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Progress in the prevention of breast cancer: concept to reality

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This article is dedicated to my friend and mentor Professor Elwood V. Jensen, the discoverer of the ER and the pioneer who fashioned the concept of the ER as a target for the treatment and ultimately the prevention of breast cancer. His guidance and inspiration when I was at the Worcester Foundation helped to turn ICI 46 474 into tamoxifen and save the lives of half a million women with breast cancer.

Abstract

In 1936, Professor Antoine Lacassagne suggested that breast cancer could be prevented by developing drugs to block estrogen action in the breast. Jensen discovered the physiologic target, the estrogen receptor, that regulates estrogen action in its target tissues and Lerner discovered the first nonsteroidal antiestrogen MER25. However, the success of tamoxifen as a treatment of breast cancer opened the door for the testing of the worth of tamoxifen to reduce breast cancer incidence in high-risk women. In 1998, Fisher showed that tamoxifen could reduce breast cancer incidence by 50%. Nevertheless, only half the women who develop breast cancer have risk factors other than age, so what can be done for women without risk factors? The recognition that nonsteroidal antiestrogens have the ability to modulate estrogen action selectively has advanced the design and development of new drug for multiple diseases. Tamoxifen and raloxifene maintain bone density and raloxifene is now used to prevent osteoporosis and is being tested as a preventive for coronary heart disease and breast cancer. The drug group is now known as selective estrogen receptor modulators (SERMs) and the challenge is to design new agents for multiple applications. If the 20th century was the era of chemotherapy, the 21st century will be the era of chemoprevention.

1. Introduction

In a lecture in 1935, at the annual meeting of the American Association for Cancer Research in Boston, Professor Antoine Lacassagne of Paris' Institute du Radium reviewed the evidence for a hormonal contribution to the pathogenesis of mammary carcinoma. He suggested that one might one day be able to identify women at specific risk and antagonize the actions of estrogenic hormones to reduce that risk. He wrote at the conclusion of his paper: 'If one accepts the consideration of adenocarcinoma of the breast as the consequence of a special heredity sensitivity to the proliferative actions of oestrone, one is led to imagine a therapeutic preventive for subjects predisposed by their heredity to this cancer. It would consist — perhaps in the very near future when the knowledge and use of hormones will be better understood — in the suitable use of a hormone, antagonistic or excretory to prevent the stagnation of oestrone in the ducts of the breast' [1].

Forty years ago Jensen proposed the ER as a mechanism of estrogen action specific to target tissues [2] and Lerner [3,4] described the first nonsteroidal antiestrogen that blocked estrogen action with no estrogen-like activity in any other species or target tissue. However, the development of a pure antiestrogen would not have allowed the implementation of Lacassagne's vision. If estrogen is essential for a woman, to maintain bone density and protect against coronary heart disease, then the long-term administration of a antiestrogen would provide no overall health benefit, despite the prevention of breast cancer. Tamoxifen was discovered by the late Dr Arthur Walpole at AstraZeneca, Macclesfield, England, who believed it would be a safe and effective chemotherapy for the palliative treatment of advanced breast cancer [5]. Serendipitously, tamoxifen was discovered to possess the properties of a SERM and has become the lead compound for drug discovery in the first century of the next millennium.

Abbreviations: ER, Estrogen receptor; SERM, Selective estrogen receptor modulator.

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With an aging population there are demands for a disease free life, as cures remain elusive. To achieve this goal Sporn first used the term chemoprevention and described a strategy to arrest or prevent the process of carcinogenesis [6,7]. Tamoxifen is the first chemopreventive for breast cancer [8], but the demonstration of proof of principle [9,10] has opened the door to prevent more than cancer. The recognition of SERM action [11–16] resulted in a paradigm shift in drug discovery in 1990 [17] that is currently having an important impact on general medicine.

> We have obtained valuable information about this group of drugs that can be applied to 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 bones 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 targeted population would be postmenopausal women in general, thereby overcoming the requirement to select a high-risk group to prevent breast cancer [17].

Raloxifene is the first SERM that holds the promise of multiple applications [18]. This paper traces the progress that has been achieved in the last 30 years to develop antiestrogenic drugs for clinical use and to apply this knowledge for the prevention of breast cancer.

