th>Age (m ± S.D.)</th>
<th>BMI (m ± S.D.)</th>
<th>HRT regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A 16</td>
<td>52.5 ± 3.43</td>
<td>23.44 ± 3.5</td>
<td>Estradiol valerate 2 mg + cyproterone acetate 1 mg; cyclic sequential</td>
</tr>
<tr>
<td>Group B 15</td>
<td>54.56 ± 4.45</td>
<td>22.83 ± 2.8</td>
<td>Estradiol 2 mg + NETA 1 mg; continuous combined</td>
</tr>
<tr>
<td>Group C 10</td>
<td>53.83 ± 5.19</td>
<td>22.00 ± 1.7</td>
<td>Estradiol 1 mg + NETA 0.5 mg; continuous combined</td>
</tr>
</tbody>
</table>

Characteristics of women enrolled in the three groups of postmenopausal women
In group C, the assays carried out after 6 months of treatment did not show significant variations for any of the following parameters:

- IGF-I (from 132.2 ± 54.8 to 124.6 ± 25.5 ng/ml; \( P = 0.75 \) Wilcoxon = 0.63); SHBG (from 68.5 ± 10.9 to 71.4 ± 14.7 nmol/ml; \( P = 0.505 \) NS; Wilcoxon = 0.486).

### 4. Discussion

The aim of this study was to evaluate whether different HRT regimens in postmenopausal women are associated with serum modifications of proteins belonging to the IGF-I system and/or of SHBG. These proteins are currently widely studied in view of their possible role as surrogate markers of breast cancer risk.

The breast is an important target of IGF-I axis activity: some studies have shown the presence of high serum IGF-I levels in breast cancer patients [12,13]. A statistically significant correlation has been observed between high serum IGF-I levels in premenopause and the lifetime risk of developing breast cancer (RR = 2.88, CI = 1.21–6.85, \( P = 0.02 \)), whereas this correlation is not statistically significant in postmenopausal women [14]. Nevertheless, data from a recent prospective Swedish study do not show any relationship between IGF-I, IGFBP1 and IGFBP3 levels and breast cancer risk among the younger women, whereas a direct relationship was observed between IGF-I and breast cancer risk among women older than 55 years of age, particularly if they were on HRT [15].

In another small study, IGF-I and IGFBP-3 appear to correlate with tumour histology, risk of relapse and survival rates. In the same study high IGF-I levels are associated with a higher risk of relapse, whereas women with levels below 120 ng/ml have better survival rates [16].

Recent data suggest that higher IGF-I levels may be found in premenopausal women with dense breasts [17]; these women also show reduced IGFBP-3 levels. Interestingly, the presence of high
breast density, which reflects the rate of epithelial and stromal glandular proliferation, is associated with a 4- to 5-fold risk of developing breast cancer.

SHBG is a plasma glycoprotein with high affinity for testosterone and dihydrotestosterone and low affinity for estradiol; therefore, circulating SHBG levels regulate plasma levels of free forms of androgens and estrogens. High serum concentrations of testosterone and free-estradiol are considered a risk factor for postmenopausal breast cancer [18], whereas an inverse association has been reported between serum SHBG levels and breast cancer [19–22].

It is now generally accepted that HRT use for more than 5 years increases breast cancer risk by 25–30%. [23,24]. Recent studies report higher risks of breast cancer for combined (estrogens + progestin) HRT as compared with estrogens alone. This is particularly evident in studies from Northern Europe [25–27], where progestins with high androgenic activity have predominantly been used. Data from the United States, where the progestin most frequently used is medroxiprogesterone acetate (MPA), are rather inconsistent [28–31], although the most recent studies show higher risks for women receiving combined treatments, either continuous or cyclic sequential [30,31]. These discrepancies have been at least partly attributed to the different biological activities of the progestins used in the different countries, mainly because of their androgenic activity, and to their differential effect on the IGF axis and SHBG levels [32].

The use of HRT after the menopause may influence both IGF-I and SHBG production; furthermore, HRT activity varies according to the route of estrogen administration and to the different progestins used. Transdermal estrogens alone do not modify circulating IGF-I and SHBG levels. On the contrary, oral estrogens are associated with a significant reduction (20–50% in different studies) of circulating IGF-I levels, as a result of hepatic exposure to high levels of estrogens [33–39]. Moreover, oral estrogens determine a sharp increase (35–160%) of SHBG levels, because of their stimulatory effect on liver metabolism [37,22].

Clinical studies show that the association of non-androgenic progestins, such as dihydrogesterone, does not interfere with the effect of oral estrogens on IGF-I and SHBG levels [32]. MPA, which has a weak residual androgenic activity, partially antagonises the reduction of IGF-I synthesis induced by oral estrogens [39]. Androgenic progestins, such as levonorgestrel and NETA, completely antagonise the effect on IGF-I and SHBG induced by oral estrogens [32].

Also in this study the sequential association of oral estradiol plus the non-androgenic progestin cyproterone acetate reduces IGF-I and increases SHBG serum levels. On the contrary, the association of an androgenic progestin, such as NETA, to oral estradiol in a continuous combined regimen, offsets the hepatocellular effect of oral estrogens on IGF-I and SHBG serum levels in a dose-independent manner. These data support the conclusions of a previous study by our own group with high dose (5 mg/day) of NETA [32].

