Short communication

The oestrogen paradox: an hypothesis

Richard J Santen

Division of Endocrinology and Metabolism, University of Virginia, Charlottesville, Virginia 22908-1416, USA

Corresponding author: Richard J Santen, RJS5Y@virginia.edu

Published: 20 December 2007
This article is online at http://breast-cancer-research.com/content/9/S2/S21

Breast Cancer Research 2007, **9(Suppl 2):**S21 (doi:10.1186/bcr1819)

Introduction

As shown in Figure 1, a wide range of epidemiologic and observational data suggest that oestrogens are associated with the development of breast cancer [1,2]. With these data as a background, it was quite surprising that recently published data suggested that women taking postmenopausal hormone therapy (MHT) with oestrogen alone for 5 to 9 years unexpectedly experienced a decrease in the risk for breast cancer [3,4]. However, when taken for more than 20 years, the risk appeared to increase [5,6]. We call this the 'oestrogen paradox' to highlight the fact that short-term oestrogen use decreases the risk for breast cancer whereas long-term use increases it. A second component of the oestrogen paradox is that high-dose oestrogen therapy in postmenopausal women with breast cancer causes tumour regression, whereas the anti-oestrogen tamoxifen is equally effective in causing remissions in similar patient groups [7-9]. It is paradoxical then that both oestrogens and anti-oestrogens cause tumour regressions.

Short-term oestrogen use and breast cancer risk

The initial publication of the Women's Health Initiative (WHI) [3] reported a 23% decrease in invasive breast cancer incidence in patients taking oestrogen alone compared with placebo, a finding which narrowly fell short of statistical significance (hazard ratio [HR] 0.77, 95% confidence interval [CI] 0.59 to 1.01). A recent exploratory analysis of updated data from this study examined subgroups to determine whether oestrogens might reduce the incidence of breast cancer significantly in women falling into certain categories [4]. Notably, this analysis reported a statistically significant 33% reduction in invasive breast cancer incidence in patients who strictly adhered to their oestrogen therapy (HR 0.67, 95% CI 0.47 to 0.97). In addition, a 31% lower incidence of localized breast cancer (HR 0.69, 95% CI 0.51 to 0.95) and a 29% reduction in ductal cancers (HR 0.71, 95% CI 0.52 to 0.99) were reported in oestrogen users. The decreases in breast cancer risk were limited to women who had not previously used MHT [4]. In a concurrent report from the Nurses Health Study [2], a significant 26% decrease in risk for breast cancer was observed in obese women, and a nonsignificant 10% decrease in all study participants, taking oestrogen alone for 5 to 9 years. Other observational studies reported a reduction in risk with oestrogen alone but of lesser magnitude and not statistically significant. For example, Schairer and colleagues [5] reported a 7% reduction in breast cancer risk at 6 years in women receiving oestrogen alone, and Lyytinen and coworkers [10] identified a similar 7% reduction. These combined results, although not conclusive, are highly suggestive of a beneficial effect of oestrogen in reducing breast cancer risk. However, this conclusion must be considered provisional until rigorous confirmation in additional studies is reported.

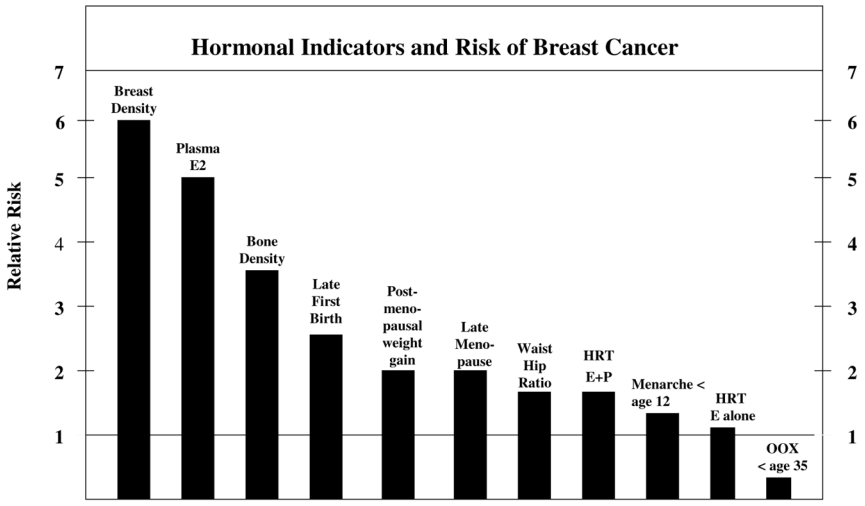
Long-term oestrogen use and breast cancer risk

