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Long-term adjuvant tamoxifen therapy for breast cancer

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Key words: antiestrogen, tamoxifen metabolites, MCF-7 cells, adjuvant therapy, node-positive breast cancer

Abstract

Tamoxifen (ICI46,474) is a competitive inhibitor of estrogen action which has found ubiquitous application in the treatment of breast cancer. The drug is the front line endocrine therapy for breast cancer and is the proven treatment of choice for the adjuvant therapy of postmenopausal women with node-positive disease. Tamoxifen is available for the treatment of premenopausal patients with advanced disease, and is being evaluated in clinical trials as an adjuvant therapy for premenopausal patients with either node-positive or node-negative disease. Laboratory studies demonstrate that tamoxifen is a tumoristatic agent and long-term treatment strategies (chemosuppression) should be considered to apply the antiestrogen to its maximal therapeutic advantage. Optimal therapy with tamoxifen may also be achieved by treatment strategies to lower circulating estrogen levels in the premenopausal patient.

Tamoxifen is a well tolerated drug, and long-term therapy does not appear to induce metabolic tolerance. Concerns about premature osteoporosis or cardiovascular disease appear to be unfounded because tamoxifen has an appropriate level of target site-directed estrogenic activity. Isolated reports about the growth or appearance of endometrial carcinoma during long-term adjuvant tamoxifen therapy must be balanced against the risks of withholding treatment to patients with a fatal disease.

Introduction

Tamoxifen (ICI46,474, Fig. 1), a non-steroidal antiestrogen [1], was originally introduced for the treatment of advanced breast cancer in postmenopausal women [2–4]. However, the demonstrated efficacy and low incidence of side effects [5] has permitted the development of tamoxifen for the effective adjuvant treatment of node-positive breast cancer [6–8]. The drug is now considered to be the front line endocrine treatment for breast cancer.

The widespread acceptance of tamoxifen by the medical community has naturally lead to the investigation of additional treatment strategies. Increasing numbers of premenopausal women with advanced breast cancer are receiving effective palliation with tamoxifen [9–11], and clinical trials are recruiting premenopausal women with both node-negative and node-positive disease [12, 13] to evaluate efficacy.

The aim of this article is to assess the progress being made to evaluate the strategy of long-term adjuvant tamoxifen therapy to suppress the recurrence of the disease following mastectomy. All the original clinical trials evaluated 1 or 2 years of

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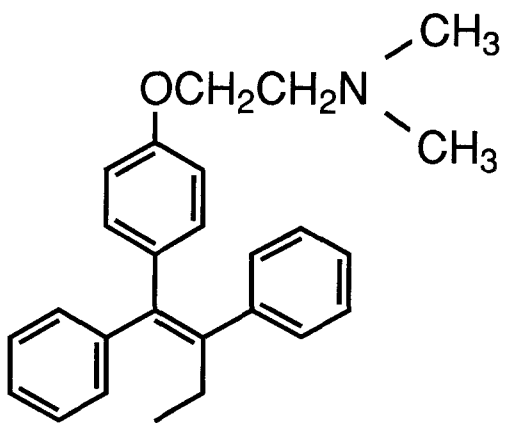

Fig. 1. The structure of tamoxifen.

tamoxifen. Although this approach has merit [14], the laboratory data [15] points to the use of tamoxifen as a long-term chemosuppressive agent. The therapeutic value of this clinical approach will be balanced against perceived long-term side effects.

Mode of action of tamoxifen in vitro

Tamoxifen inhibits the binding of [^{3}H]estradiol to estrogen receptors derived from human breast tumors [16]. Tamoxifen is a competitive inhibitor of estrogen binding *in vitro* [17], and increasing concentrations of estradiol will reverse the inhibitory action of tamoxifen on the growth of hormone-responsive MCF-7 breast cancer cells [18]. Nevertheless, large concentrations of antiestrogen will produce estrogen-irreversible effects on breast cancer cell growth *in vitro* [19, 20]. It remains speculative that estrogen receptor independent actions play a significant role in the antitumor actions of tamoxifen in patients.

Estrogen-stimulated growth has been the subject of intense investigation during the past 20 years. Progress has been facilitated by the development of estrogen-responsive breast cancer cell lines [21–23] and suitable culture conditions to study estrogen action [24]. There is still considerable debate about the direct or indirect effects of estradiol. However, the concepts of estrogen-regulated growth are evolving with the application of autocrine and paracrine growth factor models [25] as a hypothesis to explain the ability of estrogen to shorten the breast cancer cell cycle.

