Selective Estrogen Receptor Modulators: An Alternative to Hormone Replacement Therapy (44204)

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Abstract. **Estrogen is a key regulatory hormone, which in addition to its role in reproduction, affects a number of physiological systems, including the skeleton and cardiovascular system. The important role of estrogen in various tissues is perhaps most evident in postmenopausal women who, in addition to menopausal symptoms, experience increases in osteoporosis and coronary heart disease as their estrogen levels decline. Estrogen replacement, while effective against osteoporosis and heart disease, produces a number of side effects associated with the breast and uterus which limits compliance. Selective estrogen receptor modulators (SERMs), such as raloxifene and tamoxifen, produce beneficial estrogen-like effects on bone and lipid metabolism, while antagonizing estrogen in reproductive tissue. SERMs can be distinguished from each other in reproductive tissue, particularly the uterus, by their activity profile. For example, while triphenylethylenes like tamoxifen behave as partial agonists, raloxifene (a benzothiophene) behaves as a complete antagonist in the uterus. The SERM profile is distinct from that of full estrogens (*i.e.* 17β-estradiol or 17α-dihydroequilenin) which behave as estrogen agonists in all tissues and pure estrogen antagonists (i.e. ICI-164,384) which exhibit only an estrogen antagonist profile in a battery of tissue types. The precise mechanism by which SERMs produce this tissue-selective pharmacology remains a question. It is clear, however, that for raloxifene, both the estrogen agonist effects on bone and cholesterol metabolism as well as the estrogen antagonist effects in uterine and mammary tissue involve high affinity interaction with the estrogen receptor. The estrogen antagonist activity is mediated via classical pharmacological competition for estrogen receptor binding. The estrogen agonist activity, in bone for example, appears to involve novel post-receptor pathways and non-classical estrogen response element(s) which are activated by SERMs. These novel response elements may represent natural pathways which respond to estrogen metabolites *in vivo*.** [P.S.E.B.M. 1998, Vol 217]

Impact of Estrogen on Women's Health

Achieving a "rectangularization of life" is an important goal for modern therapeutics. Over the last 50 years, medical advances such as antibiotics for bacterial infections and current therapies for cardiovascular diseases have led some to foresee a society where nearly all individuals survive to an advanced age, then succumb rather abruptly over a narrow age range (1, 2). This rectangularized survival curve, a plot of cumulative survival versus age, highlights the importance of targeting new therapies to additional disease. Thus, as the global population ages, diseases associated with frailty will become of greater consequence, particularly those associated with significant disability such as osteoporosis. A key goal of therapeutics aimed at such chronic disabling diseases will be to prevent the underlying causes of morbidity. Therefore, clinically effective medicines, with excellent safety profiles that insure compliance, will be essential for therapeutic regimens targeted at these disease states.

As women enter the menopause, a number of hormonal changes occur. Most notable of these changes is the dramatic fall in circulating levels of 17β-estradiol and estrone (3). Progestin pulsatility decreases, and pituitary gonadotrophin levels (FSH, LH) increase (4). These hormonal

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0037-9727/98/2171-0045$10.50/0

changes are associated with loss of menstrual cyclicity and a number of symptoms associated with the decline in estrogen (i.e., hot flushes, mood swings, and sleep disorders). Important chronic diseases, such as heart disease and osteoporosis, have also been attributed to (or associated with) long-standing estrogen deprivation. Heart disease is the leading cause of death in postmenopausal women, as the apparent benefit of estrogen on $LDL_{cholesterol}$ and other markers of cardiovascular disease is lost in the postmenopausal period (5). Postmenopausal women also experience a sharp rise in bone turnover that leads to a net loss of bone mass due to excessive bone resorption by osteoclasts (6). Whereas a number of various fractures are associated with postmenopausal bone loss, the most severe outcome of postmenopausal osteoporosis is hip fracture. Over one-half of the women who experience a hip fracture become dependent upon someone else for all or part of their daily care (7). The eventual cost of osteoporosis to the health care system in the United States in 1995 was estimated to be $13.8 billion, and as the postmenopausal population increases, this cost should increase as well (8).