2. Tamoxifen: the first antiestrogen for the treatment of breast cancer

Tamoxifen blocks the binding of tritiated estradiol to the ER derived from rat uterus [19–22] or human tumor [23,24]. However, initial clinical studies with tamoxifen were conducted exclusively on unselected populations of postmenopausal women with advanced breast cancer [25,26] and it was only in 1977 that it was noted that tamoxifen was more likely to be effective in ER positive breast cancer [27]. Tamoxifen is currently used as a palliative therapy in the treatment of pre and postmenopausal patients with ER positive advanced (Stage IV) breast cancer. By contrast, the application of the concept of adjuvant therapy has revolutionized the treatment of breast cancer. Systemic adjuvant therapy is used following breast surgery to destroy undetected micrometastases around a woman's body.

Adjuvant studies with tamoxifen have proved to be successful in increasing survival [28–30] but, perhaps most importantly, the interaction between laboratory and clinical research endeavors has ultimately elucidated both the principal mechanism of action of tamoxifen as an antitumor agent in women and identified those women most likely to benefit from adjuvant tamoxifen treatment.

The 1998 Oxford Overview Analysis [31] involved any randomized trial that was started before 1990. The analysis included 55 trials of adjuvant tamoxifen versus no tamoxifen before recurrence. The study population was 37 000 women with node positive and node negative breast cancer, thus comprising 87% of world evidence of known randomized clinical trials. Of these women, fewer than 8000 had a very low or zero level of ER and 18 000 were classified as ER positive. The ER status of the remaining nearly 12 000 women was unknown, but based on the normal distribution of ER in random populations; the authors estimated that two-thirds would be ER positive.

This clinical trial data base [31] has been used to answer the questions raised over the past two decades by laboratory results and hypotheses. In the 1970's three laboratory observations emerged that merited evaluation in clinical trial: (1) tamoxifen blocks estrogen binding to the ER so patients with ER positive disease would be more likely to respond than those with ER negative disease [32]; (2) tamoxifen prevents mammary cancer in rats [33,34] so the drug could reduce the incidence of primary breast cancer; and (3) long term treatment was better than short term treatment to prevent rat mammary carcinogenesis, so longer adjuvant therapy with tamoxifen should be superior to short term adjuvant therapy [35–37], i.e. 5 years of tamoxifen should be superior to 1 year of tamoxifen. By the late 1980's, tamoxifen had been shown in the laboratory to block estrogen stimulated breast tumor growth but to encourage the growth of human endometrial cancer implanted in the same athymic mouse [16,38]. The clinical question therefore became 'are patients, who are receiving long term adjuvant tamoxifen therapy, at risk for an increased incidence of endometrial cancer?' [16].

The process of evaluating the impact of translational research is important to establish what works, and achieves clinical progress, and what does not. A clinical trial should not be started without a strong hypothesis and the incorporation of the relevant scientific results. The results of the Overview Analysis have been discussed in detail elsewhere [18] but will be summarized briefly.

The Overview shows that the proportional mortality reductions were similar for women with node positive or node negative disease [31]. However, the absolute reductions in mortality were much greater in node

positive than node negative disease. Additionally, patients with ER positive disease have an increased reduction in death rate with longer duration of tamoxifen treatment whereas patients who are ER negative do not benefit from tamoxifen, regardless of the duration of therapy. The value of a long duration of treatment is most important for the premenopausal patient (Fig. 1). This latter finding is new, as the results for premenopausal women could not be ascertained with certainty in earlier Overviews [30]. The Oxford Overview Analysis has established the veracity of the laboratory concepts that tamoxifen would be most effective in ER positive disease, longer duration would be more beneficial, and tamoxifen would prevent primary breast cancer, in this case contralateral disease [32–37].

Overall, the absolute improvement in recurrence was greater during the first 5 years following surgery but improvement in survival increased steadily throughout the first 10 years. This is an important finding because the patient is clearly benefiting from tamoxifen despite stopping therapy. There is an accumulation of the tumoristatic/tumoricidal actions of tamoxifen for at least the first 5 years of treatment, but the benefit continues after therapy stops. This is also true for the reduction in contralateral breast cancer; the breast seems to be protected so the value remains after therapy stops. This observation is extremely important for the application of tamoxifen as a preventive because a 5-year course of tamoxifen would be expected to protect a woman from breast cancer for many years afterwards.

