The endometrial effects of SERMs

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The ideal selective oestrogen receptor modulator (SERM) would retain an oestrogen-like effect on the bones, the heart and cardiovascular apparatus, and the central nervous system, while acting as an anti-oestrogen on the breast and the genital tract. It seems, however, that such a compound is not available for clinical use yet. The uterine tissue, and particularly the endometrium, defines an area of special interest in the SERM action, since endometrial hyperplasia and cancer has been linked to agonistic oestrogen effects. Additionally, tamoxifen, the SERM which accumulates most of the clinical experience, has been associated with stimulatory effects on endometrium, including the development of cancer. In contrast, the more recent benzothiophenes, led by raloxifene, seem to operate as endometrial antagonists, thus providing an interesting alternative for clinical use. This review analyses the endometrial action of tamoxifen, including the information gathered from laboratory models, the observed endometrial effects in women using tamoxifen, and the epidemiological and molecular data which link the use of tamoxifen with endometrial cancer. A parallel examination of the raloxifene data presents the available experimental and clinical information, suggesting the endometrial neutrality of this compound.

Key words: cancer/endometrium/raloxifene/SERMs/tamoxifen

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Introduction

The modern concept of selective oestrogen receptor (ER) modulation is attractive, since it provides the option of designing drugs that retain oestrogen agonism at the desired target tissues, such as bone, heart or brain, while lacking stimulation potential at tissues where oestrogens may harbour any oncogenic potential, endometrium and breast. Pharmacological research has yielded a series of compounds in the last years whose properties in vitro and in vivo have allowed them the consideration of selective oestrogen receptor modulators (SERMs). Much of the basic and clinical information supporting the notion of SERMs has accumulated from the study of the family of triphenylethylene derivatives, and particularly tamoxifen, together with the benzothiophenes and some other more recent compounds (Goldstein et al., 2000). However, the ideal SERM seems a still elusive objective. Although tamoxifen does act as a SERM, its action on the endometrium is a matter of controversy, with a wealthy pool of observations supporting an oncogenic, oestrogen-like effect. There is less experience with the group of benzothiophenes, although both the experimental and the still incipient clinical data obtained with raloxifene seem consistent in supporting an antagonistic endometrial action. This review analyses the basic clinical information on the action of SERMs on the endometrium. Both the triphenylethylene and the benzothiophene families are considered with reference to their most studied representatives, tamoxifen and raloxifene.

Mechanism of action in the endometrium

As detailed in another paper of this symposium (Cano, 2000), the agonist/antagonist profile of a SERM is determined at the tissue level. There are obvious differences in molecular structure between the triphenylethylene and the benzothiophene derivatives, what defines an a priori distinct conformational change of the ligand–receptor complex when both are bound to the ER (Figure 1).

The ER consists of six functional domains transcribed by eight exons (Figure 2). The A/B domain, also called the regulatory domain, contains the transcription activating function-1 (TAF-1). TAF-1 can stimulate transcription in the absence of agonist binding. The C region constitutes the DNA-binding domain (DBD), which mainly consists of two zinc fingers. The D region, also called the hinge, is a site of rotation, whereas the E region

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includes the ligand-binding domain (LBD). The E region also contains the transcription activating function-2 (TAF-2), a dimerization domain, and a heat shock protein 90 binding function. Finally, the E region, or C-terminal segment, is supposed to participate in the modulation of gene transcription by agonists and antagonists. Unlike TAF-1, which is constitutively active, TAF-2 is induced upon ligand binding to the receptor. A great deal of the actual knowledge of the molecular basis of TAF-2 induction has been gained through the recent determination of the crystal structure of the LBD of the ER (Brzozowski et al., 1997). The presence of an α-helix made up by the residues 538–552 is crucial for TAF-2 activity. The use of mutant receptors has identified additional residues in helices H3, H5/6 and H11.