The ability of estrogen, either alone (estrogen replacement therapy or ERT) or in combination with one of several progestin regimens (hormonal replacement therapy or HRT), to prevent many of the symptoms and diseases associated with the menopause speaks to the central role of estrogen in the pathophysiology of these disease states. ERT and HRT arrest bone loss due to the menopause (9, 10) and reduce the rate of major cardiovascular events such as myocardial infarction and cerebrovascular accidents (11, 12). Preliminary evidence suggests a potential benefit of estrogen use on cognitive function (13). Unfortunately, ERT and/or HRT are associated with a number of side effects including: resumption of menses, breast tenderness, and abdominal bloating, among others (14). Recent studies show that in addition to these side effects, additional concerns over increased relative risk for breast and uterine cancer exist. Use of HRT for 5 years was recently associated with a 40% increased risk of developing breast cancer (15). Others have suggested no real increase in relative risk for breast cancer in estrogen users (16). However, in reviewing the numerous epidemiological data available on the relationship between HRT and breast cancer, consensus groups have concluded that long-term use of HRT may be associated with up to 35%–40% increased incidence of breast cancer (17). Duration of exposure may be a key determining factor (16). Addition of the progestin (HRT) is required in women with an intact uterus to counteract the uterine stimulatory effect of estrogen and subsequently reduce the risk for uterine cancer. While this is likely true for short-term HRT regimens aimed at treating menopausal symptoms, a recent case-controlled study in the United States suggests that depending upon the progestin schedule used and the duration of therapy, an increased risk of developing endometrial cancer can be detected in postmenopausal women using HRT (18).

The ultimate impact of the side effect profile with chronic estrogen use and concern over cancer risk is a severe limitation to compliance with ERT or HRT for the prevention of chronic diseases such as osteoporosis or coronary heart disease, as these disease states are typically asymptomatic until either the occurrence of a fracture or cardiovascular event. It is clear, particularly in the case of osteoporosis, that once estrogen therapy is discontinued, the benefit of estrogen is lost over time (19). Thus, there exists a need for, what we have termed, "the ideal estrogen." The ideal estrogen is one that produces the beneficial effects of estrogen on bone mass, intermediate endpoints of cardiovascular disease (i.e., cholesterol reduction), and perhaps cognition. With the ideal estrogen, these positive effects occur in the absence of estrogen-like stimulation of reproduction-associated tissue, most notably, the breast and uterus. These key factors should be accompanied by an excellent overall safety profile in postmenopausal women in order to insure long-term compliance for a preventative medicine.

The first clues to the possibility of ideal estrogens were provided by compounds that were associated traditionally with estrogen antagonist activity for breast cancer, and thus, were categorized as "anti-estrogens." These agents, exemplified by tamoxifen, a triphenylethylene, (Fig. 1) and raloxifene, a benzothiophene, (Fig. 1) were later demonstrated to exhibit either full or partial estrogen agonist effects at various tissue sites (20, 21). Since both the estrogen agonist and antagonist activities of these compounds involve high

