Conjugated estrogens and breast cancer risk

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Abstract

Available epidemiologic data suggest the possibility that the use of oral conjugated equine estrogens (CEE) 0.625 mg/day as a first-choice dose could be associated with a very limited (if any) breast cancer risk increase. Some biological peculiarities of oral CEE back the possibility of a limited detrimental effect on breast tissue, due to either direct or indirect actions. Direct actions. Some experimental findings suggest that the 17α-dihydroderivatives of equilenin and equilin (15% of the CEE components) have a non-estrogenic or even an anti-estrogenic effect on breast tissue. This could partially counterbalance the stimulatory action of the other CEE components. Indirect actions. Oral estrogens, through their metabolic and hepatocellular effects (emphasized by the first liver passage) cause a sharp increase in sex hormone binding globulin (SHBG) level which is followed by a lower quantity of both estrogen and androgen in the free, bioavailable, form. More importantly, they cause a decrease in circulating insulin-like growth factor I (IGF-I) activity, due to both a reduction in IGF-I synthesis by the liver and an increase in IGF-binding protein-1 level. A strong relationship between breast cancer risk and the concentration of circulating IGF-I in premenopausal women has been recently found. Actually, estrogens and IGF-I have a synergistic effect on cell proliferation, and IGF-I is necessary for maximum estrogen-receptor activation in breast cancer cell lines. The possibility does exist that the SHBG level increase and the IGF-I bioavailability decrease, caused by oral CEE, balance the increased estrogen stimulation on breast tissue.

Introduction

Ovarian hormones, by stimulating mammary epithelial cells, cause an increase in breast cancer risk. This is confirmed by the fact that the incidence rate of breast cancer, particularly if expressed in the logarithmic way, shows a diminished slope after the menopause. Early menopause is associated with a reduced risk of breast cancer: women who stop menstruating before the age of 40, either naturally or through surgical intervention, have half the risk of breast cancer of those who continue to menstruate to the age of 50. For every 1 year increase in age at menopause the risk of breast cancer increases by approximately 3%. Furthermore, in postmenopausal women, endogenous estrogen levels are associated with a higher risk of breast cancer. The use of hormone replacement therapy (HRT) increases the level of estrogens in postmenopausal women. However, the impact on breast tissue, and breast cancer risk, could be reduced when conjugated equine estrogens (CEE) are used, due to some biological peculiarities of this estrogen preparation.
EPIDEMIOLOGIC DATA ON CONJUGATED EQUINE ESTROGENS AND BREAST CANCER RISK

In the last 30 years more than 50 epidemiologic studies have been published evaluating the incidence of breast cancer in women using HRT as compared to those who have never used hormones.

A comprehensive re-analysis of these studies has recently been carried out by the Collaborative Group on Hormonal Factors in Breast Cancer (CGHFBC). The main finding of this study is a moderate but significant increase in relative risk associated with long duration of use in current and recent users. For women who were current users and had taken HRT for 5 years or longer (11 years on average) the relative risk was 1.35 (95% CI 1.21–1.49) compared with those who had never used it. No risk increase was found, with either less than 5 years duration of use or 5 or more years after cessation of use. The absolute numbers of breast cancers due to long-term HRT use have also been calculated. At the age of 70 the estimated cumulative incidence of breast cancer in 1000 women is 63 if they never used HRT, and 65 and 69, respectively, if they used HRT for 5 and 10 years after the menopause.

Some aspects of the CGHFBC study deserve attention. Women included in the main analysis had their breast cancer diagnosed on average in 1985; obviously, many long-term users began treatment in the Seventies. In the US, from which the majority of epidemiologic data are derived, the CEE have been used for a long time at the dosage of 1.25 mg per day. The 0.625 mg per day dose, until then considered the low one, began to prevail only at the end of the Eighties (Figure 1). Actually, in the group of breast cancer patients on long-term HRT of the CGHFBC study, for every 100 women using the low doses, 167 used the high ones. The distribution of different doses is similar to that of the prescriptions dispensed in the US in the period 1977–1980.