What are the data regarding use of oestrogen alone for more than 20 years? The Nurses Health Study [2] also evaluated women using oestrogen alone for more than 20 years and found a statistically significant 41% increase in breast cancer risk in women 50 years of age or older, and a 77% increase in the subset of lean women. Earlier studies by Magnusson [11] and Schairer [5] and their colleagues also identified significantly increased breast cancer risks in women taking oestrogen alone for more than 10 years (odds ratio 2.7) and 16 years (relative risk 1.6), respectively. The Million Women Study [6] also reported a linear increase in breast cancer risk over time in women receiving MHT with oestrogen alone over a period of 10 years. In contrast to the other studies reported, however, the Million Women Study found a nonsignificant increased risk for breast cancer, even in women receiving this therapy for less than 5 years.

High-dose oestrogens as breast cancer treatment

A second component of the oestrogen paradox is that women with hormone-dependent breast cancer respond to high-dose oestrogens with objective tumour regressions. This form of therapy was the mainstay of hormonal treatment of

Figure 1



Hormonal risk factors associated with an increased risk of breast cancer and related to oestrogen exposure. For references supporting the validity of this figure, see Santen [1]. E, oestrogen; E_2, oestradiol; HRT, hormone replacement therapy; OOX, oophorectomy; P, progesterone. Reproduced with permission from Santen RJ: Endocrine-responsive cancer. In *Williams Textbook of Endocrinology*. Edited by Larsen PR, Kronenberg HM, Melmed S, Polonsky KS. Philadelphia, PA: WB Saunders Company; 2007:1763-1801. © Elsevier 2007.

breast cancer from the late 1940s until the early 1980s [7-9]. When compared in randomized trials with tamoxifen, high-dose oestrogens were equally efficacious [7] and in one study they were associated with significantly enhanced survival [8] compared with an anti-oestrogen. Extensive studies demonstrated that only specific subgroups of women respond to high-dose oestrogen [9,12]. Premenopausal women and those less than 1 year postmenopausal do not respond at all. Women who had undergone menopause many years earlier frequently experienced objective tumour regressions; the longer the duration of the period after cessation of menses, the greater the response rate. Only oestrogen receptor (ER)-positive tumours regress in women receiving high-dose oestrogens [12].

Possible mechanisms to explain the oestrogen paradox

Our preclinical data demonstrate that long-term deprivation of oestradiol causes this sex steroid to trigger cell death through apoptosis (Figure 2a), whereas wild-type cells with a normal oestrogen milieu exhibit reduced apoptosis (Figure 2b) [13-21]. The postmenopausal women receiving MHT with oestrogen alone may be considered to be in a state of long-term oestradiol deprivation. Extensive review of autopsy studies provides strong evidence that there is a reservoir of undiagnosed breast cancer in postmenopausal women (Table 1) [22,23]. The short-term reduction in breast cancer in the patients with undiagnosed occult breast tumours may be due to oestrogen-induced apoptosis of tumour cells. Similarly, the effect of oestrogen in inducing tumour regressions in patients with known breast cancer may reflect a similar phenomenon. We suggest that the increased risk for breast cancer results from long-term use of oestrogens alone because the risk from MHT may occur via different mechanisms [24,25]: the genotoxic effects of oestradiol metabolites and the ER-mediated proliferative effects of oestradiol. The following sections of this treatise will review the evidence for each of these statements.