Finally, the risk/benefit ratio of tamoxifen therapy can be stated to be strongly in the benefit category. The risk of endometrial cancer, a concept derived from laboratory studies [16,38], is of concern, but the benefits clearly outweigh the risks. In contrast, early concerns about the carcinogenic effects of tamoxifen in the rat liver [39] do not translate to the clinic as there is no evidence from the Overview Analysis of an increase in either liver or colorectal cancer in-patients who take tamoxifen [31].

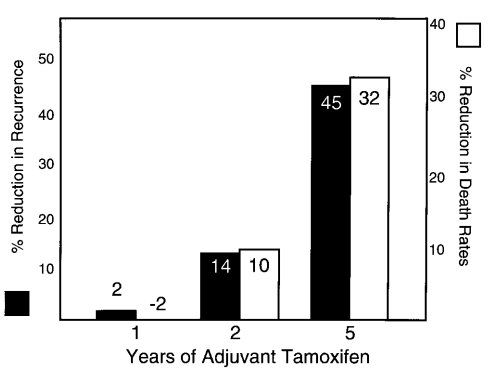

Fig. 1. The relationship between the duration of adjuvant tamoxifen therapy in ER-positive premenopausal patients and the reduction in recurrence and death rate. A longer duration of treatment has a dramatic effective on patient survival. Data adapted from [31].

3. Tamoxifen: the first antiestrogen for the prevention of breast cancer

Tamoxifen has been studied in mouse models of carcinogenesis to provide a basis for clinical testing of the concept of prevention. Early, long-term tamoxifen therapy inhibits mouse mammary tumorigenesis [12,13] and the therapy is superior to early oophorectomy. However, this is only one piece of laboratory evidence was used as a rationale to support the use of tamoxifen as a preventive treatment.

The administration of antiestrogens for different times around the time of carcinogen administration in rats can alter carcinogenesis [34,40,41]. The co-administration of carcinogens and antiestrogens to female rats prevents mammary carcinogenesis [34]. Short-term (4 week) administration of tamoxifen a month after carcinogen administration only delays carcinogenesis, but it does reduce the number of mammary tumors produced [36]. In contrast, long-term treatment with low dosages of tamoxifen after the carcinogenesis insult can almost completely prevent the development of mammary tumors [42]. These data, plus the finding that tamoxifen has a good safety profile [43] as well as the observation that tamoxifen halves the incidence of contralateral breast cancer, served as the incentive to initiate clinical trials.

Dr Trevor Powles at the Royal Marsden Hospital, England, initiated the first pilot study of tamoxifen in high-risk women [44–46]. However, the results were inconclusive [47] because the study was too small and not designed to be a chemoprevention study.

In contrast, the specific aim of NSABP Breast Cancer Prevention Trial P-1 was to test the value of tamoxifen as a preventive for breast cancer. This prospective clinical trial closed after accruing 13,388 women because of the exceptionally high-risk status of the participants, which made the projected events adequate to establish statistical significance. The study design is illustrated in Fig. 2. Those eligible for entry included any women over the age of 60 and women between the ages of 35 and 59 whose 5 year risk of developing breast cancer, as predicted by the Gail Model [48] was equal to that of a 60-year-old woman. In addition, any woman older than 35 years with a diagnosis of lobular carcinoma in situ (LCIS) was eligible for entry into the study. In the absence of LCIS, the risk factors necessary to enter the study varied with age. A 35-year-old woman had to have a relative risk of 5.07, whereas the required relative risk for a 45-year-old woman was 1.79. Routine endometrial biopsies were also performed to evaluate the incidence of endometrial carcinoma in both arms of the study.

