Vaginal estriol to overcome side-effects of aromatase inhibitors in breast cancer patients


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Key words: BREAST CANCER, VAGINAL ESTRIOL, AROMATASE INHIBITOR, VAGINAL DRYNESS, DYSPAREUNIA

ABSTRACT

Objective Aromatase inhibitors are essential as endocrine treatment for hormone receptor-positive postmenopausal breast cancer patients. Menopausal symptoms are often aggravated during endocrine treatment. We investigated whether vaginal estriol is a safe therapeutic option to overcome the urogenital side-effects of aromatase inhibitors. Serum hormone levels were used as the surrogate parameter for safety.

Methods Fasting serum hormone levels of ten postmenopausal breast cancer patients receiving aromatase inhibitors were prospectively measured by electro-chemiluminescence immunoassays and gas chromatography/mass spectrometry before and 2 weeks after daily application of 0.5 mg vaginal estriol (Ovestin® ovula), respectively.

Results Two weeks of daily vaginal estriol treatment did not change serum estradiol or estriol levels. However, significant decreases in levels of serum follicle stimulating hormone (p = 0.01) and luteinizing hormone (p = 0.02) were observed. Five out of six breast cancer patients noticed an improvement in vaginal dryness and/or dyspareunia.

Conclusions The significant decline in gonadotropin levels, indicating systemic effects, has to be kept in mind when offering vaginal estriol to breast cancer patients receiving an aromatase inhibitor.

INTRODUCTION

Estrogens are clearly involved in the pathogenesis and progression of breast cancer. They exert strong pro-mitotic effects on mammary tissue and are a major proliferative stimulus for hormone-dependent tumors. Antiestrogenic therapy, either by blocking the estrogen receptor (tamoxifen) or by estrogen deprivation (aromatase inhibitor), has become the most effective treatment for endocrine-responsive breast cancer1,2. These observations and, furthermore, a randomized trial regarding safety of hormone therapy (HT) after breast cancer (HABITS-trial)3 have lead to the recommendation that women with breast cancer should not receive or continue HT even after a long period of initial therapy4,5. As shown by the LIBERATE study group6, tibolone, a synthetic steroid that is pharmacologically and clinically different from conventional sex steroids, relieves menopausal symptoms in breast cancer survivors but increases the risk of recurrence of breast cancer.

It has been suggested that up to 70% of breast cancer patients develop menopausal symptoms, either naturally or as a result of breast cancer-related treatment7. Chemotherapy and/or ovarian ablation can lead to premature ovarian failure and consequently to menopausal symptoms8,9. Endocrine therapy of pre- and postmenopausal women enhances menopause-related symptoms especially hot flushes and vaginal dryness as well as dyspareunia5. With regard to aromatase inhibitors, particularly high numbers of urogenital symptoms have been observed. The Arimidex, Tamoxifen, Alone or in Combination Trial (ATAC)10 reported vaginal dryness and dyspareunia in 18.5% and 17.3%, respectively. Even more, re-analysis of the Tamoxifen Exemestan Adjvant Multicenter

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trial (TEAM)\textsuperscript{11} revealed vaginal dryness and decreased libido in more than 40\% of patients treated with exemestane. As aromatase inhibitors have become a mainstay of endocrine breast cancer treatment, the amelioration of side-effects such as local menopausal symptoms is a major issue in breast cancer follow-up. In order to overcome those symptoms of estrogen deprivation, vaginal moisturizers or poorly absorbed topical estrogens are used in daily clinical practice. Though non-hormonal vaginal moisturizers improve urogenital symptoms, their benefit is not statistically superior when compared to placebo and is significantly lower compared to vaginal estrogens\textsuperscript{12,13}. In contrast to oral HT, local application of estrogens was expected to be safe in postmenopausal breast cancer patients\textsuperscript{14}. Recently, the report of a small prospective observation\textsuperscript{15} advised caution with the use of vaginal estradiol in breast cancer patients receiving aromatase inhibitors, as an increase of serum estradiol levels was found that potentially counteracts endocrine breast cancer treatment. According to the publication, practical guidelines for managing menopausal symptoms after breast cancer published in 2008\textsuperscript{16} dissuaded from the use of vaginal estradiol in patients treated with aromatase inhibitors, but recommend the application of vaginal estriol. However, safety data of vaginal estriol in breast cancer patients receiving aromatase inhibitors are missing. To throw more light on this issue, we prospectively investigated the safety of vaginal estriol in breast cancer patients receiving aromatase inhibitors by measuring serum hormone levels before and 2 weeks after daily use of vaginal estriol tablets.

METHODS

We prospectively analyzed fasting serum hormone levels of 10 postmenopausal breast cancer patients receiving aromatase inhibitors before and 2 weeks after daily application of 0.5 mg vaginal estriol. This study was approved by the ethical committee of the Medical University of Vienna, Vienna, Austria.