The members of the triphenylethylene and the benzothiophene families bind to the LBD of the ER, whose particular crystal structure with oestradiol or raloxifene has been revealed (Brzozowski et al., 1997). Once inserted into the binding cavity, oestradiol makes direct hydrogen bonds with its A-ring to the carboxylate of Glu 353, the guanidinium group of Arg 394, and a water molecule, and of its D-ring with His 524 (Brzozowski et al., 1997) (Figure 3). Raloxifene is also anchored to the same three amino acids as oestradiol by direct hydrogen bonds, but also interacts with Asp 351 (Figure 3). The final orientation of raloxifene within the binding pocket determines that its side-chain displaces helix 12. As a consequence of the oestradiol insertion within the LBD, the large helix 12 of the ER folds over and traps the steroid, thus exposing three specific amino acids, 540, 543, and 547, critical within the TAF-2 region for binding co-activators (Tzukerman et al., 1994). In the case of raloxifene, helix 12 becomes reoriented, and cannot seal the pocket containing the ligand (MacGregor and Jordan, 1998). The repositioned TAF-2 region impairs the formation of a transcription complex by co-activators, and the signal transduction is blocked.

Tamoxifen, together with other members of the triphenylethylene family, has been shown to act as a TAF-2 antagonist, a trait shared with raloxifene. However, tamoxifen and other triphenylethylene derivatives act as partial agonists in the uterus, an effect that seems opposite to that of raloxifene. It may, therefore, be postulated that at the endometrial level (where both tamoxifen and raloxifene share the same tissue and promoter context), the contrasting action of those compounds may be due to particular details of the conformational change induced on the ligand–receptor complex, at either the TAF-2 or other domain.

Tamoxifen and the endometrium

The original report describing endometrial carcinomas in breast cancer patients who were treated with tamoxifen (Killackey et al., 1985), marked the beginning of several larger clinical trials and case reports measuring the incidence of endometrial carcinoma. Some of those trials detected an increase in the rate of endometrial cancers among patients receiving tamoxifen (Fornander et al., 1989; Fisher et al., 1994; Rutquist et al., 1995). Analysis of the design and conclusion of those studies has raised some controversy, which has been partially overcome by new data obtained in studies on healthy patients receiving tamoxifen for breast cancer prevention. To examine the available data on the action of tamoxifen on the endometrium, this review will first focus on the action of the drug on animals, cell lines and transplants, followed by the analysis of clinical data in the human.

Laboratory models

Several investigators have used rodent models, mainly ovariectomized rats or mice, in an attempt to better understand the action of tamoxifen in the human uterus. Extrapolation of animal data, however, is complicated by a variety of significant differences existing among species, e.g. (i) anatomy and organ structure; (ii) physiology and cellular biochemistry, and (iii) metabolism and bioactivation of tamoxifen and its metabolic intermediates (Lien et al., 1991; MacMahon, 1997). This caution is particularly pertinent in the case of SERMs, whose specific action is strongly influenced by the environment defined by the

![Figure 1](image1.png)

**Figure 1.** Molecular structures of tamoxifen and raloxifene, the most relevant compounds of the triphenylethylene and benzothiophene families, respectively. The structure of 17β-oestradiol has been included for comparison.

![Figure 2](image2.png)

**Figure 2.** The oestrogen receptor (ER) consists of six functional domains, transcribed by eight exons. The main functional activity ascribed to each domain is detailed in the text. TAF = transcription activating function.
local conditions of the tissue. To further increase this complexity, qualitative and quantitative differences in endometrial response to tamoxifen have been detected as a function of age, dose, and species. This lack of a uniform picture in the effect of SERMs on the uterus of animals makes it difficult to obtain valid conclusions for the human uterus.

To illustrate those varied responses, tamoxifen induces hypertrophy of the stroma and hyperplasia of the endometrial glands and lumen epithelia in immature rodents (Gallo and Kaufman, 1997; Poulet et al., 1997). In contrast to those uterotrophic effects, studies of tamoxifen administration to oophorectomized rats indicate mixed responses, with reports describing uterine atrophy and the absence of any histopathological reports of endometrial neoplasia (Greaves et al., 1993; Yoshida et al., 1998), increases in uterine weight and epithelial cell hypertrophy (Carthew et al., 1999), or even no detectable change (Kafkasli et al., 1998). To further complicate things, a recent study shows differences in the uterine response of Sprague–Dawley and Fischer 344 rats to tamoxifen; that study found a time-dependent reduction of endometrial glands, that disappeared after 12 weeks of treatment, without an associated decrease in cell proliferation (Karlsson et al., 1998).

