Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 58; Numer/Number 3/2007
ISSN 0423–104X

The oestrogen paradox: a hypothesis

Hipoteza paradoksu estrogenowego

Richard J. Santen

Division of Endocrinology and Metabolism, University of Virginia, Charlottesville, Virginia, USA

Abstract

Epidemiological and observational studies suggest that oestrogens, when used as hormonal therapy in post-menopausal women, can increase the risk of breast cancer if used long term. However, more recent data suggest that short-term use in sub-groups of post-menopausal women significantly decreases the risk of breast cancer. This beneficial effect is also observed when high-dose oestrogen is administered to post-menopausal women with breast cancer to cause tumour regression, a phenomenon which commonly occurs. We consider these divergent responses to oestrogen to represent a "paradox". Data from our own and other investigative groups suggest a hypothesis to explain this paradox. Deprivation of oestradiol in model systems causes cells to adapt and to undergo apoptosis in response to oestrogen. This occurs through the Fas/Fas ligand death receptor pathway and through alterations in apoptotic mechanisms mediated by mitochondria. This process of programmed cell death may explain the regression of established breast cancer with oestrogen administration and the diminution in the rate of new breast cancer diagnoses recently reported. Our hypothesis is based upon pathological data indicating the presence of a "reservoir" of undiagnosed breast cancer in the population of women who would be starting on oestrogens as menopausal hormonal therapy. The long-term increased risk of breast cancer may then reflect different mechanisms. Oestrogens can cause mutations through enhancement of the rate of cell division and concomitantly the error rate in DNA replication. In addition, oestrogens can be metabolised to directly genotoxic compounds. These carcinogenic processes take much longer, since a number of mutations must accumulate before resulting in breast cancer. These hypotheses regarding oestrogen-induced apoptosis in the short term and carcinogenesis in the long term now require rigorous verification but would serve to explain the "oestrogen paradox".

(Pol J Endocrinol 2007; 58 (3): 222–227)

Key words: *oestrogens, breast cancer risk, menopausal hormonal therapy*

Streszczenie

Badania epidemiologiczne i obserwacyjne sugerują, że estrogeny stosowane przez długi okres u kobiet w ramach hormonalnej terapii zastępczej, mogą zwiększać ryzyko wystąpienia raka piersi. Jednak ostatnie dane wskazują, że estrogeny stosowane krótko w podgrupie kobiet w okresie pomenopauzalnym, mogą to ryzyko istotnie zmniejszać. Ten sam korzystny efekt obserwowano również podczas podawania wysokich dawek estrogenów kobietom z rakiem piersi znajdującym się w okresie pomenopauzalnym, powodując regresję guza nowotworowego. Autorzy rozważyli odmienne wzorce reakcji na podane estrogeny. Zjawisko to można nazwać mianem paradoksu. Dane własne autorów oraz innych zespołów badawczych sugerują hipotezę, która mogłaby tłumaczyć powyższy „paradoks". W układzie modelowym deprywacja wpływu estradiolu powoduje, że komórki adaptują się i rozpoczynają proces apoptozy w odpowiedzi na działanie estrogenów. Zjawisko to przebiega poprzez receptor ścieżki śmierci dla liganda Fas/Fas oraz poprzez modyfikację mechanizmów apoptotycznych pośredniczonych przez mitochondria. Opisany powyżej proces programowanej śmierci komórek może tłumaczyć zarówno zjawisko regresji istniejącego już raka piersi po podaniu estrogenów, jak również opisywane ostatnio zmniejszenie częstości wykrywania nowych przypadków raka piersi. Hipoteza ta bazuje na danych wskazujących na istnienie w populacji dużej grupy kobiet, u których rak piersi pozostaje niezdiagnozowany, a które dopiero zaczną stosować estrogeny w ramach hormonalnej terapii zastępczej w okresie pomenopauzalnym. Obserwowany po dłuższym czasie wzrost częstości zachorowań na raka piersi u kobiet stosujących estrogeny, musi odzwierciedlać inny mechanizm działania tych związków. Estrogeny mogą powodować mutacje poprzez wzrost wskaźnika częstości podziałów komórki

