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TAMOXIFEN: Toxicities and Drug Resistance During the Treatment and Prevention of Breast Cancer

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ABSTRACT

Tamoxifen, a nonsteroidal antiestrogen, is the endocrine therapy of choice for all stages of breast cancer. There are six million women-years of experience with tamoxifen, and the drug has produced survival advantages in node-positive and node-negative patients who have had 2–5 years of adjuvant tamoxifen therapy. A low incidence of side effects has been reported with tamoxifen, resulting in the proposal to use the antiestrogen as a preventive agent for breast cancer. Three separate clinical trials are currently under way—in the United States, Italy, and the United Kingdom. Current concerns about tamoxifen are the development of rat liver tumors during long-term treatment and an increased incidence of endometrial carcinomas observed in patients. Another concern is the development of drug resistance to long-term tamoxifen therapy. There is increased interest in both determining the mechanism of drug resistance and evaluating new antiestrogens that may be more beneficial as a preventive, as an adjuvant therapy, or for the treatment of advanced breast cancer.

INTRODUCTION

Tamoxifen is a nonsteroidal antiestrogen (1–4) that exhibits antitumor properties in laboratory animals (5–9). Although many compounds were investi-

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gated in the 1960s and 70s (10), only tamoxifen was successfully developed in the laboratory for the treatment of breast cancer (10–12). The initial focus of anti–breast cancer drug development in the 1970s was as a palliative therapy for advanced breast cancer. However, adjuvant therapy and the exploration of long-term tamoxifen therapy (13, 14) has revolutionized breast cancer treatment. An overview analysis of randomized clinical trials of adjuvant tamoxifen therapy demonstrates that long-term (i.e. more than one year) adjuvant tamoxifen is an appropriate strategy to control the recurrence of both node-positive and node-negative breast cancer (15). Tamoxifen confers a survival advantage to those women with breast cancer who are treated for more than two years. Tamoxifen is the hormonal treatment of choice for all stages of breast cancer.

The use of tamoxifen as a treatment for breast cancer has over six million women-years of experience. The drug has a low reported incidence of serious side effects (16), and five years of therapy is commonplace (17).

The success of tamoxifen as a treatment for breast cancer has fueled interest in the drug as a preventive agent. Estrogen is known to promote the development of breast cancer, so it is only natural that an antiestrogenic drug that has been extensively clinically tested would be the leading candidate for evaluation. The pharmacology of tamoxifen is complex (18, 19), but the scientific rationale for testing tamoxifen has merit. An important component of the drug's pharmacology is the target-site specificity of tamoxifen; the drug can act as an antitumor agent (probably as an antiestrogen) in the breast but can also be estrogenic at physiological sites to prevent bone loss and decrease circulating cholesterol.

Animal studies demonstrate that tamoxifen prevents mammary carcinogenesis (5, 7, 20–22), and clinical studies show that adjuvant tamoxifen therapy decreases the incidence of second primary breast cancers by 40% (15). Postmenopausal bone density is maintained by tamoxifen treatment (23–25), which could ultimately lead to the prevention of osteoporosis. Tamoxifen also decreases low-density lipoprotein cholesterol levels in postmenopausal women (26–28). This positive property of tamoxifen may be responsible for the decrease in hospital visits for the treatment of any cardiac condition (29) and the significant decrease in fatal myocardial infarction for women treated for five years with tamoxifen (30, 31). These data provide a scientific basis for placebo-controlled clinical trials to test tamoxifen's ability to prevent breast cancer.

PREVENTION STUDIES

Unlike the laboratory models of mammary tumorigenesis, where all animals develop tumors and the efficacy of tamoxifen is readily demonstrated, targeting

the appropriate population at risk for breast cancer is difficult. Numerous risk factors have been identified, and these have been reviewed elsewhere (32). However, because the incidence of breast cancer is small in the general population, women volunteers with a high-risk profile must be recruited. It is essential to design a double-blind, placebo-controlled trial, but the large numbers of volunteers required and the long time period necessary to obtain a statistically significant result mandates data management, compliance monitoring, and an enormous clinical trials effort.

