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An estrogen receptor basis for raloxifene action in bone☆

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Abstract

Although controversy remains regarding direct effects of estrogen on bone, *in vivo* data clearly show that estrogens suppress bone turnover, resulting in decreased bone resorption and formation activity. Selective estrogen receptor modulators (SERMs), such as raloxifene, produce effects on bone which are very similar to those of estrogen. *In vitro*, both raloxifene and estrogen inhibit mammalian osteoclast differentiation and bone resorption activity, but only in the presence of IL-6. Data from a number of ovariectomized rat model manipulations (i.e. hypophysectomy, low calcium diet and drug combinations) demonstrate a strong parallel between the antiosteopenic effects of raloxifene and estrogen. A characteristic action of estrogens on the skeleton is inhibition of longitudinal bone growth, an effect which is not observed with other resorption inhibitors, including calcitonin and bisphosphonates. Consistent with an estrogen-like mechanism on bone, raloxifene inhibits longitudinal bone growth in growing rats. In addition to the overall similarity of the bone activity profile in animals, estrogen and raloxifene also produce similar effects on various signaling pathways relative to the antiosteopenic effect of these two agents. For example, IL-6, a cytokine involved in high turnover bone resorption following estrogen deficiency in rats, is suppressed by both raloxifene and estrogen. Raloxifene and estrogen also produce a similar activation of TGF-β3 (a cytokine associated with inhibition of osteoclast differentiation and activity) in ovariectomized rats. Like 17β-estradiol, raloxifene binds with high affinity to both estrogen receptor-α (ERα) and estrogen receptor-β (ERβ). Crystal structure analyses have shown that 17β-estradiol and raloxifene bind to ERα with small, but important, differences in three dimensional structure. These subtle differences in the conformation of the ligand:receptor complex are likely the basis for the key pharmacological differences between estrogens and the various SERMs (i.e. raloxifene vs tamoxifen). Raloxifene also produces estrogen-like effects on serum cholesterol metabolism and the vasculature. Thus, while raloxifene exhibits a complete estrogen antagonist in mammary tissue and the uterus, it produces beneficial effects on the cardiovascular system and prevents bone loss via an estrogen receptor mediated mechanism.
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Keywords: Raloxifene; Estrogen receptor; SERM

1. Introduction

Estrogenic hormones play an important role in a wide range of physiologic processes, including actions on mammary and uterine tissue, the skeleton, the cardiovascular system, the central nervous system and the immune system, to list a few. While the precise mechanism for estrogen action in many of the tissues remains elusive, it is clear that the estrogen receptor (ER) is central to most of these effects. The ER is a nuclear transcription factor which dimerizes and migrates to the nucleus upon ligand binding. The ER:ligand complex then interacts with specialized sequences of DNA in the promoter region of certain genes, with subsequent activation or inhibition of transcription [1]. Genomic sequences capable of binding the ER:ligand complex include a palindromic sequence known as the estrogen response element (ERE) as well as more recently described alternative sequences [2, 3]. Through these interactions with the ER, estrogens produce agonist effects to varying degrees in estrogen responsive tissues. Various nonsteroidal ligands also bind the ER, however, these compounds produce distinct ER:ligand complex conformations [4]. These molecules, known as selective estrogen receptor modulators (SERMs), interact with

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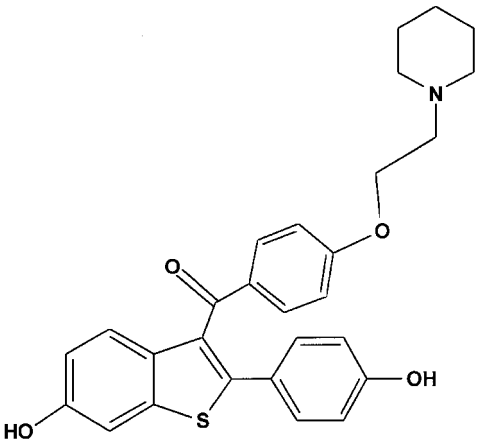
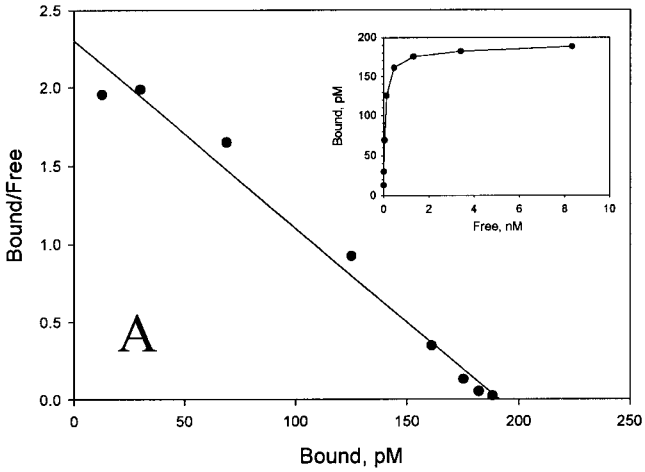