Prof. Dr. Richard J. Santen
Division of Endocrinology Department of Medicine
University of Virginia, Charlottesville
PO Box 800379, Virginia 22908, USA
e-mail: RJS5Y@hscmail.mcc.virginia.edu

oraz następczego wzrostu liczby błędów podczas replikacji DNA. Ponadto estrogeny mogą zostać metabolizowane bezpośrednio do związków genotoksycznych. Opisane powyżej procesy karcynogenezy wymagają dłuższego czasu, ponieważ warunkiem koniecznym dla rozwoju raka piersi jest kumulacja wielu mutacji. Powyższa hipoteza dotycząca indukowanej apoptozy poprzez podawane w krótkim okresie czasu estrogenów oraz karcynogenezy spowodowanej podawaniem tych samych związków przez dłuższy czas wymaga ścisłej weryfikacji, jednak stara się wyjaśnić zjawisko „paradoksu estrogenowego".

(Endokrynol Pol 2007; 58 (3): 222–227)

Słowa kluczowe: *estrogeny, ryzyko raka piersi, hormonalna terapia zastępcza w okresie pomenopauzalnym*

Introduction

A wide range of epidemiological data suggest that oestrogens are associated with the development of breast cancer [1]. As shown in Figure 1, bilateral oophorectomy before the age of 35 decreases the risk of breast cancer. On the other hand, the use of menopausal hormone therapy that includes an oestrogen plus a progestin for more than five years increases the risk. Other factors known to be associated with an increase in long-term exposure to oestrogens also enhance the chances that a woman will develop breast cancer. These include early menarche, late menopause, weight gain of more than 20 kg as an adult, increased bone density and plasma oestrogen levels. A high degree of breast density, which could reflect tissue oestrogen levels, is also a major risk factor for breast cancer. Observational data suggest that long-term oestrogen use increases risk to as much as 70% in thin women taking oestrogen alone as MHT for more than 20 years [2].

With these epidemiological and observational data as a background, it was quite surprising that recently published data suggested that women taking post-menopausal hormone therapy (MHT) with oestrogen alone for 5–9 years unexpectedly experienced a decrease in the risk of breast cancer [3, 4]. However, when the hormone therapy was 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 of breast cancer, whereas long term use increases it. A second component of the oestrogen paradox is that high-dose oestrogen therapy in post-menopausal 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) reported a 23% decrease in invasive breast cancer incidence in patients taking oestrogen alone compared to placebo, a finding which narrowly missed statistical significance (HR 0.77; 95% CI 0.59–1.01) [3]. A recent exploratory analysis of updated data from this

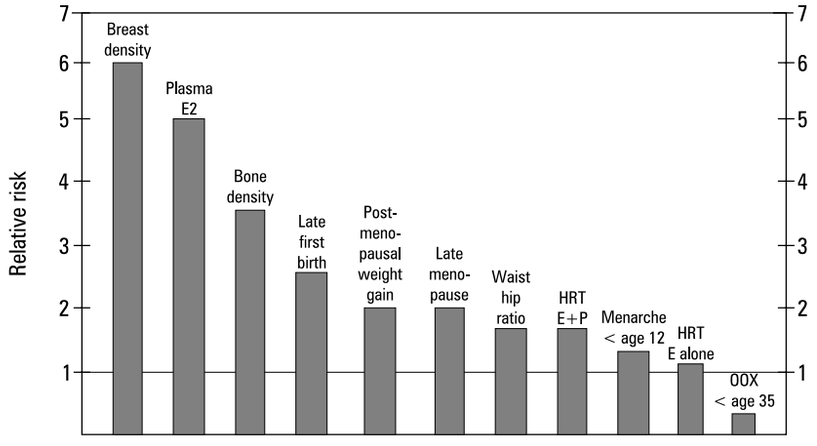

Figure 1. *Hormonal risk factors associated with an increased risk of breast cancer and related to oestrogen exposure (for references supporting the validity of this figure, see Williams RH, Textbook of Endocrinology, 10th Edition [1]*