Three clinical trials are currently recruiting and following volunteers to test tamoxifen's ability to prevent breast cancer. The first trial was begun at the Royal Marsden Hospital in 1986 (33, 34). The initial goal was to recruit 2000 women as a Vanguard study and monitor the progress of the volunteers. Healthy women aged 30–70 are eligible provided they have a family history of breast cancer on the maternal side, with at least one first-degree relative (sister, mother, daughter) under the age of 45 years who has developed breast cancer or bilateral breast cancer or with a first-degree relative and at least one other maternal relative affected. The women are randomly assigned to receive 20 mg of tamoxifen or a placebo daily for at least eight years. At five years (by June 1993) compliance for the 2012 women assigned to the tamoxifen arm of the study was greater than 70% (35).

There is a significant increase in hot flashes (34% vs 20%), vaginal discharge (16% vs 4%), and menstrual irregularities (14% vs 9%) for tamoxifen- vs placebo-treated women. Safety monitoring shows no obvious effects on radial bone mineral density, but fibrogen, antithrombin III, and cholesterol levels decrease out to five years in the tamoxifen-treated women.

Most importantly, the Marsden study demonstrates an increased incidence of uterine fibroids and benign ovarian cysts as a result of tamoxifen treatment. An in-depth study (36) of the postmenopausal women demonstrated that tamoxifen causes potentially malignant changes in the endometrium, but transvaginal ultrasonography can be used to identify those women who should be monitored. These findings resulted in approval by the Department of Health (July 1993) to recruit an additional 15,000 women volunteers from sites around the United Kingdom. Recruitment of additional volunteers has also been conducted for more than a year in Australia.

The second prevention trial began recruiting volunteers from throughout North America in May 1992. The study, funded by the National Cancer Institute, will recruit 16,000 women who will be randomly assigned to be treated with tamoxifen (20 mg daily) or a placebo for five years. Those eligible for entry into the study include any woman over the age of 60 or women between the ages of 35 and 59 years whose five-year risk of developing breast cancer, as predicted by the Gail model (37), equals that of a 60-year-old woman. Any woman over 35 years of age, with a diagnosis of lobular carci-

noma in situ (LCIS) treated with biopsy alone, is eligible for entry. In the absence of LCIS, the risk factors for entry vary with age, so a 35-year-old woman must have a relative risk of at least 5, while a 45-year-old woman's relative risk must be 1.8 to be eligible for entry.

Seven thousand women were recruited in the first year. Pretrial concerns that younger women at risk would not volunteer for the trial are not substantiated by the population distribution. About one third of the volunteers are 35–50 years old, with relative risks ranging on average between 10 for the youngest participants to 4 for the 50-year-olds.

By December 1993, 11,000 women had been recruited to the prevention trial. Administrative concerns about the development of uterine carcinoma during tamoxifen treatment halted recruitment to National Surgical Adjuvant Breast Project (NSABP) trials for several months during 1994, but recruitment was reinitiated in June 1994 after the Food and Drug Administration had reviewed the concerns.

The final tamoxifen prevention trial was initiated in Italy (38) by the European Institute of Oncology and the Milan Cancer Institute. Up to 20,000 volunteers who are over the age of 45 but who have already had a hysterectomy will be recruited. The aim of these restrictions is to avoid the complications of both pregnancy and endometrial cancer. Volunteers are being randomly assigned to tamoxifen (20 mg daily) or a placebo for five years. There were more than 3000 volunteers recruited by July 1993.

For the first time, the clinical trials community is evaluating a therapy to prevent breast cancer. Although the majority of women recruited to the trials will not develop breast cancer, they will experience symptoms and side effects from tamoxifen. The evaluation of the toxicity of tamoxifen in the trials is extremely important, not only to determine the therapeutic value of the intervention but also to assess whether compliance can be maintained by the study population. Extra attention is being paid to chronic toxicities.

TOXICITIES OF TAMOXIFEN

Considerable concern has been expressed about the potential toxic effects of tamoxifen that could become critical in any evaluation of the drug given to women without breast cancer. These concerns are listed in Table 1 and have been the subject of a recent commentary (39). Ocular problems and the small increase in thromboembolic disease has been adequately reviewed (16), but the potential of tamoxifen to be carcinogenic is a serious risk.