Estrogen (Fig. 2) appears to be able to initiate a cascade of subcellular events, e.g. induction of progesterone receptor [26], increase in transforming growth factor α (TGFα) mRNA [27], and decrease in transforming growth factor β (TGFβ) [28]. Antiestrogens like tamoxifen can block these actions (Fig. 3). In fact, it has been suggested that TGFβ could act as a paracrine inhibitory factor on hormone independent breast cancer cells [28]. Estrogen receptor negative cells, unlike receptor positive cells, are particularly sensitive to the inhibitory actions of TGFβ [29]. Therefore, in a breast tumor that is predominantly estrogen receptor positive, it could be possible that TGFβ produced in the receptor positive cells will control the growth of estrogen receptor negative clones. Unfortunately this hypothesis has proved to be difficult to test successfully *in vivo* [30].

Mode of action of tamoxifen in animal models

Tamoxifen was initially evaluated for a whole range of clinical indications [31–33]. However, the efficacy in the treatment of breast cancer coupled with the parallel studies in animal models of breast cancer has established the drug as the 'gold standard' for endocrine therapy [34]. Two experimental systems, the dimethylbenzanthracene (DMBA)-induced rat mammary carcinoma model and the immune deficient (athymic) mouse implanted with breast cancer cell lines, have provided valuable information to support the use of long-term adjuvant tamoxifen therapy as a chemosuppressive strategy in breast cancer.

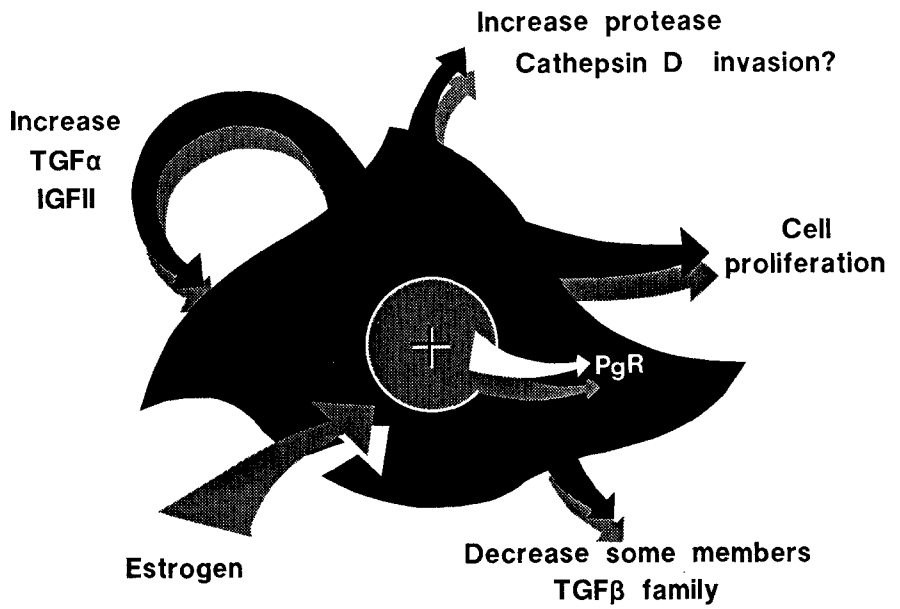

Fig. 2. The subcellular effects of estrogen in causing the replication of breast cancer cells (usually MCF-7) *in vitro*. Progesterone receptor (PgR), transforming growth factor α (TGFα), insulin-like growth factor II (IGFII), transforming growth factor β (TGFβ).

Carcinogen-induced rat mammary carcinoma models

Mammary tumors can be induced by a single feeding (20 mg in 2 ml peanut oil) of DMBA to 50 day old female Sprague Dawley rats [35]. Palpable hormone responsive tumors appear about 3–6 months later. Tamoxifen inhibits the initiation [36, 37] and growth of established DMBA-induced tumors [38–42]. However, the value of this test system has been to determine whether short term tamoxifen therapy after the DMBA would produce a tumoricidal effect and cure the animals. The administration of tamoxifen at various doses or at various times after the carcinogenic insult [43, 44] only causes a delay in tumor appearance and, ultimately, the majority of animals have mammary tumors. The administration of continuous daily tamoxifen therapy after DMBA causes a complete suppression of tumorigenesis [45–47], but when treatment with tamoxifen is stopped tumor growth recurs [48].