Rycina 1. *Hormonalne czynniki ryzyka związane z ekspozycją estrogenową a zwiększone ryzyko wystąpienia raka piersi (źródła potwierdzające zasadność danych przedstawionych w powyższych rycinie patrz: Williams RH, Textbook of Endocrinology, 10th Edycja 10 [1])*

study examined sub-groups 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 0.47–0.97). In addition, a 31% lower incidence of localised breast cancer (HR 0.69, 95% CI 0.51–0.95) and a 29% reduction in ductal cancers (HR 0.71, 95% CI 0.52–0.99) 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 a significant 26% decrease in risk of breast cancer was observed in obese women and a non-significant 10% decrease in all study participants taking oestrogen alone for 5–9 years [2]. Other observational studies report a reduction in risk with oestrogen alone but of lesser magnitude and not statistically significant. For example, Schairer et al. [5] reported a 7% reduction in breast cancer risk at 6 years in women receiving oestrogen alone and Lyttinen et al. [10] reported a similar 7% reduction. These combined results, while not conclusive, are highly suggestive of a beneficial effect of oestrogen in reducing breast cancer risk. However, this conclusion must be considered provisional until confirmation has been obtained from rigorously conducted additional studies.

Long-term oestrogen use and breast cancer risk

What are the data regarding the use of oestrogen alone for more than 20 years? The Nurses' Health Study also evaluated women using oestrogen alone for more than 20 years and found a statistically significant 41% increase in breast cancer risk in women of 50 years of age or older and a 77% increase in the sub-set of lean women [2]. Earlier studies by Magnusson et al. [11] and Schairer et al. [5] also reported 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 also reported a linear increase in breast cancer risk over time in women receiving MHT with oestrogen alone over a period of 10 years [6]. In contrast to the other studies reported, however, the Million Women study found a non-statistically significant increased risk of 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 breast cancer from the late 1940s until the early 1980s [7–9]. When compared in randomised trials to tamoxifen, high-dose oestrogens were equally efficacious [7] and in one study were associated with statistically significantly enhanced survival [8] compared to the anti-oestrogen. Extensive studies demonstrated that only specific sub-groups of women respond to high dose oestrogen [9, 12]. Pre-menopausal women and those less than one year after the menopause do not respond at all. Women who had undergone the menopause many years earlier frequently experienced objective tumour regressions. The longer the period after the cessation of menses, the greater is the response rate. Only ER-positive tumours regress in women receiving high-dose oestrogens [12]. In contrast to high-dose oestrogens, tamoxifen is equally effective in pre-menopausal and post-menopausal women. We also consider the fact that women respond in a similar fashion to high-dose oestrogens as to anti-oestrogens to be an "oestrogen paradox".

Possible mechanisms to explain the oestrogen paradox

Our pre-clinical data demonstrate that long-term deprivation of oestradiol causes this sex steroid to trigger cell death through apoptosis. (Fig. 2A), whereas wild-type cells with a normal oestrogen milieu exhibit reduced apoptosis (Fig. 2B) [13–21]. The post-menopausal woman receiving MHT with oestrogen alone may be considered to be in a state of long-term oestradiol deprivation. An extensive review of autopsy studies provides strong evidence that there is a reservoir of undiagnosed breast cancer in post-menopausal women (Table I) [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 of breast cancer from the long-term use of oestrogens alone as MHT may occur through a different mechanism, the genotoxic effects of oestradiol metabolites [24, 25]. The next 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 there have been at least eight studies assessing the frequency of occult malignant disease, primarily ductal carcinoma *in situ* (DCIS),

