Abstract

This paper describes the laboratory discovery and clinical testing of the first nonsteroidal antiestrogen, MER-25 (ethamoxytriphethol). The compound blocks estrogen action in all species tested and has only slight but transient estrogenic effects. No other antiestroidal actions are noted. MER-25 is antiiestrogenic in primates and was investigated in the clinics in a wide range of gynecological conditions, including breast and endometrial cancer. Unfortunately toxic side effects (hallucinations, etc.) precluded further investigation. A derivative of triphenylethylene, clomiphene, has some partial agonist (estrogen-like) actions in laboratory animals and following clinical evaluation is now an established agent for the induction of ovulation in subfertile women. Although clomiphene is active in advanced breast cancer, it was not developed further. In the late 1960s a related compound, tamoxifen, was evaluated to treat a number of estrogen-responsive disorders but was successfully introduced in the 1970s for the treatment of advanced breast cancer. Although there was only modest initial interest in the palliative use of tamoxifen, an enormous investment in the development of antiestrogens has led to a new antiestrogen with different pharmacological properties for other potential clinical applications.

Introduction

Estrogen is involved in a large number of physiological phenomena throughout the body. The mechanism by which this hormone produces its effects is complex and may not be universal for all of the activities reported to be estrogenic. Estrogen is also involved in some pathologies. Here too, its role has not been completely defined. The removal of estrogen or interference with its action is helpful in elucidating the role of estrogen and the mechanism of its activity in both physiological and pathological processes. Perhaps the earliest association of reproductive tissue and behavioral responses to a hormonal secreting organ was the cessation of estrus in domesticated animals following ovariectomy and the diminution of aggressive behavior in male animals and humans after castration. Indeed, almost 100 years ago there was recognition that the ovary was involved in mammary carcinoma and that removal of that endocrine gland could be beneficial to the patient (1).

Ablation of other endocrine organs including adrenalectomy (2) and hypophysectomy (3) were also found to be beneficial in some breast cancers. Surgical removal of these glands or the ovaries, however, did more than decrease or eliminate endogenous estrogen from the body. A number of hormones and nonhormonal substances were removed, some of which were not involved in the pathology. However, it is reasonable to expect a complex tissue such as the mammary gland, which requires a number of hormones and probably growth factors for its normal growth and function, to have the involvement of more than one hormone in its pathology.

Certainly there are a number of possible ways of interfering with estrogen interaction with its target tissue other than by removing the steroid-secreting glands. Reduction of pituitary gonadotropin secretion by sex steroids or by blocking the bioconversion of androgen to estrogen are ways of decreasing the endogenous pool of estrogens. Biologically available estrogen can also be reduced by increased catabolism or altering the plasma level of estrogen-binding protein. Another way to interfere with estrogen activity is to block it at the level of its receptor in estrogen target tissues.

Development of Nonsteroidal Estrogens

The most common of the wide spectrum of biological activities associated with estrogens, regardless of chemical classification or source, is the uterotrophic response and the cornification of vaginal epithelium. These activities have been incorporated into the bioassays that have defined every natural and synthetic compound designated as an estrogen. Development of a sensitive bioassay in rodents by Allen and Doisy (4) enabled characterization and classification according to potency of the natural steroidal estrogens and nonsteroidal plant estrogens. This formed the basis to identify the structural requirements for activity, as well as potency.

Cook et al. (5) found that the phenanthrene nucleus was not necessary for estrogenic activity. The laboratory of Dodds and his collaborators therefore synthesized a large number of diphenolic and triphenolic compounds resulting in the development of potent, clinically important estrogenic substances such as diethylstilbestrol (6), hexestrol, and dienestrol (7). At this same time in the mid-1930s, Robson and Schonberg reported that triphenylethylene had estrogenic activity (8) and that oral potency and prolongation of activity could be achieved by bromination (9). This finding led, in the 1940s, to the synthesis of a number of triphenylethylene derivatives including more potent halogenated compounds by the William S. Merrell Co. in the United States (10, 11) and by Imperial Chemical Industries in the United Kingdom.

While all of the compounds called estrogens increase uterine weight and stratify and cornify the vaginal epithelium, the spectrum of other biological activities associated with estradiol may or may not be present in all of these estrogens. Moreover, the quantitative aspect of one of the activities may not be related to other activities of the same compound. Knowledge of structure-activity relationships allows for the rational design of chemical agents with greater specificity for a particular pharmacological action.

Some of the physiological interactions of the steroidal hor-
mones were known in the first half of the 20th century. These interactions could be additive, augmentative, synergistic, or antagonistic depending upon the particular steroids, dosage of each, order and time course of administration, as well as the end point and species of animal under study. A small dose of an estrogen administered to an immature rabbit prior to treatment with progesterone allows for maximal growth and development of the uterus.

It is now known that this effect is a result of increased estrogen-induced uterine hyperemia and induction of progestation receptors. Large doses of the estrogen, however, can reduce the subsequent effect of the gestational agent in the rabbit.

**Estrogen Antagonism**

Treatment of an immature or ovariectomized rodent with a progestogen, androgen or corticoid can antagonize the uterotrophic effect of estrogens (12-16). Antagonism, however, is closely linked to the relative and absolute dose of the agonist and the antagonist.

The first estrogens that demonstrated antagonism of the stimulation of another estrogen were estriol (17) and 16-epiestriol (18). These weak estrogenic steroids partially inhibited the uterotropic effect of estradiol in the rat. This antiestrogenic activity was manifested only over a narrow range of doses (19), and at lower doses the two estrogens induced additive uterine growth responses (20). Moreover, for the end point of vaginal cornification in rats and mice, estriol was always additive with estradiol (21).

The nonsteroidal synthetic estrogens and their structurally related weak or inactive relatives were an attractive source in the search for modulators or inhibitors of estrogenic activity. Several weakly estrogenic stilbene and triphenylethylene compounds demonstrated partial antagonism of estrone- or estradiol-induced uterotropic activity in mice in my laboratory (L. J. L.). At a presentation of data (22) on the antiestrogenic activity of one of the diphenolic compounds Dr. Roy Hertz from the NIH remarked that the findings were interesting but a clinically useful antiestrogen would have to be much more potent.