Molecular effects of tamoxifen on the endometrium

The proliferative effect of tamoxifen on the endometrium has been supported by molecular data. The expression of both ER (Figure 4) and progesterone receptors (PR) was found to be consistently positive in endometria from women treated with tamoxifen for 1 month (A.Cano et al., in preparation). That positivity has been reported to be even higher than that found in a control group of premenopausal women (Kommoss et al., 1998). Tamoxifen also mimicked oestradiol treatment in up-regulating ER, c-fos and glyceraldehyde phosphate dehydrogenase mRNAs, together with other oestrogen-induced genes (Rivera-González et al., 1998; Robertson et al., 1998). The bromo-deoxyuridine index, an indicator of cell mitogenesis, was shown to be increased in endometrial cells from tamoxifen-treated uteri (Karlsson et al., 1998; Carthew et al., 1999). In this connection, the expression of markers of proliferation, e.g. Ki67, was potentiated by tamoxifen in human endometrium (Elkas et al., 1998). An increased susceptibility to genetic lesions associated with carcinogenesis linked to tamoxifen was suggested by a study on endometrium of surgically post-menopausal cynomolgus macaques, where the drug induced p53 positivity, although at a lower level than conjugated oestrogens (Isaksson et al., 1999).

There are also indications suggesting that 17β-oestradiol and tamoxifen induce uterine hypertrophy and hyperplasia by different molecular mechanisms. In contrast to the efficient counterbalancing effect of progestins on most 17β-oestradiol responses, only some of the agonistic responses of tamoxifen in the uterus can be antagonized by progestins (Bigsbys and Li, 1994; Powles et al., 1998a). These data clearly indicate that a simple explanation of tamoxifen agonism is not possible. In fact there is experimental evidence in favour of an antagonistic effect of tamoxifen on calmodulin (Hardcastle et al., 1996) and on protein kinase C (O’Brian et al., 1985), two actions possibly independent of the interaction of tamoxifen with ER.

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**Figure 3.** A detailed representation of the interaction of (A) oestradiol and (B) raloxifene with relevant amino acids in the ligand-binding pocket of the oestrogen receptor (from Brzozowski et al., 1997, with permission).
Clinical impact of tamoxifen on the endometrium

Ultrasound and hysteroscopy findings

The endometrial effects of tamoxifen therapy in post-menopausal women have been monitored using a wide range of techniques including: transvaginal sonography (Lahti et al., 1993b; Goldstein, 1994; Achiron et al., 1995; Bowne, 1995; Hann et al., 1997; Friedrich et al., 1998; Ozsener et al., 1998; Tesoro et al., 1999); dilatation and curettage (Gibson et al., 1994); endometrial sampling (Gal et al., 1991); and hysteroscopy (Neven et al., 1989, 1990; Neven, 1993; Consomni et al., 1995).

A ‘thickened endometrial stripe’ has been observed by vaginal sonography in 40–69% of tamoxifen-treated women (Hann et al., 1997; Friedrich et al., 1998; Mourits et al., 1999; Tesoro et al., 1999). Endometrial atrophy, the expected histological status in post-menopausal women whose endometrium has not been stimulated, generates a thin, regular line, whose thickness is <4–5 mm (Osmers et al., 1990; Varner et al., 1991; Langer et al., 1997).

Several cases of thick endometria observed in women receiving tamoxifen are due to oedema and enlargement of the stromal cells (Achiron et al., 1995). This subendometrial thickening has been further confirmed with the use of sonohysterography, a technique which injects fluid into the uterine cavity while performing vaginal endosonography (Goldstein, 1994). It is possible then to observe irregular sonolucencies beneath a thin endometrium. The thickened area sometimes forms cystic and polypoid abnormalities. In a cohort of 235 normal post-menopausal women recruited from a randomized, double-blind controlled trial, the Pilot Breast Cancer Prevention Trial at the Royal Marsden Hospital (Powles et al., 1998a), the use of tamoxifen was associated with 7% of cysts, 3% of polyps, and 8% of both cysts and polyps. Hysteroscopic examination often revealed tough, fibrous changes, which were ascribed a stromal origin.

The thickened endometrium observed with vaginal sonography in women under tamoxifen differs, therefore, from the sonographic findings in patients who are taking continuous unopposed oestrogens, where the sonolucencies are mainly endometrial in origin. There is real hypertrophy and hyperplasia confined to the endometrium in women under oestrogens, a difference which also underlines the distinct biological behaviour of tamoxifen from oestradiol. The hysteroscopic image is described as a smooth white but hypervascularized and transparent thin endometrial mucosa with patches of pseudocystic endometrial stroma (Neven et al., 1998a).