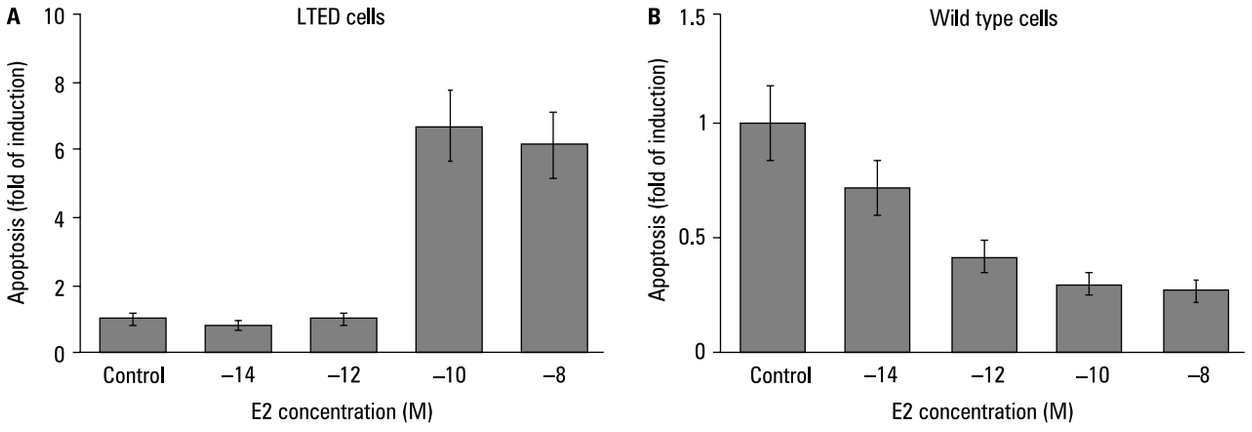

Figure 2. *Long-term oestradiol-deprived (LTED) MCF-7 cells respond to oestradiol with an increase in apoptosis (2A), whereas wild-type MCF-7 cells respond to the same dose of oestradiol with a reduction in apoptosis (2B).*

Rycina 2. *Długotrwale pozbawione wpływu estradiolu (LTED, Long-term oestradiol-deprived) komórki MCF-7 odpowiadają na estradiol nasileniem procesów apoptozy (2A), podczas gdy natywne (*wild type*) komórki MCF-7 odpowiadają na tę samą dawkę estradiolu redukcją apoptozy (2B).*

Table I
Reservoir of occult breast cancer found at autopsy

Tabela I
Odsetek ukrytych przypadków raka piersi stwierdzonych podczas autopsji

Occult DCIS or invasive breast cancer at autopsy			
Author	**#patients**	**%DCIS**	**%IBC**
1962 Ryan	#100	0%	0%
1973 Kramer	#70	4.3%	1.4%
1975 Wellings	#67	4.5	0%
1984 Nielsen	#77	14.3%	1.3%
1985 Alpers	#101	8.9%	0%
1985 Bathal	#207	12.1%	1.4%
1987 Bartow	#221	0%	1.8%
1988 Nielsen	#109	14.7%	0.9%
Total cases	#952	7%	1.1%

Table derived from report of Welch et al. [22] and Ryan [23]

found at autopsy in women with no history of breast cancer [22] (Table I). The frequency of occult DCIS varied considerably between these studies (range 0–15%), most likely reflecting methodological differences. Variation aside, approximately 7% of the 952 combined cases from these studies contained occult DCIS and 1%, occult invasive breast cancers (IBCs) [22]. On the basis of these results it is probably reasonable to assume that 5–10% of the women entering the WHI and Nurses' Health Studies had occult breast cancer when they were initially enrolled.

Evidence for oestradiol-induced apoptosis

Recent *in vitro* studies from our laboratory have shown that hormone-dependent breast cancer cells deprived of oestrogen in the long term undergo adaptive changes which paradoxically cause oestrogen to stimulate apoptosis [13–15] (Fig. 2A). Whereas wild-type MCF-7 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 et al. demonstrated that long-term tamoxifen exposure also results in adaptation and development of oestrogen-induced apoptosis [16–21] (Fig. 3, 4). Apoptotic mechanisms in adapted cells involve up-regulation of the 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 and down-regulation of the anti-apoptotic factor N-F-Kappa B [14, 15, 18].