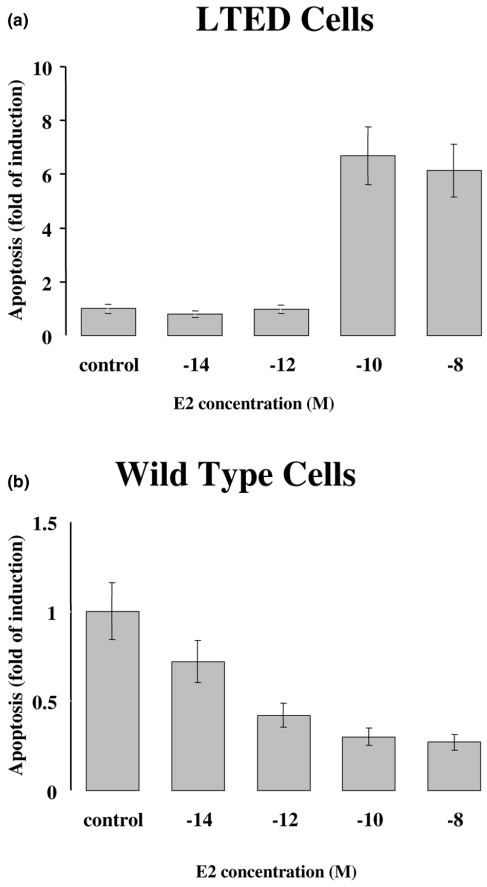
Occult pre-existing breast cancers in women

Over the past three decades at least eight studies have assessed the frequency of occult malignant disease, primarily ductal carcinoma *in situ* (DCIS), found at autopsy in women with no history of breast cancer [22] (Table 1). The frequency of occult DCIS varied considerably among these studies (range 0% to 15%), most likely reflecting methodological differences. Variation aside, approximately 5% of the 1,052 combined cases from these studies included occult DCIS and 1% occult invasive breast cancers [22]. Based on these findings, it is reasonable to assume that 5% to 10% of the women entering the WHI and Nurse's Health Study had occult breast cancer when they were initially enrolled.

Evidence for oestradiol-induced apoptosis

Recent *in vitro* studies from our laboratory showed that hormone-dependent breast cancer cells deprived of oestrogen in the long term undergo adaptive changes that cause oestrogen to paradoxically stimulate apoptosis [13-15] (Figure 2a). Whereas wild-type MCF7 cells respond to oestradiol with a reduction in apoptosis, those deprived of oestrogen in the long term exhibit an increase in programmed cell death. Similarly, Jordan and collaborators [16-21]

Figure 2



Effect of oestradiol on apoptosis in wild type and long-term oestradiol-deprived cells. **(a)** Long-term oestradiol deprived (LTED) MCF7 cells respond to oestradiol (E_2) with an increase in apoptosis, whereas **(b)** wild type MCF7 cells respond to the same dose of oestradiol with a reduction in apoptosis. Reproduced with permission from Oxford University Press from Song RX, Mor G, Naftolin F, McPherson RA, Song J, Zhang Z, Yue W, Wang J, Santen RJ: Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17beta-estradiol. J Natl Cancer Inst 2001, 93:1714-1723.

demonstrated that long-term tamoxifen exposure also results in adaptation and development of oestrogen-induced apoptosis. Apoptotic mechanisms in adapted cells involve upregulation of death receptor as well as mitochondrial pathways. Specific molecular events include activation of the Fas death receptor/Fas ligand complex, the release of cytochrome C from the mitochondria, alterations in Bcl-2, and downregulation of the anti-apoptotic factor nuclear factor-κ [14,15,18].

Long-term oestradiol deprivation in the Women's Health Initiative and the Nurse's Health Study

At the time of enrolment, participants in the WHI trial were 63 years old on average and menopausal for more than 10 years [3]. Plasma oestradiol levels fall precipitously at menopause from 50 to 600 pg/ml to levels of 5 to 10 pg/ml. Even though breast tissue levels might not precisely reflect plasma concentrations, one would still expect substantial reduction in breast tissue levels and adaptation to this reduction. If our hypothesis were correct, then exposure to oestrogen therapy as MHT would induce apoptosis and shrink or even eradicate the occult tumours, which would reduce the detection of a cancer by mammography or palpation over the next several years. This scenario could explain the reduction in breast cancers diagnosed in the WHI and Nurses Health Study in women receiving oestrogen alone as MHT for 5 to 9 years [2,4]. This hypothesis would also explain why women who had received MHT before entering the WHI study did not experience a reduction in breast cancer risk [4].