Pathological changes

The vast majority of the available literature supports an agonistic effect of tamoxifen on endometrium, with a higher rate of endometrial and cervical polyps, and distinct forms of structural changes denoting hypertrophy or hyperplasia.

The incidence of endometrial polyps in breast cancer patients receiving tamoxifen oscillates between 12–25% (Neven et al., 1989; Gal et al., 1991; Lahti et al., 1993b; Gibson et al., 1994; Cosgrove et al., 1994; Pinheiro et al., 1994; Consomni et al., 1995; Tesoro et al., 1999), which exceeds the ~4% observed in patients with breast cancer not receiving tamoxifen.

The reported increase of endometrial hyperplasia is actually a subject of some debate, since a part of the incidence derives mainly from the thickened endometrium identified by vaginal sonography. The diagnosis of hyperplasia in women treated with tamoxifen has been then inferred from the ultrasonographic observations, not confirmed histologically. In all studies in which endometrial biopsies have been obtained by curettage, aspiration, or hysteroscopy, or direct observations of the

Figure 4. Expression of oestrogen receptors in frozen sections obtained from endometrium of normal post-menopausal women treated with 20 mg/day of tamoxifen for 1 month prior to hysterectomy (A and B). The high positive staining and proliferative epithelial development contrasts with the results obtained in untreated post-menopausal women (C). Scale bars = 75 µm (A) and 50 µm (B and C). (A.Cano et al., in preparation).
endometrium made by hysteroscopy, a discordance between the sonographic findings and the endometrial findings has been reported (Neven et al., 1989, 1990; Lahti et al., 1993b; Goldstein, 1994; Achiron et al., 1995).

The histological counterpart of the white, but hypervascularized, endometrial mucosa observed by hysteroscopy is a flattened or cuboidal epithelium overlying fibrous and condensed stroma containing cystic dilated endometrial glands. This is a description that some investigators consider similar to the senile cystic atrophy commonly seen in post-menopausal untreated women (Neven et al., 1998a,b; McGonigle et al., 1998; Mourits et al., 1999), but the glandular crowding plus the frequent proliferative activity of both epithelial and stromal cells has also been read as a form of endometrial hyperplasia (Ismail, 1994, 1998).

The senile cystic endometrial atrophy is not, however, a universal endometrial pattern in women treated with tamoxifen. In a study on 16 breast cancer patients receiving the drug, seven women had mild proliferative endometrium after 16 months of treatment (Neven et al., 1990). The same group ultimately reported that five out of 57 women receiving tamoxifen developed clear endometrial hyperplasia or cancer (Neven et al., 1998a); the endometrium remaining atrophic in 24 women. Although there was no control group, one interesting aspect of both studies was that all women underwent hysteroscopy before tamoxifen administration, and all were diagnosed with atrophic endometrium. An increased incidence of endometrial hyperplasia has also been detected by some investigators in samples obtained from breast cancer women treated with tamoxifen, either compared (Gal et al., 1991; Lahti et al., 1993a, Tesoro et al., 1999), or not (Ozsener et al., 1998; Kennedy et al., 1999) with breast cancer women not receiving the drug. In a cohort of 61 normal post-menopausal women recruited from the Pilot Breast Cancer Prevention Trial (Kedar et al., 1994), the use of tamoxifen was associated with 39% (24 women) of histologically active endometrium compared with only 10% in the control. Atypical hyperplasia was detected in 16% of the tamoxifen women.

Tamoxifen has also been observed to play a permissive role, if not inductive role, in some oestrogen-promoted diseases, e.g. endometriosis (Ford et al., 1988; Cano et al., 1989; Hajjar et al., 1993; Cohen et al., 1994; Morgan et al., 1994; Thylans, 1995) or leiomyomata (Cohen, 1997). In conclusion, it seems that the behaviour of tamoxifen in human endometrium involves an inconsistent, particular form of agonistic profile that, additionally, does not completely overlap with the effect of oestrogens.

Tamoxifen and endometrial cancer

Since unopposed oestradiol and exogenous oestrogens induce hyperplasia and, in the long term, increase the risk of endometrial carcinoma, it was to be expected that any compound that is an agonist of the ER and is given at a high enough dose would follow the same steps. The observation of endometrial hyperplasia in some women treated with tamoxifen, as detailed above, has contributed to create concern on a potential oncogenic action of tamoxifen on human endometrium.