In the laboratory, tamoxifen can stimulate the growth of human endometrial carcinoma but can block the growth of a breast tumor transplanted in the same immune-deficient mouse (40). This possibility was demonstrated in patients when a 40% decrease in second primary breast cancers but a threefold increase

Table 1 Potential long-term toxicities from tamoxifen therapy

Ocular problems
Thromboembolic disorders
Endometrial cancers
Liver cancer

in endometrial cancer was observed in an adjuvant clinical trial of tamoxifen (41). Seventeen endometrial cancers were reported for the 1000 patients taking tamoxifen after eight years of evaluation. The rate of detection is two per thousand per year (42). A similar rate is reported by the NSABP (43). However, the NSABP study reports 6 deaths out of the 23 patients who developed endometrial carcinoma over the 6 years of evaluation for the 2639 women (43). The causes of death and the association with the duration of tamoxifen treatment are shown in Table 2. It is clear that women must be monitored for the development of endometrial carcinoma, but perhaps most importantly, patients must be screened before therapy to ensure that preexisting endometrial carcinoma is not present.

Investigators are currently interested in determining whether tamoxifen produces a higher-grade, more aggressive endometrial carcinoma. Initial reports from the Yale–New Haven tumor registry suggested that women were "at risk for high-grade endometrial cancers that have a poor prognosis" (44). Current comparisons of histological grade and stage in patients treated with tamoxifen (42, 43, 45) demonstrate the same proportions noted for the general population.

Table 2 Patients randomly assigned to the tamoxifen arms of NSABP B_{14} who died of EC[a]

Patient	Age	Time on tamoxifen (months)	Diagnosis of EC after tamoxifen (months)	Cause of death
1	66	Never took tamoxifen	—	EC
2	68	5	0	CV disease[b]
3	63	9	0	EC
4	58	22	73	EC
5	54	42	23	EC
6	68	65	0	Pulmonary embolism

[a] Endometrial cancer
[b] Cardiovascular disease

Nevertheless, tamoxifen does not retard the development of endometrial carcinoma, and clonal selection may result in premature changes in pathology. These findings contrast with the effects of estrogens that only promote growth.

In contrast, the concern about hepatocellular carcinoma is based upon laboratory studies alone. Numerous reports show that large doses of tamoxifen produce liver tumors in rats (46–48). Tamoxifen produces DNA adducts in rat liver (48–50) and protein adducts in vitro (51). It is hypothesized that tamoxifen can become metabolically activated through selective hydroxylation to form an unstable alkylating species (52). Nevertheless, even though DNA adducts can be formed by human liver microsomes in vitro (53), no practical demonstration of DNA adduct formation has occured in humans. Indeed, there have been no reports of hepatocellular carcinoma in women taking 20 mg tamoxifen daily and only two reports of hepatocellular carcinoma in women who took 40 mg daily (41). Part of the problem is that the tumor is so rare—5 per 100,000 of the population—that even a tenfold rise would be difficult to detect.

The striking differences observed for the toxicology for tamoxifen in the rat may be species and dose related. Tamoxifen is used therapeutically at a dose of 250 μg/kg in both humans and rats (14). In contrast, rats are given 12 mg/kg of tamoxifen for half their lifetime to produce liver tumors. This regimen (48) is approximately 40 times the human therapeutic dose given for about 8 times as long as the relative human duration, which is five years or 6% of a woman's life. The excessive doses of tamoxifen given to the rat could be overwhelming the capacity of the liver to such a degree that the rat must invoke unique metabolic routes to cope with the overdosing schedule. The equivalent experiment in humans would be a woman taking 800 mg tamoxifen daily for 40 years.

The relationship of liver carcinogenesis and tamoxifen is undoubtedly an important area of toxicology, not only to protect the treatment population but also to determine the relevance of certain animal models to human disease processes.

Overall, the broad use of tamoxifen, both as a treatment and as a preventive agent in clinical trials has necessitated increased vigilance by the clinical community to identify untoward side effects as rapidly as possible. Tamoxifen is an effective therapy for breast cancer, and it has become an important cornerstone of treatment strategies. However, drug resistance is the inevitable result of any long-term therapy. The discovery of the processes of growth deregulation will provide additional therapeutic opportunities for the future.