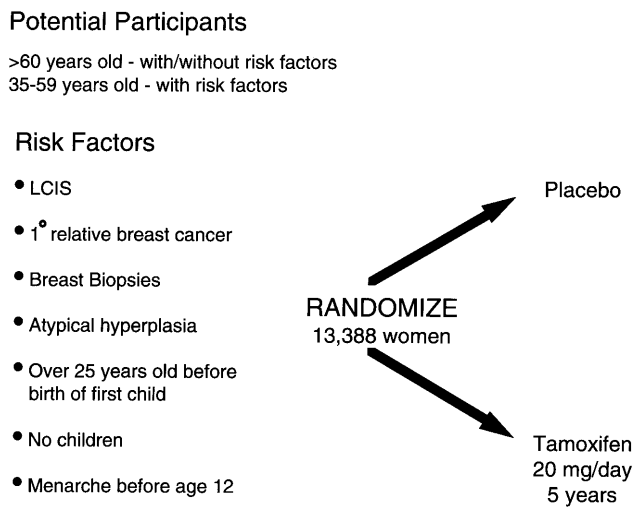

Fig. 2. Eligibility and design of the National Surgical Adjuvant Breast and Bowel Project tamoxifen breast cancer prevention trial. Originally, the recruitment goal was 16 000 volunteers, but the actual calculated risk of the recruited group was higher than anticipated and resulted in a changed in recruitment goals. A total of 13 388 women were recruited by summer, 1997, and the preliminary results were reported in April 1998 [8]. A full report was presented in September 1998. LCIS, lobular carcinoma in situ.

The first results of the NSABP study were reported after a mean follow-up of 47.7 months [8]. A total of 368 cases of invasive and noninvasive breast cancer occurred among the participants, 124 in the tamoxifen group and 244 in the placebo group. A 47% reduction in the risk of invasive breast cancer and a 50% reduction in the risk of noninvasive breast cancer were observed in women taking tamoxifen. A subset analysis of women at risk due to a diagnosis of LCIS demonstrated a 56% reduction in this group. The most dramatic reduction was seen in women at risk due to a diagnosis of atypical hyperplasia for whom risk was reduced by 86%.

The benefits of tamoxifen were observed in all age groups. The relative risk of breast cancer ranged from 0.45 in women 60 and older, to 0.49 for those in the 50- to 59-year age group, and to 0.56 for women aged 49 and younger (Fig. 3). A benefit of tamoxifen was also observed for women with all levels of breast cancer risk within the study, a finding that indicates that the benefits of tamoxifen are not confined to a particular lower- or higher-risk subset. Benefits were observed in women at risk on the basis of family history and those whose risk was due to other factors.

As expected, the effect found for tamoxifen was on the incidence of tumors positive for estrogen receptor (ER), which was reduced by 69% per year. The rate of ER-negative tumors in the tamoxifen group (1.46 per 1000 women) did not significantly differ from the rate in the placebo group (1.20 per 1000 women) (Fig. 4). Tamoxifen use reduced the rate of invasive cancers of

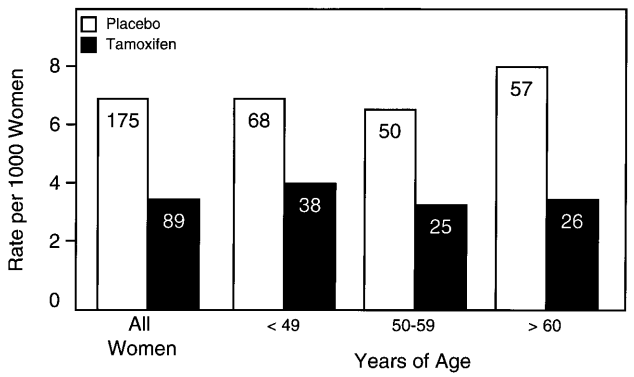

Fig. 3. The overall reduction in invasive breast cancer observed in the NSABP tamoxifen prevention trial P-1 in women at high risk for the disease, recruited to receive either tamoxifen (20 mg daily) or placebo. The women were also subdivided into age groups, and the same reduction in the incidence of breast cancer was observed. The numbers of breast cancers are shown on the top of each histogram for each treatment arm. Data adapted from [8].

all sizes, but the greatest differences were seen in the incidence of tumors 2.0 cm or less in size. Tamoxifen also reduced the incidence of both node-positive and node-negative breast cancers. The beneficial effects of tamoxifen were observed for each year of follow-up study. After year 1, the risk was reduced by 33%, and in year 5, it was reduced by 69%.