**Discovery and Development of Ethamoxytriphetol (MER-25)**

In 1954, a compound related to triphenylethylene and chloro-rotarianisene was synthesized at Merrell to study its possible cardiovascular effects, but it was found to be inactive. The structural similarities of that compound to the triphenylethylenes that were being studied for antiestrogenic activity in my laboratory (L. J. L.) prompted us to test its endocrine activity.

That compound, 1-p-2-(diethylamino)ethoxyphenyl-2-(p-methoxyphenyl)-1-phenylethanol was later given the generic name of ethamoxytriphetol and the reference designation of MER-25 (Fig. 1). The initial dose of MER-25 was selected on the basis of experience that showed that even the most active of the compounds tested up to that time were of low potency and only partially inhibited the uterotropic effect of estrone or estradiol.

Weanling mice were given s.c. injections of MER-25 in olive oil for 3 days at a daily dose of 1.7 mg (170 mg/kg). One-half of the animals were also given daily injections of estradiol benzoate (0.1 µg/animal). The results of this experiment were surprising since the uterine weights of the mice that had been given MER-25 with or without estradiol benzoate were identical to those of animals given injections of the olive oil alone. In addition, uterine vascularity and intraluminal fluid, which also increases with estrogen treatment, were absent in the MER-25-treated groups. Moreover, body weight remained normal. The study was repeated several times and the complete blockade of the estrogen-induced uterotrophic response was confirmed.

The inhibition of estradiol action in the mouse uterus by MER-25 was dose related and simultaneous administration of graded doses of estradiol was able to prevent the antiestrogenic effects of MER-25 in a dose-related manner (Fig. 2). This study demonstrated that MER-25 is a competitive antagonist of estrogen action.

The nature of the competitive mechanism of this and derivative antiestrogens was elucidated when Jensen et al. (23, 24) found that they prevented binding of labeled estradiol to the rat uterus. Numerous studies in vivo and in vitro have confirmed that the nonsteroidal antiestrogens act at the level of the estrogen receptor. However, the exact nature of the mechanism(s) is still incompletely understood. Moreover, other activities have been documented for this class of pharmacological agents. These include inhibition of estrogen-induced protein synthesis, thymidine incorporation, DNA synthesis, cell cycle blockage at the G1 phase, inhibition of calmodulin, and non-estrogen-related events.

Not all of these activities, however, have been found universally with all antiestrogens and results from studies in vivo do not necessarily support findings in vitro. Some of these effects may be a result of the relative estrogenic to antiestrogenic activities of the compound studied or to the biological half-life of each activity. A comparison of an estrogenic antiestrogen
(partial agonist) with a nonestrogenic antiestrogen (pure antagonist) for each of the activities may help resolve this problem.

The antiuterotrophic effect of MER-25 was also demonstrated in immature, ovariectomized, and adult rats (25–30). In the immature rat, a MER-25:estradiol benzoate ratio of 400:1 reduced the uterotropic effect of the estrogen by approximately 45% and complete suppression of the estrogen activity was obtained with a ratio of 10,000:1 (Fig. 3). Uterine as well as oviductal growth stimulated by estradiol was inhibited in a dose-related fashion as were a number of biochemical parameters elevated by estrogen. These included nucleic acids, phospholipids, glucose-6-phosphate dehydrogenase and other enzyme activities, nitrogen, and water.

These initial studies prompted my colleagues and I (L. J. L.) to investigate the pharmacological spectrum of activities of MER-25 in different species in various stages of maturity. Our studies evaluated the antihormonal and antifertility properties of MER-25 in acute and long-term studies (15, 26). Each study brought new information but also raised new questions. MER-25 was an antiuterotrophic agent regardless of the route of administration or the estrogen used as the agonist, but it had little or no activity as an antagonist of androgen or progesterone-induced uterotrophic activity.

MER-25 was also a complete inhibitor of the effect of endogenous estrogen or administered estrogen at the level of the vagina. Cornification of vaginal epithelium by estrogen was blocked and MER-25 did not induce any cornification when administered to immature or ovariectomized mice or rats, demonstrating a lack of inherent estrogenicity.

Further Studies with MER-25

There was some evidence that this compound did possess very weak and short-lived estrogenic activity at other end points. At relatively low doses, it induced a weak uterotropic effect which, many times, was only borderline in significance. When a single dose of this agent was administered to immature mice, a slight increase in uterine weight was noted 48 h after treatment but was not seen on subsequent days (26). Those doses that were weakly uterotropic also elevated uterine alkaline phosphatase (27).

In immature rats, lower doses induced minimal increases in uterine weight (28). Low doses of MER-25 induced a short-lived increase in glucose-6-phosphate dehydrogenase, glucose isomerase, and lipids (30). In the immature rabbit pretreated with estrogen to allow for progesterone-induced endometrial development, MER-25 effectively inhibited the estrogen activity (presumed induction of progesterone receptors), thereby blunting the effect of the progesterone (26). MER-25, however, when administered instead of estradiol, permitted a weak uterine growth response to subsequent progesterone treatment. Other indications of weak estrogenic activity were the acceleration of vaginal opening in prepuberal rats and mucification of the adult rat vagina (26, 27). Offspring of rats treated with MER-25 during the last third of gestation demonstrated early opening of their vaginas (27).

MER-25 has only weak antigonadotropin activity, but it effectively blocks estrogen- or castration-induced pituitary hyper trophy and gonadotroph degranulation (26). It was found to be devoid of any other hormonal activity.

Mouse uteri stimulated by testosterone were unaffected by simultaneous treatment with this estrogen antagonist (26, 27). High doses of MER-25, however, partially inhibited androgen-stimulated uterine weight increase and biochemical changes in the immature rat (29). Immature castrated male rats given injections of MER-25 and testosterone propionate demonstrated no antagonism of the androgen-induced changes in the seminal vesicles although there was a partial inhibition of the

**Fig. 2.** Uterotrophic and antiestrogenic activities of MER-25 in immature mice. Groups of animals were treated for 3 days with (A) estradiol benzoate (0.3 μg) and MER-25 (O) or MER-25 alone (△) or (B) different doses of estradiol benzoate with (○) or without (●) 5 mg MER-25. All doses are 3-day total dosages. Data in parentheses, number of animals exhibiting full (F), partial (P), or no (N) uterine intraluminal fluid.