Table 1

Occult breast cancers found at autopsy

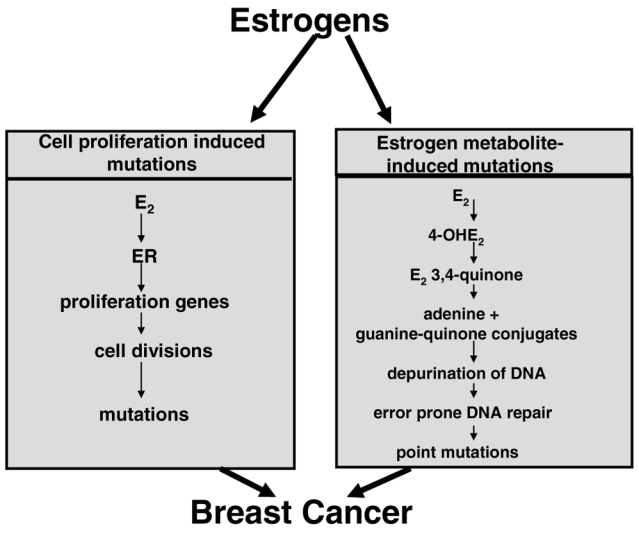
Study	Number	%
1962 Ryan	200	0%
1973 Kramer	70	4.3%
1975 Wellings	67	1.9%
1984 Neilsen	77	14.3%
1985 Alpers	101	8.9%
1985 Bhathal	207	12.1%
1987 Bartow	221	0%
1988 Neilsen	109	14.7%
Total cases	1,052	5% DCIS and 1% IBC

Derived from the reports of Welch and coworkers [22] and Ryan [23]. DCIS, ductal carcinoma *in situ*; IBC, invasive breast cancer.

Long-term exposure to oestradiol

Why would oestrogen increase the risk for breast cancer when it is given for more than 20 years? The commonly accepted explanation for the carcinogenic effect of oestrogen is that this sex steroid stimulates breast cancer proliferation genes, increases the rate of breast cell divisions, and thereby enhances the chances for development of mutations [25]. An additional and more controversial mechanism suggests that metabolites of oestradiol are directly genotoxic [24,25] (Figure 3). Recent studies demonstrate that oestradiol is converted to 4-OH-oestradiol in human breast tissue via the cytochrome p450 1B1 enzyme, and it is then oxidized to quinone metabolites. These metabolites are highly reactive and covalently bind to adenine and guanine on DNA, resulting in depurination, error-prone DNA repair, and point mutations [24]. Other recent studies have shown that 4-OH-oestradiol is directly mutagenic in cellular mutagenesis assays [26-29]. In addition, 4-OH-oestradiol can transform ER-negative benign breast epithelial cells into serially transplantable

Figure 3



Two pathways potentially responsible for oestradiol induced carcinogenesis. E_2, oestradiol; ER, oestrogen receptor.

carcinomas in immune deficient mice [28]. Finally, an ER knockout model of breast cancer forms tumours in response to increasing doses of exogenous oestradiol in previously castrated animals [24,30]. These combined observations suggest that directly genotoxic as well as ER-mediated mechanisms may be responsible for the long-term carcinogenic effects of oestradiol [24]. In time, the pro-carcinogenic effects of oestradiol would outweigh the pro-apoptotic effects.

Conclusion

A variety of data are congruent with our 'oestrogen paradox' hypothesis; however, additional confirmatory studies are needed to prove this contention. Specifically needed are more comprehensive autopsy studies to determine precisely the magnitude of the reservoir of occult breast cancers and their precursor lesions. The ability of highly sensitive imaging strategies, such as digital mammography and magnetic resonance imaging, should be evaluated for their abilities to detect occult breast cancers in women initiating MHT. Direct demonstration of oestrogen-induced apoptosis in occult breast cancers in women will also be critical.

