LY353381.HCl: A Novel Raloxifene Analog with Improved SERM Potency and Efficacy In Vivo

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ABSTRACT

Body weight, uteri, serum cholesterol and bones were shown previously in vivo to be sensitive to circulating levels of estrogen, as well as to synthetic, nonsteroidal ligands termed selective estrogen receptor modulators (SERM). In this study, we examined the in vivo effects of a new potent SERM on these tissues in 6-month-old, ovariectomized rats that were orally dosed with 0.0001–10 mg/kg/day LY353381.HCl for 5 weeks. LY353381.HCl prevented the ovariectomy-induced increase in body weight and serum cholesterol levels of treated rats and lowered them to below sham levels in a dose dependent manner, with maximum efficacy similar to estrogen or raloxifene. However, LY353381.HCl was consistently more potent than raloxifene, with maximum efficacy similar to estrogen or raloxifene. LY353381.HCl efficacy on reproductive and nonreproductive tissues was evaluated in vivo to examine whether this compound behaves as an estrogen agonist or estrogen antagonist on body weight, uterus, serum cholesterol and bone. The ovariectomized rat model was chosen to model postmenopausal osteoporosis, based on previous bone studies (Kalu, 1991; Wronski and Yen, 1991; Frost and Jee, 1992). Specifically, ovariectomized rats have been used for bone efficacy studies of estrogens (Turner et al., 1987; Wronski et al., 1988; Durbridge et al., 1990), tamoxifen (Turner et al., 1988; Kalu et al., 1991; Moon et al., 1991), and raloxifene (Black et al., 1994; Sato et al., 1994, 1995, 1996b) with results that have largely paralleled clinical bone studies in humans (Weiss et al., 1980; Quigley et al., 1987; Love et al., 1992; Delmas et al., 1997). Additionally, an immature rat model was used to clarify the estrogen antagonist activity of LY353381.HCl in the uterus, as previously shown (Black et al., 1983).

Estrogens have been shown to effectively minimize bone loss of bone due to ovariectomy with an ED50 of about 0.01 mg/kg with maximal efficacy observed at 0.1–1 mg/kg/day. Maximally attainable bone mineral density and content with LY353381.HCl were not significantly different from Sham or ovariectomized rats treated with estrogen or raloxifene. Interestingly, assessment of bone quality by biomechanical analyses showed that LY353381.HCl preserved the strength of the femur neck and midshaft, while improving the Young’s modulus of cortical bone to beyond estrogen, raloxifene or sham levels. In uteri of immature rats treated with estrogen, LY353381.HCl antagonized the estrogen-induced elevation in uterine weight down to vehicle-dosed control levels with ED50 of 0.03 mg/kg/day. Therefore, LY353381.HCl was 30–100 times more potent than raloxifene in preventing ovariectomy effects on body weight, serum cholesterol and bone, while maintaining estrogen antagonist effects on the uterus. These animal data suggest that LY353381.HCl may have advantages over estrogen or raloxifene in the prevention of bone loss and treatment of other tissues in postmenopausal women.

LY353381.HCl is a novel benzothiophene analog with selective estrogen receptor modulator (SERM) activity similar to, but not identical with, raloxifene (Black et al., 1994; Sato et al., 1994, 1995, 1996b), see figure 1. Recent structure-activity relationship studies directed at the carbonyl of raloxifene identified a precursor with an ether linked basic side chain as a highly potent estrogen antagonist (Palkowitz et al., 1997). Additional substitution of the 4-hydroxyl group to a methoxy was shown to improve oral bioavailability of LY353381.HCl (Bryant et al., 1997).

LY353381.HCl efficacy on reproductive and nonreproductive tissues were evaluated in vivo to examine whether this compound behaves as an estrogen agonist or estrogen antagonist on body weight, uterus, serum cholesterol and bone. The ovariectomized rat model was chosen to model postmenopausal osteoporosis, based on previous bone studies (Kalu, 1991; Wronski and Yen, 1991; Frost and Jee, 1992). Specifically, ovariectomized rats have been used for bone efficacy studies of estrogens (Turner et al., 1987; Wronski et al., 1988; Durbridge et al., 1990), tamoxifen (Turner et al., 1988; Kalu et al., 1991; Moon et al., 1991), and raloxifene (Black et al., 1994; Sato et al., 1994, 1995, 1996a, 1996b) with results that have largely paralleled clinical bone studies in humans (Weiss et al., 1980; Quigley et al., 1987; Love et al., 1992; Delmas et al., 1997). Additionally, an immature rat model was used to clarify the estrogen antagonist activity of LY353381.HCl in the uterus, as previously shown (Black et al., 1983).