**Fig. 3.** Uterotrophic and antiestrogenic activities of MER-25 in ovariectomized immature rats. Groups of animals were treated for 4 days with a total dose of 2.0 μg estradiol benzoate (⋯⋯⋯⋯), MER-25 (●●●●), estradiol benzoate plus MER-25 (○○○○) or injection vehicle (sesame oil) alone (⋯⋯⋯⋯). Animals were killed on day 5 and blotted uteri were weighed wet.
augmented weight and nucleic acids of the ventral prostate (32). Antiestrogenic activity was also found in the chick. MER-25 inhibited estrogen-induced oviductal growth and elevated plasma phospholipids (26). MER-25 did not alter these same estrogen target parameters at any dose used, indicating a lack of estrogenicity in this species. Tamoxifen and other triphenylethylene antiestrogens (33, 34) were later also found to be devoid of estrogen activity in the chick oviduct. This is confirmatory evidence for a species-related hormonal profile for this chemical class of compounds.

MER-25, if it were to have clinical potential, had to have antiestrogenic activity in a primate. Ovariectomized Macaca mulatta monkeys, maintained on estradiol, were periodically given varying p.o. doses of MER-25. The antagonist induced a sharp reduction in the cornified vaginal cells of these estrogenized animals and at higher doses the decrease in cornified cells was to the castration level. Accompanying this was a lessening of the sex skin swelling and reddening and eventually estrogen withdrawal bleeding (26). Other studies in intact cycling monkeys demonstrated that endogenous estrogen was inhibited by antagonism or via reduced gonadotropin secretion since the menstrual cycles were lengthened and the percentage of cornified cells in the vaginal smear decreased.

These studies paralleled those in intact rats where a daily p.o. dose of 1 mg lengthened the estrous cycle and 5 mg/day stopped cycling (26). The rats resumed normal cycling of 2-6 days following the last dose.

This prototype antiestrogen was further investigated in order to determine its pharmacological properties especially in relation to those biological phenomena where endogenous estrogen or gonadotropins had been shown to be involved.

**Effect of Antiestrogens on Reproduction**

The reproductive system and all of its accessory tissues require a changing balance of hormones for their development and function. Changes in the absolute and relative quantities of these hormones may lead to functional disorders and pathology. Treatments that increase or decrease the available pool of the particular hormone or alter its activity may correct the problem. Similarly a physiological process such as ovulation can be interrupted by addition of hormones.

It was interesting to explore the actions of MER-25, an antiestrogen having weak gonadotropin-inhibitory and very weak selective estrogenic activities, on reproduction. The compound was administered to female rats at various times prior to or after mating (26, 27). None of the pretreated animals mated, since none of the animals exhibited estrus behavior or showed vaginal cornification. Treatments that were initiated after mating on day 1 of pregnancy probably inhibited nidation or altered ova transport since no fetuses or products of conception were found. Administration of the compound on days 4-7 postmating prevented blastocyst implantation. When treatment was delayed until the 13th day of pregnancy, gestation continued and live pups were delivered, but parturition was delayed and was difficult with several of the young dying probably due to the effects of estrogen deprivation on uterine contraction.

The postcoital antifertility effect of MER-25 was confirmed by other investigators (35, 36) and similar results were obtained for other triphenylethylene antiestrogens including clomiphene and MRL-37 in my laboratories (L. J. L.) and by other investigators (37, 38). None of the antiestrogenic agents were sufficiently efficacious in primates, although ovum implantation in the monkey uterus was inhibited by several (39) and at least one such agent was developed for postcoital antifertility utility (40), but it was never marketed.

MER-25 was also shown to augment pregnancy maintenance by progesterone in ovariectomized rats similar to that obtained with estrone (27). However, in rats fed a protein-free diet where estrone helps maintain pregnancy, the administration of MER-25 negated the protective effect of the estrogen (27).

The activities of MER-25 were expressed relative to the hormonal and developmental state of the animal. In adult cycling rats, this compound decreased the weights and function of the ovaries, pituitary, and uterus. It also antagonized the effect of estrogen on the latter two organs (26). Administration of MER-25 to pubertal rats, however, produced different results. Ovarian weights, nucleic acids, and enzyme activities were elevated after 4 days of treatment (41). The increases in the ovaries were similar to that obtained by treatment with human chorionic gonadotropin (41). Estradiol treatment at the same time prevented the MER-25-induced augmentation of ovarian weight and biochemical activities.

The increase in ovarian activity following MER-25 treatment of the pubertal rat may parallel the ovarian stimulation in women after administration of antiestrogens including MER-25 and clomiphene citrate.

**Utility of Antiestrogens for Ovulation Induction**

In the 1950s Dr. James H. Leathem used the human chorionic gonadotropin-treated hypothyroid rat as a model for polycystic ovaries. His work stimulated us to study the effect of MER-25 in this model. The antiestrogen blocked the uterine hypertrophy induced by the hyperactive ovary and reduced ovarian weight; however, cystic follicles were still evident. These studies were repeated and extended by Leathem and his graduate students. MER-25 prevented the induction of cystic ovaries and alteration in ovarian ascorbic acid concentration (42). Furthermore, pretreatment with MER-25 prior to induction of the hypothyroid state followed by gonadotropin administration was also effective in blocking cystic development (43).

A preliminary study in nymphomaniac cows bearing cystic ovaries was performed by W. Hansel and R. M. Melampy using a low use of MER-25 that I (L. J. L.) had suggested. These cows promptly resumed normal behavior; however, no measurement of ovarian follicular size or ovulation was recorded.

These findings in animals encouraged us to initiate exploratory studies on selected patients to determine the effects of MER-25 on menstrual or ovarian disorders. Kistner and Smith (44) reported that patients treated for endometrial hyperplasia and carcinoma with MER-25 demonstrated higher urinary excretion of estrogen and follicle-stimulating hormone and had temperature changes indicative of ovulation. These investigators also induced ovulation with this compound in patients with Stein-Leventhal syndrome (45). At this same time Tyler et al. (46) reported that 6 of 18 patients with severe anovulatory problems ovulated within 7 days of starting MER-25 therapy.

This antiestrogen, however, induced undesirable and unexpected central nervous system symptoms leading to cessation of its use. Fortunately my laboratory (L. J. L.) had been developing, at the same time, another related but more potent antiestrogen that had some qualitative differences from MER-25. That compound, MRL-41 or clomiphene citrate (47) (originally called chloramphene) (Fig. 1), was, in comparison to MER-25, weakly estrogenic (25). Clomiphene was provided to R. W. Kistner, E. T. Tyler, and R. B. Greenblatt to study its ability to induce ovulation. Shortly thereafter Greenblatt et al.

