Distinct mechanisms of action of selective estrogen receptor modulators in breast and osteoblastic cells

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Distinct mechanisms of action of selective estrogen receptor modulators in breast and osteoblastic cells. Am J Physiol Cell Physiol 279: C1550–C1557, 2000.—Raloxifene and idoxifene are selective estrogen receptor modulators (SERMs) that exhibit tissue-specific agonist or antagonist properties via interactions with the estrogen receptor (ER). Both compounds are similarly osteoprotective in the ovariectomized rat in vivo as assessed by measurement of bone mineral density, urinary pyridinium cross-links, and serum osteocalcin, suggesting a similar mechanism of action. However, we have identified a fundamental difference in this mechanism via the estrogen response element (ERE) in osteoblast-like cells. With the use of ERE-luciferase reporter constructs, raloxifene, like the complete ER-antagonist ICI-182780, acts as an antagonist via the ERE in osteoblastic cells. In contrast, idoxifene, like 17β-estradiol itself and 4-OH-tamoxifen, acts as an agonist in osteoblastic cells via an ERE-mediated mechanism. Both ICI-182780 and raloxifene inhibited the ERE-dependent agonist activity of 17β-estradiol and idoxifene in osteoblastic cells. In contrast, in breast cells, raloxifene, idoxifene, 4-OH-tamoxifen, and ICI-182780 had no agonist activity and, indeed, raloxifene and idoxifene were potent antagonists of ERE-mediated 17β-estradiol action, indicating an ERE-dependent mode of action in these cells. Although these SERMs exhibit a similar antagonist activity profile in breast cells, they can be distinguished mechanistically in osteoblastic cells.

raloxifene; selective estrogen receptor modulator; estrogen; bone; breast

The protective effects of 17β-estradiol on the skeleton, and thus its utility in postmenopausal osteoporosis, have attracted considerable clinical attention (13, 18). Estrogen can effectively prevent the rapid bone loss that occurs in postmenopausal women and in clinically relevant animal models, such as the ovariectomized (Ovx) rat (2, 26). Although clinically useful in preventing bone loss, estrogen therapy has been reported to be linked to an increased risk of tissue-specific side effects including endometrial hyperplasia, which may result in uterine cancer, and proliferative effects in mammalian tissue, which may result in an increased risk of breast cancer (1, 4, 7, 8). Because of the known and suspected risks of estrogen therapy, it has been estimated that in the United States <40% of women on estrogen replacement therapy will continue treatment beyond 1 yr (18, 31).

The ideal postmenopausal “estrogen” would reproduce the beneficial effects of estrogen on vasomotor symptoms, skeletal tissue, and the cardiovascular system without producing the adverse effects of estrogen on reproductive tissues (15). This concept has led to the development of selective estrogen receptor modulators (SERMs) (16, 41). A SERM is defined as a compound that exhibits estrogen agonism in one or more target tissues such as bone or liver and exhibits antagonism and/or minimal agonism (i.e., clinically insignificant) in reproductive tissue (15, 16, 31, 39, 41). Although a number of SERMs have reached later stages of clinical development, only raloxifene is approved for the treatment and prevention of osteoporosis. Since the failure of idoxifene and levormeloxifene in the clinic was associated with unwanted reproductive tissue side effects, we decided to investigate the mechanism for the tissue-selective activity of raloxifene with idoxifene and other estrogen receptor (ER) ligands.

We reported previously (26) that idoxifene, like raloxifene, lowers cholesterol levels in Ovx rats, decreases uterine weight in intact rats, and is osteoprotective in vivo. We have now extended these studies to directly compare the SERMs idoxifene and raloxifene in the Ovx rat in vivo. The effects of idoxifene and raloxifene in vivo were examined by dual-energy X-ray absorptiometry (DXA) measurement of bone mineral density, serum osteocalcin, and urinary pyridinium cross-link excretion as markers of bone turnover. In addition, we also have evaluated the molecular mechanism of action of raloxifene and idoxifene as well as that of 17β-estradiol, 4-OH-tamoxifen, and the pure antagonist ICI-182780 (37) in osteoblast-like and breast cells in vitro.