DRUG RESISTANCE

The potential molecular mechanisms of resistance to antiestrogen therapy have recently been surveyed (54), but several new developments deserve comment.

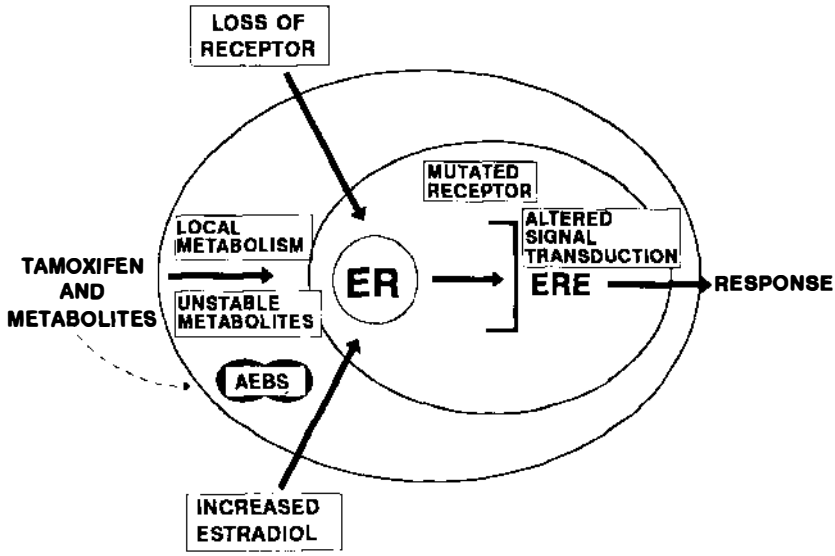

Figure 1 The potential mechanisms of drug resistance against tamoxifen in human breast cancers. AEBP = antiestrogen binding protein. ER = estrogen receptor.

Three major areas of concern are increased estrogen levels observed in premenopausal women during tamoxifen therapy, local tumor metabolism to unstable compounds that stimulate tumor growth, and the isolation of mutant estrogen receptors from tumors stimulated to grow by tamoxifen. These possibilities are illustrated in Figure 1.

High Estrogen Levels

Tamoxifen causes an elevation in circulating estrogen levels in premenopausal patients (55–58), and a high estrogen environment could reverse the antitumor actions of tamoxifen. Tamoxifen is most effective in vitro in a low-estrogen environment; however, in vivo, tamoxifen is converted to a range of antiestrogens with high affinity for the receptor. The reversal of the actions of tamoxifen as an antitumor agent is complicated. Studies in athymic mice demonstrate that high levels of circulating estradiol (>1600 pg/ml) will partially reverse the growth inhibitory effects of low levels (40 ng/ml) of circulating tamoxifen (59). Clinical experience demonstrates that tamoxifen alone is effective in the control of Stage I and IV breast cancer in premenopausal patients (15, 60–62). Nevertheless, if patients with Stage IV disease initially respond and then fail treatment, there is a 30% probability of a second response to oophorectomy (63).

Local Metabolism

The pharmacokinetics and metabolism of tamoxifen have been extensively studied in patients (64–66), and there is no evidence that high levels of estrogenic metabolites are produced during long-term therapy. However, the tumor cells or the stromal component could locally metabolize tamoxifen to potent estrogens that could stimulate tumor growth. In the laboratory long-term tamoxifen treatment will eventually cause the growth of MCF-7 breast cancer cells transplanted into athymic mice (67, 68). The tumors are estrogen-receptor positive and grow in either athymic rats or athymic mice in response to either estradiol or tamoxifen (68, 69). Pure antiestrogens will block tamoxifen-stimulated growth; therefore, tamoxifen must be converted to estrogens locally to stimulate growth through the estrogen receptor (70).