Fig. 1. Chemical structure of raloxifene (LY139481).

the ER to produce tissue dependent estrogen agonist or antagonist responses [5]. Raloxifene, a benzothiophene structure depicted in Fig. 1, is a prototypical SERM that produces estrogen-like effects in bone and on cholesterol metabolism in ovariectomized rats [6], ovariectomized monkeys [7] and postmenopausal women [8]. Raloxifene, however, fails to produce estrogen agonist effects on the uterus of ovariectomized rats or postmenopausal women [6, 8], permitting raloxifene to completely antagonize estrogen effects in the uterus [9].

This lack of significant estrogen agonist activity in the uterus with raloxifene is the central key feature distinguishing it from other SERMs, such as those of the triphenylethylamine family (i.e. tamoxifen, droloxifene, idoxifene) which are partial estrogen agonists in the uterus [9].

The tissue selective estrogen agonist/antagonist profile observed for raloxifene is best explained by its high affinity interaction with the ER. For example, this ability to bind ER is directly responsible for the estrogen antagonist profile of raloxifene in mammary tissue and the uterus, where direct competition with estrogen for the ER prevents subsequent ERE activation. Additionally, as reviewed here, the high affinity interaction with ER by raloxifene is also responsible for the estrogen agonist profile in the skeleton, via activation of non-ERE genomic sequences. The rationale behind an ER-based mechanism for the skeletal response to raloxifene is the subject of this review. The data summarized include: (1) the high affinity interaction of raloxifene with ER, (2) the similar profile of estrogen and raloxifene action in the skeleton of estrogen deficient animals and women, (3) similarity of response shifts in estrogen or raloxifene treated ovariectomized rats subjected to similar manipulations of the model and (4) similar effects of estrogen and raloxifene on key cytokines which regulate bone resorption. Finally, important structural activity relationships within the raloxifene molecule will also be considered.



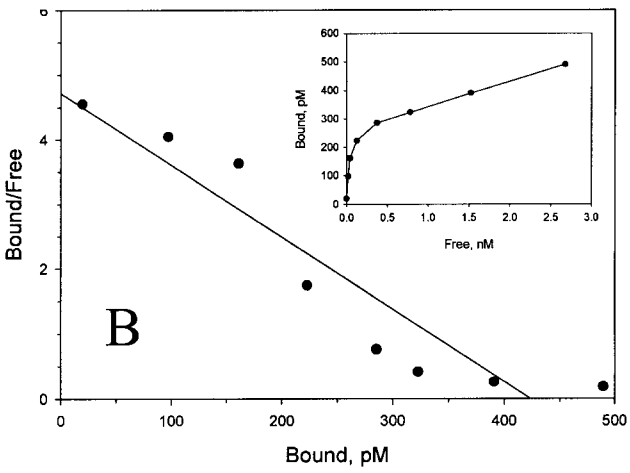


Fig. 2. Saturation binding of compounds to native human estrogen receptor (hER). Serial dilutions of ^{3}H-17β-estradiol (a) or ^{3}H-raloxifene HCl (b) were mixed with 0.25 mg/ml MCF-7 cell lysate containing native hER in the absence or presence of 1 or 2 μM (respectively) cold competitor in binding buffer. After 24 h incubation at 4°C, unbound radioligand was extracted with dextran-coated charcoal and bound radioligand was determined by scintillation counting. Bound and free radioligand were calculated (inset) and plotted according to Scatchard. (a): K_D = 83 pM, R^2 = 0.98; for (b): K_D = 90 pM, R^2 = 0.88.

1.1. Raloxifene binds with high affinity to ER

Raloxifene was similar to 17β-estradiol in exhibiting a high affinity, subnanomolar, binding affinity to the ERα [10]. Scatchard analyses of [^{3}H]-raloxifene or [^{3}H]-17β-estradiol binding to recombinant human ERα revealed that these two ligands bind a single high affinity site with respective K_d values of 90 and 83 pM (Fig. 2). As shown in Table 1, raloxifene does not bind significantly to other major steroid hormone receptors. In competitive inhibition binding assays, raloxifene

Table 1
Raloxifene does not bind to major nonestrogen receptor steroid hormone receptors

	% Inhibition at:			Internal Standard		
Receptor	1 nM	0.1 uM	10 uM	standard	IC_{50} (nM)	K_d (nM)
Glucocorticoid (human)	7	−15	17	dexamethasone	38	4.3
Progesterone (bovine)	−8	1	22	progesterone	1	0.3
Androgen (rat)	3	7	8	testosterone	8	0.4

completely inhibited binding of [^{3}H]-17β-estradiol to MCF-7 derived ERα, with a relative binding affinity of 0.37 ± 0.02 (relative binding affinity for 17β-estradiol = 1; [10]). These competitive binding assays showed that raloxifene and 17β-estradiol compete for a single ligand binding domain on ERα.