These principles have been confirmed in the N-nitrosomethyl urea (NMU) model of rat mammary carcinogenesis [49]. A short course of tamoxifen therapy after NMU delays the appearance of mammary tumors [50] but continuous therapy suppresses tumorigenesis [52]. Again, tumors appear if tamoxifen treatment is stopped [51].

Athymic animal models of hormone dependent breast cancer

Primary breast cancers can be transplanted into athymic (immune deficient) mice [51], but it has proved to be difficult to develop a reproducible model to study hormone-dependent primary breast cancer. Hormone-responsive breast cancer cell lines, which in the main have been established in culture from pleural effusions, can be grown into solid tumors in estrogen-supplemented ovariectomized athymic mice [53, 54]. The hormone-responsive tumors do not grow without estrogen treatment, while hormone-independent breast cancer cell lines do not require estrogen for growth. Tamoxifen and its metabolites will inhibit the estrogen-stimulated growth of MCF-7 tumors [55, 56], but once tamoxifen treatment is stopped tumor growth continues in the face of unopposed estrogen stimulation [55, 57].

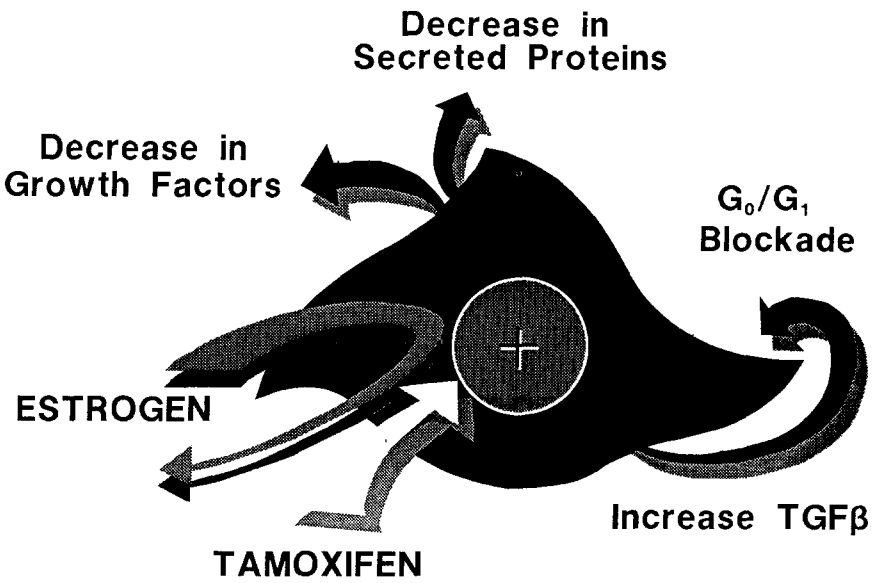

Fig. 3. The competitive action of tamoxifen to inhibit the binding of estrogen to nuclear estrogen receptors (+). The key to the figure is the same as in Figure 2.

The question can be posed whether the action of tamoxifen can be reversed with increasing levels of circulating estrogen. Indeed, these studies have just been completed in our laboratory, and the results demonstrate the competitive nature of the relationship of tamoxifen and estradiol *in vivo* [Fig. 4, 58]. Tamoxifen can inhibit estradiol-stimulated growth; however, if the level of circulating estrogen is increased 4–5 fold, the effect of tamoxifen as an antitumor agent is reversed.

Alternatively, the question can be asked whether, in the absence of circulating estradiol, tamoxifen can destroy implanted MCF-7 cells. Tamoxifen treatment of animals for up to six months does not result in the demonstration of a tumoricidal action of the drug. Estrogen can re-activate tumor growth after 6 months of tamoxifen therapy [59]. In fact, tumor growth may resume despite continuous tamoxifen therapy [60, 61]. Repeated transplantation of the growing tumors into tamoxifen-treated mice results in a tumor variant (MCF-7 TAM) that is facilitated to growth by tamoxifen. Growth is, however, reduced when tamoxifen treatment is stopped [62]. The tumors have estrogen receptors, and the tamoxifen-facilitated growth may be caused by its weak estrogen-like qualities [63]. Pure antiestrogens such as ICI164,384 [64] will inhibit tamoxifen-stimulated MCF-7 tumor growth [62].

