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## Estrogen-induced apoptosis – a new option for prevention and treatment of breast cancer?

The following paradox has been known for many decades, although not fully understood: estrogen therapy in postmenopausal women can either result in breast cancer cell growth or breast cancer regression. In the WHI trial, the mono-arm with conjugated estrogen caused a decrease in the incidence of breast cancer after 11.8 years median follow-up. In the Million Women Study, current users had little increase in breast cancer if estrogen-alone use was started more than 5 years after menopause (RR = 1.05), but, if it was begun straight after menopause, there was an increase in breast cancer (RR = 1.43). A similar pattern, although with higher RR was recorded for the estrogen–progestogen users (RR = 1.53, RR = 2.04, respectively). The reason for this result remains unclear. The estrogen receptor (ER) is extremely promiscuous in its desire to bind with a wide spectrum of phenolic ligands either to switch off or to switch on the ER signal transduction pathway, which may then under certain circumstances trigger breast cancer cell apoptosis. Jordan [1] has raised an interesting hypothesis, based on experimental and clinical data, that long-term estrogen deprivation may convert breast cancer cells vulnerable to estrogen-induced apoptosis. Based on clinical and experimental data Jordan proposed that 'A 5-year gap is necessary after menopause to permit the selection of estrogen-deprived breast cancer cell populations to cause them to become vulnerable to apoptotic cell death. Earlier treatment with estrogen around menopause encourages growth of ER-positive tumor cells, as the cells are still dependent on estrogen to maintain replication within the expanding population.'

**Comment**

Estrogen as an antitumor agent was proposed in the early 1940s by Haddow [2] who showed that metastatic breast and prostate cancer were responsive towards high-dose estrogen therapy. Several further clinical results suggest that a certain period of time after menopause seems to be necessary to expose an antitumor efficacy of high-dose estrogen in metastatic breast cancer. In the last 10–15 years, several mechanism(s) have been revealed in different experimental models such as the long-term, estrogen-deprived MCF-7 cell population (LTED), probably responsible for the anticancer action of estrogen, especially the apoptotic property of estrogens. Estrogens can be apoptotic by activating the intrinsic (mitochondrial) as well as the extrinsic (death receptor) apoptotic pathway. Essential for estrogen-induced apoptosis is the estrogen receptor and several *in vitro* experiments have revealed that the apoptotic actions of estrogens are mediated by a genomic pathway.

Not all estrogen-like compounds exhibit this apoptotic property or show at least a time-dependence, because they differ in their structure, and only planar compounds such as estradiol seem to be able to trigger apoptosis within a rather short time in contrast to angular estrogens such as triphenylethylene derivatives.

Some factors have been identified which seem to play a role in estrogen-induced apoptosis such as protein kinase C alpha (PKCα) and the oncogene cSRC. PKCα is associated with antiestrogen resistance and, in an animal model, interestingly ER-positive cells transfected with PKCα are resistant to tamoxifen treatment, but estrogen causes rapid tumor regression that can be blocked by the steroidal antiestrogen fulvestrant [3]. cSRC is critically involved in the phosphorylation of Y537 that regulates ER turnover and accumulation, and inhibitors of cSRC have attracted some attention as potential therapeutic agents in breast cancer. However, cSRC inhibitor blocks estrogen-induced apoptosis in LTED breast cancer cells [4]. Also of interest are findings showing that glucocorticoids such as dexamethasone can inhibit estrogen-induced apoptosis and that medroxyprogesterone acetate, exhibiting partial glucocorticoid activity, can modify estrogen-induced apoptosis [5].

The proposed concept by Jordan is certainly very interesting, since it may in part explain the observed results of the WHI mono-arm in which most of the treated patients were older than 60 years [6]. Results of this only placebo-controlled study should have a biological plausibility, whereas at least some results of the Million Women Study [7] can be judged as questionable, especially regarding the time of hormone exposure [8]. However, according to Jordan's concept, only a special patient population, i.e. patients who present a breast cancer cell population that are resistant to long-term estrogen deprivation, may be vulnerable for estrogen therapy in the metastatic setting, and up to now screening of this vulnerable population is not possible.

Supporting this 'gap-hypothesis' is a study by Fournier and colleagues [9] showing a reduced breast cancer risk for combined hormone therapy, when starting after a 3-year gap after the menopause. However, another study suggests a reduced breast cancer risk for estrogen-only therapy for up to 10 years also when starting early in postmenopausal women [10)], but with a relatively small patient number.

Since resistance to endocrine therapy in the postmenopause might be resolvable by switching to high-dose or even low-dose estrogen therapy, as shown in a small clinical trial [11], estrogens may develop carcinoprotective mechanisms which work without any time gap. Jordan's hypothesis of an estrogenic, anti-cancerogenic property is based on the pro-apoptotic effect of estrogens. However, anti-cancerogenic estrogen action might also be explained by the presence of estradiol metabolites that can act antiproliferatively and antioxidatively such as 2-hydroxestradiol and 2-methoxyestradiol, especially if present in high concentrations [12]. Especially the latter metabolite has a strong antiproliferative and apoptotic property which may work without any 'time-gap' [13]. Thus, different mechanisms might be responsible for an anti-cancerogenic effect of estrogen therapy.

One criticism on the 'gap hypothesis' might be that, starting from the first new cancer cell, defensive mechanisms like apoptosis or radical capture, e.g. supported by certain protective estrogen metabolites, could work immediately, but, if defensive mechanisms have been successful, can be manifested or be proven clinically only after about 10 years, according to the time of the development of clinical cancer, which can be calculated from the volume of the cells and the doubling time on the basis of the most malignant cancer types [14]. Starting new hormone therapy in the case of already pre-existing and, to some extent, already proliferated cancer, this time of course can be shorter. However, any 'gap', longer or shorter, might only reflect the time for clinical detection of the protective effects which, however, may start early during estrogen therapy, rather than the gap reflecting mechanisms which only work after a gap of time. Different results in clinical studies might be explained by different treated populations – if (various) protection mechanisms (not only apoptosis!) work stronger and/or faster than proliferation mechanisms, the statistical result calculated for the whole study population will be a decrease in clinically observed breast cancer. The observation of a gap (yes or not, duration of a gap) would be dependent on the relation of proliferation to protective effects and the amount of already pre-existing cancer cells. Since progestogens can increase the estrogen-induced proliferation, the research on these mechanisms and investigation of whether there is a dependency on type and dosage of the progestogens are of greatest importance [15]. The new concept of combining estrogens not with progestogens, but with SERMs might lead to a reduction of the proliferative effects, and protective mechanisms during estrogen treatment might been observed earlier, without a time gap.

In summary, in our view it remains unclear whether timing of hormone therapy relative to menopause is really important regarding the risk for breast cancer. It may be that a 'time gap' is not needed to achieve protection with estrogen therapy according to biological effects; it may only be dependent on the study population and type of hormone therapy, decisive for the ratio of proliferation to protective effects, and what we see is a statistical result for the study population and cannot express the individual risk of breast cancer during hormone therapy. More experimental and clinical studies are needed to prove the ability of estrogens to trigger apoptosis and other protective mechanisms under certain circumstances, especially according to Jordan's 'gap' hypothesis.

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