MCF-7 breast adenocarcinoma cells are a transformed human cell line, which naturally overexpress the human ERα (10^6 receptors/cell). Other binding analyses with recombinantly expressed human ERα containing the steroid binding domain, produced similar relative binding affinity with raloxifene [11]. Raloxifene also exhibited high affinity binding interactions with the recently described ERβ [11]. Analyses of the molecular structure of crystals of ERα complexed to raloxifene or 17β-estradiol have confirmed that raloxifene and estrogen interact with the ligand binding domain with minor differences in conformation [12]. Recently, through the use of a battery of benzothiophene analogs of raloxifene with varying affinities for the ERα, Kauffman *et al.* demonstrated that ER binding is also critical for the reduction of serum cholesterol produced by raloxifene in ovariectomized rats [13].

1.2. Raloxifene produces estrogen-like effects on bone

Perhaps one of the most distinctive effects of estrogen action on the skeleton is inhibition of longitudinal and radial bone growth (bone modeling), an effect observed in rodents and humans [14, 15]. While not of clinical relevance to postmenopausal women, the inhibition of bone elongation in the rat appears to be the result of a direct effect on chondrogenesis in the growth plate, an effect not ascribed to other inhibitors of bone turnover such as bisphosphonates or calcitonin. As depicted in Fig. 3, estrogen deficiency following ovariectomy in rats leads to increased longitudinal growth of the femur, while raloxifene produces an estrogen-like suppression of this bone growth [15]. The mechanistic basis for estrogen regulation of chondroblast differentiation, proliferation and synthetic activity remains to be elucidated, however, the remarkable similarity between raloxifene and estrogen on bone modeling is a strong indication of an estrogenic mechanism for raloxifene effects on the skeleton.

The estrogen agonistic effects of raloxifene on bone have been the subject of intensive experimentation. The ovariectomized rat is a useful model for the assessment of antiosteopenic agents. In ovariectomized

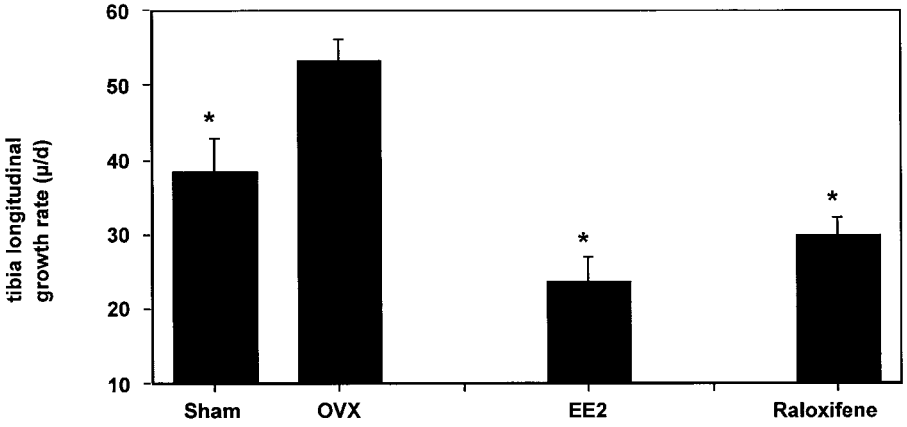


Fig. 3. Inhibition of longitudinal bone growth in growing ovariectomized (OVX) rats by ethynyl estradiol (EE2; 0.1 mg/kg) or raloxifene (3 mg/kg). * = $p < 0.05$ vs OVX control [16].

rats, raloxifene produces a clear, estrogen-like, prevention of bone loss [6]. *In vivo* dual energy X-ray absorptiometry (DEXA) analysis of the lumbar vertebrae (L1-4) of ovariectomized rats dosed daily for 4–6 weeks with either oral raloxifene or subcutaneous 17β-estradiol revealed quantitatively similar protective effects on bone mineral density [16]. Raloxifene showed a dose-related effects to preserve L1-4 bone mineral density in ovariectomized rats, with an ED_{50} value of 0.1–1 mg/kg. These observations were confirmed by subsequent quantitative computed tomography analysis of the proximal tibia in 6 month old ovariectomized rats [17]. This beneficial effect of raloxifene on bone mineral density of the axial and appendicular skeleton in ovariectomized rats was extended to 1 year with continuous daily oral treatment, with beneficial effects on bone that were quantitatively similar to those of an orally delivered estrogen (17α-ethynyl estradiol; [18, 19]). These beneficial effects of raloxifene to prevent estrogen deficiency-induced bone loss are also observed following 2 years of daily oral dosing in OVX monkeys [7] and in postmenopausal women [8].