Tamoxifen also reduced the overall incidence of osteoporotic fractures of the hip, spine, and radius by 19% [8]. This difference approached, but did not reach, statistical significance. Reduction was greatest in women aged 50 and older at study entry. No difference in the risk of myocardial infarction, angina, coronary artery bypass grafting, or angioplasty was noted between groups [8]. These were secondary end points

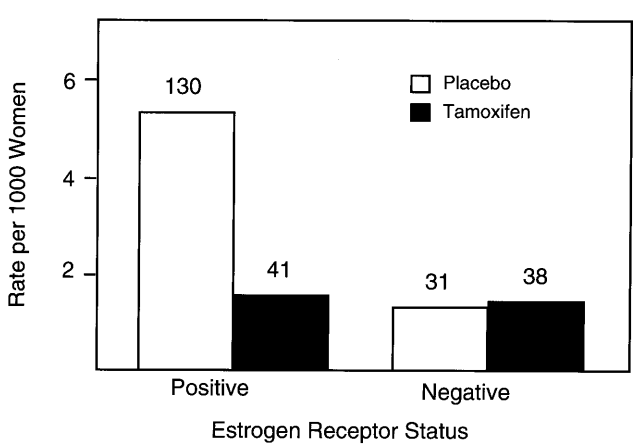

Fig. 4. Incidence of estrogen receptor (ER)-positive and ER-negative breast cancer in the placebo- and tamoxifen- treated groups of the NSAPB tamoxifen breast cancer prevention trial. The antiestrogen reduced the risk of developing ER-positive breast cancer, but no change was seen in the incidence of ER-negative breast cancer. The number of invasive breast cancers is shown at the top of the histogram for each treatment group. Data adapted from [8].

monitored in low-risk populations, however, so a major benefit would not be anticipated.

This study confirmed the association between tamoxifen use and endometrial carcinoma [31,49]. The relative risk of endometrial cancer in the tamoxifen group was 2.5. The increased risk was seen in women aged 50 and older, whose relative risk was 4.01. All endometrial cancers in the tamoxifen group were grade 1, and none of the women on tamoxifen died of endometrial cancer. An endometrial cancer death occurred in the placebo group. Although no doubt exists that tamoxifen increased the risk of endometrial cancer, one must recognize that this increase translates to an annual incidence of 2.3 women per 1000 who develop endometrial carcinoma.

More women in the tamoxifen group than in the placebo group developed deep vein thrombosis [8]. Again, this excess risk was confined to women aged 50 and older. The relative risk of deep vein thrombosis in the older group was 1.71 (95% confidence interval, 0.85–3.58). An increase in the occurrence of pulmonary emboli was also seen in the older women taking tamoxifen, who had a relative risk of approximately 3. Three deaths from pulmonary emboli occurred in the tamoxifen arm of the study, but all were in women with significant comorbidities. An increased incidence of stroke (relative risk, 1.75) was also seen in the tamoxifen group, but this did not reach statistical significance.

An assessment of the incidence of cataract formation was made using patient self reporting. A small increase in cataracts was noted in the tamoxifen group — a rate of 24.8 women per 1000 compared to 21.7 per 1000 in the placebo group. Risk of cataract surgery also increased in the women on tamoxifen. These differences were marginally statistically significant and were observed in the older patients in the study. These findings emphasize the need to assess the patient's overall health status before making a decision to use tamoxifen for breast cancer risk reduction.

An assessment of quality of life showed no difference in depression scores between groups [50]. Hot flashes were noted in 81% of the women on tamoxifen compared with 69% of the placebo group; the tamoxifen-associated hot flashes appeared to be of no greater severity than those in the placebo group. Moderately bothersome or severe vaginal discharge was reported by 29% of the women in the tamoxifen group and by 13% of the women in the placebo group. No differences in the occurrence of irregular menses, nausea, fluid retention, skin changed or weight gain, or loss, were reported.

In 1998, tamoxifen became the first drug to be approved for the reduction of risk for breast cancer.