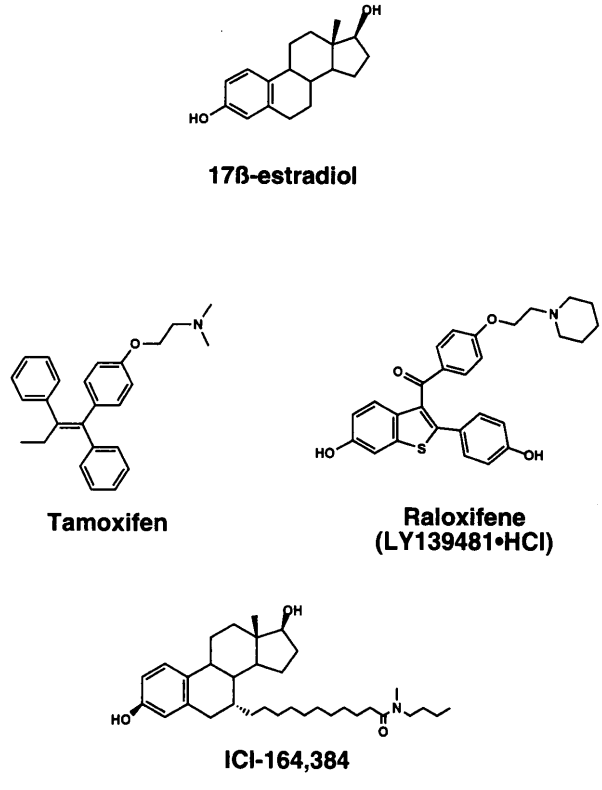

Figure 1. Chemical structures of representative ligands for the estrogen receptor.

affinity interaction with the estrogen receptor, agents displaying this tissue selective profile were later classified as selective estrogen receptor modulators (SERMs) (22). As will be discussed here, SERMs exhibit a distinct pharmacological profile from full estrogens (i.e., ICI-164,384; Fig. 1). While the discussions herein will focus on tamoxifen and raloxifene as representative SERMs, there now exist a large number of agents with a SERM-profile (23), which include: tamoxifen-like triphenylethylenes (droloxifene, idoxifene, toremifene, clomiphene), benzopyrans (centchroman), dihydronaphthylenes (trioxifene, LY326315), tetrahydronaphthylenes (CP-336,156), and substituted benzothiophenes (LY317783 · HCl). In this review we will summarize the SERM activity profile in bone, cholesterol metabolism, uterus, and mammary tissue. We will also describe potential mechanisms for the tissue selective activity.

SERM Tissue Activity Profile

Bone. Estrogen deficiency in postmenopausal women increases the rate of bone turnover with bone resorption outpacing bone formation and resulting in the net loss of bone mineral density, particularly at trabecular rich bone sites such as the metaphyses of long bones, vertebrae, and hip (5). Postmenopausal bone loss can be reproduced in ovariectomized (OVX) rats that exhibit osteopenia in as little as 5 weeks (24). The effects of various estrogenic agents at preventing bone loss induced by ovariectomy in rats are depicted in Figure 2. As shown, an orally available estrogen (ethynyl estradiol) exhibits a dose-dependent anti-osteopenic effect that approaches the level of bone mineral density measured for ovary-intact controls. The SERMs, tamoxifen and raloxifene, exhibit a similar anti-osteopenic effect, albeit with reduced potency. However, the pure estrogen antagonist, ICI-164, 384, has no effect on bone mineral density in OVX rats (25).

As with estrogen, the antiosteopenic effects of raloxifene and tamoxifen are associated with suppression of bone resorption, which can be shown both by dynamic bone histomorphometry (26) as well as by measurement of biochemical markers of bone metabolism (27). With raloxifene, the beneficial effects on bone mineral density are associated with an improvement in the biomechanical properties of bone in OVX rats as well (28). Positive effects of raloxifene or tamoxifen have also been described in postmenopausal women, either as the result of placebo-controlled trials (29) or as an observation in women under treatment for breast cancer (30).

Cholesterol Metabolism. As a woman enters menopause, an increase in $LDL_{cholesterol}$ levels is observed that approaches those measured in males (4). Estrogen lowers $LDL_{cholesterol}$ and increases $HDL_{cholesterol}$ (31). The OVX rat model is quite useful for assessing the $LDL_{cholesterol}$ lowering capacity of estrogenic agents (32) although it suffers limitations in terms of modeling $HDL_{cholesterol}$ effects. Because both $LDL_{cholesterol}$ and $HDL_{cholesterol}$ in rats is cleared via hepatic LDL receptor, cholesterol levels in the rat can be used to predict $LDL_{cholesterol}$ lowering efficacy of estrogen-like substances. As shown in Figure 3, ethynyl estradiol, tamoxifen, and raloxifene all produce hypocholesterolemic effects of similar magnitude in OVX rats, with ethynyl estradiol showing the greatest potency. As with bone, ICI-164,384 showed no significant estrogen agonist activity (25). The hypocholesterolemic activity with raloxifene occurs via interaction with the estrogen receptor, based on extensive testing of chemically related benzothiophenes for estrogen receptor binding affinity (*in vitro*) and their ability to lower cholesterol *in vivo* (33). The ability of SERMs to lower $LDL_{cholesterol}$ has also been demonstrated in postmenopausal women. Daily administration of raloxifene for 2 months to postmenopausal women in a placebo-controlled, double-blind study resulted in a significant reduction of $LDL_{cholesterol}$ and treatment of postmenopausal breast cancer patients with tamoxifen produced similar lowering of cholesterol (29).