When dosing is initiated at or near the time of ovariectomy (as in the studies described above), both raloxifene and estrogen maintain bone density at or near sham control levels. However, in ovariectomized rats with established osteopenia (i.e. 5–12 week postovariectomy delay prior to initiation of dosing), administration of estrogen produces only slightly higher bone mass relative to ovariectomized controls. Raloxifene tested under similar conditions produces an effect very similar qualitatively and quantitatively to that of estrogen [20]. The inability of either raloxifene or estrogen to add new bone in ovariectomized rats with established osteopenia is distinct from the effects of anabolic agents, such as parathyroid hormone or slow release-fluoride, which can restore bone to varying degrees in rats with established osteopenia (unpublished observations, [21, 22]).

While bone mineral density provides much valuable information as to the effectiveness of drugs for osteopenia, it is also important to consider bone architecture and quality. Histomorphometric data are particularly useful in this regard. In ovariectomized rats treated orally for 5 weeks with either raloxifene or 17α-ethynyl estradiol, raloxifene prevented trabecular bone loss by mimicking the action of estrogen as an inhibitor of bone resorption as evidenced by lowering of osteoclast number and eroded perimeter [15]. The effects of raloxifene on static trabecular bone parameters and measurements of bone resorption paralleled those of estrogen. Interestingly, in 75 day old rats, raloxifene and 17α-ethynyl estradiol did diverge in their effects on certain bone formation parameters in trabecular bone (i.e. estrogen suppressed bone formation rate and mineral apposition rate in ovariectomized rats while raloxifene produced less severe reductions of these endpoints [15]). However, the apparent discrepancy in effects on bone formation may be related to the growth status of the animals, as subsequent testing of raloxifene and 17α-ethynyl estradiol in older animals, using a model with greater relevance to bone formation, indicated that raloxifene produced an estrogen-like suppression of bone formation in addition to the antiresorptive effect [23]. The ability of raloxifene to produce an estrogen-like suppression of bone resorption, and bone turnover in general, is also indicated by parallel effects of raloxifene and estrogen on biochemical markers of bone metabolism in the serum or urine of ovariectomized rats. To this regard, both 17α-ethynyl estradiol and raloxifene caused a reduction in urinary collagen cross-links and serum osteocalcin levels in ovariectomized rats [24].

With regard to bone strength, raloxifene and 17α-ethynyl estradiol produced equivalent beneficial effects on biomechanical properties of rat vertebrae (load to fracture) and femoral neck (shear to failure) as compared to ovariectomized controls in studies carried out to 1 year in duration [18, 19]. In ovariectomized cynomolgus macaques, a significant correlation of lumbar bone mineral density to lumbar vertebrae ultimate force was observed for both OVX controls and raloxifene treated animals, as well as OVX controls and conjugated equine estrogen treated animals [7]. Consistent with the similarity of these two agents in bone, the slope and *y*-intercept for the raloxifene vs conjugated equine estrogen lines were superimposable.

Thus, it is clear that the effects of raloxifene on bone in ovariectomized rats in terms of bone density, bone histomorphometry, biochemical markers of bone metabolism and bone biomechanical strength mimic those of estrogen.

1.3. Similarity of estrogen and raloxifene effects on bone in other rat models

In addition to comparison of the skeletal profile on ovariectomized animals treated with estrogen or raloxifene, the use of various manipulations of the ovariectomized rat model and combinations of drug treatments has furthered our understanding of the close similarity of the effects of raloxifene and estrogen on bone. As described below, the parallel shifts of pharmacological response in these various model manipulations confirm the similar nature of the effect of raloxifene and estrogen on bone.