All patients included in this study were hormone receptor-positive and received anastrozole (Arimidex\textsuperscript{\textregistered}) for breast cancer treatment. Patients were excluded if they had an actual or history of thromboembolic disease, postmenopausal uterine bleeding, any hormonal, natural (phytoestrogens) or herbal products for treatment of menopausal symptoms within at least 1 year and respiratory, cardiac, liver or kidney insufficiencies. A full gynecological examination, including vaginal ultrasound and PAP smear, was performed at least 6 months before the treatment period. The study design comprised a pretreatment visit (T1), a treatment period lasting 14 days and a post-treatment visit (T2) exactly 15 days after T1. At T1 and T2, blood was drawn at the outpatient clinic between 07.00 and 08.00. Blood was taken after a fasting period of at least 8 h to rule out any interference of insulin and sex hormone binding globulin (SHBG). At T2, blood was taken about 8–12 h after the last application of vaginal estriol. A questionnaire concerning general medical history and in particular menopausal symptoms was completed. Menopausal symptoms were scored from 0, meaning not existent, to 10, meaning worst affected. All participating patients agreed to insert vaginal estriol tablet (Ovestin\textsuperscript{\textregistered}, 0.5 mg) as instructed once daily in the evening for 14 days.

Hormone measurements

Chemiluminescence immunoassay

After blood was allowed to clot for 1 h at room temperature, serum was separated and aliquots were stored at −80°C. Estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH) and SHBG were measured with kits for electro-chemiluminescence immunoassays purchased from Roche Diagnostics (Germany) on a ‘Modular-170 < EEE >’ auto-analyzer. Insulin and C-peptide were analyzed by an Immulite 2000 system by chemiluminescence immunoassays from Siemens-DPC, USA. The detection limit for estradiol was as low as 10 pg/ml to detect even small increases in our group.

Gas chromatography-mass spectrometry

In order to obtain results with high specificity and adequate sensitivity, serum levels of estriol and estradiol were measured by gas chromatography-mass spectrometry (GC-MS).

Chemicals and reagents

Disodium hydrogen phosphate, potassium dihydrogen phosphate, methanol, ammonium iodide and ethyl acetate (analytical grade) were obtained from Merck (Darmstadt, Germany). Amberlite XAD-4 was purchased from Rohm and Haas (France). Ultrapure water was produced by a Milli-Q plus 185 water purification system with a Q-PAK 1 cartridge purchased from Millipore Corp. (Bedford, MA, USA). Reference compounds estriol and estradiol were obtained from Steraloids (Newport, USA). The internal standard ethinylestradiol-d4 was purchased from Ehrensdorfer (Augsburg, Germany). N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) was supplied from Machery-Nagel (Düren, Germany). Ethylthio-trimethylsilane was purchased from Sigma-Aldrich (Vienna, Austria). A trimethylsilyl iodide (TMSI) stock solution was prepared by adding 5 ml MSTFA to 100 mg ammonium iodide and 300 μl ethylthio-trimethylsilane. The TMSI working solution was obtained by diluting 1 ml of TMSI stock solution with 9 ml MSTFA.

GC/MS analysis

The GC-MS system consisted of a Trace GC 2000 gas chromatograph coupled to a quadrupole mass spectrometer Voyager (Thermo Quest Finigan, Manchester, UK) operated in electron impact mode at 70 eV. The mass detector was operated in selected ion monitoring mode. Separation was performed on a rtx-1 ms fused-silica capillary column, 15 m × 0.25 mm internal diameter, 0.1 μm film thickness.

Sample preparation

For the extraction of steroids, 1 ml serum was used; 10 μl internal standard (ethinylestradiol-d4, con-
centrations: 4 μg/ml), 1 ml phosphate buffer and 1 ml XAD suspension (ca. 1 g/ml) were added to the sample aliquot and shaken overnight. After filtration and washing XAD with deionized water, the steroids were eluted with ethylacetate : methanol in the ratio 1 : 1. The organic phase was brought to dryness and derivatized with 100 μl MSTFA/TMSI. For calibration, bovine serum was spiked in the range 0.2–10 ng/ml. Male human serum was used as a blank sample.

Statistics

A non-parametric Wilcoxon signed rank test was used to identify differences between mean hormone serum levels before and after vaginal estriol treatment. For all analyses, a p value < 0.05 was considered statistically significant. SPSS version 17.0 statistical software system was used for all calculations.