Acknowledgement

This article has been published as part of Breast Cancer Research Volume 9 Supplement 2, 2007: Controversies in Breast Cancer. The full contents of the supplement are available online at http://breast-cancer-research.com/supplements/9/S2.

References

1. Santen RJ: **Endocrine-responsive cancer.** In *Williams Textbook of Endocrinology*. Edited by Larsen PR, Kronenberg HM, Melmed S, Polonsky KS. Philadelphia, PA: WB Saunders Company; 2007:1763-1801.
2. Chen WY, Manson JE, Hankinson SE, Rosner B, Holmes MD, Willett WC, Colditz GA: **Unopposed estrogen therapy and the risk of invasive breast cancer.** *Arch Intern Med* 2006, **166:** 1027-1032.
3. Anderson GL, Limacher M, Assaf AR, Bassford T, Beresford SA, Black H, Bonds D, Brunner R, Brzyski R, Caan B, *et al.*; Women's Health Initiative Steering Committee: **Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial.** *JAMA* 2004, **291:**1701-1712.
4. Stefanick ML, Anderson GL, Margolis KL, Hendrix SL, Rodabough RJ, Paskett ED, Lane DS, Hubbell FA, Assaf AR, Sarto GE, *et al.*; WHI Investigators: **Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy.** *JAMA* 2006, **295:** 1647-1657.
5. Schairer C, Lubin J, Troisi R, Sturgeon S, Brinton L, Hoover R: **Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk.** *JAMA* 2000, **283:**485-491. [Erratum appears in *JAMA* 2000, **284:**2597.]
6. Beral V, Million Women SC: **Breast cancer and hormone-replacement therapy in the Million Women Study.** *Lancet* 2003, **362:**419-427. [Erratum appears in *Lancet* 2003, **362:** 1160.]
7. Ingle JN, Ahmann DL, Green SJ, Edmonson JH, Bisel HF, Kvols LK, Nichols WC, Creagan ET, Hahn RG, Rubin J, Frytak S: **Randomized clinical trial of diethylstilbestrol versus tamoxifen in postmenopausal women with advanced breast cancer.** *N Engl J Med* 1981, **304:**16-21.
8. Peethambaram PP, Ingle JN, Suman VJ, Hartmann LC, Loprinzi CL **Randomized trial of diethylstilbestrol vs. tamoxifen in postmenopausal women with metastatic breast cancer: an updated analysis.** *Breast Cancer Res Treat* 1999, **54:**117-122.
9. Carter AC, Sedransk N, Kelley RM, Ansfield FJ, Ravdin RG, Talley RW, Potter NR: **Diethylstilbestrol: recommended dosages for different categories of breast cancer patients. Report of the Cooperative Breast Cancer Group.** *JAMA* 1977, **237:**2079-2078.
10. Lyytinen H, Pukkala E, Ylikorkala O: **Breast cancer risk in postmenopausal women using estrogen-only therapy.** *Obstet Gynecol* 2006, **108:**1354-1360.
11. Magnusson C, Baron JA, Correia N, Bergstrom R, Adami HO, Persson I: **Breast-cancer risk following long-term oestrogen- and oestrogen-progestin-replacement therapy.** *Int J Cancer* 1999, **81:**339-344.
12. Santen RJ, Manni A, Harvey H, Redmond C: **Endocrine treatment of breast cancer in women.** *Endocr Rev* 1990, **11:**221-265.
13. Song RX, Zhang Z, Mor G, Santen RJ: **Down-regulation of Bcl-2 enhances estrogen apoptotic action in long-term estradiol-depleted ER+ breast cancer cells.** *Apoptosis* 2005, **10:**667-678.
14. Song RX, Santen RJ: **Apoptotic action of estrogen.** *Apoptosis* 2003, **8:**55-60.
15. Song RX, Mor G, Naftolin F, McPherson RA, Song J, Zhang Z, Yue W, Wang J, Santen RJ: **Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17beta-estradiol.** *J Natl Cancer Inst* 2001, **93:**1714-1723.
16. Jordan VC, Lewis JS, Osipo C, Cheng D: **The apoptotic action of estrogen following exhaustive antihormonal therapy: a new clinical treatment strategy.** *Breast* 2005, **14:**624-630.
17. Jordan VC, Liu H, Dardes R: **Re: Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17 beta-estradiol and the two faces of Janus: sex steroids as mediators of both cell proliferation and cell death.** *J Natl Cancer Inst* 2002, **94:**1173-1175.
18. Lewis JS, Meeke K, Osipo C, Ross EA, Kidawi N, Li T, Bell E, Chandel NS, Jordan VC: **Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation.** *J Natl Cancer Inst* 2005, **97:**1746-1759.
19. Lewis JS, Osipo C, Meeke K, Jordan VC: **Estrogen-induced apoptosis in a breast cancer model resistant to long-term estrogen withdrawal.** *J Steroid Biochem Mol Biol* 2005, **94:**131-141.
20. Liu H, Lee ES, Gajdos C, Pearce ST, Chen B, Osipo C, Loweth J, McKian K, De Los Reyes A, Wing L, Jordan VC: **Apoptotic action of 17beta-estradiol in raloxifene-resistant MCF-7 cells in vitro and in vivo.** *J Natl Cancer Inst* 2003, **95:**1586-1597.