The hypothesis of a possible association between use of tamoxifen and induction of endometrial carcinoma emerged from observations made in the laboratory. The human endometrial cancer line EnCa 101 may partially grow in athymic mice in response to tamoxifen (Satyaswaroop et al., 1984). When two separate tumours, one a hormone-sensitive endometrial tumour (EnCa 101) and the other a hormone-sensitive breast tumour (MCF-7), were implanted into a nude mouse, the endometrial tumour grew, whereas the MCF-7 tumour diminished, when the animal received tamoxifen (Gottardis et al., 1988). The difference in response of the two human tumours transplanted into the same animal discounted the influence of the species in the experiment, and was eloquent on the agonist effect of tamoxifen.

These experimental observations contributed to initiate investigations on an eventual clinical association between the use of tamoxifen as an adjuvant in breast cancer patients and the development of endometrial cancer. There is a long list of studies, including both randomized trials and case control studies (MacMahon, 1997), which have identified about 400 cases of endometrial cancer associated with the use of tamoxifen. Although unanimity is absent, in both types of studies the evidence leans toward the view that there is an association between the use of tamoxifen and the risk of endometrial cancer. The magnitude of the association oscillated in most studies around a relative risk of 2–4.

However, evidence from the literature is incomplete and inconclusive. Previous analyses (Cohen, 1997; MacMahon, 1997), showed that it is difficult to conclude from the studies on breast cancer women treated with tamoxifen whether its association with endometrial cancer, if real, is a causal one. A number of reasons indicate that the available findings in the randomized trials do not warrant a definite conclusion (MacMahon, 1997). These include: (i) three randomized trials in which no increase in endometrial cancer was noted; (ii) in one of the largest studies (Fisher et al., 1994), the women randomized to not receive tamoxifen have a low risk of endometrial cancer; (iii) the confounding variables of prior hysterectomy or hormone replacement therapy have not been adequately dealt with in any of the positive studies; and (iv) none of the studies addresses the issue of detection bias, i.e. the fact that the induction of signs such as vaginal discharge will probably lead to repeated screening of these women, and therefore, to increased detection rates.

Also the case control studies suffer from distinct defects which decrease the weight of their conclusions. Selection or information bias reduces the valid data of those from two studies, the Netherlands study and the Washington State study, the former positive and the latter negative (MacMahon, 1997).

Together with above-mentioned difficulties, a common flaw in the clinical studies is the lack of a clear association between the duration of tamoxifen use and the risks of developing endometrial carcinoma (MacGregor and Jordan, 1998). The long genesis of cancer in humans makes it likely that short courses of tamoxifen may condition the build-up of cancers which become clinically detectable later.

To conclude, the available evidence suggests an association between the use of tamoxifen and endometrial cancer in women who already have cancer in the breast, although the data are still inconclusive. The fact that, because of their breast malignancy, this type of woman is also at increased risk for endometrial tumours, has contributed to interest in clinical studies on normal women, where tamoxifen has been used as a chemopreventive.
The results of three trials have been published (Fisher et al., 1998; Powles et al., 1998b, Veronesi et al., 1998), although only two, the American Breast Cancer Prevention Trial (BCPT) (Fisher et al., 1998) and the British Royal Marsden Hospital Trial (Powles et al., 1998b) included non-hysterectomized women.

The sample size of the BCPT was 13,388 women, amounting 46,858 woman-years of follow-up, whereas the corresponding figures in the British study were 2471 and 12,355. The total number of endometrial cancers detected in the tamoxifen arms of both studies was 40, of whom 36 corresponded to the BCPT and four to the Royal Marsden trial. In contrast, the placebo arm of each study included only 15 cases (BCPT) and one case (Royal Marsden). As already mentioned for the data obtained from observational studies in women treated with tamoxifen because of breast cancer, the increase in incidence after tamoxifen administration was observed early in the follow-up period (Figure 5).