RESULTS

The patient characteristics are shown in Table 1. All patients received aromatase inhibitor therapy for at least 1 year (median 2.18 years, range 1.1–4 years). No patient stopped or modified therapy with the aromatase inhibitor or vaginal estriol during study participation. Gynecological examination of the patients, including vaginal ultrasound and PAP smear, revealed no pathology (data not shown). Nine patients were given breast-conserving surgery, and one patient had undergone mastectomy. Four lymph node-positive patients received adjuvant chemotherapy before starting endocrine therapy.

Table 1  Patient demographics and characteristics

<table>
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<tr>
<th>Patient characteristic</th>
<th>Median</th>
<th>Range</th>
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<tr>
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<td>65</td>
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<td>Body mass index (kg/m²)</td>
<td>28.2</td>
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<tr>
<td>Age at menarche (years)</td>
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<td>12–16</td>
<td>10</td>
</tr>
<tr>
<td>Age at menopause (years)</td>
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<tr>
<td>&lt; 20</td>
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<td>&gt; 20</td>
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<table>
<thead>
<tr>
<th>Nodal status</th>
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<tr>
<td>Negative</td>
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<table>
<thead>
<tr>
<th>ER/PR status</th>
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<tbody>
<tr>
<td>ER+/PR−</td>
</tr>
<tr>
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<tr>
<td>ER−/PR+</td>
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<table>
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</tr>
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<td>Grade 2</td>
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<tr>
<td>Grade 3</td>
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</table>

| ER/PR, estrogen receptor/progestogen receptor |

Hormone levels

As indicated in Table 2, hormone serum levels measured by radioimmunoassay and/or GC/MS were in the normal postmenopausal range and remained there after 2 weeks of daily vaginal estriol treatment. Before treatment with vaginal estriol, no patient had a serum estriol level above 0.2 ng/ml or a serum estradiol level above 10 pg/ml. After 2 weeks of vaginal estriol use, no increase of serum estrogens could be demonstrated, either by use of radioimmunoassay or by GC/MS. In all ten patients, serum levels of estriol and estradiol measured by GC/MS remained under the detection limits of 0.2 ng/ml and 0.5 ng/ml, respectively, after 2 weeks of treatment with vaginal estriol. Comparing fasting serum levels of LH and FSH before and after 2 weeks of treatment with vaginal estriol, a significant decrease in both gonadotropins could be observed (LH -10.8%, p = 0.02; FSH -12.8%, p = 0.01) (Figure 1).

Vaginal symptoms

Six out of ten patients reported vaginal symptoms including vaginal dryness (6/10, 60%) and dyspareunia (5/10, 50%). Four patients (40%) did not indicate vaginal symptoms but suffered from flushes and/or fatigue. Five out of six breast cancer patients (83%) noticed a distinct improvement in vaginal dryness during the treatment period. Three out of five patients (60%) with dyspareunia reported obvious relief after 2 weeks of daily vaginal estriol. No worsening of the complaints was reported.

DISCUSSION

Aromatase inhibitors have become the mainstay in the adjuvant treatment of endocrine-responsive postmenopausal breast cancer17. Any aromatase inhibitor-containing regimen

Table 2  Fasting serum hormone levels before and after 2 weeks of treatment with vaginal estriol

<table>
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<th></th>
<th>Timepoint 1</th>
<th>Timepoint 2</th>
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<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Estriol (ng/ml)</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td></td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td></td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>0.40–0.97</td>
<td>0.35–0.67</td>
<td>0.14</td>
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<tr>
<td>LH (mU/ml)</td>
<td>32.4–52.0</td>
<td>28.9–45.1</td>
<td>0.02</td>
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<tr>
<td>FSH (mU/ml)</td>
<td>75.7–100.9</td>
<td>66.0–100.9</td>
<td>0.01</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>48.0–103.5</td>
<td>46.0–100.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>12.8–28.1</td>
<td>12.34–19.5</td>
<td>0.96</td>
</tr>
<tr>
<td>C-peptide (μg/l)</td>
<td>2.6–4.3</td>
<td>2.6–4.3</td>
<td>0.79</td>
</tr>
</tbody>
</table>

LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin.
has demonstrated superior efficacy when compared to tamoxifen alone\textsuperscript{18}. Hence, the vast majority of postmenopausal estrogen receptor-positive breast cancer patients will be offered an aromatase inhibitor as endocrine therapy except those presenting with rare contraindications. Though aromatase inhibitors are well tolerated, menopausal symptoms are aggravated during endocrine treatment and thereby impair a patient’s quality of life\textsuperscript{19,20}. Two out of three most reported menopausal symptoms, i.e. hot flushes, vaginal dryness, dyspareunia, could be overcome by the use of vaginal estrogens. Several studies\textsuperscript{21–23} have confirmed the efficacy of vaginal estradiol in showing significant improvement in urogenital symptoms. But, since an increase in serum estradiol following vaginal application in breast cancer patients receiving aromatase inhibitors has been reported\textsuperscript{15}, this therapy is contraindicated for those patients. Estriol is an estrogen of low potency, which seems to exert anti-estrogenic effects in an estradiol-rich environment and is not converted back to the highly potent estradiol\textsuperscript{24}. In contrast to topical estradiol, vaginal estriol does not contribute to endometrial stimulation\textsuperscript{25}. In a meta-analysis of 214 women using vaginal estriol cream\textsuperscript{26}, a total of 337 biopsies generated at various stages of treatment revealed only atrophic endometrium. Vaginal estriol has been shown to be a potent and safe treatment for urogenital estrogen deficiency symptoms. In our prospective trial, the majority of patients also reported distinct improvements in urogenital complaints.