We utilized reporter constructs containing estrogen response elements (ERE X5, ERE X3) upstream of the...
luciferase reporter gene as an indicator of the estrogenic agonist activity of the ER ligands. This approach measures directly the effects of estrogen agonists or antagonists on gene expression and extends classic ER binding studies, since receptor binding does not necessarily correlate with the modulation of gene expression in a given tissue (14). We report that both idoxifene and raloxifene are similarly osteoprotective in vivo. Interestingly, the results in vitro support data previously reported for a lack of raloxifene activity via the ERE (15, 40). Our data provide important information that distinguishes the molecular mechanism of action of idoxifene as an ERE-mediated agonist from that of raloxifene and point to differences suggesting that the clinically acceptable profile of raloxifene in bone may be due to non-ERE-mediated effects.

MATERIALS AND METHODS

In Vivo Experiments

All procedures were reviewed and approved by the Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals. Virgin female Sprague-Dawley rats (Charles River) were used at the age of 8–9 mo following an acclimation period of at least 1 mo. Before either sham operation or ovariectomv was performed, proximal tibial bone mineral density (BMD) was determined by DXA with the use of a Hologic QDR-1000 (Hologic, Waltham, MA) equipped with high-resolution scanning software. Animals were maintained anesthetized with isoflurane while placed prone on an acrylic block. The hind legs were maintained in exterior rotation with adhesive tape, and hock joints were arranged at a 90° angle to aid reproducibility. Surgery was done after the collection of 24-h urine samples for the determination of baseline pyridinium cross-link excretion by ELISA (product no. 8001; Metra Biosystems, Palo Alto, CA).

Groups of Ovx rats (n = 8/group) were given either idoxifene (0.5 mg/kg), raloxifene (1.0 mg/kg), or vehicle [1% aqueous solution (wt/vol) of carboxymethyl cellulose] by oral gavage once daily for 28 days. The doses chosen were based on the previously reported maximum effective dose (3, 26). A group of sham-operated rats was dosed with vehicle.

During the course of the following measurements were made: proximal tibial BMD (expressed as a percentage of the baseline BMD for each animal), serum osteocalcin (Biomedical Technologies, Stoughton, MA), and pyridinium cross-link excretion (measurements normalized to creatinine).

In Vitro Experiments

Cell culture and materials. All chemicals were purchased from Sigma (St. Louis, MO) unless otherwise stated. The cell lines used in this study were human breast cancer cells MCF-7 (36), human osteosarcoma cells MG-63 (6), and rat osteosarcoma cells Ros 17/2.8 (20). All cell lines were grown routinely in Dulbecco’s modified Eagle’s medium (DMEM; Gibco-BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco-BRL) supplemented with penicillin (10 U/mL) and streptomycin (100 mg/mL) (Gibco-BRL) and 1.1 mM calcium chloride and 25 mM HEPES. All experiments were performed in phenol red-free Eagle’s modified minimal essential medium (MEM) containing 10% heat-inactivated, charcoal-dextran-stripped fetal bovine serum (HyClone, Logan, UT) and supplemented as described above. All cell lines were cultured at 37°C in a humidified atmosphere of 95%/5% CO2. At confluence, cells were subcultured after exposure to trypsin-EDTA (Gibco-BRL). The MMTV-ERE(X5)-Luc and C3-ERE(X3)-Luc constructs were a kind gift from D. McDonnell (Duke University, Durham, NC). The MMTV-ERE(X5)-Luc construct comprises the mouse mammary tumor virus promoter in which the glucocorticoid response elements have been replaced with five copies of a 33-base pair vitellogenin estrogen response element [positive strand of oligonucleotide: 5′-AATTCAAGTCTAGTTCTAGCTACCCATG GCCAGTAAGGCCAGGCAGTTATGC (upstream of the luciferase reporter gene MMTV-ERE(X5)-Luc) (38). The C3-ERE(X3)-Luc construct is the natural complement 3 containing three nonconsensus EREs cloned upstream of the luciferase reporter gene (25). These constructs were used for transfection in both osteoblast-like and breast cells. The Renilla luciferase vector was used to correct for transfection efficiency using the dual-luciferase detection method (Promega, Madison, WI). The osteocalcin promoter (POCZCAT) was a kind gift from J. Lian (University of Massachusetts, Worcester, MA). This construct was transfected into Ros 17/2.8 cells as described in Transient transfections, and chloramphenicol acetyltransferase (CAT) activity was determined with the use of a CAT ELISA (Boehringer Mannheim, Indianapolis, IN) as described in the manufacturer’s protocol. Results are expressed as absorbance units (405 nm). ICI-182780 and raloxifene were synthesized at SmithKline Beecham.

