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Gene expression changes induced by estrogen and selective estrogen receptor modulators in primary-cultured human endometrial cells: signals that distinguish the human carcinogen tamoxifen

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Abstract

Tamoxifen has long been the endocrine treatment of choice for women with breast cancer and is now employed for prophylactic use in women at high risk from breast cancer. Other selective estrogen receptor modulators (SERMs), such as raloxifene, mimic some of tamoxifen's beneficial effects and, like tamoxifen, exhibit a complex mixture of organ-specific estrogen agonist and antagonistic properties. However, accompanying the positive effects of tamoxifen has been the emergence of evidence for an increased risk of endometrial cancer associated with its use. A more complete understanding of the mechanism(s) of SERM carcinogenicity and endometrial effects is therefore required. We have sought to compare and characterise the transcript profile of tamoxifen, raloxifene and the agonist estradiol in human endometrial cells. Using primary cultures of human endometria, to best emulate the in vivo responses in a manageable in vitro system, we have shown 230 significant changes in gene expression for epithelial cultures and 83 in stromal cultures, either specific to 17β-estradiol, tamoxifen or raloxifene, or changed across more than one of the treatments. Considering the transcriptome as a whole, the endometrial responses to raloxifene or tamoxifen were more similar than either drug was to 17β-estradiol. Treatment of endometrial cultures with tamoxifen resulted in the largest number of gene changes relative to control cultures and a high proportion of genes associated with regulation of gene transcription, cell-cycle control and signal transduction. Tamoxifen-specific changes that might point towards mechanisms for its proliferative response in the endometrium included changes in retinoblastoma and c-myc binding proteins, the APCL, dihydrofolate reductase (DHFR) and E2F1 genes and other transcription factors. Tamoxifen was also found to give rise to the

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highest number of gene expression changes common to those that characterise malignant endometria. It is anticipated that this study will provide leads for further and more focused investigation into SERM carcinogenicity.

Keywords: SERM; Estrogen; Endometrium; Arrays; Tamoxifen; Raloxifene; Endometrial cancer

1. Introduction

Selective estrogen receptor modulators (SERMs) comprise a group of compounds that are capable of estrogen agonist and antagonist effects in a tissue-specific manner throughout the body. There are two main families of SERMs, the triphenylethylene derivatives such as tamoxifen (and analogues such as toremifene and idoxifene) and the benzothiophene derivatives, mainly represented by raloxifene.

Tamoxifen has been used for the treatment of breast cancer since 1971 and is now the most prescribed antineoplastic agent in the world (Powles, 1997). Tamoxifen is used for pre- and post-menopausal patients with all stages of breast cancer including the treatment of advanced breast cancer. As an adjuvant therapy, tamoxifen behaves as an antiestrogen but has been shown to have additional clinical estrogen-like benefits on estrogen-regulated systems throughout the body, such as the maintenance of bone density and cardioprotective qualities (Resch et al., 1998; Rosano and Sarrel, 1994). Tamoxifen is now licensed for use as a prophylactic for women at a high risk of developing breast cancer in the US. Results from the multiple outcomes raloxifene evaluation (MORE) trial, suggest that raloxifene may also be effective in preventing breast cancer (Cummings et al., 1999).

Tamoxifen has been shown to affect the endometrium of post-menopausal women, leading to benign changes such as hyperplasia and proliferative polyposis (Neven and Vergote, 2001; Vosse et al., 2002) and more severely, to endometrial carcinoma (Curtis et al., 1996; Fisher et al., 1994,1998; Rutqvist et al., 1995; van Leeuwen et al., 1994). Between 40% and 50% of post-menopausal women receiving tamoxifen have some form of endometrial proliferative abnormality (Ismail, 1996; Kedar et al., 1994; Neven and Vernaeve, 2000). In 1996, the International Agency for Research on Cancer (IARC) concluded that there was a strong and statistically significant association between tamoxifen therapy and risk of developing endometrial cancer (IARC, 1996). As a result, IARC classified tamoxifen as a group 1 (human) carcinogen. The increased risk of endometrial carcinoma in women on tamoxifen therapy in various trials is proposed to be between 1.6 and 7.5-fold, compared to women in the general population. The general consensus appears to be that the benefits of tamoxifen therapy for women, who have had breast cancer, far outweigh the potential side effects. However, the use of tamoxifen in a preventative capacity means healthy women will be exposed to a higher risk of endometrial cancer.

The mechanism whereby tamoxifen causes endometrial cancer remains unclear. Endometrial cancer is an estrogen-dependent pathology (type I endometrioid endometrial carcinoma) and unopposed estrogen is a confirmed risk factor. It has therefore been suggested that the estrogen-like properties of tamoxifen in the uterus may well be responsible for the carcinogenicity seen. Tamoxifen is reported to stimulate proliferation of endometrial epithelium, with an increased distribution of the cells into S, G2 and M phase of the cell-cycle (Boccardo et al., 1981; Mourits et al., 2002). Analysis of steroid receptors and growth regulatory genes after tamoxifen treatment also reveal levels similar to those found in the proliferative (estrogen-dependent) phase of the menstrual cycle (Elkas et al., 2000).

In animal models, tamoxifen's effects on the uterus are more complex than simply estrogen-like proliferation. Comparisons of murine endometria from neonates treated with tamoxifen and estradiol showed that all tamoxifen-treated mice had rapid induction of uterine adenomyosis, characterised by disordered location of endometrial glands and stroma into the myometrium of the uterus, whereas none of the mice treated with estradiol showed this effect (Parrott et al., 2001). In neonatal rats, tamoxifen has been shown to cause endometrial and vaginal cancers, in the absence of endometrial hyperplasia, again suggesting an estrogen agonist effect is not essential for tamoxifen carcinogenicity in the