Perhaps the strongest linkage of the antiosteopenic effect of raloxifene and estrogen can be made on the basis of observations made in hypophysectomized–ovariectomized rats [25]. While estrogen prevented bone loss in ovariectomized rats, in simultaneously tested

ovariectomized–hypophysectomized rats, the antiosteopenic effect of estrogen was completely lost. However, the uterine stimulatory capacity of estrogen was unaffected in the hypophysectomized–ovariectomized animals, thereby providing strong indication that the mechanism for the bone and lipid effects of estrogen are fundamentally different from the effect in reproductive tissue. As with the estrogen treated rats, ovariectomized rats given raloxifene exhibited the expected antiosteopenic effect, while ovariectomized–hypophysectomized rats given raloxifene did not show a bone response. These observations are consistent with the notion that raloxifene and estrogen function through similar mechanisms with respect to their effects on bone. Interestingly, the antiosteopenic effect of a bisphosphonate (alendronate) was not lost in the ovariectomized–hypophysectomized rat, indicating the estrogen/raloxifene mechanism for inhibiting resorption of bone, is distinct in part from that of bisphosphonate antiresorptives. Thus, while questions remain as to the precise mechanism for the antiosteopenic effect of raloxifene and the role of pituitary hormones in this effect, these studies demonstrate two critical points: (1) the effects of estrogen in bone and the uterus are mechanistically distinct and (2) raloxifene and estrogen both prevent bone loss in ovariectomized rats via a mechanism which involves at least one pituitary hormone, which is not required for the antiosteopenic effect of nonestrogenic antiresorptive agents.

A second manipulation of the ovariectomized rat model which demonstrated parallel shifts in activity with raloxifene and estrogen were observed in studies involving low vs normal dietary calcium intake. Reducing the dietary calcium content was associated with a greater degree of bone loss following ovariectomy, likely due to an enhanced level of bone resorption [26]. In each dietary group, estrogen and raloxifene exhibited quantitatively similar antiosteopenic effects, suggesting that raloxifene did not differ from estrogen for its dependence upon dietary calcium for the beneficial activity on bone.

Drug combination studies have provided further insight into the estrogenic nature of the antiosteopenic effect of raloxifene. When combined with bone active agents which manifest their effects through nonestrogen receptor dependent mechanisms, raloxifene and estrogens exhibit a pattern of activity indicative of similar mechanism of effect on bone. For example, combination of estrogen with maximally effective doses of medroxyprogesterone produces an additive effect on bone mineral density of the distal femur, leading to bone mass levels which exceed those of sham surgery controls [27]. A similar upward shift in the raloxifene dose response curve for distal femur bone mineral density was observed when raloxifene was given in combination with medroxyprogesterone [27]. Of note, when maximally effective doses of estrogen and raloxifene were combined in ovariectomized rats, no additivity or synergism was observed, suggesting that estrogen and raloxifene act via the same mechanism in bone. Finally, raloxifene mimics the characteristic effect of estrogen in growing orchiectomized male rats to increase bone mineral density to levels above those observed in nonorchiectomized (sham surgery) controls [28].

1.4. Similar effects of raloxifene and estrogen on bone signaling pathways

A key cytokine which mediates the increase in bone resorption which occurs following estrogen deficiency is IL-6 [29]. When IL-6 is used to stimulate the differentiation and resorptive activity of mammalian osteoclasts *in vitro*, raloxifene and estrogen produce antiresorptive effects of similar potency and magnitude of effect [30]. Interestingly, in the absence of IL-6, raloxifene and estrogen had minimal effects on the resorption activity of fully differentiated mammalian osteoclasts. This dependence on cytokine induction of resorption is not required for nonestrogenic antiresorptives such as bisphosphonates or calcitonin. As an *in vivo* correlate, serum IL-6 levels are elevated following ovariectomy in rats. Both raloxifene and 17α-ethynyl estradiol produced parallel reductions of IL-6 in ovariectomized rats to levels observed in sham controls [31].

A second estrogen regulated cytokine, which suppresses bone resorption, is TGFβ-3 [33]. TGFβ's are cytokines that are directly incorporated in the latent form into the bone matrix through binding proteins. In rats, ovariectomy is associated with a reduction in TGFβ-3 expression in bone while 17β-estradiol treatment produces an increase in bone TGFβ-3 mRNA levels [32]. As with estrogen, raloxifene administration to ovariectomized rats also increased bone TGFβ-3 mRNA levels [32]. *In vitro* studies have further shown that estrogen metabolites, rather than 17β-estradiol itself, likely mediate these effects on TGFβ-3 expression levels, through an ER-dependent mechanism [3]. TGFβ-3 elevation by raloxifene can also be produced in cultured cells transfected with ERα or ERβ [11, 32]. The key role of ER in this response is evident, as omission of ER from the cell culture assay prevents raloxifene induced elevation of TGFβ-3. Thus, for two key local regulators of the bone microenvironment, raloxifene and estrogen produce parallel effects.