4180
were suggested for evaluation with MER-25. These included,

1) precocious development, 2) anti-fertility, 3) habitual abortion,

a chemical compound that demonstrates an estrogen-like activity

utilities based on the particular pharmacological spectrum of

demonstration of the efficacy of an antiestrogen in breast

catalogue of factors required for cellular growth, development,

widespread use in in vitro fertilization technology.

Effect of Antiestrogens on Lipids

Estrogens are known to regulate the synthesis and metabolism of lipids. This produces a change in the relative concentration of various lipid fractions in the plasma and disposition in tissues. Indeed endogenous estrogen is a normal protective regulator for the cardiovascular system and the decreased synthesis of these steroids at menopause is associated with an increase in atherosclerosis and coronary disease. Men are more prone to these disorders than are premenopausal women. A chemical compound that demonstrates an estrogen-like activity on lipids without affecting the reproductive system would therefore be desirable. Another possible partitioning of the biological spectrum of activities of estrogen might result from the administration of an estrogen such as estradiol and of a "nonestrogenic" antiestrogen.

An experiment in 1-week-old chicks showed that estradiol benzoate could increase oviducal weight by 1500% while doubling the plasma phospholipid concentration. MER-25 administration did not alter either end point even at doses 300 times larger than that of the estrogen. When both substances were administered to these birds the estrogenic effect on the plasma phospholipids as well as on the oviduct were blocked (26).

Even though MER-25 failed to show an estrogen-like effect on the chick plasma phospholipids, this antiestrogen and a number of other compounds having different spectra of estrogenic and/or antiestrogenic activities were investigated in the rat. MER-25, clomiphene citrate, and a related compound, triparanol, were hypocholesterolemic (41) with the latter compound being 5 times more potent than the other two. Studies in vitro with rat liver homogenates demonstrated that MER-25 and clomiphene inhibited the conversion of mevalonate to cholesterol whereas triparanol produced its major blocking effect after mevalonic acid (41, 49).

The nonestrogenic antiestrogen 2,2′′′-(1-methyl-4,4-
diphenylbutylidene)bis(p-phenyleneoxy)bis triethylamine oxalate lowered plasma cholesterol in rats fed normal and hyperlipemic diets by blocking cholesterol biosynthesis between mevalonate and lanosterol (49). It also reduced serum free fatty acids and triglycerides.

It may be interesting to investigate the relationship of cholesterol biosynthesis inhibition and anticancer potential of the antiestrogens in light of present knowledge concerning the catalogue of factors required for cellular growth, development, regulation, and multiplication.

Demonstration of the Efficacy of an Antiestrogen in Breast Cancer

Laboratory studies with MER-25, clomiphene, and many other related compounds suggested a variety of potential clinical utilities based on the particular pharmacological spectrum of each compound. In mid-1956 a list of some of these utilities was suggested for evaluation with MER-25. These included, "1) precocious development, 2) anti-fertility, 3) habitual abortion, 4) fertility in the female, 5) dysmenorrhea, 6) all kinds of menstrual disturbances including menorrhagia or prolonged menstrual periods, 7) menopause, 8) endometriosis, 9) acne, 10) cancer—a) to test for or substitute for adrenalectomy in cancer, b) as a substitute for ovariectomy, c) mammary carcinoma, d) prostatic cancer—alone or in combination with an estrogen, in an attempt to maintain the effects of estrogen on the prostate and avoid side reactions of estrogens, 11) benign prostatic hypertrophy, 12) postpartum breast engorgement, and 13) precipitate the menopause when desirable.”

While that list was compiled largely on the basis of laboratory data on MER-25 presented by L. J. L., it is interesting that the anticancer indications were not derived from animal tumor models since such models were not available. The stimulation for such evaluation in human cancers came largely from the availability of a unique compound that could block the effect of estrogen completely and also have weak gonadotropin inhibitory activity.

Several well known competent clinical investigators were suggested for each of the areas. The two who were asked to investigate MER-25 in breast disorders including cancers were Dr. Roy Hertz at the NIH and Dr. Robert Kistner at Harvard University. The dosages suggested were derived from the laboratory studies in animals including those performed in the monkey. Dr. Kistner also investigated other diseases of the breast, uterus, and ovaries.

Both investigators confirmed antiestrogenic activity in their patients. Kistner and Smith (44) reported that patients with chronic cystic mastitis not only had pain relief but also had reduced glandular proliferation and breast size. He also found that two of four patients with breast carcinomas experienced pain relief and a reduction of calcium excretion while on MER-25 therapy. Hertz also treated several patients with metastatic breast cancer and obtained similar favorable results. These studies demonstrated the utility of an antiestrogen in breast cancer.

Unfortunately the investigation of MER-25 as a therapeutic agent had to be discontinued because Drs. Hertz, Kistner, and Tyler reported that their patients were experiencing hallucinations, psychotic episodes, nightmares, disturbed sleep, or other indications of undesirable central nervous system activity.

Clomiphene which was the next most advanced in preclinical investigation or another of the structurally related antiestrogens could have been evaluated for anticancer activity in the early 1950s. However, the clinical evaluation of these compounds for those indications was never undertaken due to the concentration of effort on the use of clomiphene in induction of ovulation in anovulatory infertile women. In fact clomiphene citrate was later shown to have efficacy in breast cancer in a few patients in 1964 (50).

Resumption of investigation of similar antiestrogens for breast cancer had to wait for more than a decade. Chemists and biologists in the United Kingdom reexplored the triphenylethlenes for antiestrogenic and antiinfertility activity and eventually for antitumor activity. The clinical efficacy of one of these compounds, tamoxifen, established antiestrogens as the standard endocrine treatment in breast cancer.

Development of Tamoxifen

Tamoxifen (ICI 46,474; Nolvadex) (Fig. 1) is a nonsteroidal antiestrogen with antifertility properties in laboratory animals (51–54). The compound exhibits a interesting species-specific pharmacology (55); it is a pure antiestrogen in chicks (33) and
an antiestrogen with partial estrogenic activity in rats (52) but, in short-term tests, it is classified as an estrogen in mice (51, 52, 56). However, it appears that the prolonged administration of tamoxifen to ovariectomized mice causes the uterus and vagina to become refractory to the stimulatory effects of estradiol (37, 57, 58).