4. A second strategy: recognition of selective estrogen receptor modulation

Throughout the 1960s and 1970s, antiestrogenic activity was correlated with antitumor activity. However, the finding that triphenylethylene-type antiestrogens expressed increased estrogenic properties, i.e. vaginal cornification and increased uterine weight in the mouse [51,52] raised questions about the reasons for the species specificity. One obvious possibility was species-specific metabolism, i.e. the mouse converts antiestrogens to estrogens via novel metabolic pathways. No species-specific metabolic routes to known estrogens have been identified [53–55], but knowledge of the mouse model created a new dimension for study, that ultimately led to the recognition of the target site specific actions of triphenylethylene-type antiestrogens. This concept is now referred to as selective ER modulation.

The ER positive breast cancer cell line MCF-7 [56] can be heterotransplanted into immune-deficient athymic mice but the cells can only grow into tumors with estrogen support. Paradoxically, tamoxifen, an estrogen in the mouse [51,52] does not support tumor growth [57] but stimulates mouse uterine growth with the same spectrum of tamoxifen metabolites present in both the uterus and human tumor [11]. To explain the selective actions of tamoxifen in different targets of the same host, it was suggested that the tamoxifen–ER complex could be interpreted as a stimulatory or inhibitory signal at different sites [11]. A similar conclusion can be drawn from the observation that long-term tamoxifen treatment prevents mouse mammary carcinogenesis in high incidence strains [12]. In contrast, tamoxifen initially causes a strong estrogen-like effect in the uterus that ultimately becomes refractory to estrogen stimulation within 8 weeks [13].

The concept of the target site specificity of antiestrogens was consolidated with experimental evidence from two further models that translated into the clinic. First, tamoxifen and raloxifene both maintain bone density in the ovariectomized rat but both compounds inhibit estradiol-stimulated uterine weight [14] and prevent carcinogen induced mammary tumorigenesis [15]. The laboratory studies on bone density and remodeling have been adequately confirmed and translated to the clinic [58,59]. Tamoxifen reduces hip and wrist fractures in postmenopausal women [8] and raloxifene prevents fractures of the spine [60]. Tamoxifen [8] and raloxifene [61] also reduce the incidence of breast cancer. Second, the finding that tamoxifen would partially stimulate the growth of a human endometrial carcinoma transplanted into athymic mice [38] allowed the question to be asked: 'if a human endometrial and breast tumor were transplanted into the same athymic mouse would tamoxifen exhibit differential pharmacology at two human target sites?' Tamoxifen demonstrated target site

specificity; breast tumor growth was blocked but endometrial tumors continued to grow [16]. Again the range of tamoxifen metabolites was the same in the tumors despite the opposite responses. Thus, the tamoxifen–ER complexes must be interpreted differently in the breast and uterus. These data also warned about the possibility of an increase risk of endometrial cancer in women taking long-term tamoxifen therapy. After a decade of investigation, there is known to be three to fourfold increase in the incidence of endometrial cancer in postmenopausal women [8,62].

5. Raloxifene as a multifunctional drug

Raloxifene is a potent antiestrogen [63,64], but maintains bone density [14,64] in laboratory animals. These properties encouraged clinical testing as a preventive for osteoporosis but with the added advantage of breast and uterine safety. Clinical trials demonstrate that raloxifene maintains bone density and prevents fractures of the spine [59,60]. The SERM has an antiestrogenic action in the breast to reduce the incidence of breast cancer [61]. Raloxifene is being tested as a breast cancer preventive in the study of tamoxifen and raloxifene (STAR) trial in North America and being evaluated in women at high risk for coronary heart disease in a study called the raloxifene use for the heart (RUTH). On the successful completion of the clinical program raloxifene may become the first multifunctional medicine [65]. Most importantly the introduction of raloxifene as a clinically useful agent again illustrates the success of translational research [17] and has opened the door to the design of new agents.

6. New agents

Newer agents have similar chemical structures to the existing SERMs, tamoxifen and raloxifene, but with slight modifications with the goal of increasing the spectrum of antitumor activity and reducing toxicity (Fig. 5). The ongoing investigation of several molecules may demonstrate advantages as breast cancer therapies and/or as a treatment for osteoporosis. Most importantly, the drugs may be multifunctional medicines and have applications for the prevention of osteoporosis, coronary heart disease and breast cancer. A diaryltetrahydronaphthalene derivative referred to as CP 336,156 reportedly has a high affinity for ER and antiuterotropic activity while preserving bone density in the rat [66]. There are two diastereometric salts. CP 336 156 is the l enantiomer that has 20 times the binding affinity of the d enantiomer. Studies demonstrated that the l enantiomer had twice the bioavailability of the d enantiomer [67].