While the cholesterol reducing effects of SERMs should produce a positive effect on cardiovascular events, it is clear that estrogen produces other, non-cholesterol-associated effects on the cardiovascular system that contribute to the overall cardioprotective profile attributed to ERT or HRT. Less information is currently available with regard to specific mechanisms for these effects of estrogen, although effects on vascular smooth muscle proliferation and migration, endothelial factors, and LDL oxidation have been hypothesized. As no clear mechanism or model exists

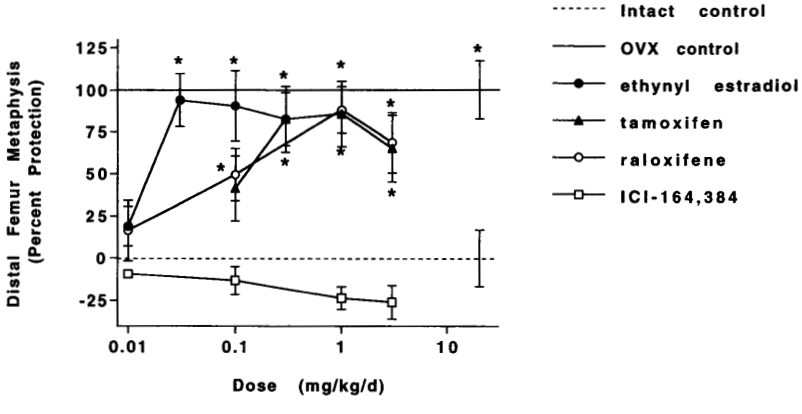

Figure 2. Effect of representative ligands for the estrogen receptor on bone mass in 75-day-old ovariectomized (OVX) rats following 35 days of oral administration (25).

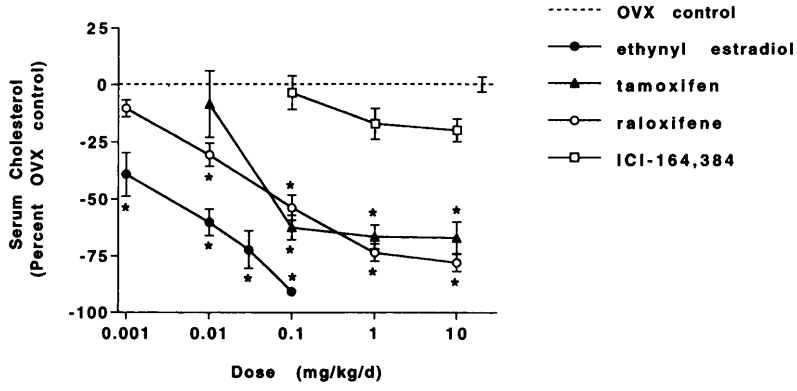

Figure 3. Effect of representative ligands for the estrogen receptor on serum total cholesterol levels in 75-day-old ovariectomized (OVX) rats following 35 days of oral administration (25). * = $p < 0.05$ vs. OVX control.

for these non-cholesterol-associated cardioprotective effects of estrogen, it is difficult to know how to model potential activities of SERMs. However, raloxifene has been shown to reduce aortic cholesterol content in rabbits (34) and inhibit LDL oxidation (35). Additionally, chronic tamoxifen treatment in cholesterol-fed mice also reduced aortic cholesterol burden (36). In breast cancer patients receiving tamoxifen therapy a significant reduction in serious cardiovascular events was observed (37).