Tamoxifen is a competitive inhibitor of estradiol binding to estrogen receptors (59, 60) and the compound inhibits the binding of estrogen to estrogen target tissues in vivo (57, 61–64).

Antiestrogens can inhibit estrogen-regulated events in cells in culture; e.g., tamoxifen slows the growth of MCF-7 breast cancer cells [in phenol red containing media (65, 66)]; however, the addition of increasing concentrations of estradiol will reverse the effects of tamoxifen and the cells will continue to grow ("estrogen rescue") (67). Nevertheless, high concentrations of antiestrogens will cause estrogen irreversible actions (68, 69). An "antiestrogen binding site" has been described (70) but its relevance to the cytotoxic actions of tamoxifen is not clear. It is clear, however, that the high affinity of tamoxifen to a variety of proteins causes ubiquitous binding of the drug in vivo and probably accounts for its long biological half-life.

Current research is focused on the action of growth factors to regulate the breast cancer cell cycle. Estrogens decrease cell cycle time and tamoxifen causes a reversible blockade at the G1 phase (71, 72). Antiestrogens inhibit estrogen-stimulated increases in transforming growth factor a, a stimulatory factor (73), and there is a complementary rise in transforming growth factor β, an inhibitory growth factor (74). Although the actual regulatory mechanisms that orchestrate the inhibitory and stimulatory actions of growth factors are unclear, this is an area of intense investigation.

The knowledge that tamoxifen is an inhibitor of estrogen action focused attempts to develop an appropriate clinical application. Tamoxifen was initially evaluated in a number of endocrine-responsive conditions (75–80) and the drug subsequently became available in some countries for the induction of ovulation in subfertile women. However, the discovery that the presence of the estrogen receptor may predict the hormone responsiveness of advanced breast cancer (81) naturally led to the evaluation of tamoxifen to control tumor growth [tamoxifen inhibits the binding of estrogen to estrogen receptors derived from breast tumors (82)]. Much of the credit for steering the use of tamoxifen toward the treatment of advanced breast cancer must go to the late Dr. Arthur L. Walpole of ICI, Pharmaceuticals Division, Macclesfield, United Kingdom. Earlier in his career Dr. Walpole had been particularly interested in carcinogenesis and cancer therapy but in the 1960s his attention was directed, as head of the fertility control program, to the development of antifertility agents (83). Much of the early preclinical antitumor work was subsequently conducted in my (V. C. J.) laboratory funded through the good offices of Arthur Walpole at ICI.

Antitumor Actions of Tamoxifen in Laboratory Models

The first systematic laboratory study of the antitumor action of tamoxifen was conducted at the Worcester Foundation for Experimental Biology in Shrewsbury, MA (82, 84–88). One of the results of this research (89, 90) was to encourage the clinical evaluation of tamoxifen in the United States by the Eastern Cooperative Oncology Group.

Most of the early research on tamoxifen used carcinogen-induced rat mammary tumor models (85–90) but during the past half-decade interest has focused on an evaluation of the antitumor action of tamoxifen in athymic mice, heterotransplanted with breast cancer cell lines (91–95).

Carcinogen-induced Models. Mammary tumors can be induced in 50-day-old Sprague-Dawley rats by a single feeding (20 mg in 2 ml peanut oil) of DMBA. Tamoxifen will inhibit the initiation (85, 87) and growth (61, 88, 96–98) of DMBA2-induced tumors. The model system could be likened to the treatment of advanced breast cancer but the tumors do not metastasize and therefore a direct analogy with breast cancer is impossible. This fact is important inasmuch as the fashion for the treatment of breast cancer was changing in the mid-1970s.

A strategy was devised to use adjuvant hormonotherapy to destroy micrometastases following mastectomy, a treatment aimed at curing a majority of patients. Unfortunately no animal model system was, or is, available to duplicate the clinical situation to test the value of this treatment strategy in the laboratory. Nevertheless the DMBA- and N-methylnitrosourea-induced rat mammary carcinoma models were adapted to evaluate whether antiestrogens could "cure" animals with a microscope tumor burden.

When DMBA is administered to Sprague-Dawley rats, the initiated mammary cells are promoted by circulating hormones to effect transformation. Palpable tumors appear 100–150 days after DMBA. There is therefore a period when microscopic disease can be treated to establish whether it is possible to cure the animals with antiestrogens. If different doses are administered for 4 weeks starting at different times after DMBA (99, 100), a delay occurs in the appearance of tumors. In contrast continuous therapy causes the majority of animals to remain tumor free (101–103). Nevertheless of tamoxifen therapy is stopped, tumor growth recurs (104).

A similar situation occurs with the carcinogen N-methylNitrosourea; a short course of tamoxifen delays the appearance of tumors but the animals remain tumor free during a continuous treatment regimen (105, 106).

Athyemic Mouse Models. Breast cancer cell lines can be heterotransplanted into athymic immunodeficient mice. Estradiol encourages the growth of hormone-responsive lines (e.g., MCF-7) but hormone nonresponsive lines (e.g., MDA-MB-231) will grow with or without estrogen treatment. Tamoxifen and its metabolites inhibit estrogen-stimulated growth of estrogen receptor-positive tumors but in general do not inhibit the growth of receptor-negative tumors (91, 92). However, this model has been used successfully to determine whether tamoxifen produces a tumorstatic or tumoricidal effect on implanted hormone-responsive breast cancer cells. If animals are treated for 1, 2, or 6 months with sustained release preparations of tamoxifen the tumor cells are not specifically destroyed. When the animals are treated with estradiol to identify surviving tumor cells, tumors regrow in every case (92, 93).

Overall the experimental evidence points to the fact that tamoxifen is a tumorstatic rather than a tumoricidal agent. Long-term adjuvant tamoxifen treatment, or treatment until relapse, is predicted to be the best therapeutic strategy to control the recurrence of breast cancer following mastectomy. This application of tamoxifen can be called chemosuppression.