**Mechanism of tamoxifen carcinogenesis in the endometrium**

Endometrial carcinoma is thought to develop as the culmination of a process of progressive alterations that occur over a prolonged time interval. Endometrial cancers are frequently preceded by endometrial hyperplasia, a stage that may regress following adequate therapy, but that may also progress and develop carcinoma. Since endometrial cancer has been linked to continuous exposure to endogenous or exogenous oestrogen (Smith et al., 1975; Ziel and Finkel, 1975; Grady et al., 1995), it was to be expected that any compound that is an agonist of the ER would be qualified to develop similar oncogenic properties. This has been the rationality of numerous experimental studies, which have received the supporting clinical evidence referred to above.

The issue of the possible mechanisms involved in the endometrial carcinogenicity of tamoxifen is, however, quite complex. The data reviewed above suggest that the molecular machinery involved in the induction of hypertrophy and hyperplasia by tamoxifen may differ from that of oestrogens. In fact, opposite to most oestrogenic responses, only some agonistic actions of tamoxifen in the uterus can be antagonized by progestins (Powles et al., 1998a).

The majority of the hypotheses on the eventual carcinogenic pathways of tamoxifen in endometrium arise from the information obtained on the mechanisms involved in rat liver carcinogenesis. Although there is no uniformity in the details of the carcinogenic potential of tamoxifen between different rat strains, there is enough evidence to conclude that tamoxifen may act as both inducer and promoter of liver tumours (Wogan, 1997). Among the mechanisms that may operate in rat hepatocarcinogenesis, mutations of the p53 gene (Vancutsem et al., 1994) or structural and numerical alterations in chromosomes (Sargent et al., 1996) have been detected, although much of the evidence involves the formation of co-valent tamoxifen–DNA adducts. The initial detection of DNA adducts in the liver of Sprague–Dawley rats (Han and Liehr, 1992) has been then widely confirmed. The focus of investigation has been the identification of the actual DNA adduct (Osborne et al., 1996). The reactive intermediate, α-acetocytamoxifen, was synthesized and was found to react with DNA in vitro (Figure 6). The products of this reaction were shown to be chromatographically identical to those isolated from DNA reacted with α-hydroxytamoxifen and to those found in the liver DNA of rats treated with tamoxifen.

However, the animal liver data are of little value in the clarification of the potential carcinogenicity of tamoxifen in human endometrium. In fact, the data obtained from rat endometrium are at variance with that described for the liver. Thus there is no evidence of uterine DNA adduct formation by tamoxifen in the rat (White et al., 1992; Li et al., 1997). The value of the liver extrapolation is further weakened when human, and not rat, endometrium is concerned, since there are wide differences between the animal and the human with regard to response to chemical exposure, maximally tolerated dose, metabolism and pharmacokinetics, and even the mechanism of carcinogenicity (Guzelian, 1997).

Studies on human endometrium have produced conflicting results. A study reported both in-vitro and in-vivo data confirming that tamoxifen does not form adducts in human endometrial cells (Carmichael et al., 1996). Another study found that the chromosomal and gene rearrangements observed in endometrial polyps associated with tamoxifen are similar to those found in patients not treated with the drug, thus discarding the suggestion that those endometrial changes could be initial stages of a stepwise process leading to overt malignancy (Dal Cin et al., 1998). Nevertheless, the use of more sophisticated technology has opened the door to doubt. The application of sensitive high-performance liquid chromatography has permitted the identification of DNA adducts in the endometrium (Hemminki et al., 1996) and white blood cells of patients under treatment with tamoxifen, although this finding could not be confirmed (Carmichael et al., 1999) using similar
technology. The trans- and cis-isomers of α-(N²-deoxyguan-oxinyl)tamoxifen (dG-N²-TAM) are a different type of adduct from those found in patients treated with tamoxifen (Shibutani, 1998; Shibutani et al., 1999). Interestingly, the dG-N²-TAM adducts have recently demonstrated miscoding potential (Shibutani and Dasaradhi, 1997), and the insertion of a single isomer of dG-N²-TAM into a single-stranded shuttle vector has shown targeted mutations in simian kidney (COS-7) cells (Terashima et al., 1999).

In summary, the available experimental information is still of little help in clarifying whether tamoxifen is genotoxic and oncogenic in human endometrium. There is, nonetheless, a great deal of debate about the actual relevance of minor concentrations of adducts (as detected by high-performance liquid chromatography and other modern technology), compared with the high concentrations induced in rat hepatic tumours, or the equally elevated concentrations formed in human DNA as a result of environmental sources (Swenberg, 1997). Accordingly, if tamoxifen is an endometrial carcinogen, as classified by the International Agency for Research on Cancer (IARC), its mechanism of action is possibly different from that reported in rat liver.