1.5. Structure activity relationships

The recent effort to solve the crystal structure for the ERα has provided important insight into the nature of raloxifene and estrogen interaction with the

Table 2
Summary of raloxifene and estrogen parallel responses in rat bone

	Estrogen Response	Raloxifene Response
Profile in OVX rat		
Longitudinal growth	↓↓	↓↓
BMD-prevention	↑↑↑	↑↑↑
BMD-treatment	↑	↑
Biomechanics	↑↑	↑↑
Histomorphometry		
- resorption	↓↓↓	↓↓↓
- formation	↓↓↓	↓↓ or no Δ
Bone turnover markers	↓↓	↓↓
Profile in rat model variations		
Drug combination studies (OVX rat)		
- estrogen/raloxifene	no Δ	no Δ
- progestin	additive	additive
Hypophysectomized/OVX	bone response lost	bone response lost
Male rats	↑ BMD	↑ BMD
Effects on bone pathways		
IL-6	inhibited	inhibited
TGFβ-3	activated	activated

Arrows indicate directionality and magnitude of response, no Δ indicates no change in response, BMD means bone mineral density and OVX ovariectomized.

ER. Both molecules bind the same site within the ligand binding domain of ERα, resulting in a similar overall homodimeric arrangement [12]. Space filling chemical modeling has demonstrated that the hydroxyl moieties of raloxifene (Fig. 1) approximate the location of the two hydroxyl groups on 17β-estradiol. The structural biology work has confirmed this, as the 6-hydroxyl constituent on the benzothiophene ring mimicked the A-ring phenolic hydroxy of 17β-estradiol by binding in the polar pocket of ERα between helices three and six [12]. An important difference between 17β-estradiol and raloxifene binding to ERα occurred with respect to the orientation of helix 12. The basic side chain of raloxifene extends out of the binding cavity, displacing helix 12, as shift which disrupts the AF-2 region of the ER [12]. The AF-2 region is important for functional transcriptional activation which is likely to account for the selective pharmacology produced by raloxifene. Behaving as a pharmacological antagonist of estrogen, raloxifene has a potent dose-related ability to block estrogen-induced stimulation of uterine weight gain in the immature rat uterus assay (ED_{50} of approximately 0.4 mg/kg; [9]). In this regard, raloxifene is unique among known SERMs in that it is able to produce a complete blockade of estrogen's effects on the uterus. Other SERMs, such as tamoxifen, which are partial estrogen agonists in the uterus, possess sufficient intrinsic activity as uterine stimulants that they produce only a partial blockade of the stimulatory effects of estrogen [9]. Of note was the discovery that in addition to blocking estrogen-induced stimulation of uterine parameters, raloxifene also completely blocked the uterine epithelial stimulatory effects of tamoxifen in ovariectomized rats [33]. This reflects a key difference in the SERM profiles of raloxifene and tamoxifen, which can be partially explained on a structural basis. The carbonyl hinge portion of raloxifene results in a nearly orthogonal orientation of the basic side chain with respect to the average plane of the three-ring system, whereas the basic side chain of the triphenylethylamine structure of tamoxifen is constrained to lie within the average plane of the stilbene nucleus [34]. As indicated by the structural biology work with ERα [12], 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 the basic side chain of raloxifene has also been associated with reduced uterine stimulation as compared to the dimethyl amino basic side chain of tamoxifen.

2. Summary

Raloxifene possesses a complex pharmacology with tissue selective estrogen agonist and antagonist effects. At the center of these effects resides the high affinity interaction of raloxifene with the ER. The ability of raloxifene to compete with estrogen for ER binding accounts for the estrogen antagonist effects of raloxifene in uterine and mammary tissue. Since the precise

mechanism for the agonist effect of estrogen on the skeleton remains uncertain, it is difficult to unequivocally cite a single estrogen-like mechanism for raloxifene in bone. However, multiple lines of evidence, as summarized in Table 2, clearly indicate that the estrogen agonist effect of raloxifene on bone and the cardiovascular system are also mediated via an interaction with ER. The data showing nonadditivity of raloxifene and estrogen effects in bone, and those showing the requirement for a pituitary hormone in the antiestrogenic action of raloxifene and estrogen are particularly important. Thus, global evaluation of the similarities and parallel responses of raloxifene and estrogen in bone and the cardiovascular system, as summarized above, strongly support a similar mechanistic basis for the agonist effects of these agents on the skeleton.

References

[1] V. Kumar, P. Chambon, The estrogen receptor binds tightly to its responsive element as a ligand induced homodimer, Cell 55 (1988) 145–156.

[2] V. Krishnan, X. Wang, S. Safe, Estrogen receptor Spl complexes mediate estrogen-induced cathepsin D gene expression in MCF-7 cells, J. Biol. Chem. 269 (1994) 15912–15917.