Long-Term Adjuvant Tamoxifen Therapy

Despite the developing laboratory data that demonstrated that tamoxifen is a tumorstatic agent (99–102), the vast ma...
mortality of clinical trials initially evaluated 1 or 2 years of adjuvant tamoxifen therapy. There were several sound reasons for this: (a) a short course of tamoxifen might produce a tumoricidal effect before the onset of resistance since it was known that patients with advanced disease respond to tamoxifen for only 1–2 years; (b) the adjuvant use of tamoxifen might encourage the early outgrowth of receptor-negative disease (the rationale was that it would be wiser to save the drug until recurrence so that physicians would have something available to treat the patient); and (c) the long-term side effects of tamoxifen were unknown (thousands of women with advanced disease had been treated, but only until relapse, 1–2 years later).

Although tamoxifen was initially used conservatively the recent overview of all randomized clinical trials (107) has demonstrated the survival advantage of postmenopausal, node-positive women who received 1 or 2 years of adjuvant tamoxifen therapy. Swayed by the encouraging clinical trial results and the laboratory findings (101, 107) clinical trials are currently focused on an evaluation of 5, 10, or in some cases indefinite adjuvant tamoxifen therapy in pre- and postmenopausal patients with node-positive and node-negative disease.

In 1977, on the basis of encouraging laboratory data (101), Dr. Douglass C. Tormey organized a pilot nonrandomized adjuvant clinical study to identify any unusual side effects produced by long-term adjuvant tamoxifen therapy (5 years) and to evaluate the potential efficacy of the treatment strategy (108). The results demonstrated the safety of tamoxifen and provided promising preliminary data to establish the Eastern Cooperative Oncology Group protocols EST 4181 and EST 5181 to evaluate chemotherapy and different lengths of tamoxifen therapy in randomized clinical trials (109).

The National Surgical Adjuvant Breast and Bowel Project has chosen a similar course of action. Based upon their successful evaluation of 2 years of adjuvant tamoxifen and chemotherapy (110) they conducted a registration study of chemotherapy and tamoxifen for 2 years followed by an additional year of tamoxifen for those patients who did not have recurrence. The results are encouraging and support the view that long-term tamoxifen therapy benefits the patient (111).

Several clinical trials have also evaluated the efficacy of long-term adjuvant tamoxifen therapy alone. A French study (112) has compared 3 years of adjuvant tamoxifen therapy versus no treatment. Tamoxifen produced a survival advantage especially in receptor-positive patients. Similarly a large clinical trial in Scotland (113) has evaluated whether it is an advantage to treat patients for 5 years with adjuvant tamoxifen. This clinical study is particularly interesting because it addresses the question of whether to use tamoxifen as an adjuvant therapy or to use the drug only when the patient develops advanced disease. In the Scottish study, patients in the control arm received tamoxifen at first recurrence. The results demonstrate that long-term adjuvant therapy with tamoxifen provides an effective control of disease recurrence and a survival advantage for the patient. There appears to be no need to delay the drug until recurrence because early drug resistance does not develop.

The National Surgical Adjuvant Breast and Bowel Project is currently conducting a trial of long-term adjuvant tamoxifen therapy (10 years) in pre- and postmenopausal patients with node-negative estrogen receptor-positive disease. The preliminary results are again encouraging (114) inasmuch as tamoxifen-treated women have an extended disease-free survival.

Overall the clinical trials community has demonstrated some advantages for long-term adjuvant tamoxifen therapy. To date the trial population has been diverse with both pre- and postmenopausal women receiving tamoxifen alone and women with node-negative disease who might be treated for decades. This expanded and extended use of tamoxifen requires an ongoing evaluation of the pharmacology and potential toxicological consequences of indefinite therapy. Obviously it is important to establish the safety of tamoxifen to reassure both physicians and patients and to avoid unwarranted restrictions on this valuable chemosuppressive therapy.

Clinical Pharmacology

The clinical pharmacology of tamoxifen was initially investigated in patients with advanced breast cancer (115–118). The drug is readily absorbed following p.o. administration and accumulates to steady-state levels by the end of the first month of treatment (116). The serum levels of tamoxifen at steady state are extremely variable (50–300 ng/ml) but blood levels are not related to response (119). The principal metabolite of tamoxifen is N-desmethyltamoxifen which produces blood levels approximately twice as high as those of tamoxifen by the end of the first month of treatment. The serum half-lives of tamoxifen and N-desmethyltamoxifen are extremely long (7 and 14 days, respectively) (120), probably because the compounds are highly protein bound.

Tamoxifen is extensively metabolized in patients (121, 122) (Fig. 4) but to date no significant quantities of estrogenic metabolites have been noted that would cause concern during long-term adjuvant tamoxifen therapy. N-Desmethyltamoxifen is further metabolized to metabolite Y which has a glycol side chain (123, 124). Tamoxifen is also converted to 4-hydroxytamoxifen but this is observed only at low concentrations in serum (118). However, this metabolite has an extremely high binding affinity for the estrogen receptor (the same as that of estradiol) (125); therefore it probably plays a significant role in supporting the antitumor action of tamoxifen. Recently 4-hydroxy-N-desmethyltamoxifen has been identified in patients (126). This metabolite has been shown in the laboratory to be...
formed from either 4-hydroxytamoxifen or N-desmethyltamoxifen (127).

The blood levels of tamoxifen and its metabolites are stable for up to 10 years of adjuvant therapy and no metabolic tolerance seems to occur. This fact and the low incidence of reported side effects (121) makes tamoxifen an ideal long-term chemosuppressive agent for the adjuvant treatment of breast cancer.

Potential Side Effects of Long-Term Adjuvant Tamoxifen Therapy

Tamoxifen therapy is being extended beyond a decade. Indeed node-negative patients with only a moderate risk of recurrence may in fact receive the drug indefinitely. This raises important questions about the potential long-term side effects of nonsteroidal antiestrogens (Table 1).

Estrogen is important in prevention of the development of osteoporosis in women; therefore the long-term administration of an "antiestrogen" would seem to imply that women may become predisposed to osteoporosis. Similarly estrogen protects women from coronary heart disease by altering the circulating levels of cholesterol. Again it would seem that the long-term administration of an "antiestrogen" might not be beneficial. However, tamoxifen exhibits some estrogen-like effects that appear to have target site specificity (55). Tamoxifen is an antiestrogen in the rat uterus (52) but displays estrogen-like effects in bone (128, 129). There are only few clinical data (130, 131) to support these beneficial laboratory findings but tamoxifen is clearly not detrimental to bone in patients. The beneficial effects of nonsteroidal antiestrogens on serum cholesterol has been noted previously in animals (41) and tamoxifen has been shown to lower serum cholesterol (mainly, low density lipoprotein) in patients (132, 133).