Surprisingly, in the CGHFBC re-analysis the low doses were associated with a similar increase of risk in long-term current and recent users (Figure 2). This finding has two possible explanations. The first is that a low estrogen stimulation is already sufficient to cause a maximal increase of the risk. The second explanation is that, as the high doses have been the first-choice for decades, the low doses have been prescribed to women thought to be at increased risk of breast cancer. This second explanation is supported by a further observation coming from the CGHFBC re-analysis. In this

![Diagram](Figure 1) Total conjugated equine estrogen prescriptions dispensed, by strength, in the United States from 1973 to 1992. ▲, 0.3 mg/day; ◦, 0.625 mg/day; ▲, 1.25 mg/day; □, 2.5 mg/day
study, after 5 or more years from the cessation of HRT use the risk returns to normal, independently from the duration of use. The only exception comes from the women treated in the past with the low doses (Figure 2). Probably, at that time these women represented a selected group with higher basal risk of breast cancer, due to family history or a previous breast biopsy or a high density mammographic profile.

Overall, the moderate increase of breast cancer risk showed by the CGHFBC study could be ascribed to the use of too high doses. The possibility does exist that the use of CEE 0.625 mg/day or less as a single hormone, could be able to counterbalance the estrogen stimulatory effects on breast tissue, through either direct or indirect actions.

DIRECT ACTIONS OF CEE

Conjugated equine estrogen contains sulfate esters of at least ten different estrogens (Figure 3). Two of these components are identical to human estrogens and represent about half of the CEE components: estrone (E1) and 17β-estradiol (17β-E2). The remaining 50% is composed of molecules produced by the fetoplacental unit of the pregnant mare: equilin (EQ), equilenin (EQN) and Δ8-E1, and by their 17-β and 17-α hydroxylated derivatives: 17β-dihydroEQ, 17α-dihydroEQ, 17β-dihydroEQN, and 17α-dihydroEQN. These non-human compounds are characterized by the ring B unsaturated structure.

After absorption E1 is partially transformed into 17β-E2, whereas EQ, EQN and Δ8-E1 are transformed into their 17β-hydroxy derivatives. The extent of this transformation is nearly 10 times higher with ring B unsaturated estrogens. Similarly, 16α-hydroxylation occurs with both types of estrogens; however, with the ring B saturated estrogens the 17-keto steroid 16α-hydroxy-E1 is the major urinary metabolite, whereas with the ring B unsaturated estrogens the 17-β-reduced steroids, such as 16α-hydroxy-17β-dihydroEQ and 16α-hydroxy-17β-dihydroEQN, are the major metabolites. This difference in metabolism may be important as it has been suggested that 16α-hydroxy-E1 (α-ketol structure) can form covalent adducts with macromolecules and that it may be oncogenic for the breast. This type of interaction will not occur with the 16α-hydroxylated 17β-reduced metabolites of ring B unsaturated estrogens.

Among the components of CEE, the 17α-hydroxy derivatives of equine estrogens (Figure 3), while maintaining favorable anti-atherogenic and anti-osteoporotic effects, seem to be characterized by a lack of estrogenic action, or even by an anti-estrogenic effect, on breast tissue. The best studied component is 17α-dihydroEQN, which has no proliferative or trophic effects on mammary gland, uterus or endometrium of ovariectomized macaques. The component 17α-dihydroEQ could have similar properties, as some preliminary data, presented some years ago, suggested an anti-estrogenic action on human breast cancer cell lines. Even if the latter data await confirmation, the possibility does exist that...
17α-derivatives have direct protective effects on breast tissue.

**INDIRECT ACTIONS**

Regarding the possible protective indirect effects, we have to go back to the CGHFBC study\(^3\). This study shows that obesity in menopausal women, who have never used HRT, is a risk factor: women with body mass index (BMI) ≥ 25 had a 35–40% risk increase compared to women with BMI < 25. This is consistent with the results of several epidemiologic studies which show that obesity, especially abdominal obesity, is a risk factor for breast cancer in menopausal women\(^9\).