Transient transfections. Cells were seeded in either six-well plates at 1.5 × 105 cells/well or in 24-well plates at 1.5 × 104 cells/well in phenol red-free medium. DNA was introduced into the cell lines by the Lipofectin method (Life Technologies, Gaithersburg, MD). Briefly, cells were cotransfected with 1 µg of either MMTV-ERE(X5)-Luc or C3-ERE(X3)-Luc per well in 24-well plates and 25 ng of the control Renilla luciferase vector (pRL-CMV) (Promega) to monitor transfection efficiency and were then incubated overnight. Transfection medium was removed, and the cells were incubated for 48 h with or without ligands. Cell lysates were prepared as described in the manufacturer’s protocol for dual-luciferase reporter assay (Promega). Briefly, cells were washed in PBS and then lysed with 100 µl/passive lysis buffer for 15 min on a rocking platform. Lysates were centrifuged for 30 s at 14,000 g, and the clear lysate was transferred to a tube before reporter enzyme analysis. Samples (20 µl) were transferred to a 96-well luminescence detection plate and reacted with 100 µl of each assay reagent (Promega). Each assay reagent was injected by using a microlumat LB96P luminometer (Wallac, Gaithersburg, MD). Results are expressed as a percentage of maximum agonism (100%) from triplicate wells for each ligand concentration.

Cell proliferation. MCF-7 cells were seeded at 1.5 × 105 cells/well in six-well plates and treated with ligands (100 nM) for 48 h. Cell counts were performed in triplicate under light microscopy by using a hemacytometer.

Statistical Analysis

Biochemical marker data from in vivo studies as well as all in vitro data were analyzed using one-way ANOVA followed by Dunnett’s multiple comparisons test. BMD data were analyzed by repeated-measures ANOVA. All statistical analyses were carried out using Statistica for Windows (version 5.1; Statsoft, Tulsa, OK).

RESULTS

In Vivo Studies With Idoxifene and Raloxifene

Four weeks after surgery, the Ovx group had ~7% lower BMD in the proximal tibia than the sham controls. This loss was significantly prevented by treat-
ment with either raloxifene or idoxifene (Fig. 1). The difference between the two treated groups was not significant, and values for either group were not significantly different from those for shams.

Ovariectomy also resulted in an increase in biochemical markers of bone turnover, including urinary pyridinoline (Fig. 2A) and serum osteocalcin (Fig. 2B). Both raloxifene and idoxifene caused a decrease in urinary pyridinoline from the Ovx level. There was no statistical difference in excreted cross-link levels between idoxifene- and raloxifene-treated animals. In addition, both compounds reduced osteocalcin to the same extent relative to the Ovx group.

![Graph 1: BMD (% baseline)](image1)

**Fig. 1.** Idoxifene and raloxifene prevent bone loss in the proximal tibia of ovariectomized (Ovx) rats. Data are bone mineral densities expressed as percent baseline values (means ± SE). Mean baseline densities were 0.260, 0.260, 0.259, and 0.261 g/cm² for the sham, Ovx, Ovx + idoxifene (I), and Ovx + raloxifene (R) groups, respectively. BMD, bone mineral density. *P < 0.01 vs. Ovx as determined by repeated-measures ANOVA.

![Graph 2: Pyr/creat (mol/mol)](image2)

**Fig. 2.** Idoxifene and raloxifene decrease bone turnover in Ovx rats. Rats were treated as described in MATERIALS AND METHODS. Pyridinyl cross-links were measured by ELISA in a 24-h urine sample collected on day 12 (A). Osteocalcin was measured in serum collected on day 14 (B). Data are presented as means ± SE. *P < 0.05 vs. Ovx as determined by Dunnett’s multiple comparisons test.