Obviously the beneficial estrogen-like effects of tamoxifen in some target sites may prove to be a disadvantage in others. Estrogens can cause an increase in thromboembolic disorders and estrogen replacement therapy has been associated with an increase in endometrial carcinoma. Tamoxifen does not, however, cause a significant increase in thromboembolic disorders when administered alone but there is a small decrease in anti-thrombin III during long-term adjuvant tamoxifen therapy (132, 134). The decrease (generally not more than 30%) is, however, not great enough to cause clinical concern. Nevertheless patients with a history of thromboembolic disease probably should not be treated with tamoxifen.

There is very little information about the effects of tamoxifen on the human uterus although tamoxifen does have some efficacy for treating endometrial carcinoma (135). Nevertheless tamoxifen has been implicated (anecdotally) in the development of endometrial carcinoma (136). Realistically, however, there is little possibility that tamoxifen could control the growth of all endometrial tumors as only about 1 of 3 are hormone responsive. Interestingly enough there is one provocative laboratory observation (137) that tamoxifen can encourage the growth of a human endometrial carcinoma in athymic mice. Indeed this effect appears to be tumor specific (138). If athymic mice are bitransplanted with the endometrial tumor EnCa101 and the breast tumor MCF-7 and treated with estrogen and tamoxifen, the endometrial tumor is stimulated to grow whereas the growth of the breast tumor is controlled by tamoxifen. One large clinical trial (139) has shown that a similar phenomenon can occur during long-term adjuvant tamoxifen therapy; i.e., second breast tumors are reduced but there is an increase in endometrial carcinoma. To date these data have not been confirmed by any other major clinical trials organization and it is important to state that adjuvant tamoxifen therapy should not be denied to a patient on the basis of this potential side effect. It should be obvious that tamoxifen is of proven benefit (107) for the treatment of breast cancer, a disease that is invariably fatal, and endometrial carcinoma, should it occur, has a good prognosis. Routine gynecological examination must be performed to monitor the health and well-being of the patients during adjuvant tamoxifen therapy.

Finally, since more and more premenopausal patients are receiving adjuvant tamoxifen therapy, it should be stressed that they are at risk for pregnancy and require counseling about barrier contraceptives. Short-term tamoxifen treatment causes an increase in ovarian estrogen synthesis (140) and premenopausal patients on long-term adjuvant tamoxifen regimens (with or without chemotherapy) continue to menstruate and have elevated circulating ovarian steroid hormone levels (141, 142). It is currently not known what the long-term effects of tamoxifen on the ovary will be; this is a topic for future clinical investigation.

Clearly the elevated levels of estrogen, a known tumor mitogen, might be able to impair the antitumor action of the antiestrogen. Tamoxifen is, however, an effective antitumor agent in premenopausal women with advanced disease (121). This clinical situation has been investigated in the athymic animal model, and similar high circulating levels of estradiol (500–800 pg/ml) do not reverse the antitumor action of tamoxifen (50 ng/ml). However, compliance issues with tamoxifen will be very important in premenopausal patients during long-term adjuvant tamoxifen. Strategies to lower circulating estrogen like oophorectomy or the administration of luteinizing hormone-releasing hormone agonists during adjuvant tamoxifen therapy should be evaluated in future clinical trials.

New Antiestrogens

A study of the structure-activity relationships of tamoxifen has provided an important insight into the mechanism of action of both estrogens and antiestrogens. Early work demonstrated that antiestrogens had a weak interaction with the estrogen receptor (59, 143) and it was widely believed that this property was essential to inhibit hormone action (144); i.e., an estrogen would bind tightly to the receptor and the resulting complex could initiate estrogen-dependent events whereas an antiestrogen would bind to the receptor to block the access of estrogen but the complex would "fall apart" before it could itself initiate estrogen action. The discovery of the pharmacological properties of monohydroxytamoxifen (4-hydroxytamoxifen) (125, 145) changed this view of antiestrogen action. 4-Hydroxytamoxifen has an affinity for the estrogen receptor similar to that of estradiol but it is a potent inhibitor of estrogen action. Subsequent confirmation of this work (146, 147) and the availability of radiolabeled material for studies in vitro and in vivo (147–150) has established the drug as a standard laboratory biochemical.

During the 1980s a number of assay systems in vitro were used to describe the structure-function relationships of anties-
trogens (151–157). These model systems are important as they provide information about the direct effects of ligands on the cell without metabolic transformation. In the future this knowledge will undoubtedly dovetail with current research on the molecular biology of the estrogen receptor to understand the complex conformation changes that occur in the receptor protein to program cell replication.

As a result of the successful use of tamoxifen to treat breast cancer, several new antiestrogens are now being evaluated in the laboratory and the clinic. The structures of several of the new compounds are shown in Fig. 5. The literature on new antiestrogens is increasing dramatically and interested readers should consult recent reviews (122, 158).

One notable advance is the investigation of nonestrogenic antiestrogens for potential clinical applications. The discovery process appears to have gone full circle (MER-25 has few estrogenic properties) to find compounds without the estrogen-like properties of the triphenylethylenes. At present only one compound (ICI 164,384) is available for evaluation and it has demonstrated activity as an inhibitor of estradiol action both in vivo and in vitro (159–162). Interestingly the steroid also has the ability to inhibit tamoxifen-stimulated tumor growth in the laboratory (Ref. 94; Fig. 6). Various pure antiestrogens are currently being evaluated in the laboratory and some are undergoing toxicology testing. When (or if) any of these new compounds become available for clinical application, they may provide some additional clues about estrogen-regulated breast cancer growth. One question has been whether the antitumor activity of tamoxifen is related to its estrogenic or antiestrogenic properties (or neither?) This could soon be resolved. It is possible that pure antiestrogens could produce longer responses in advanced disease or be useful as a second line therapy after long-term adjuvant tamoxifen treatment. This presupposes that a significant proportion of patients who eventually fail tamoxifen therapy do so because of the estrogen-like properties of tamoxifen. Unfortunately the potential of pure antiestrogens to produce osteoporosis or early atherosclerosis may preclude their use as a front line long-term adjuvant therapy in node-negative disease.