Tamoxifen is metabolized to 4-hydroxytamoxifen in the mouse (71). This metabolite is a potent antiestrogen (72) that is known to have antitumor activity in the athymic mouse model (73). However, the potent antiestrogenic Z isomer is unstable (74, 75) and can be converted to the weakly antiestrogenic E isomer (76–78). If the isomerization occurs locally, the net antiestrogenicity of tamoxifen will decrease, but this would not in itself account for increased tumor growth because an estrogenic stimulus is required. Minute amounts of Metabolite E (tamoxifen without the dimethylaminoethane side chain) have been detected in human tumors during tamoxifen therapy (79). Fortunately, this metabolite of tamoxifen is too weakly estrogenic to promote tumor growth alone (64, 78). Nevertheless, the metabolite is unstable and can isomerize to a potent estrogen (78). If large quantities of this estrogenic metabolite can accumulate in the tumors, they could be the stimulus for tamoxifen-stimulated tumor growth. The hypothesis (80, 81) that tamoxifen-stimulated growth depends upon the simultaneous isomerization of metabolites to weak antiestrogens and potent estrogens is illustrated in Figure 2.

We recently addressed the question of metabolite isomerization as the mechanism of tamoxifen-stimulated growth by determining the ability of tamoxifen derivatives, which cannot isomerize, to cause tumor growth. We found that a fixed-ring version of tamoxifen (Figure 2) can adequately support and develop ligand-stimulated tumor growth (82). Osborne and coworkers (83) have confirmed our finding but also report that the related compound toremifene (see section on new agents) stimulates tumor growth, as does a tamoxifen derivative lacking the ether oxygen in the alkylaminoethoxy side chain. This latter compound cannot form Metabolite E, so the hypothesis is untenable. At present it is unclear how tamoxifen-stimulated tumor growth occurs within the cell, but one possibility is that the development of mutated estrogen receptors could alter the pharmacology of the antagonist to an agonist.

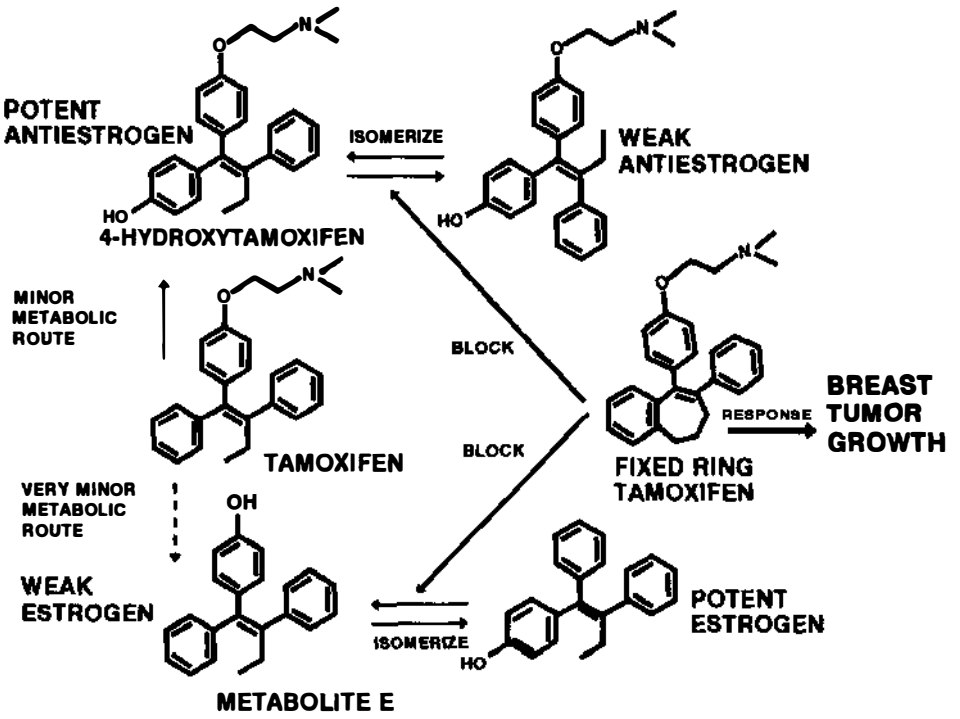

Figure 2 A proposed scheme for the metabolism of tamoxifen in breast tumors that could cause tamoxifen-stimulated growth. Tamoxifen could be converted to the potent antiestrogen 4-hydroxytamoxifen and the weak estrogen referred to as Metabolite E. The key event in the hypothesis is the ability of the metabolites in the tumor cells to isomerize to a weak antiestrogen and a potent estrogen. The fixed-ring derivative of tamoxifen that cannot isomerize is biologically active at promoting growth of tamoxifen-dependent breast tumors. This observation makes the proposed scheme unlikely to be the major mechanism for tamoxifen-stimulated growth.