An alternative oncogenic mode of action of tamoxifen on human endometrium may derive from its proliferative activity. It is possible that the increase in the rate of mitoses in a given tissue entails an augmented risk of mutation, the first step towards malignancy (Ames and Gold, 1990). Therefore, it is possible that the oncogenetic attributes of oestrogens in tissues where they induce a proliferative effect (e.g. breast or endometrium), might be influenced by the mutagenic potential derived from a higher mitotic activity. This might also be the main pathway for the oncogenic action of tamoxifen in the uterus, where a study has shown that the increase in uterine weight by tamoxifen was accompanied by a doubled uterine expression of insulin-like growth factor-I (IGF-I), whereas the opposite occurred when the pure anti-oestrogen ICI 182780 was used instead of tamoxifen (Huynh and Pollak, 1993). In a subsequent study, the same investigators showed that the expression of insulin-like growth factor-binding protein (IGFBP)-3, the principal quantitative binder of IGF-I, was similarly suppressed by oestradiol and tamoxifen (Huynh and Pollak, 1994). More recently, a study on endometrium has confirmed that tamoxifen up-regulates the expression of Ki67, a marker of proliferation, and that of IGF-I (Elkas et al., 1998). In the same study, concomitant decreases in the expression of IGFBP-1 were also detected in endometrium of women treated with tamoxifen.

The dysregulation of transforming growth factor-β (TGFβ) has also been suggested as an additional epigenetic (non-genotoxic) mechanism of tamoxifen carcinogenicity (Carmichael et al., 1998). It has been postulated that the tamoxifen-induced dysregulation of the TGFβ signalling pathway may create an environment which selects for cells with genetic alterations in that signal system (Carmichael et al., 1998). Cells with mutations in
this pathway become refractory to mitosis inhibitory signals, thus developing into end-stage tumours.

**Raloxifene and the endometrium**

Crystallographic studies have confirmed that a critical difference in the anti-oestrogenic action of raloxifene lies in the interaction of the alkylaminoethoxy side-chain with the amino acid aspartate at position 351. The peculiar orientation of this side-chain of the raloxifene molecule, an essential determinant of the anti-oestrogenic properties (Clark and Jordan, 1976), is believed to account for its lack of endometrial activity (Clark and Jordan, 1976; Grese et al., 1997; Bryant and Dere, 1998).

Biochemical data support the lack of agonistic activity of raloxifene on uterine tissue (Somjen et al., 1996). Raloxifene has exhibited little uterotrophic activity in rodents (Black and Goode, 1980, 1981; Black et al., 1983), and has not resulted in increases of uterine weight or in stimulation of epithelial cell height and eosinophilic infiltration (Bryant and Dere, 1998). In other experiments on ovariectomized rats, raloxifene was similar to the no-treatment controls with regard to uterine epithelial cell height, myometrial thickness, and stromal expansion (Black et al., 1994; Sato et al., 1996). There are, however, discrepant data showing increases in uterine weight and uterine epithelial thickness in ovariectomized (Sato et al., 1996) or immature (Ashby et al., 1997) rats. Interestingly, raloxifene has been shown to block the stimulating endometrial effects of oestrogen and tamoxifen (Black et al., 1994; Sato et al., 1996; Kleinman et al., 1996; Bryant and Dere, 1998), an effect confirmed on an endometrial carcinoma cell line grown in athymic mice (Gottardis et al., 1990).