Tamoxifen-stimulated tumor growth has not been described routinely in the clinics, but it is possible that this may occur after many years of adjuvant therapy. An interesting feature of the animal model is the finding that the MCF-7 tumor will also grow with estrogen treatment [62]. Therefore, if this form of tamoxifen drug resistance should occur in the clinics, it will not be sufficient to withdraw tamoxifen alone; the patient's own estrogen could support tumor growth. Nonestrogenic antiestrogens are currently being tested for clinical application. These new drugs may be of value as second line therapies [62, 64]. Indeed, the principle may already have been demonstrated with the finding that patients with advanced disease who respond, but then fail tamoxifen therapy, can respond to aminoglutethimide therapy [65]. The reverse is uncommon. If it is possible for a tumor to be dependent on tamoxifen for growth then a withdrawal response should be observed, similar to that documented for high-dose estrogen therapy [66]. The fact that this is rarely observed is probably a

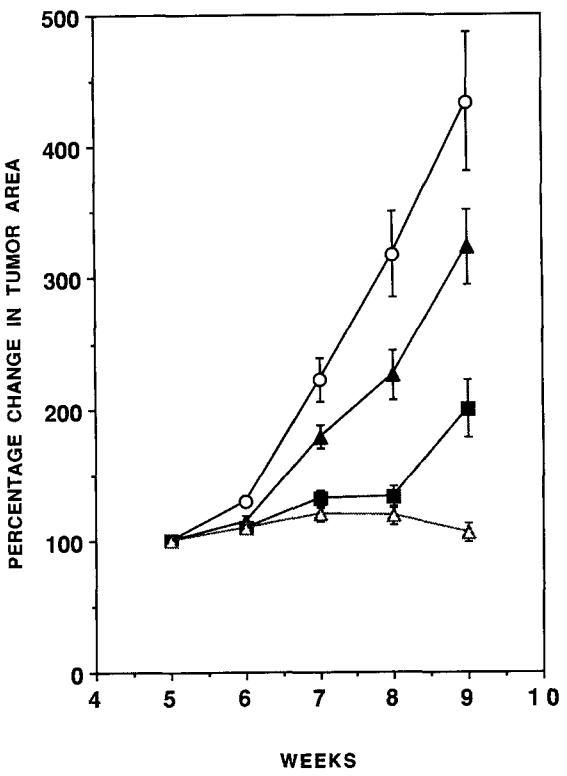

Fig. 4. The reversal of the action of tamoxifen on estradiol-stimulated MCF-7 tumor growth in athymic mice. Breast tumors were grown to approx. 0.5 cm^2 with a 1 cm silastic implant of estradiol before tamoxifen (2 cm silastic implant producing tamoxifen levels of 50 ng/ml) was implanted. Tamoxifen inhibits the growth of tumors. However, the implantation of delivery systems that produce higher circulating levels of estradiol causes increased tumor growth. The groups are: △ tamoxifen alone (no E_2), 9 ± 4 pg/ml; ○, E_2 alone, 755 ± 152 pg/ml; ■ tamoxifen + E_2, 543 ± 42 pg/ml; ▲ tamoxifen + E_2, 1950 ± 558 pg/ml.

reflection of the prolonged biological half life of tamoxifen.

Clinical pharmacology

Tamoxifen is readily absorbed following oral administration. The recommended daily dose (depending upon the country) is 10 or 20 mg bid or 10 mg tid. However, the long plasma half-life (7 days) at steady state (achieved after 30 days of administration) probably makes it unnecessary to use divided daily doses.