Mutated Estrogen Receptors

There is considerable interest in determining the biological relevance of mutant steroid hormone receptors. Laboratory models have demonstrated that specific mutations of the androgen (84) and progesterone receptors (85) can change the biological properties of antiandrogens and antiprogestins to full agonist molecules. Therefore, mutations in the estrogen receptor that change the pharmacology of an antiestrogen to an estrogen could explain tamoxifen-stimulated growth in tumors.

The screening of clinical tumor material and cell lines has resulted in the identification of several mutations of the estrogen receptor (86–88), but the

biological relevance of these findings is unclear. However, the impact of point mutations in the estrogen receptor on the pharmacology of antiestrogens can be examined under laboratory conditions. If MDA-MB-231 breast cancer cells (receptor negative) are transfected with either a wild-type estrogen receptor (ER) gene or an ER gene with a glycine-to-valine mutation at amino acid 400, the resulting transfected clones will respond to estrogen by decreasing growth (89). This laboratory model then becomes a test of the estrogenicity of any ligand receptor complex under controlled conditions. Pure antiestrogens prevent the inhibitory effect of estradiol in both wild-type and mutant cDNA (HEO) transfectants (89).

In contrast, the antiestrogens that are normally partial agonists in assays involving wild-type receptors only express estrogenic activity in HEO transfected cells (90, 91). We have proposed a model that describes the changes in the folding of the mutant receptor around the antiestrogen that produces an estrogenic coupling (91). However, the HEO mutant is known to be a laboratory cloning artifact, and until recently, no single-point mutations of the receptor had been observed in nature.

Tamoxifen-stimulated MCF-7 breast tumors that grow in athymic mice appear to have normal estrogen receptors. However, a screen of mRNAs for estrogen receptors in tamoxifen-stimulated tumors, using first reverse transcriptase and polymerase chain reaction followed by single-stranded conformational polymorphism, revealed a tumor line containing an estrogen receptor with a mutation (92). The error is a single-point mutation in the codon, which converts an aspartate to a tyrosine at AA351 in the steroid-binding domain (93). Preliminary studies with the cDNA for the novel mutant receptor demonstrate high activity for the protein when MDA-MB-231 cells are transfected with the gene and, most importantly, a conversion of the pharmacology of nonsteroidal antiestrogens to potent estrogenic activity (94). Although the development of drug resistance to tamoxifen is clearly not caused by mutation of the estrogen receptor alone, this mutation may be one of the many mechanisms that come into play during long-term tamoxifen treatment.

A drug development program is well under way by the pharmaceutical industry to find either new first-line antiestrogens, which are less toxic than tamoxifen, or new second-line antiestrogenic agents to be used after tamoxifen treatment failure.

CONCLUSIONS AND FUTURE DEVELOPMENTS

The enormous success of tamoxifen as a first-line endocrine therapy for all stages of breast cancer has encouraged a search for alternative antiestrogens that might ultimately replace, or at least compliment, tamoxifen. Extensive clinical testing of a number of tamoxifen derivatives is under way. Toremifene