Uterus. The uterus is very sensitive to estrogens and represents a key organ for clinical safety concerns with chronic use of estrogenic agents. Certain SERMs distinguish themselves from traditional estrogens in terms of uterine stimulation. In assessing the uterine profile of various ligands for the estrogen receptor, it is important to consider their activities both in the absence and the presence of estrogen in order to determine relative estrogen agonist and estrogen antagonist capacities.

In OVX rats, reduction in circulating 17β-estradiol levels results in uterine atrophy within a few days. As shown in Figure 4, administration of ethynyl estradiol markedly stimulates the uterus as measured by uterine weight gain. Tamoxifen similarly increases uterine weight in OVX rats. In contrast, raloxifene produces no consistent or dose-related increases of uterine weight. Consistent with its lack of estrogen agonist activity in other tissues, ICI-164,384 also fails to increase uterine weight (25). A similar pattern is observed for other estrogenic responses in the uterus of OVX rats given raloxifene, including no stimulation of endothelial cell height and eosinophil infiltration (24). Postmenopausal women given raloxifene for a 2-month period demonstrated no signs of uterine stimulation as determined by histologic procedures, whereas similar women in the same study given conjugated equine estrogens displayed marked stimulation of the uterus (29). Endometrial stimulation of women treated chronically with tamoxifen represents a significant safety concern: an increase in uterine carcinomas was observed in women following 1 year of continuous exposure to tamoxifen in a breast cancer prevention trial (38).

The estrogen antagonist properties of SERMs are also very important in understanding the uterine profile of these agents. In immature rats given maximal stimulatory doses of estrogen, raloxifene completely antagonizes the effects of estrogen, as does ICI-182,780 (Fig. 5). By contrast, tamoxifen partially blocks the action of estrogen on the uterus, as at higher dose levels the intrinsic uterine stimulation produced by tamoxifen itself is a limiting factor (39). Thus, in the uterus, tamoxifen behaves as a classical partial agonist at the estrogen receptor whereas raloxifene and ICI-182,780 are complete antagonists. Further work distinguishing the uterine effects of raloxifene and tamoxifen has shown that raloxifene can completely block tamoxifen-induced elevation of uterine epithelial cell height in ovariectomized rats (40). The differences in the uterine profile of raloxifene and tamoxifen can also be explained on a structural basis, as the carbonyl hinge portion of raloxifene results in a more planar orientation of the basic side chain that moves this moiety

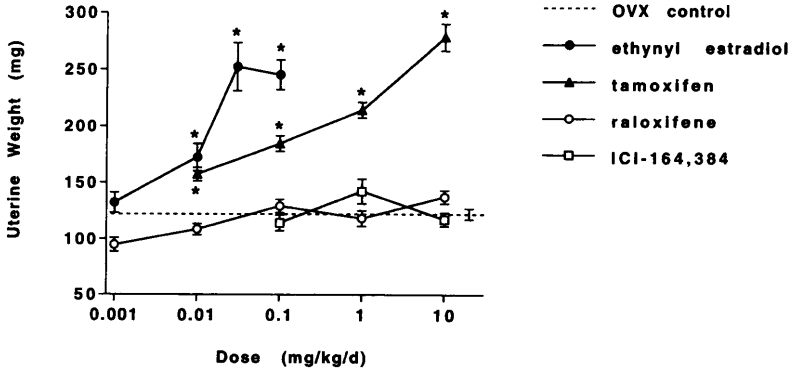

Figure 4. Effect of representative ligands for the estrogen receptor on uterine weight in 75-day-old ovariectomized (OVX) rats following 35 days of oral administration (25). * = $p < 0.05$ vs. OVX control.