In addition to the experimental studies, over the last few years abundant clinical data has been collected which suggest that the endometrial profile of raloxifene falls in the inactive side. Two studies published in 1997 were unanimous in confirming the lack of endometrial activity of raloxifene in post-menopausal women. In an 8-week study on 208 post-menopausal women, raloxifene was compared with 0.625 mg/day of unopposed conjugated equine oestrogens and with placebo. While 77% of oestrogen-treated women and 15% of placebo-treated women demonstrated significant oestrogenic effects on their endometrial biopsy specimens, none of the 54 women receiving raloxifene modified their baseline inactive endometrium (Boss et al., 1997). The second study included 444 European women who were investigated with transvaginal ultrasonography at baseline and at least once thereafter over a 24-month period when receiving either raloxifene or placebo. There was no difference in endometrial thickness between the two groups of women (Delmas et al., 1997). The measurement of endometrial thickness by endovaginal sonography or saline infusion sonohysterography (SIS), together with the assessment of endometrial histology, were the end-points of two studies performed by Goldstein et al. (1997) on a total of 551 healthy post-menopausal women for a period of 12 months. Raloxifene improved the results obtained by a continuous combined formulation of 0.625 mg of oral conjugated oestrogens plus 2.5 mg of medroxyprogesterone acetate in a first study on 136 women, since hormone replacement therapy but not raloxifene was associated with a higher incidence of vaginal bleeding and with an increase of endometrial thickness and uterine volume. In the second study on 415 women, raloxifene also compared favourably with placebo or 0.625 mg of conjugated oestrogens with respect to endometrial thickness, uterine volume, and endometrial histology. The recently published 3-year follow-up of the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, a multicentre, randomized, double-blind trial in which a total of 7705 women were subjected to either raloxifene or placebo, additionally confirms that raloxifene does not increase the risk of endometrial cancer or, in the subset of women who underwent endometrial biopsy, endometrial hyperplasia (Cummings et al., 1999).

The antagonistic effects of raloxifene on endometrium, however, seem substantially reduced in presence of high concentrations of circulating oestrogens, as suggested by a study in premenopausal women, where only subtle antagonistic changes were observed. The concentrations of ovarian steroids and gonadotrophins were not affected, nor was the length of the cycle (Baker et al., 1998). These findings suggest that raloxifene may be of little help in the treatment of oestrogen-dependent diseases, e.g. endometriosis.

In conclusion, it seems that raloxifene differs from tamoxifen in its impact on uterine tissue. In contrast with the particular stimulatory profile of tamoxifen, both the experimental and the clinical data suggest that raloxifene is inert for both the endometrium and the myometrium. However, the clinical experience with raloxifene is still limited, and data on its long-term effects of the uterus are required. This has been, in fact, one of the observations raised by the Advisory Committee for Reproductive Health Drugs in the evaluation of the data submitted for Food and Drug Administration (FDA) approval of raloxifene (Curtis, 1999).

**Other SERMs and the endometrium**

There is very limited experience with other SERMs regarding endometrial effects. Idoxifene, a parent molecule of tamoxifen, exhibits less agonistic endometrial effect than tamoxifen in laboratory studies with the ovariectomized rat model (Chander et al., 1991; Nuttall et al., 1998). Droloxifene (3-hydroxytamoxifen), has a shorter half-life and a higher affinity for the ER than tamoxifen. In studies in ovariectomized rats, droloxifene exerts no significant uterotrophic effects (Ke et al., 1997). Toremifene, finally, is another triphenylethylene derivative which in rat models has been reported to show less (Hamm, 1997) or similar (Karlsson et al., 1998) uterotrophic effect than tamoxifen. A study on 31 women who were randomized for either toremifene or tamoxifen showed that, after 12 months, both drugs had a similar uterotrophic effect. Together with the growth of uterine fibroids and development of intrauterine polyps, endometrial thickness increased during treatment (Tomas et al., 1995). In this connection, endometrial tumours implanted on athymic, ovariectomized mice grew similarly when the animals were treated with either tamoxifen or toremifene (O’Regan et al., 1998).

In short, the available evidence of all the other SERMs is much lower than for raloxifene or tamoxifen. There is less data, most of which is restricted to the experimental model of the ovariectomized rat. Accordingly, only the information gathered on the uterine effects of tamoxifen and raloxifene may be used at present to draw valid conclusions on clinical grounds. The clinical data suggest that tamoxifen has the potential to induce a
characteristic pattern of endometrial abnormalities, where the inclusion of adenocarcinoma cannot be discarded.Raloxifene activity, in contrast, seems comparable with that of a placebo, but this should still be confirmed with more prolonged clinical studies.

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Brzozowski, A.M., Pike, A.C.W., Dauter, Z. et al. (1998) An epigenetic mechanism of tamoxifen-induced endometrial changes mimicking endometrial abnormalities, where the inclusion of adenocarcinoma cannot be discarded. Raloxifene activity, in contrast, seems comparable with that of a placebo, but this should still be confirmed with more prolonged clinical studies.


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