However, changes in hormone serum levels due to vaginal estriol application have been reported\textsuperscript{27–29}; this implies systemic and not only local effects of this hormonal therapy. Hence, the nearly total suppression of the level of serum estrogen, which is warranted by use of an aromatase inhibitor, might be counteracted by vaginal estriol. Our prospective study investigated serum hormone levels of breast cancer patients receiving aromatase inhibitors before and after 2 weeks of daily vaginal application of 0.5 mg estriol. We observed no increase in levels of serum estriol and estradiol, by analyzing fasting serum 8–12 h after the last application of vaginal estriol. This is of interest, since any increase in the level of serum estrogen would potentially be deleterious for breast cancer patients on aromatase inhibitors. Several studies have reported a long-term increase in serum estradiol and estrone levels in postmenopausal women after vaginal application of estradiol. Estradiol serum levels increase between 4- and 20-fold after vaginal application and stay increased, indicating that vaginal estradiol cannot be recommended for hormone receptor-positive breast cancer patients receiving aromatase inhibitors\textsuperscript{15,30}. As vaginal estriol is poorly absorbed and cannot be converted to estradiol, several scientific communities...
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recommend this estrogen for amelioration of postmenopausal urogenital symptoms. This is underlined by our study. However, a short-term increase in serum estriol levels after vaginal application has been reported. Keller and co-workers, as well as Mattsson and Cullberg, demonstrated peak levels of serum estriol 1–2 h after vaginal application of estriol, reaching pre-treatment levels after 8–24 h. No change in serum estradiol levels after application of vaginal estriol has been reported. We are not able to rule out a short-term increase in serum estriol between 1 and 8 h after vaginal application in our group, but we demonstrate here that serum estriol levels do not exceed our detection limit of 0.2 ng/ml 8–12 h after the last insertion. It is questionable whether a short-term increase in serum estriol for less than 8 h is able to impact on breast cancer recurrence risk, but it cannot be ruled out entirely. Interestingly, we and others were able to demonstrate significantly reduced FSH and LH levels after 2 weeks of vaginal estriol treatment. Schiff and co-workers reported a decline in FSH by 17% and in LH by 45% after vaginal estriol, which could not be seen after orally administered estriol. In their study, the decline of gonadotropins was paralleled by an increase in unconjugated estriol, thereby suggesting that serum estriol suppressed LH and FSH serum levels. Likewise, Mattsson and Cullberg supposed a relationship between the decline of serum gonadotropins and increased estriol serum levels, as they observed a decrease of LH and FSH 6 h after vaginal estriol insertion. In our study, the significant decline in levels of gonadotropins, 10.8% for LH and 12.8% for FSH, was not paralleled by increased serum estriol or even estradiol levels. Nevertheless, a possible short-term increase in estriol 1–8 h after application could be stimulus enough to down-regulate FSH and LH. It is speculative and unlikely that the down-regulation of gonadotropins directly impacts on breast cancer recurrence.

Our study has several limitations. Our detection limit for estriol was 0.2 ng/ml and for estradiol 10 pg/ml. We cannot rule out significant increases of these hormones beyond these detection limits, which might have caused our observed decline of gonadotropins after 2 weeks of vaginal estriol treatment. Second, desirable changes in the atrophic mucosa by vaginal estriol might lead to greater ability to absorb considerable amounts of hormone into the systemic circulation, an effect which possibly occurs long after 2 weeks of treatment. However, as the treatment dosage is routinely reduced from 0.5 mg vaginal estriol daily to once weekly after 2 weeks, this is unlikely, but cannot be ruled out entirely. Third, our results concerning the relief of urogenital complaints are limited as this is not a controlled study and no standardized scales for the evaluation of symptoms were used.

In conclusion, our prospective study investigating postmenopausal breast cancer patients receiving aromatase inhibitors demonstrates that vaginal estriol does not lead to a long-term elevation of serum estrogen levels. Nevertheless, the significant decline in gonadotropins indicating systemic effects has to be kept in mind when offering vaginal estriol to breast cancer patients on aromatase inhibitors.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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References