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Estrogens have been shown to effectively minimize bone
LY353381•HCl

Fig. 1. Chemical structure for LY353381.HCl. LY353381.HCl is a new benzothiophene analog that is structurally related to raloxifene. LY353381.HCl is classified as a selective estrogen receptor modulator (SERM), based on its effects in vivo on estrogen target tissues.

loss following ovariectomy or menopause for substantial periods of time and to reduce fracture risk in humans (Weiss et al., 1980; Lindsay et al., 1980; Quigley et al., 1987; Grodstein et al., 1997). There are additional benefits to estrogen therapy, including a reduction in cardiovascular disease (Hammond et al., 1979; Stampfer et al., 1991; Grodstein et al., 1997; Col et al., 1997). However, estrogens administered without progestin also substantially increase the incidence, but not the mortality, of endometrial cancer (Ziel and Finkle 1975; Smith et al., 1975; Vesey 1984), with controversial effects on the incidence of breast cancer (Steinberg et al., 1991; Dupont and Page, 1991; Col et al., 1997). This multiplicity of estrogen effects has led researchers to search for compounds with estrogen agonist activity in bone and in serum lipids, but antagonist activity or no activity in reproductive tissues. One compound recently described to show this selective pharmacology is raloxifene, which was recently approved by the FDA to prevent osteoporosis (Bryant et al., 1996; Kaufman and Bryant, 1996; Sato and Bryant, 1996).

Our studies were conducted using a prevention model of postmenopausal osteoporosis and an immature rat model used to evaluate estrogen antagonism in the uterus. LY353381.HCl effects in vivo were compared to those for 17α-ethyl estradiol, or raloxifene. The data shows improved bone efficacy and benefits in the uterus for LY353381.HCl.

Methods

Rat groups and dosing regimens. For bone studies, 3 separate sets of experiments (experiments 1, 2 and 3) were conducted with 6-month-old, virgin Sprague-Dawley female rats (Harlan, IN) weighing about 270 g and maintained on a 12 hr light/dark cycle at 22°C with ad lib access to food (TD 89222 with 0.5% Ca and 0.4% P, Teklad, Madison, WI) and water. Bilateral ovariectomies were performed using isoflurane anesthesia, except on Sham-operated controls. Rats were grouped into treatment units of 5 within 5 days of surgery and dosing continued for 5 weeks post-surgery before sacrifice, as indicated in specific tables and figures. Rats were dosed within 5 days of surgery and dosing continued for 5 weeks post-surgery before sacrifice, as indicated in specific tables and figures.

Antiestrogen activity in experiment 4 was evaluated in 21-day old Sprague Dawley rats (Charles River Labs, Portage, MI). Animals were housed and maintained on a 12/12 light/dark cycle at room temperature (22°C). The animals had ad libitum access to both food and tap water. 17α-Ethynyl estradiol at 0.1 mg/kg/day was used as the estrogenic stimulus to increase uterine weight in these rats. LY353381.HCl (0.001–10 mg/kg) or raloxifene (1 mg/kg) were administered by oral gavage in a volume of 0.2 ml, 15 min prior to the EE2 gavage. Dosing with test compounds was continued for 3 consecutive days. Animals were fasted overnight, following the final dose. On the following morning, rats were weighed, euthanized by CO2 asphyxiation and uteri were excised and weighed, as described below. All test compounds and 17α-ethyl estradiol were dissolved in 20% cyclodextrin vehicle. Nonestrogenic controls were given vehicle alone. Uterine weight/body ratios (UWR) were calculated for each animal. The percent inhibition of the estrogen-induced response was then calculated by the following formula: % inhibition = 100 x ([UWRtest - UWRcontrol] / UWRcontrol).

All animal procedures were reviewed before implementation by an internal animal welfare committee, to ensure compliance with NIH guidelines.