Long-term oestradiol deprivation in the WHI and Nurses' Health Studies

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 50–600 pg/mL to levels of 5––10 pg/mL. Even though breast tissue levels might not precisely reflect plasma concentrations, one would still expect a substantial reduction in breast tissue levels and adaptation to this reduction. If our hypothesis were correct, then exposure to oestrogen therapy as MHT

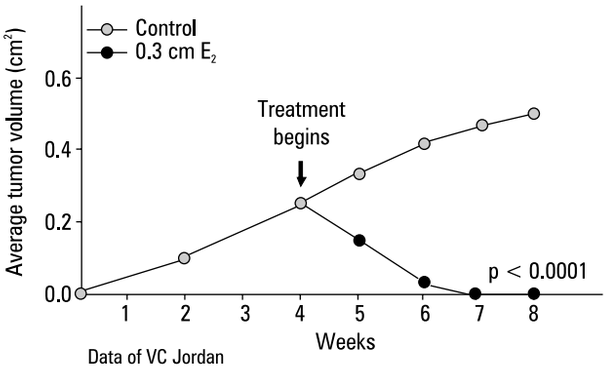

Figure 3. *MCF-7 cells treated with tamoxifen in the long term were implanted as xenografts into nude mice. At growth for 4 weeks, they are exposed to oestradiol as shown by "treatment begins". Slide adapted from the data of Jordan VC et al. [16–21]*

Rycina 3. *Komórki MCF-7 wystawione przez długi okres czasu na działanie tamoksifenu zostały implantowane jako ksenografty do szczepu myszy. Po 4-tygodniowym okresie wzrostu rozpoczęto podawanie estradiolu (punkt oznaczony na wykresie mianem „treatment begins" — początek leczenia). Rycina zaadaptowana z danych Jordan VC i wsp. [16–21].*

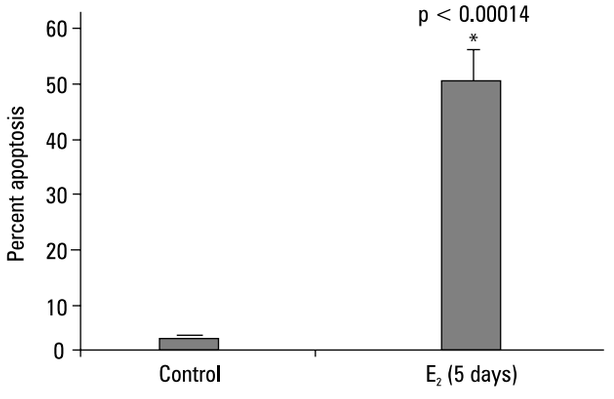

Figure 4. *MCF-7 cells were exposed to tamoxifen long term and then implanted into nude mice as xenografts. After exposure to oestradiol as in Figure 3 above they were excised and examined histologically for apoptosis. Data adapted from the data of Jordan et al. [16–21]*

Rycina 4. *Komórki MCF-7 wystawione przez długi okres czasu na działanie tamoksifenu zostały implantowane jako ksenografty do szczepu myszy. Po okresie ekspozycji na estradiol, jak to zostało przedstawione na rycinie 3, zostały one wycięte i poddane badaniu histologicznemu w celu oceny nasilenia apoptozy. Dane zaadoptowane z VC i wsp. [16–21]*

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 Studies in women receiving oestrogen alone as

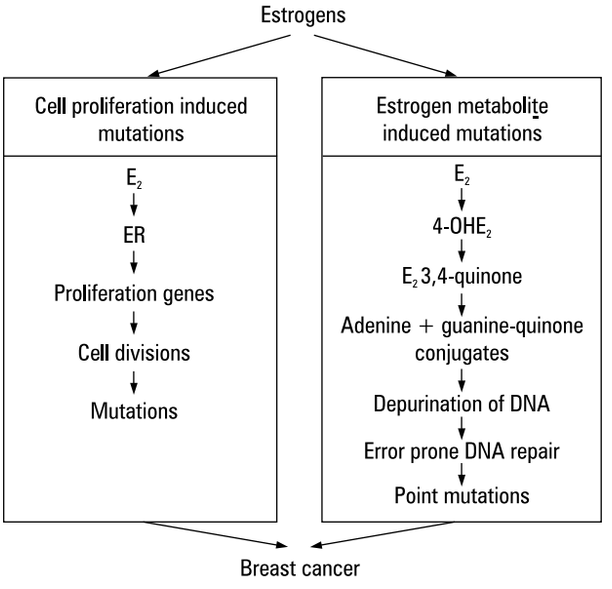

Figure 5. *Two pathways potentially responsible for oestradiol-induced carcinogenesis*

Rycina 5. *Dwie patogenetyczne ścieżki potencjalnie odpowiedzialne za indukowane przez estradiol procesy karcynogenezy*

MHT for 5–9 years [2, 4]. This hypothesis would also explain why women who had received MHT earlier in the WHI study did not experience a reduction in breast cancer risk [4].