21. Yao K, Lee ES, Bentrem DJ, England G, Schafer JI, O'Regan RM, Jordan VC: **Antitumor action of physiological estradiol on tamoxifen-stimulated breast tumors grown in athymic mice.** *Clin Cancer Res* 2000, **6:**2028-2036.
22. Welch HG, Black WC: **Using autopsy series to estimate the disease 'reservoir' for ductal carcinoma in situ of the breast: how much more breast cancer can we find?** *Ann Intern Med* 1997, **127:**1023-1028.
23. Ryan JA, Cody CJ: **Intraductal epithelial proliferation in the human breast-a comparative study.** *Can J Surg* 1962, **5:**12-19.
24. Cavalieri E, Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, Muti P, Rogan E, Russo J, Santen R, *et al.*: **Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention.** *Biochim Biophys Acta* 2006, **1766:**63-78.
25. Yager JD, Davidson NE: **Estrogen carcinogenesis in breast cancer.** *N Engl J Med* 2006, **354:**270-282.
26. Fernandez SV, Russo IH, Russo J: **Estradiol and its metabolites 4-hydroxyestradiol and 2-hydroxyestradiol induce mutations in human breast epithelial cells.** *Int J Cancer* 2006, **118:**1862-1868.
27. Russo J, Russo IH: **The role of estrogen in the initiation of breast cancer.** *J Steroid Biochem Mol Biol* 2006, **102:**89-96.
28. Russo J, Fernandez SV, Russo PA, Fernbaugh R, Sheriff FS, Lareef HM, Garber J, Russo IH: **17-Beta-estradiol induces transformation and tumorigenesis in human breast epithelial cells.** *FASEB J* 2006, **20:**1622-1634.
29. Zhao Z, Kosinska W, Khmelnitsky M, Cavalieri EL, Rogan EG, Chakravarti D, Sacks PG, Guttenplan JB: **Mutagenic activity of 4-hydroxyestradiol, but not 2-hydroxyestradiol, in BB rat2 embryonic cells, and the mutational spectrum of 4-hydroxyestradiol.** *Chem Res Toxicol* 2006, **19:**475-479.
30. Devanesan P, Santen RJ, Bocchinfuso WP, Korach KS, Rogan EG, Cavalieri E: **Catechol estrogen metabolites and conjugates in mammary tumors and hyperplastic tissue form estrogen receptor alpha knock-out (ERKO)/Wnt1 mice: implications for initiation of mammary tumors.** *Carcinogenesis* 2001, **22:**1573-1576.