[3] N.N. Yang, M. Venugopalan, A.L. Glasebrook, Identification of an estrogen response element activated by metabolites of 17β-estradiol and raloxifene, Science 273 (1996) 1222–1225.

[4] D.P. McDonnel, D.L. Clemm, T. Hermann, M.E. Goldman, J.W. Pike, Analysis of estrogen receptor function *in vitro* reveals three distinct classes of antiestrogens, Mol. Endocrinol. 9 (1995) 659–669.

[5] R.F. Kauffman, H.U. Bryant, Selective estrogen receptor modulators, Drug News Perspect. 8 (1995) 531–539.

[6] L.J. Black, M. Sato, E.R. Rowley, D.E. Magee, A. Bekele, D.C. Williams, G.J. Cullinan, R. Bendele, R.F. Kauffman, W.R. Bensch, C.A. Frolik, J.D. Termine, H.U. Bryant, Raloxifene (LY139481 HCl) prevents bone loss and reduces serum cholesterol without causing uterine hypertrophy in ovariectomized rats, J. Clin. Invest. 93 (1994) 63–69.

[7] C.P. Jerome, C.J. Lees, T.C. Registar, M. Stancill, Iliac and lumbar vertebral histomopometry, bone densitometry and bone biomarker data from raloxifene-treated macaques, J. Bone Mineral Res. 12 (Suppl. 1) (1997) S347.

[8] P.D. Delmas, N.H. Bjarnason, B.H. Mitlak, A.-C. Ravoux, A.S. Shah, W.J. Huster, M. Draper, C. Christiansen, Effects of raloxifene on bone mineral density, serum cholesterol concentrations, and uterine endometrium in postmenopausal women, New Engl. J. Med. 337 (1997) 1641–1647.

[9] H.U. Bryant, P.K. Wilson, M.D. Adrian, H.W. Cole, D.L. Phillips, J.A. Dodge, T.A. Grese, J.P. Sluka, A.L. Glasebrook, Selective estrogen receptor modulators: pharmacological profile in the rat uterus, J. Soc. Gynecol. Invest. 3 (1996) 152A.

[10] A.L. Glasebrook, D.L. Phillips, J.P. Sluka, Multiple binding sites for the antiestrogen raloxifene, J. Bone Mineral Res. 8 (Suppl. 1) (1993) S268.

[11] E.A. Gize, M. Venugopalan, A.L. Glasebrook, N.N. Yang, Characterization of raloxifene binding and transactivation properties of the estrogen receptor-beta, J. Bone Mineral Res. 12 (Suppl. 1) (1997) S428.

[12] A.M. Brzozowski, A.C.W. Pike, Z. Dauler, R.E. Hubbard, T. Bonn, O. Engstrom, L. Ohman, G.L. Greene, J.-A. Gustafsson, M. Garlquist, Molecular basis of agonism and antagonism in the oestrogen receptor, Nature 389 (1997) 753–758.

[13] R.F. Kauffman, W.R. Bensch, R.E. Roudebush, H.W. Cole, J.S. Bean, D. Phillips, A. Monroe, G.J. Cullinan, A.L. Glasebrook, H.U. Bryant, Hypocholesterolemic activity of raloxifene (LY139481): Pharmacological characterization as a selective estrogen receptor modulator (SERM), J. Pharmacol. Exp. Ther. 280 (1997) 146–153.

[14] R.T. Turner, B.L. Rigs, T.C. Spelsberg, Skeletal effects of estrogen, Endocrine Rev. 15 (1994) 275–300.

[15] G. Evans, H.U. Bryant, D. Magee, M. Sato, R.T. Turner, The effects of raloxifene on tibia histomorphometry in ovariectomized rats, Endocrinology 134 (1994) 2283–2288.

[16] M. Sato, C. McClintock, J. Kim, C. Turner, H.U. Bryant, D.E. Magee, C.W. Slemenda, Dual-energy X-ray absorptiometry of raloxifene effects on the lumbar vertebrae and femora of ovariectomized rats, J. Bone Mineral Res. 9 (1994) 715–724.

[17] M. Sato, J. Kim, L.L. Short, C.W. Slemenda, H.U. Bryant, Longitudinal and cross-sectional analysis of raloxifene effects on tibiae from ovariectomized aged rats, J. Pharmacol. Exp. Ther. 272 (1995) 1252–1259.

[18] C.H. Turner, M. Sato, H.U. Bryant, Raloxifene preserves bone strength and bone mass in ovariectomized rats, Endocrinology 135 (1994) 2001–2005.