Fig. 5. Structures of new antiestrogens that are being evaluated in the laboratory and the clinics.

Fig. 6. Effect of the nonestrogenic antiestrogen ICI 164,384 on tamoxifen-stimulated growth of endometrial tumor EnCa 101 in the athymic mouse. Animals were treated with a sustained release preparation of tamoxifen (2-cm Silastic capsule) (□), a control capsule (O), or ICI164,384, 1 mg/0.1 ml peanut oil s.c. 3 times weekly (■) or tamoxifen plus ICI164,384 (●).

Current and Future Uses of Antiestrogens

In the 30 years since the first publication of the pharmacological properties of MER-25, the clinical application of antiestrogens has proved to be a remarkable success. Tamoxifen is now the antihormonal treatment of choice for advanced breast cancer (pre- and postmenopausal). As valuable as this application is as a palliative treatment, the remarkable advances have undoubtedly been made in the application of antiestrogens to treat all stages of breast cancer. Tamoxifen is the antihormonal treatment of choice for the adjuvant therapy of postmenopausal node-positive women. Two years of treatment is known to produce a survival advantage, and longer treatment schedules are being evaluated to exploit the known tumorstatic qualities of tamoxifen. We already know that long-term adjuvant tamoxifen therapy (5 years) is beneficial compared to saving tamoxifen until first recurrence, so 10-year and indefinite schedules are being evaluated.

The proven efficacy and low incidence of side effects have encouraged the evaluation of adjuvant tamoxifen therapy in pre- and postmenopausal women with node-negative disease. At present the long-term toxicological concerns appear to be negligible compared to the known course of breast cancer without treatment. Hundreds of thousands of women could be being treated indefinitely with tamoxifen by the turn of the century. Indeed it has even been suggested that antiestrogens could be used to prevent breast cancer. However, the timing and cause of the carcinogenic insult are unknown; therefore the use of antiestrogens as true preventives seems impractical. A better term, also applicable to adjuvant therapy, is chemosuppressive; i.e., the drug can be used to suppress subclinical disease and prevent the appearance of a primary tumor (163). In some adjuvant trials tamoxifen inhibits the development of second primary breast tumors (114, 139, 164) and an early clinical evaluation of the toxicology of tamoxifen in normal women has been reported (165) as a first step in a prevention study. Nevertheless there is a real concern about being able to target the right population. We cannot predict who will develop breast cancer; we can only guess at the probability. Furthermore “high risk” women are, in fact, only a minority of those who will develop breast cancer so any success must be balanced against as yet unknown accumulative toxicities.

Is this the end of the possible applications for antiestrogens? Certainly not. We have obtained valuable clinical information
about this group of drugs that can be applied in other disease states. Research does not travel in straight lines and observations in one field of science often become major discoveries in another. Important clues have been garnered about the effects of tamoxifen on bone and lipids so it is possible that derivatives could find targeted applications to retard osteoporosis or atherosclerosis. The ubiquitous application of novel compounds to prevent diseases associated with the progressive changes after menopause may, as a side effect, significantly retard the development of breast cancer. The target population would be postmenopausal women in general, thereby avoiding the requirement to select a high risk group to prevent breast cancer.

Acknowledgments

This paper is not intended to be an encyclopedic review article but is offered only as a personal recounting of research conducted in our respective laboratories during a period that has spanned almost 40 years. We sincerely acknowledge the support of colleagues, students, and assistants in our respective laboratories. We also acknowledge the important additional contributions made to the understanding of antiestrogen action by James H. Clark, Kathryn B. Horwitz, Benita and John Katzenellenbogen, Marc E. Lippman, C. Kent Osborne, Robert I. Nicholson, Henri Rocheft, Robert L. Sutherland, and Alan E. Wakeling. The influence of our teachers the late James H. Leatham and the late James B. Allison (L. J. L.) and Edward R. Clark (V. C. J.) was important for the direction and development of our investigative approach. Dr. Lerner is ever grateful for the encouragement he received as a developing scientist from Roy O. Greep, Roy Hertz, the late Albert Segaloff, and the late Ralph Dorfman. Dr. Jordan wishes to acknowledge William L. McGuire, Jack Gorski, Elwood V. Jensen, the late Arthur L. Walpole, and colleagues at ICI for their invaluable help and support and Paul P. Carbone, Richard R. Love, Bernard Fisher, Sydney E. Salkind, and Douglas C. Tormey for the clinical application of many of the concepts and results described here. The Enca101 tumor was kindly provided by Dr. P. G. Satyaswaroop, Department of Obstetrics and Gynecology, Hershey School of Medicine, Hershey, PA, for the experiment in Fig. 6 performed by Dr. Marco M. Gottardis and Michelle Ricchio.

References


ANTIESTROGENS AND BREAST CANCER


ANTIESTROGENS AND BREAST CANCER

159. Wakeling, A. E., and Bowler, J. Steroidal pure antioestrogens. J. Endocri-

160. Cormier, E. M., and Jordan, V. C. Contrasting ability of antiestrogens to
inhibit MCF-7 growth stimulated by estradiol or epidermal growth factor.

161. Jordan, V. C., and Koch, R. Regulation of prolactin synthesis in vitro by
estrogenic and antiestrogenic derivatives of estradiol and estrone. Endocri-

162. Robinson, S. P., and Jordan, V. C. The paracrine stimulation of MCF-7
cells by MDA-MB-231 cells: possible role in antiestrogen failure. Eur. J.

163. Jordan, V. C. Chemosuppression of breast cancer with tamoxifen: labora-
tory evidence and future clinical investigations. Cancer Invest., 6: 5–11,

164. Cuzik, J., and Baum, M. Tamoxifen and contralateral breast cancer. Lancet,

165. Powles, T. J., Hardy, J. R., Ashley, S. E., Farrington, G. H., Cosgrove, D.,
Davey, J. B., Dowsett, M., McKinna, J. A., Wash, A. G., Sinnett, H. D.,
Tillyer, C. R., and Treleaven, J. G. A pilot trial to evaluate the acute toxicity
and feasibility of tamoxifen for prevention of breast cancer. Br. J. Cancer,
Development of Antiestrogens and Their Use in Breast Cancer: Eighth Cain Memorial Award Lecture

Leonard J. Lerner and V. Craig Jordan


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/14/4177

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/50/14/4177. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.