Long term exposure to oestradiol

Why would oestrogen increase the risk of breast cancer when 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 division 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] (Fig. 5). Recent studies demonstrate that oestradiol is converted to 4-OH-oestradiol in human breast tissue via the cytochrome p450 1B1 enzyme and is then oxidised 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 oestrogen receptor (ER)-negative benign breast epithelial cells into serially transplantable carcinomas in immune deficient mice [28]. Finally, an ER knock-out 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 carcinogenetic effects of oestradiol [24]. In time, the pro-carcinogenic effects of oestradiol would outweigh the pro-apoptotic effects.

Conclusions

Additional studies are needed to confirm our hypothesis regarding the oestrogen paradox. Specifically, more comprehensive autopsy studies to determine precisely the magnitude of the reservoir of occult breast cancers and their precursor lesions are needed The ability of highly sensitive imaging strategies, such as digital mammography and MRI, should be evaluated in terms of their ability to detect occult breast cancers in women initiating MHT. Direct demonstration of oestrogen-induced apoptosis in breast cancers in women will also be critical.

References

1. Larsen R et al. Williams Textbook of Endocrinology. Santen RJ, editor. Endocrine Responsive Cancer. 19, 1797–1833. 2002. Philadelphia, Pennsylvania, W B Saunders Company.
2. Chen WY, Manson JE, Hankinson SE et al. Unopposed estrogen therapy and the risk of invasive breast cancer. Arch Intern Med 2006; 166: 1027–1032.
3. Anderson GL, Limacher M, Assaf AR et al. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial [see comment]. JAMA 2004; 291: 1701–1712.
4. Stefanick ML, Anderson GL, Margolis KL et al. 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 et al. Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk [see comment] [erratum appears in JAMA 2000 Nov 22–29; 284: 2597]. JAMA 2000; 283: 485–491.
6. Beral V, Million Women SC. Breast cancer and hormone-replacement therapy in the Million Women Study [see comment] [erratum appears in Lancet. 2003 Oct 4; 362 (9390): 1160]. Lancet 2003; 362: 419–427.
7. Ingle JN, Ahmann DL, Green SJ et al. 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 et al. 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 et al. Diethylstilbestrol: recommended dosages for different categories of breast cancer patients. Report of the Cooperative Breast Cancer Group. JAMA 1977; 237: 2079–2080.
10. Lyytinen H, Pukkala E, Ylikorkala O. Breast cancer risk in postmenopausal women using estrogen-only therapy [see comment]. Obstet Gynec 2006; 108: 1354–1360.
11. Magnusson C, Baron JA, Correia N et al. Breast-cancer risk following long-term oestrogen- and oestrogen-progestin-replacement therapy. Inter J Can 1999; 81: 339–344.
12. Santen RJ, Manni A, Harvey H et al. Endocrine treatment of breast cancer in women. [Review] [282 refs]. Endocr Rev 1990; 11: 221–265.
13. Song RX, Zhang Z, Mor G et al. 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 (1): 55–60.
15. Song RX, Mor G, Naftolin F et al. Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17 beta-estradiol [see comment]. J Natl Cancer Inst 2001; 93: 1714–1723.
16. Jordan VC, Lewis JS, Osipo C et al. The apoptotic action of estrogen following exhaustive antihormonal therapy: a new clinical treatment strategy. [Review] [43 refs]. 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 [comment]. J Natl Cancer Inst 2002; 94: 1173–1175.
18. Lewis JS, Meeke K, Osipo C et al. 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 et al. 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 et al. 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 et al. Antitumor action of physiological estradiol on tamoxifen-stimulated breast tumors grown in athymic mice. Clin Can Research 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, Coady 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 et al. Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. Bioch 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 et al. 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 et al. 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. 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. Santen RJ BW, Korach K, Rogan EG CE, editors. Carcinogenesis 2001; 22: 1573–1576.