Tissue collection. After treatment, anesthetized rats were subjected to cardiac puncture and asphyxiated by CO2 inhalation. Uteri were removed and wet weight were determined on a Mettler balance to evaluate ovariectomy and efficacy of treatment with estrogen. Uteri were then fixed in 10% formalin, embedded in paraffin and processed for histology. Blood samples were allowed to clot at 4°C for 2 hr before centrifugation at 2000 g for 10 min. Sera were collected and stored at −70°C before analysis. Serum cholesterol was assayed using a high performance colorimetric assay (Boehringer Mannheim Biochemicals, Indianapolis, IN). Tibia and femora were removed, cleaned of soft-tissue, fixed in 50% ethanol/saline, and stored at 4°C.

X-Ray bone densitometry of excised rat bones. The metabolism of proximal tibiae or distal femora were scanned in plastic tubes (Falcon) in 50% ethanol/saline, using a 960A pQCT loaded with Dichte software version 5.1 (Norland/Stratec, Ft. Atkinson, WI). Cross-sectional area (X-Area), volume, voxel number, mineral content (BMC, mg), and volumetric bone mineral density (BMD, mg/cm³) were quantitated for the whole cross-section of the metaphysis, using voxel dimensions of 148 × 148 × 1200 μm. For respective tables and figures, volume can be calculated by multiplying X-Area by the slice thickness of 1.2 mm. The fibula was used as a positioning aid for the proximal tibia, while the condyles were used as positioning aids for the distal femora, with precision of 2% for proximal tibia and 1% for the distal femora, respectively (Sato, 1995; Sato et al., 1995). Because multiple scans (3–5) were typically necessary for positioning purposes, scan and analysis typically required 20–30 min per site.

Uterine histology and morphometrics. Measurements of uterine epithelial cell height were derived from four transverse sections that were cut using a Jung Supercut 2065 microtome (Ma-gee Scientific Inc., Dexter, MI) from each paraffin-embedded uteri with medial and distal aspects relative to the cervix. Sections were stained with Mayer’s hematoxylin and eosin. Slides were evaluated in random order with the operator “blinded” as to treatment group. Images were captured with a COHU CCD camera (San Diego, CA) attached to a Nikon Optiphot (Melville, NY) and quantitated using NIH Image 1.59 (NIH, Bethesda, MD). The average pixel line length was measured for multiple operator defined epithelial regions of uteri, using a 20× objective (Nikon).

Biomechanical analyses. Bone strength was measured at one time for the midshaft and femoral neck of preserved femora. Cortical bone strength was measured for intact femora using a three-point bending test. Load was applied midway between two supports that were 15 mm apart. The femora were positioned so that the loading
Results

LY353381.HCl effects on body weight, uteri and cholesterol. LY353381.HCl, 17α-ethynyl estradiol (EE2), or raloxifene (RA) effects in vivo were evaluated in 6-month-old, ovariectomized rats that were dosed for 5 weeks post-surgery and compared with OVX and Sham controls. Ovariectomy increased body weight significantly above Sham by 12%, as shown previously in this model, figure 2 (Sato et al., 1994). Dose response analyses with EE2 conducted previously showed that 0.1 mg/kg EE2 is fully efficacious in preventing ovariectomy stimulated weight gain and lowered body weight to significantly below OVX and Sham levels (Sato et al., 1996b). RA at 0.1 to 10 mg/kg lowered body weight to significantly below OVX and Sham. LY353381.HCl lowered body weight to below that of OVX and Sham, in a dose dependent manner, with half maximal efficacy ED₅₀ of 0.001 mg/kg and maximal efficacy observed at 0.01 mg/kg. LY353381.HCl was as efficacious as estrogen or raloxifene with respect to controls in preventing gain of body weight in this model.

Ovariectomy was confirmed to lower the uterine wet weight to 27% of Sham levels in this rat model, figure 3 (Sato et al., 1994). Dose response analyses with EE2 conducted previously showed that 0.1 mg/kg EE2 is fully efficacious in maintaining uterine weight at Sham levels in this model (Sato et al., 1995). Raloxifene (0.01–10 mg/kg) was confirmed to increase uterine weight to 36% of Sham which was significantly greater than OVX for 1 mg/kg but not 10 mg/kg (Black et al., 1994; Sato et al., 1995, 1996b). Uterine weights of LY353381.HCl were 27–36% of Sham levels for the range of 0.0001 to 10 mg/kg/day, which was significantly greater than OVX for 0.01, 1 and 10 mg/kg but not 0.1 mg/kg. LY353381.HCl uterine effects were significantly less than EE2 effects and not significantly different from RA effects, suggesting marginal effects on the uterus in this model.