In the CGHFBC re-analysis, the increase in breast cancer risk associated with long-term HRT is lacking in women with BMI ≥ 25\(^3\). The finding that HRT does not further increase the risk in overweight women has two possible explanations. The first is that the higher estrogen activity, shown by several studies in women with abdominal obesity\(^1\), causes an already maximal estrogen stimulation. However, data from the Nurses’ Health Study suggest that the difference in estrogenization is very little: the mean 17β-E2 concentration is 5 pg/ml in women with BMI < 21 and 10 pg/ml in women with BMI > 29\(^10\). The second explanation is that oral estrogens oppose other features of obesity that are potentially involved in breast cancer, so balancing the increased estrogen stimulation (Figure 4)\(^11\). The increase of insulin levels due to insulin resistance is the key point of these biological features of obesity\(^12,13\). This causes an increase in insulin-like growth factor I (IGF-I) activity, due to a reduction of IGF binding protein–1 levels, and a decrease in sex hormone binding globulin (SHBG) levels, which determines a higher quantity of both estrogen and androgen in the free, bioavailable, form. Oral estrogens, through their metabolic action, oppose insulin resistance; more importantly, through their hepatocellular actions, emphasized by the first liver passage, they cause a sharp increase in SHBG levels and a decrease in circulating IGF-I activity\(^14\).

The latter modification seems to be particularly relevant to breast cancer risk\(^15\). Circulating IGF-I may vary in adults from 50 to 300 ng/ml. Data from the Nurses’ Health Study show that having an IGF-I level in the top tertile represents a strong breast cancer risk factor in premenopausal (hence estrogenized) women (Figure 5)\(^16\). This observation has some biological explanations. First of all, IGF-I is a potent mitogen for breast tissue and breast cancer cells\(^15,17\). Furthermore, estrogen and IGF-I have mutual favoring actions on breast cancer cell proliferation. The 17β-E2 increases the number of IGF-I receptors and enhances IGF-I activity\(^18\). IGF-I stimulates enzymatic activities favoring intracellular 17β-E2 production and is required for a maximal activation of estrogen receptors in breast cancer cells\(^19\). Moreover, 17β-E2 and IGF-I are responsible for inducing the expression of two different genes, *fos* and *jun*, whose activities are required for maximal cell proliferation\(^17\). Overall, the IGF-I decrease induced by oral estrogen administration could have a real protective effect on breast tissue. In our series the IGF-I decrease induced by CEE 0.625 mg/day is sharper in women with higher basal levels (Figure 6)\(^20\). Oral estrogen could also decrease IGF-I activity by increasing the hepatic synthesis of IGF binding protein–1\(^21,22\).
CONSEQUENCES OF THE PROGESTIN ASSOCIATION

Progestin can oppose the estrogen stimulating effect in different ways, especially by reducing the estrogen receptor production, or by opposing the enzymatic steps which increase the $17\beta$-E2 level in breast cells\textsuperscript{23,24}. Studies \textit{in vitro} suggest that, after an initial stimulatory phase of breast cell replication, progestins block the proliferation induced by estrogens\textsuperscript{25}. In a recent study of post-menopausal women, topical application to the breast of a gel containing $17\beta$-E2 for 14 days enhanced the number of cycling epithelial cells, whereas a gel with a combination of $17\beta$-E2 and progesterone significantly limited this proliferative effect\textsuperscript{26}. However, in two different studies of ovariectomized monkeys, Cline and colleagues\textsuperscript{27} observed that the long-term continuous combined addition of medroxyprogesterone acetate (MPA) caused a breast cell proliferation significantly higher than that observed with the use of CEE\textsuperscript{27}. This could be due to the fact that the slightly androgenic MPA contrasts with the estrogen effect on circulating IGF-I level: in a large cross-sectional study MPA partially opposed the IGF-I reduction induced by CEE\textsuperscript{28}, while in a longitudinal study MPA completely inhibited the IGF-I decrease induced by CEE\textsuperscript{29}. On the contrary, the addition of dydrogesterone, a progestin devoid of androgenic effects, did not interfere with either the IGF-I decrease or the SHBG increase induced by CEE\textsuperscript{30}.

**CONCLUSION**

In conclusion, available data suggest that, through their direct and indirect actions potentially balancing the estrogen stimulatory effect, CEE at the right low doses with the addition of a non-androgenic progestin could be associated with a very limited (if any) increase in breast cancer risk.

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