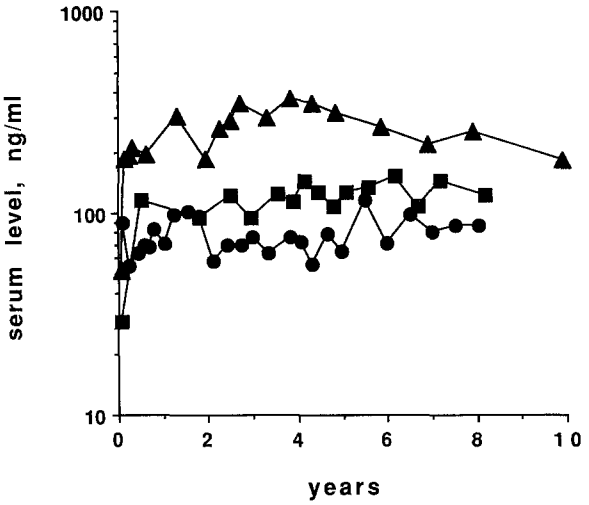

Fig. 5. The serum tamoxifen levels in patients receiving 10 mg bid for the long-term adjuvant treatment of node-positive breast cancer. Three patients (■, ▲, ●) have been treated for up to 10 years with tamoxifen.

The range of tamoxifen serum levels is extremely large (50–300 ng/ml), and this variability is important if patient compliance becomes an issue during long-term adjuvant tamoxifen therapy. A single determination from a patient can only really provide information about whether the patient is taking the drug or not. Several determinations must be made over a period of months to establish the steady state level for the individual.

In conjunction with Dr. Douglass Tormey we have monitored the serum levels of patients receiving adjuvant tamoxifen therapy (10 mg bid) to prevent the recurrence of node-positive breast cancer. Serum levels are stable for the ten years of therapy (Fig. 5) and there are no unusual estrogenic metabolites. We have monitored 10 patients for up to 10 years and we cannot detect any evidence of metabolic tolerance.

Tamoxifen [67] is extensively metabolized in patients (Fig. 6). The principal metabolite is N-desmethyltamoxifen [68] which may be further converted to Metabolite Y [69]. A minor metabolite is 4-hydroxytamoxifen; however, this compound has a binding affinity for the estrogen receptor 20–30x that of tamoxifen [70]. Figure 7 shows the relative levels of various metabolites during the long-term adjuvant treatment with tamoxifen for node-posi-

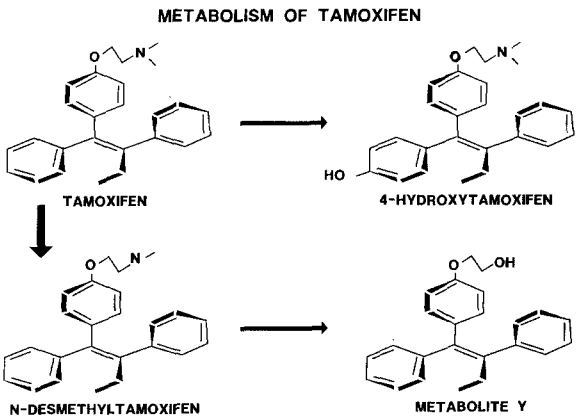

Fig. 6. The metabolism of tamoxifen in patients.

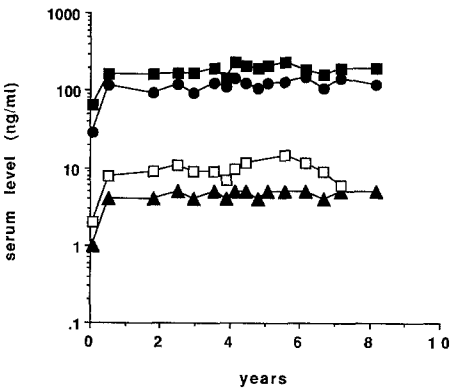

Fig. 7. The steady state serum levels of tamoxifen and its metabolites during the long-term adjuvant treatment of node-positive breast cancer. ▲ 4-hydroxytamoxifen, □ Metabolite Y, ● tamoxifen, ■ N-desmethyl-tamoxifen.

tive breast cancer. Each of these metabolites exhibits antiestrogenic properties which probably contribute to the overall efficacy of tamoxifen as an antitumor agent [71, 72].

Long-term adjuvant tamoxifen therapy

The majority of the clinical trials that were established to test the efficacy of tamoxifen therapy chose to use one or two years of treatment. This strategy was the result of several factors: 1) the short period of therapy might produce a tumoricidal effect before the onset of drug resistance; 2) there was a general belief that extended tamoxifen therapy would only encourage the outgrowth of aggressive receptor-negative disease; and 3) there were uncertainties about the long-term toxicological effects of tamoxifen.