To clarify possible stimulatory effects of LY353381.HCl on the endometrium, epithelial cell height of these uteri were evaluated at higher resolution by histological techniques. EE2 was confirmed to increase epithelial cell height by 260% compared to OVX which was not different from Sham (fig. 4). RA at 1 mg/kg increased epithelia by 26% which was not significantly different from OVX. LY353381.HCl at 0.01 mg/kg increased cell height by 45% which was greater than OVX; however, epithelia for 0.1–10 mg/kg LY353381.HCl were not significantly different from OVX. For the range of 0.01–10 mg/kg, LY353381.HCl epithelia were significantly

Fig. 2. LY353381.HCl effects on body weight of ovariectomized rats. 6-month-old rats were ovariectomized (except for Sham controls, solid line) and treated with vehicle (OVX controls, dashed line). LY353381.HCl (353381, ▼), raloxifene (RA, ▽) or 17α ethynyl estradiol (EE2, ■) at the indicated doses for 5 weeks post-surgery. Body weight (mean ± standard error) were measured and plotted with group size of n = 6–7 from experiment 1. Comparisons were made to Sham and OVX controls; significant differences (P < .05) from OVX are designated “o”, while significant differences from Sham are designated “s” (Fisher’s PLSD). Both EE2 and RA lowered body weight to below OVX and Sham. LY353381.HCl lowered body weight in a dose dependent manner.

Fig. 3. LY353381.HCl effects on uterine wet weight of ovariectomized rats. 6-month-old rats were ovariectomized (except for Sham controls, solid line) and treated with vehicle (OVX controls, dashed line). LY353381.HCl (353381, ▼), raloxifene (RA, ▽) or 17α ethynyl estradiol (EE2, ■) at the indicated doses for 5 weeks post-surgery. Plotted are mean ± standard error with group size of n = 6–7 from experiment 1. Comparisons were made to Sham and OVX controls; significant differences (P < .05) from OVX are designated “o”, while significant differences from Sham are designated “s” (Fisher’s PLSD). Uteri from Sham and 0.1 mg/kg EE2 were significantly greater than OVX. LY353381.HCl or RA had smaller effects on uteri.
less than 0.1 mg/kg EE2, but not different from 1 mg/kg RA, indicating marginal stimulation of the endometrium, Fig. 4. Uterine effects of LY353381.HCl were further analyzed in immature, 21-day-old rats dosed with 0.1 mg/kg EE2. EE2 induced a substantial 312% increase in uterine weight ratio, which was antagonized by RA at 1 mg/kg to nearly vehicle control levels (CDX), as shown previously, figure 5 (Black et al., 1983). LY353381.HCl completely antagonized EE2 stimulation of the uterus in a dose dependent manner down to vehicle control levels (CDX), figure 5. The dose response relationship suggested half maximal antagonism, ED\text{50}, of 0.03 mg/kg with maximal antagonism at 0.1 mg/kg which was not different from CDX control levels.

Ovariectomy in 6-month-old rats significantly increased cholesterol levels to 114% of Sham, as shown previously (Sato et al., 1994), figure 6. Both 17α ethynyl estradiol (EE2) and raloxifene (RA) lowered cholesterol to significantly below OVX and Sham control levels (Sato et al., 1995). LY353381.HCl lowered cholesterol to below OVX and Sham in a dose dependent manner, with half maximal efficacy ED\text{50} of 0.001 mg/kg and maximal efficacy observed at 0.01 mg/kg. LY353381.HCl was as efficacious as EE2 or RA in lowering serum cholesterol levels with respect to controls in this model.

Bone effects of LY353381.HCl in aged ovariectomized rats. Bone effects of LY353381.HCl on the metaphysis of proximal tibia were analyzed in cross-section by pQCT, table 1. A significant 17–22% reduction in BMD and BMC was observed for OVX controls compared to SHAM, table 1. As shown previously, 0.1 mg/kg EE2 or 1.0 mg/kg RA largely prevented this loss of bone (Black et al., 1994; Sato et al., 1994, 1995, 1996b). LY353381.HCl prevented ovariectomy effects on BMD and BMC in a dose dependent manner, with ED\text{50} of 0.01 mg/kg and maximal efficacy observed at about 0.1 mg/kg. Maximal efficacy in the metaphysis appeared to be similar to EE2 or RA. Ovariectomy was not observed to consistently affect X-Area or voxel number (Voxels, or vol-