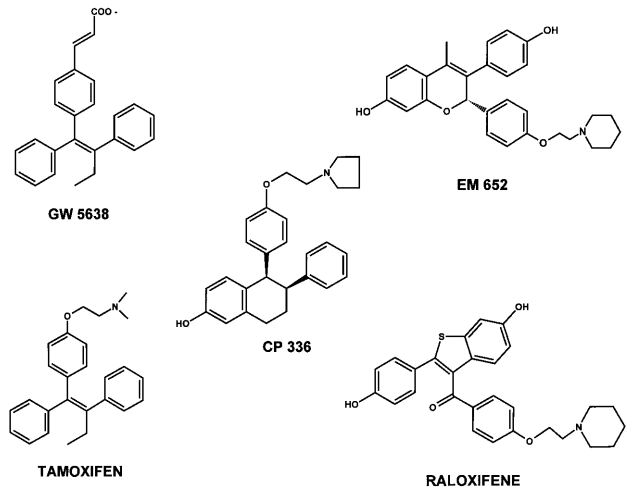

Fig. 5. Formulae of novel SERMs referred to in the text and compared with the formulae of tamoxifen and raloxifene.

GW 5638, discovered by Willson in 1994 at Glaxo Wellcome, is effective in preservation of bone density with minimal antiuterotropic activity in animal models [68]. The compound departs from the usual tertiary amino antiestrogenic side chain with a shorter allyl carboxylic group on a triphenylethylene molecule. The molecule induces a complex with properties similar to pure antiestrogens [69,70]. This interesting new SERM is currently being tested in animal models in an attempt to establish a lack of cross resistance with tamoxifen without enhancement of endometrial cancer growth. As it is a tamoxifen analog, laboratory research must also examine any potential role for GW 5638 in rat liver carcinogenesis so as to permit clinical trials in well women and to facilitate broader applications.

The compound EM 800 and its active metabolite EM 652 are orally active agents with virtually no uterotropic activity and could be described as orally active pure antiestrogens because the molecule silences both AF-1 and AF-2 in ERα [71,72]. The location of the antiestrogenic side chain is similar to that of the steroidal pure antiestrogens, but would seem too short for optimal activity. EM 800 is an antitumor agent in the DMBA model [73], and has antiestrogenic activity in mice with none of the estrogenic activity seen with tamoxifen [74]. The drug is extremely potent against breast cancer cells in culture and prevents the growth of estrogen-stimulated tumor xenografts in athymic mice [75]. However unlike other pure antiestrogens, EM 800 does not decrease bone density in the rat. As an orally active pure antiestrogen, EM 800 could be used as a second line therapy following tamoxifen failure. Based on the structural similarity with other raloxifene analogues, EM 652 appears to be a SERM with potential cross resistance with tamoxifen. A recent report demonstrates that EM 652 and raloxifene both have the

antiestrogenic side chain interacting with aa351 in the ER. The D351Y mutant converts both EM 652 and raloxifene to an estrogenic complex whereas a pure antiestrogen, ICI 182,780 is unaffected [76]. Therefore, EM 800 may fail as a second line agent after tamoxifen treatment and may be more beneficial as first line therapy. However, EM 652 may have broad application as a raloxifene-like drug.

7. Conclusion

The development of current compounds and the design of new agents based on the emerging understanding of SERM action [77] will exploit novel targets to control a broad range of diseases. The discovery of a new ER known as ERβ [78] has introduced a new tier of complexity for targeting SERMs [79,80]. Indeed the recent resolution of the X-ray crystallography of ligand binding domain of ERα with raloxifene and 4OHT [81,82] and ERβ with raloxifene [83] has already provided an insight into the similarities and differences in the complexes that can be exploited.

It is clear that the challenge for the future is to exploit these differences to design molecules that act exclusively as agonists or antagonists at a particular receptor. This may be achieved through ligand engineering but additionally, the realization that the surface of the complexes can present additional targets [70,84,85] could prove invaluable to the future of chemoprevention.

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