The recent overview of randomized clinical trials has established that tamoxifen therapy for up to two years can result in an increase in the survival of node-positive postmenopausal patients [14]. The situation with regard to premenopausal women is less encouraging, as tamoxifen does not seem to provide benefit over and above combination chemotherapy (most trials use tamoxifen with chemotherapy in premenopausal patients).

Swayed by the encouraging clinical trials data and the laboratory studies [15], most clinical trials organizations are focused upon an evaluation of 5 or 10 years of adjuvant tamoxifen therapy for node-positive (and in some cases, node-negative) disease.

In 1977, based upon the encouraging laboratory data [43, 45], Dr. Douglass Tormey organized a pilot (nonrandomized) clinical study to evaluate the toxicology and efficacy of long-term tamoxifen in node-positive breast cancer patients. Three groups of women were selected (pre- and postmenopausal women with no bias towards receptor status): chemotherapy, chemotherapy plus tamoxifen, or chemotherapy and then tamoxifen alone up to five years [73]. The results demonstrated the safety of tamoxifen and provided the preliminary data to establish the ECOG trials EST 4181 and EST 5181 to evaluate the efficacy of long-term adjuvant tamoxifen therapy in randomized clinical trials [74].

The NSABP has compared 5-FU, L-PAM, and tamoxifen as an adjuvant for two years with a registration arm of the same treatment but with an additional year of tamoxifen alone. The results are encouraging and support the view that long-term tamoxifen therapy benefits the patient [75].

Several clinical trials have evaluated the efficacy of tamoxifen alone. Delozier and coworkers [76] compared three years of adjuvant tamoxifen therapy vs no treatment in a small randomized clinical study. The tamoxifen-treated patients who were receptor-positive had a survival advantage when

compared with either receptor-negative patients or those not receiving tamoxifen.

A much larger randomized clinical study has evaluated five years of adjuvant tamoxifen therapy against a control arm that received tamoxifen therapy upon first recurrence [77]. The Scottish trial has demonstrated a survival advantage for patients receiving adjuvant tamoxifen therapy, thereby dispelling the fears that early resistance to tamoxifen will occur during an extended treatment schedule. The patient population for the Scottish trial contains a significant number of premenopausal women and node-negative patients. The NSABP has taken the view that the logical direction for their clinical trials is to evaluate the efficacy of long-term adjuvant tamoxifen therapy in pre- and postmenopausal node-negative patients who had ER-positive primary tumors. The preliminary results of their investigation are encouraging [13], as tamoxifen produces an increase in disease-free survival. Nevertheless, the large potential patient population (up to half a million women could be taking tamoxifen forever, in the US alone, by the turn of the century), and the improved survival, requires an evaluation of the perceived risks of long-term tamoxifen therapy to be balanced against the benefits in delaying recurrence of a fatal disease.

Antiestrogenic side effects of tamoxifen

Estrogen is required for the maintenance of bone and to provide a beneficial lipid profile in postmenopausal patients. Osteoporosis and developing cardiovascular disease are serious complications of the advancing years following menopause. Estrogen replacement therapy has provided a clear benefit for the long-term health and wellbeing of women [78]. Obviously the long-term administration of an 'antiestrogen' would be expected to cause premature osteoporosis, atherosclerosis, and an increase in myocardial infarction.

Tamoxifen is an antiestrogen [6] but there appears to be a target site specificity to its action. Studies in laboratory animals have demonstrated that tamoxifen has an estrogen-like effect upon bone [79, 80], and in fact tamoxifen can inhibit estrogen-stimulated increases in ovariectomized rat uterine wet weight but produce an additive estrogenic effect on bone density [81]. These encouraging laboratory results suggest that tamoxifen may in fact have a beneficial effect on patient bone density. This appears to be true from the results of preliminary clinical studies [82, 83].

Similarly, tamoxifen appears to produce an estrogen-like effect upon the lipid profile in postmenopausal women [84]. The LDL cholesterol fraction has a significant decrease during tamoxifen adjuvant therapy [85, 86]. Whether these positive effects produced by tamoxifen in women will be identified as the overall cause of the apparent increased survival of patients (regardless of survival from breast cancer) remains to be established [87].