![Graph 3: % Agonist Activity](image3)

**Fig. 3.** Effects of 17β-estradiol, idoxifene, raloxifene, tamoxifen, and ICI-182780 on estrogen response element (ERE)-dependent transcriptional activity in rat osteoblast-like cells (Ros 17/2.8). Ros 17/2.8 cells were transfected with the MMTV-ERE(X5)-Luc construct as described in MATERIALS AND METHODS. Cells were treated with either vehicle (dimethylformamide [DMF]), 17β-estradiol (E), idoxifene (I), raloxifene (R), 4-OH-tamoxifen (T), or ICI-182,780 (ICI) (10 nM–10 μM) for 48 h. 17β-Estradiol, idoxifene, and 4-OH-tamoxifen showed significant increases in reporter gene activation as ERE agonists, while raloxifene and ICI-182780 exhibited no activity. These observations are averages of duplicate experiments, and the maximum range was ±11%. Results are typical of at least 6 independent experiments.

![Graph 4: % Agonist Activity](image4)

**Fig. 4.** Effects of 17β-estradiol, idoxifene, raloxifene, tamoxifen, and ICI-182780 on complement 3-ERE-dependent transcriptional activity in Ros 17/2.8 cells. Ros 17/2.8 cells were transfected with the C3-ERE(X3)-Luc construct as described in MATERIALS AND METHODS. Cells were treated with either vehicle (DMF), 17β-estradiol, idoxifene, raloxifene, 4-OH-tamoxifen, or ICI-182,780 (100 nM) for 48 h. 17β-Estradiol, idoxifene, and 4-OH-tamoxifen showed significant increases in reporter gene activation as ERE agonists, while raloxifene and ICI-182780 exhibited no activity. Data are presented as means ± SD; n = 3. *P < 0.05 compared with vehicle alone. Results are typical of at least 3 independent experiments.
In Vitro Studies With ER Ligands

Raloxifene and the pure antagonist ICI-182780 had no demonstrable direct agonist activity on the MMTV-ERE(X5)-Luc construct transfected into Ros 17/2.8 cells (Fig. 3). In contrast, idoxifene and 17β-estradiol were both potent agonists via the ERE in Ros 17/2.8 cells (Fig. 3). We reported previously (26) that idoxifene, like 17β-estradiol, was an agonist through this response element in both rat and human osteoblast-like cells (26). 4-OH-tamoxifen appeared to be a mixed agonist/antagonist, showing a biphasic effect with a decrease in agonist activity at higher concentrations (Fig. 3). We confirmed that these observations were not dependent on the number of EREs in the construct by using the endogenous complement 3 promoter that contains three nonconsensus EREs (Fig. 4).

With the C3-ERE(X3)-Luc construct, idoxifene, 17β-estradiol, and 4-OH-tamoxifen were all agonists, whereas raloxifene and ICI-182780 had no demonstrable agonist activity (Fig. 4). These observations are similar to reports by us and others using the endogenous ERE from the complement 3 gene linked to luciferase (25, 26). To confirm the ERE-dependent agonist activity of idoxifene, we transfected Ros 17/2.8 cells with a control luciferase reporter and the osteocalcin promoter construct (pOCZCAT) lacking an ERE. 1,25-Dihydroxyvitamin D3 was used as a positive control because it has been shown to directly upregulate this promoter via the osteocalcin vitamin D responsive element (42). No increase in reporter gene (CAT) activity was observed in the presence of 100 nM 17β-estradiol, idoxifene, raloxifene, 4-OH-tamoxifen, or ICI-182780, suggesting that an intact ERE is required for transcriptional activity in these cells (Fig. 5A). This concentration of ligand was effective at inducing ERE-dependent transcription (Fig. 3). No transcriptional activation was observed with idoxifene when the same reporter vector lacking the ERE was used (Fig. 5B). We next examined the effects of these ER ligands in human osteoblast-like (MG-63) cells transfected with the MMTV-ERE(X5)-Luc construct, where the profile was similar to that observed in rat osteoblast-like cells, suggesting that these effects are not species specific (Fig. 6).

In contrast to the effects seen in osteoblast-like cells, 17β-estradiol was the only ligand exhibiting measurable agonist activity in human breast cancer cells (MCF-7) transfected with the ERE reporter (Fig. 7). 17β-Estradiol was a potent agonist through the ERE in human breast cells.

To confirm the estrogen selectivity in breast cancer cells, we also examined MCF-7 proliferation. 17β-Estradiol, but not the other ligands, stimulated breast cancer cell proliferation (Fig. 8). These data may suggest that ERE-dependent activity is directly responsible for the increase in MCF-7 cell proliferation (Fig. 8).
Although there is a correlation between ERE-dependent agonist activity and cell proliferation, these effects could also be through non-ERE-containing genes. None of the ligands, including 17β-estradiol, had any effect on the proliferation of either rat or human osteoblast-like cells in vitro (data not shown).