[19] H.U. Bryant, C.H. Turner, C.A. Frolik, D.E. Magee, H.W. Cole, C.E. Brown, M.K. Rippy, M. Sato, Long term effects of raloxifene (LY139478 HCl) on bone, cholesterol and uterus in ovariectomized rats, Bone 16 (Suppl.) (1995) 116S.

[20] H.U. Bryant, H.W. Cole, M.D. Adrian, P.K. Wilson, M. Sato, Raloxifene and estrogen preserve PTH induced bone mass in ovariectomized rats, FASEB J. 10 (1996) A469.

[21] Y. Jiang, J. Zhao, R. Van Audekercke, J. Dequeker, P. Geusens, Effects of low dose long term sodium fluoride preventative treatment on rat bone mass and biomechanical properties, Calcif. Tissue Int. 58 (1996) 30–39.

[22] D.W. Dempster, F. Cosman, M. Parisien, V. Shen, R. Lindsay, Anabolic actions of parathyroid hormone on bone, Endocrine Rev. 14 (1993) 690–709.

[23] G. Evans, D. Magee, H.U. Bryant, R.T. Turner, Raloxifene is as effective as estrogen in preventing further bone loss in mature rats with established bone loss, Endocrinology 137 (1996) 4139–4144.

[24] C.A. Frolik, H.U. Bryant, E.C. Black, D.E. Magee, S. Chandrasekhar, Time dependent changes in biochemical bone markers and serum cholesterol in ovariectomized rats: effects of raloxifene HCl, tamoxifen, estrogen and alendronate, Bone 18 (1996) 621–627.

[25] H.U. Bryant, D.E. Magee, H.W. Cole, S. Chandrasekhar, H.M. Hsuing, M.L. Heiman, M. Sato, Beneficial effects of ethynyl estradiol and raloxifene in the ovariectomized rat are dependent on an intact pituitary, J. Bone Mineral Res. 9 (Suppl. 1) (1994) S135.

[26] D.E. Magee, H.W. Cole, C.E. Brown, H.U. Bryant, Raloxifene and estrogen retain antiosteopenic activity in ovariectomized rats fed a low calcium diet, J. Bone Mineral Res. 9 (Suppl. 1) (1994) S198.

[27] H.U. Bryant, L.J. Black, E.R. Rowley, M. Sato, G.J. Cullinan, Medroxyprogesterone augments the bone effects of estrogen or raloxifene in the ovariectomized rat, Endocr. Soc. Abstr. 77 (1995) 597.

[28] P.K. Shetler, E.R. Rowley, H.W. Cole, M.D. Adrian, D.E. Magee, T. Pell, M. Sato, H.U. Bryant, Raloxifene, estrogen, LY117018 and testosterone in the steroid hormone deficient male rat, J. Bone Mineral Res. 11 (Suppl. 1) (1996) S446.

[29] R.L. Jilka, G. Hangoi, G. Girasole, G. Passeri, D.C. Williams, J.S. Abrams, B. Boyce, H. Broxmeyer, S.C. Manolagas,

Increased osteoclast development after estrogen loss: mediation by interleukin-6, Science 257 (1992) 88–91.

[30] M. Sato, H.U. Bryant, Raloxifene efficacy in nonreproductive and reproductive tissues in rat models *in vivo* and *in vitro*. in: H. Kuramoto, E. Gurpide (Eds.), *In Vitro Biology of Sex Steroid Hormone Action*, Churchill Livingstone, Tokyo, 1996, pp. 406–421.

[31] A.L. Glasebrook, L.L. Short, H.W. Cole, M. Sato, H.U. Bryant, Regulation of serum IL-6 by raloxifene in an ovariectomized rat model, Bone 19 (Suppl. 1) (1995) 99S.

[32] N.N. Yang, S. Hardikar, M. Sato, R.J.S. Galvin, A.L. Glasebrook, H.U. Bryant, J.D. Termine, Estrogen and raloxifene stimulate transforming growth Factor-β3 expression in rat bone: a potential mechanism for estrogen or raloxifene-mediated bone maintenance, Endocrinology 137 (1996) 275–2084.

[33] R. Fuchs-Young, D.E. Magee, H.W. Cole, L. Short, A.L. Glasebrook, M.K. Rippy, J.D. Termine, H.U. Bryant, Raloxifene is a tissue-specific antiestrogen that blocks tamoxifen or estrogen-stimulated uterotrophic effects, Endocr. Soc. Abstr. 0 (1995) 57.

[34] T.A. Grese, J.P. Sluka, H.U. Bryant, H.W. Cole, J.R. Kim, D.E. Magee, E.R. Rowley, M. Sato, Benzopyran selective estrogen receptor modulators (SERMs): pharmacological effects and structural correlation with raloxifene, Biorog. Med. Chem. Lett. 6 (1996) 903–908.