Estrogenic side effects of tamoxifen

The estrogen-like actions of tamoxifen are well documented in postmenopausal patients. There is a partial decrease in circulating gonadotrophins [88], an increase in sex hormone binding globulin [89], an estrogen-like change in circulating proteins [90], and an estrogen-like effect on vaginal cytology [91].

There are, however, two principal concerns, liver carcinogenesis and endometrial carcinoma, which may involve the estrogenic component of tamoxifen's action. Animal studies have adequately documented the role of estrogen as a promoter of liver carcinogenesis. The estrogens in oral contraceptives are extremely potent promoters [92]. In contrast, only very large (200 mg/kg) daily doses of tamoxifen can promote liver carcinogenesis in rats [93]. To date, no evidence of an increase in tamoxifen-induced hepatomas has emerged from randomized clinical trials.

Unopposed estrogen administration to postmenopausal women causes an increase in endometrial carcinoma [94]. At present, there is really no information concerning the long-term effects of tamoxifen therapy on the human endometrium. Nevertheless, tamoxifen has been used successfully to treat endometrial carcinoma [95], so that its action is unlikely to be as a full estrogen.

The concern about the estrogen-like actions of tamoxifen to support the growth of occult endometrial cancer comes from the observation that a human, hormone-responsive endometrial carcinoma will grow more rapidly in athymic mice during tamoxifen treatment [96]. A number of anecdotal reports [97–99] have linked tamoxifen with an increase in endometrial carcinoma. However, this is only to be expected, as tamoxifen could only inhibit the growth of about $^1/_3$ of the tumors that might occur in patients with breast cancer. To date, only one randomized clinical trial has reported an increase in endometrial carcinoma during long-term tamoxifen therapy [100]. No increase in endometrial carcinoma has been found in the Scottish trial [101] or been reported by the ECOG or NSABP clinical trials organizations in the United States.

The Swedish study [100] is particularly interesting because tamoxifen causes a significant decrease in the numbers of second primary breast cancers. This again illustrates the target site specificity of the drug observed in animals; tamoxifen causes human endometrial carcinomas to grow but prevents the growth of bitransplanted breast tumors (MCF-7) in the athymic mouse [102]. Overall, it should be obvious that the benefits of tamoxifen to inhibit the recurrence (or prevent the appearance of primary breast tumors) of a fatal disease outweigh the potential concerns about endometrial carcinoma, a curable disease, in node-positive breast cancer.

The application of tamoxifen to prevent the recurrence of node-negative disease should be monitored carefully by the physician, and suspicious bleeding and discharge should be investigated immediately.

Ovarian actions of tamoxifen

The trend towards treating large populations of premenopausal, node-negative women with long-term adjuvant tamoxifen therapy [13, 77] must take into account the known effects of tamoxifen upon ovarian steroidogenesis. Tamoxifen induces ovulation in premenopausal patients [32, 33], and there is a known effect of tamoxifen to increase ovarian steroidogenesis [103–105]. Clearly, patients must be counseled to use barrier contraceptives, as tamoxifen therapy is not recommended during pregnancy. The increases in ovarian estrogen production could possibly reduce the efficacy of tamoxifen either if the patient is non-compliant or if the drug is rapidly cleared (reflected by low serum concentrations). However, the antitumor action of tamoxifen is not easily reversed in the athymic animal model [58]. Nevertheless, for optimal efficacy, a low-estrogen environment would probably be beneficial for tamoxifen. Physicians could consider ovariectomy or administration of sustained release preparations of synthetic luteinizing hormone-releasing hormone [106] to prevent rises in ovarian estrogen and to prevent the long-term effects of ovarian stimulation (which are as yet unknown).

Conclusions

Tamoxifen has proved to be the front line endocrine therapy for breast cancer. The drug has proven efficacy to improve survival in postmenopausal, node-positive patients (approx. 2 years of adjuvant therapy), and long-term treatment (5–10 years) schedules are being evaluated in clinical trial. Overall, the drug is remarkably nontoxic (compared with other cancer therapeutic agents), and very few side effects of significance have been noted that should deter the physician from treating node-positive postmenopausal disease. There is a growing clinical experience with premenopausal patients, especially those that are node-negative. Physicians must use their clinical judgement whether to offer their patients tamoxifen to prevent the recurrence of an invariably fatal disease or to withhold a relatively nontoxic therapy (because of possible long-term side effects) until recurrence.

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