Because of the lack of agonism of idoxifene and raloxifene in MCF-7 cells in vitro, we next tested the ability of idoxifene and raloxifene to antagonize 17β-estradiol-induced ERE-dependent transcriptional activity (Fig. 9). Both raloxifene and idoxifene were able to completely suppress 100 nM 17β-estradiol-induced transcriptional activation of the MMTV-ERE(X5)-Luc construct in MCF-7 cells (Fig. 9). In addition, both raloxifene and idoxifene suppressed 17β-estradiol-stimulated proliferation of MCF-7 cells (data not shown). This is similar to the antagonism of 17β-estradiol shown previously for both 4-OH-tamoxifen and ICI-182780 (10, 27). Our data indicate that idoxifene functions as a full agonist via the ERE in osteoblast-like cells (Fig. 3) and is a functional antagonist in breast (Fig. 9).

Given the clear cell-specific differences between raloxifene and idoxifene, we next examined the ability of idoxifene and raloxifene to antagonize 17β-estradiol-stimulated transcriptional activity in osteoblast-like Ros 17/2.8 cells. Idoxifene had no effect on the agonist activity of 17β-estradiol (100 nM), whereas raloxifene...
dose-dependently inhibited the ERE-dependent transcriptional activity of 17β-estradiol in osteoblast-like cells (Fig. 10).

To investigate this difference in antagonism/agonism further, we compared the ability of raloxifene and idoxifene to antagonize 17β-estradiol-stimulated transcription compared with that of the pure antagonist ICI-182780. Raloxifene and ICI-182780 (500 nM) both inhibited the agonist activity of 100 nM 17β-estradiol in osteoblast-like cells (Fig. 11) similarly to their antagonist activity in MCF-7 cells (Fig. 9). In contrast, idoxifene (500 nM) had no effect on the agonist activity of 17β-estradiol in osteoblast-like cells (Fig. 11). With the use of submaximal agonist concentrations of 17β-estradiol (<0.1 nM), idoxifene was able to enhance the observed 17β-estradiol-stimulated transcription (data not shown). It is likely that idoxifene is stimulating target gene activation in the presence of low concentrations of estradiol by simply increasing the amount of available ligand of the receptor and thereby enhancing the occupancy of the receptor. In addition, both raloxifene and ICI-182780 (500 nM) completely inhibited 100 nM idoxifene-stimulated transcriptional activity in osteoblast-like cells (Fig. 11), indicating that both raloxifene and ICI-182780 are acting as antagonists of idoxifene- and 17β-estradiol-stimulated transcription. These data highlight the mechanistic differences between raloxifene and idoxifene and suggest that the osteoprotective effects of both may be mediated by both ERE- and non-ERE-dependent mechanisms.

**DISCUSSION**

It was shown previously that raloxifene, idoxifene, 4-OH-tamoxifen, and ICI-182780 bind to the ER (17, 21) and that binding of ligands to nuclear receptors produces either agonist or antagonist effects, depending on the specific cellular context (14, 21). The fact that raloxifene and idoxifene are able to function as dose-dependent antagonists of 17β-estradiol-stimulated transcription in human breast cancer cells and appear to have different activities in osteoblast-like cells suggests that this in vitro system is a viable model in which to investigate cell-specific differences between these ER ligands. The differences in potency of estrogen between breast and osteoblast cells suggest that there could be other important differences between the
mechanism of action of this ER ligand in these different tissues. Together, these data support the notion that both raloxifene and idoxifene, like 4-OH-tamoxifen and the pure antagonist ICI-182780, may have potential utility in estrogen-responsive breast cancer treatment (7).