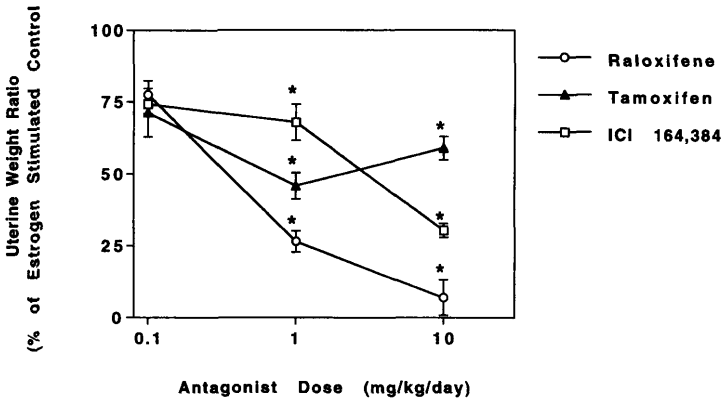

Figure 5. Estrogen antagonist effect of representative ligands for the estrogen receptor on uterine weight in estrogen-supplemented (ethynyl estradiol, 0.1 mg/kg; PO) immature rats following 3 days of oral administration (39). * = $p < 0.05$ vs. ethynyl estradiol control.

out of the average plane of the three-ring system (41). The basic side chain of the triphenylethylene structure of tamoxifen, however, lies within the average plane of the stilbene nucleus. The orientation of this basic side chain is an essential element for determining whether other SERMs produce a more raloxifene-like or tamoxifen-like profile in the uterus. The piperidyl constituent of raloxifene's basic side chain also has been associated with reduced uterine stimulation, as compared to the dimethyl amino basic side chain of tamoxifen (41).

Mammary Tumors. Tamoxifen and raloxifene were originally developed as antagonists of estrogen-dependent tumors of mammary tissue. *In vitro,* estrogen induces proliferation of MCF-7 cells (human breast cancer cell line), an effect that is potently antagonized by raloxifene with an IC_{50} of 0.2 nM (42). The putative active metabolite of tamoxifen, 4-hydroxy-tamoxifen, and ICI-182,780 inhibit estrogen-induced MCF-7 cell proliferation with a similar potency and maximal efficacy level (42). Various *in vivo* models have shown the ability of tamoxifen and raloxifene to blunt growth of established mammary tumors induced by carcinogens such as dimethylbenzanthrocene (43) or in tumor cell xenografts in athymic mice (44). ICI-164,384 produces similar anti-tumor effects in animal models of breast cancer (45). Work in animal models has also shown that both tamoxifen and raloxifene are effective at preventing mammary tumors induced by the carcinogen, nitrosomethylurea (46). Tamoxifen has proven clinical utility as a treatment for breast cancer (47).

SERMs: Multiple Pathways for Estrogen Responses

Efforts aimed at understanding the mechanism for selectivity of estrogen agonist versus estrogen antagonist activity for SERMs are currently the subject of intensive research. The estrogen receptor itself lies at the center of this mechanism. Recent evidence generated primarily with the SERM raloxifene has shown the presence of distinct post-receptor binding pathways that are responsible for some of the tissue-selective activities of SERMs. The estrogen receptor is a nuclear transcription factor that is activated by ligand-binding to assume a conformation that permits binding to specific DNA sequence and subsequent activation or inhibition of gene expression (48). Modulation of the estrogen receptor plays a central role in the actions of SERMs on the skeleton and cholesterol metabolism as well as on reproductive tissue. Scatchard plot analyses of [^{3}H]-raloxifene or [^{3}H]-17β-estradiol binding to recombinant human estrogen receptor reveal that these two ligands compete for a single high-affinity binding site with respective K_d values of 54 and 86 pM (49). A similar high affinity binding of 4-hydroxy-tamoxifen to the estrogen receptor has been shown (49).

The mechanism by which raloxifene produces estrogen antagonistic effects in reproductive tissues is that of a classical pharmacological antagonist. By virtue of its ability to compete with estrogen for binding to the estrogen receptor, raloxifene prevented transcriptional activation of estrogen-response element (ERE)-containing genes, such as vitellogenin, thus preventing stimulation by estrogen (50). In the absence of estrogen, raloxifene produced no stimulatory effects on transcriptional activity of vitellogenin (50). These observations are very similar to the complete antagonist profile observed with raloxifene in uterine and mammary tissue *in vivo.*