Circulating IGFBPs levels may also be influenced by different HRT regimens; however, inconsistent results have been reported in the small studies investigating this point, particularly if associations of estrogens and progestins are taken into consideration (Table 3) [40–48]. All studies show a significant increase of IGFBP-1 levels associated with the use oral estrogens alone [41–43,48] and no change associated with transdermal estrogens alone [41,43,48]. Similarly, IGFBP-3 levels are not modified by transdermal estrogens [40,41], whereas they can be reduced [40,42] or stay unchanged [41] with oral estrogens alone. It has been hypothesised that, since IGFBP-3 is the main IGF-I transferring protein in the serum, a fall of serum IGF-I levels could produce a secondary reduction of the specific binding protein as any unbound IGFBP-3 is rapidly cleared from the circulation [41].

Most of the data pertaining the combined therapy show that the association of a progestin (MPA or NETA) levels off the increase of IGFBPs induced by oral estrogens [42,44–46], with the sole exception of the study by Cardim et al. [41]. For instance, a randomised cross-over study [42] shows a significant decrease of circulating IGF-I and increase of IGFBP-1 from baseline with conjugated estrogen alone; these effects were progres-
sively opposed by progestin of increasing androgenic activity (MPA, Desogestrel, NETA).

Our data on IGFBP-1 showing stable values with respect to basal measurements both with ciproterone acetate and NETA at different doses, are thus in line with those of the literature. No change of both IGFBP-1 and IGFBP-3 [41,44,46] has been detected in users of combined regimens with transdermal estrogens, excepting in a single study by Raudakoski et al. [45]; the IGFBP-1 inducing action exerted in the endometrium by the IUD containing levonorgestrel used in this study has been suggested to explain such finding. All studies evaluating a possible effect of various combinations of oral estrogens and progestins on IGFBP-3 levels, do not report any variation with respect to basal values [41,42,44–46], similarly to what we found in the three different groups treatment of our study.

However, it is difficult to understand the clinical relevance of the variations of IGFBP-3 levels, since the role of every single IGFBP has not been clarified.

Alterations in total plasma IGF-I levels may not necessarily reflect alterations in IGF-I bio-availability or bioactivity. IGF-I bio-availability is regulated by interactions with at least six specific binding proteins [49], of which the most important are IGFBP-1 and IGFBP-3. In the circulation, the most of the IGF-I forms a ternary complex with IGFBP-3 and an acid-labile subunit, which limits IGF-I activity [34,35]. Moreover, IGFBP-3 can be modified by a specific circulating protease that decreases the affinity for IGF-I and potentially increases free IGF-I [50]. Proteolytic activity that modifies IGFBP-3 has been found to be elevated in many cancer patients. A study has shown that high dose MPA (160 mg daily) causes a moderate but significant increase in total IGFBP-3 and an inhibition of IGFBP-3 protease in patients with advanced breast cancer [51]. Furthermore, recent data show that IGFBP-3 exerts a direct anti-proliferative action on breast cancer cells that could be mediated by the interaction with specific IGFBP receptors at the cell surface [52]. Several studies have indicated that premenopausal breast

Table 3
Modifications of serum IGF-1 and IGF binding proteins in relation to type of HRT

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of HRT</th>
<th>IGF-1</th>
<th>IGFBP-1</th>
<th>IGFBP-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cano, 1999 [44]</td>
<td>Oral E2 + MPA</td>
<td>↓</td>
<td>⏫</td>
<td>⏫</td>
</tr>
<tr>
<td>Kam, 2000 [40]</td>
<td>Oral CE</td>
<td>↓</td>
<td>≳</td>
<td>≳</td>
</tr>
<tr>
<td>Cardim, 2001 [41]</td>
<td>Oral CE</td>
<td>↓</td>
<td>⏫</td>
<td>⏫</td>
</tr>
<tr>
<td>Campagnoli, 2002 [47]</td>
<td>Oral E2 + Dydr</td>
<td>↓</td>
<td>⏫</td>
<td>⏫</td>
</tr>
</tbody>
</table>
cancer patients have reduced IGFBP-3 levels [53,54], whereas this correlation is not evident in postmenopausal patients.

IGFBP-1 seems to be the main regulator of IGF-I bio-availability in response to acute changes of insulin and glucose levels [55]. In addition to inhibiting IGF effects, it has been shown that IGFBP-1 may also diminish basal motility in a metastatic cell line of breast cancer, MDA-231BO [56]. In a recent study, high IGFBP-1 levels are significantly associated with distant recurrence and death for breast cancer: these effects persisted after adjustment for tumour-related variables and treatment but were not independent of insulin levels [57].

5. Conclusions

The association of cyproterone acetate, a progestin devoid of androgenic action, to oral estradiol determines a significant reduction of IGF-I levels and an increase of SHBG synthesis; nevertheless, it does not seem to influence the serum levels of the main IGF-I binding proteins.

The treatment with oral continuous combined estrogens plus androgenic progestins, even at low doses, produces minor changes in the circulating levels of IGF-I and SHBG, both of which after 6 months of treatment are not significantly modified as compared with basal values. With this regimen, also no significant serum variations of IGFBPs have been observed. Such findings could have clinical implications, but need to be replicated in a large population-based study.

References