Interestingly, although both raloxifene and idoxifene are osteoprotective in vivo, their mechanism of action in bone cells appears quite distinct. The fact that both compounds are ER ligands suggests a similar mechanism of action in bone. However, raloxifene is an antagonist and idoxifene is a potent agonist via the ERE in osteoblast-like cells. At all doses examined here, raloxifene antagonized 17β-oestradiol-stimulated transcriptional activity in osteoblast-like cells (Figs. 9 and 10). We confirmed the data generated through the MMTV-ERE(5)-Luc construct using the complement 3 gene promoter, which contains three non-consensus EREs and has been shown to be regulated by ER ligands in vivo (25). It is therefore possible and, given the clinical data, highly likely that there are distinct mechanisms for the bone-sparing effects of both raloxifene and idoxifene. These observations suggest that, although raloxifene and idoxifene are osteoprotective, there may be fundamental mechanistic differences in the reproductive tissue. The lack of agonism by raloxifene via the ERE in Ros 17/2.8 and MG-63 cells suggests that either raloxifene is substantially less potent than idoxifene (concentrations of raloxifene higher than those reported here could not be used due to inherent cell toxicity) or that the molecular mechanism of action of raloxifene is different from that of either idoxifene or 17β-oestradiol (15, 40). The data presented here provide mechanistic support for the clinical efficacy of raloxifene. These data indirectly support the existence of the raloxifene response element (RRE) or related elements through which raloxifene has been shown to exert its effects in osteoblast-like cells (40, 41). Raloxifene has been shown to act as an agonist via a non-ERE sequence located in the 5'-untranslated region of the human transforming growth factor TGFβ3 promoter (41) and, potentially, other target genes. In human and rat osteoblast-like cells, raloxifene, in contrast to idoxifene, inhibited ERE-containing promoter activity and exhibited pure estrogen antagonism. This distinguishes the mechanism of action of raloxifene from that of idoxifene. These data demonstrate that idoxifene acts as an agonist in osteoblast-like cells via the ERE, similarly to the agonist activity of 17β-oestradiol in these cells (Figs. 3 and 6).

The activity of 4-OH-tamoxifen in osteoblast-like cells is in agreement with the clinical data of ER-mediated partial agonist activity in bone and the cardiovascular system (19, 34). 4-OH-tamoxifen also has been shown to be both an agonist and antagonist of 17β-oestradiol action in osteoblast-like cells (11, 30). It would be interesting to examine whether bone-specific transcription factors modulate the actions of these ER ligands in a tissue-specific manner, as has been previously reported for the effects of Cbfa 1 on estrogen effects in osteoblast-like and non-osteoblast-like cells (33).

We propose a model (Fig. 12) to illustrate the mechanistic differences between raloxifene and idoxifene in osteoblast-like cells. Our data suggest that idoxifene acts as an agonist via the ERE, whereas raloxifene is an antagonist via this element (Fig. 12). The osteoprotective action of raloxifene (agonism) is presumably mediated by the RRE or other non-ERE elements. The model highlights the different mechanistic pathways that ultimately contribute to the osteoprotective effects of raloxifene and idoxifene and provides insight into the features that distinguish the two SERMs clinically.

This mechanistic explanation for the activity of ER ligands has been further complicated by the discovery of another ER isoform (ERβ), which has different ligand specificities and tissue distribution (9, 23). Recent studies on conformation of the isoforms of the ER have revealed that ligand binding induces distinct conformational changes to the receptors (14, 22, 29). The design of ligands and the elucidation of the reasons for tissue-specific expression of functional activity are areas of considerable research interest (22). In addition, the identification of nuclear hormone coreactivators and corepressors greatly impacts the tissue selectivity of the SERMs (35). There is also increasing evidence of estrogen(s) having a non-genomic mechanism of action (5) as well as activity through nonclassic EREs such as AP-1 (28). Evidence is also accumulating to support the existence of a plasma membrane ER (32) and involvement of estrogen in proteosome-dependent degradation of the ER (24). Added to this are data suggesting that estrogens and the SERMs can induce osteoclast apoptosis and thereby inhibit bone resorption (12, 26). It is possible that SERMs could exert their effects through a variety of mechanisms and have multiple target cells, which may contribute to tissue selectivity, and therefore the in vivo effects on bone could be through a variety of transcriptional and nontranscriptional mechanisms in multiple cell types that cannot be accurately represented as in vitro models. Nonetheless, idoxifene utilizes a classic ERE-dependent pathway in osteoblast-like cells and breast cells, whereas raloxifene, which has a similar mechanism of action in breast cells, has at least in part a distinct non-ERE-dependent mechanism of action in osteoblast-like cells. There are clearly mechanistic cell-specific differences between ER ligands that result ultimately in the same physiological end point of bone mass preservation.

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