Emerging evidence from several groups indicate the presence of multiple transcriptional pathways for ligand-bound estrogen receptor and the existence of multiple estrogen receptor subtypes. The estrogen receptor contains multiple transcriptional activating functions (i.e., AF-1 and AF-2) that account for some of the tissue-selective effects of tamoxifen (51). Furthermore, various ligand-induced receptor conformations may be responsible for the range of pharmacological effects observed for SERMs, including raloxifene (52). As a consequence of the unique estrogen receptor:raloxifene conformation, this complex binds to sequences of the DNA distinct from the ERE in tissues where raloxifene exerts estrogen-agonist effects. Recently, a novel mechanism for the transcriptional activation of the non-ERE containing gene, TGFβ3, by raloxifene was described. TGFβ is an abundant bone matrix protein with anti-osteoclastic properties whose expression *in vitro* was induced by SERMs but not by 17β-estradiol (53). In an *in vitro* cell-based transcriptional reporter assay, raloxifene in-

Table I. Summary of Tissue-Associated Estrogen Activities of Various Estrogen Receptor Ligands Based on Preclinical Studies

Compound class examples	Profile in bone	Profile on cholesterol metabolism	Profile in uterus	Profile in mammary tissue
17β-estradiol 17α-dihydroequilenin 17α-ethynyl estradiol	agonist	agonist	agonist	agonist
tamoxifen	agonist	agonist	partial agonist	antagonist
raloxifene	agonist	agonist	antagonist	antagonist
ICI-164,384 ICI-182,780	antagonist	antagonist	antagonist	antagonist

duced TGFβ3 expression relative to 17β-estradiol by a mechanism requiring the estrogen receptor, but not its DNA-binding domain. Since the TGFβ3 promoter lacks a classical ERE, these data suggest the presence of a novel pathway for estrogen receptor mediated activation of cellular function, which was termed the "raloxifene response element" or RRE (50). From these observations, one can infer that the unique conformation induced by the raloxifene:estrogen-receptor complex recruits other transcription factor(s) for DNA (RRE-specific) binding. Of note was the observation that *in vivo,* raloxifene and 17β-estradiol produced equivalent, dose-related increases in TGFβ3 mRNA obtained from femurs of treated ovariectomized rats (53). These observed discrepancies *in vitro* and *in vivo* suggest that an estrogen metabolite may be responsible for transcriptional activation of RRE-containing genes *in vivo.* Indeed, several estrogen metabolites were shown to be potent activators of the RRE pathway (50), suggesting that this pathway may represent a natural pathway for mediating the effects of estrogen in bone, distinct from the pathway that mediates the stimulatory effects of estrogen on reproductive tissues. Activation of TGFβ in vascular tissue may be important for cardiovascular effects of SERMs as well. In cholesterol-fed mice, tamoxifen reduced aortic atherosclerotic lesion size while elevating aortic levels of TGFβ (36).

Summary

Various classes of chemical ligands for the estrogen receptor are summarized in Table I. In order to understand how SERMs are positioned among the other compounds that bind to the estrogen receptor, it is essential to consider the activity profile in various tissue types. 17β-Estradiol and related steroidal estrogens (i.e., 17α-dihydroequilenin) (54) behave only as estrogen agonists in bone, uterus, and mammary tissue and on cholesterol metabolism. In no case with these complete estrogens does one see antagonistic effects. The complete estrogen antagonists (i.e., ICI-164,384 or ICI-182,780), on the other hand, lack the ability to mimic estrogen effects at these targets and produce only estrogen antagonist effects in reproductive associated tissues. Of note was the observation that ICI-182,780 also behaves as an estrogen antagonist on bone (55), and cholesterol metabolism (56). SERMs are those agents that depending upon the tissue type, produce either estrogen agonist or estrogen antagonist activities. Tamoxifen and raloxifene produce estrogen-like effects on bone and cholesterol metabolism and are estrogen antagonists in mammary tissue. However, in the uterus, raloxifene and tamoxifen diverge in their pharmacological profiles, tamoxifen behaves as a partial estrogen agonist whereas raloxifene acts as a complete estrogen antagonist. SERMs, like raloxifene, which have an improved safety profile in reproductive tissue, represent a potentially important alternative to chronic ERT or HRT in postmenopausal women for the prevention and treatment of osteoporosis and cardiovascular disease.

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