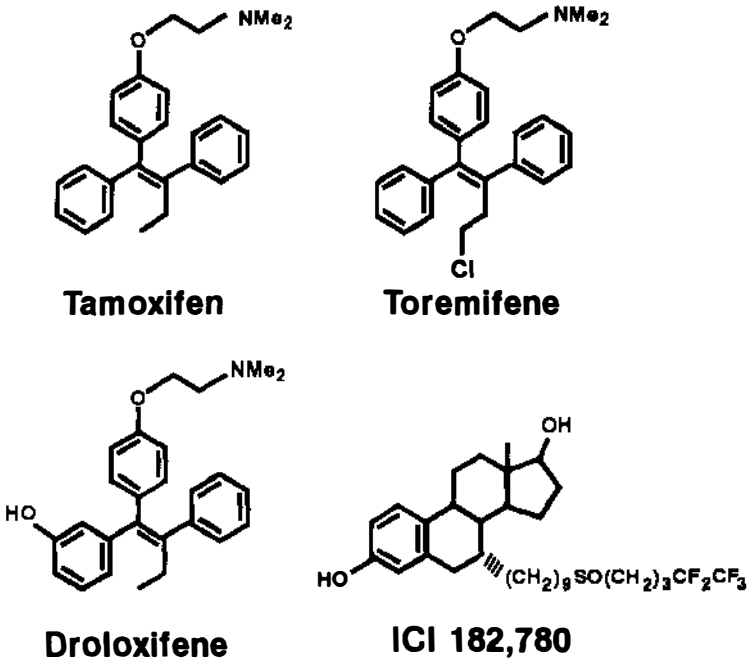

Figure 3 The formulae of new antiestrogens for breast cancer therapy.

(95, 96) and droloxifene (97) are completing phase III trials against tamoxifen in postmenopausal women (Figure 3). Idoxifene (98) is entering phase II trials in the United Kingdom.

The pure antiestrogen ICI 182,780 (Figure 3) is in clinical trials in the United Kingdom (99) and offers the advantage that it could be used as a second-line therapy if long-term adjuvant treatment results in tamoxifen-stimulated tumor growth. This principle has been demonstrated in the laboratory (70). The pure antiestrogens have a complete inhibitory effect on estrogen action in the primate uterus (100), but most importantly, the mode of action appears to be different than the nonsteroidal antiestrogens. Pure antiestrogens cause the loss of estrogen receptor from tumors and estrogen target tissues (101–103); thus the tissue becomes refractory to additional estrogenic stimulation.

Finally, new antiestrogens could be targeted for novel applications. The nonsteroidal antiestrogen keoxifene (now renamed raloxifene) (Figure 4) preserves bone density in the ovariectomized rat (104, 105), and large doses will prevent the development of rat mammary tumors (21). The compound also decreases circulating cholesterol in the rat (105) and has only a weak agonist

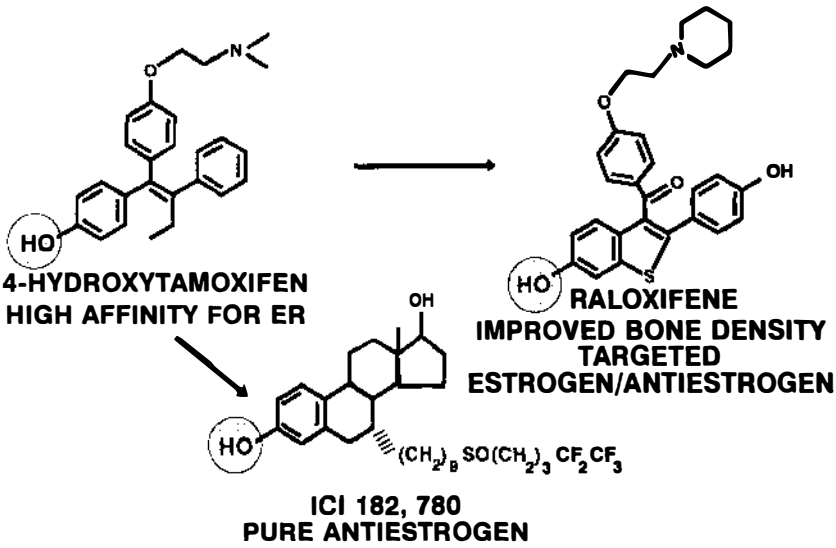

Figure 4 The new antiestrogen strategies to be developed based on the knowledge about the high binding affinity of 4-hydroxytamoxifen for the estrogen receptor. Each of the new agents has high binding to the estrogen receptor, but unlike the pure antiestrogen ICI 182,780, the antiestrogen raloxifene has target site–specific effects and will be used to treat osteoporosis.

effect in the rat and mouse uterus (105). One could also predict that there is a low probability of rat liver carcinogenesis.

Raloxifene is being developed as a treatment for osteoporosis in postmenopausal women. The large numbers of postmenopausal women who would be treated with raloxifene to prevent osteoporosis might also be protected from coronary heart disease and breast cancer and have with a low probability of developing endometrial carcinoma and hepatocellular carcinoma. A future decrease in the incidence of breast cancer may occur as a positive side effect from the prevention of osteoporosis by an antiestrogen with targeted estrogenic properties.

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