Fig. 4. LY353381.HCl effects on the endometrium. Uteri from 6-month-old ovariectomized rats treated with the indicated agent for 5 weeks were processed for histomorphometry in experiment 3. LY353381.HCl effects on epithelial cell height were compared to RA at 1 mg/kg, which was shown previously to be a fully efficacious dose in lowering body weight, preventing bone loss, and lowering serum lipids (Sato et al., 1996b). Each point represents mean epithelial cell height ± SEM value for 5–15 rats. Significant differences from OVX are designated "o", (P < .05, Fisher’s PLSD). Epithelial cell height for Sham and 0.1 mg/kg EE2 were significantly greater than OVX. LY353381.HCl at 0.1–10 mg/kg or 1 mg/kg RA had smaller effects on epithelial cell height.

Fig. 5. LY353381.HCl effects on the uterus of immature rats dosed with estrogen. Uterine effects were further analyzed in experiment 4 of 21-day-old rats dosed with vehicle (CDX), 0.1 mg/kg EE2, 0.1 mg/kg EE2 plus 1 mg/kg raloxifene, or 0.1 mg/kg EE2 plus LY353381.HCl at the indicated doses. Uterine weight/body ratios (UWR) were calculated for each animal. Specifically, the percent inhibition of the estrogen-induced response was then calculated by the following formula: % inhibition = 100 × [(UWR\text{EE2} – UWR\text{test agent})/UWR\text{EE2} – UWR\text{control}]. Significant differences from controls dosed with 0.1 mg/kg EE2 was designated ** (P < .05; Fisher’s PLSD). EE2 induced a three-fold increase in uterine weight over controls dosed only with vehicle (CDX). Raloxifene at 1 mg/kg suppressed the EE2 stimulation by 78%. LY353381.HCl antagonized the EE2 stimulation of the uterus in a dose dependent manner down to CDX levels.

Fig. 6. LY353381.HCl effects on serum cholesterol in ovariectomized rats. 6-month-old rats were ovariectomized (except for Sham controls, solid line) and treated with vehicle (OVX controls, dashed line), LY353381.HCl (353381, ▼), raloxifene (RA, ◊) or 17α ethynyl estradiol (EE2, □) at the indicated doses for 5 weeks post-surgery. Plotted are mean ± standard error with group size of n = 6–7 for experiment 1. Comparisons were made to Sham and OVX controls; significant differences (P < .05) from Ovx are designated “o”, while significant differences from Sham are designated “s” (Fisher’s PLSD). Ovariectomy increased cholesterol levels above Sham. EE2 at 0.1 mg/kg lowered cholesterol to significantly below OVX and Sham. Both LY353381.HCl and RA lowered cholesterol to below OVX and Sham, in a dose dependent manner.
Biomechanical analyses of the effects LY353381.HCl on the femoral neck and mid-shaft

<table>
<thead>
<tr>
<th>Groups</th>
<th>BMD</th>
<th>BMC</th>
<th>X-Area</th>
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<tr>
<td>Sham</td>
<td>mg/ml</td>
<td>mg</td>
<td>mm²</td>
</tr>
<tr>
<td>OVX</td>
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<td>18.7 ± 0.5</td>
<td>21.8 ± 0.7</td>
</tr>
<tr>
<td>0.0001 mg/kg</td>
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<td>15.5 ± 0.6</td>
<td>23.1 ± 0.6</td>
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<tr>
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<td>16.6 ± 0.6</td>
<td>22.8 ± 0.7</td>
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<tr>
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<td>21.9 ± 0.5</td>
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<tr>
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<td>18.5 ± 0.4</td>
<td>21.3 ± 0.8</td>
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<tr>
<td>1.0 mg/kg</td>
<td>692 ± 12</td>
<td>17.9 ± 0.7</td>
<td>23.1 ± 1.1</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>744 ± 25</td>
<td>17.7 ± 0.7</td>
<td>19.9 ± 0.5</td>
</tr>
<tr>
<td>EE2 0.1 mg/kg</td>
<td>699 ± 19</td>
<td>17.6 ± 0.6</td>
<td>21 ± 0.5</td>
</tr>
<tr>
<td>RA 1 mg/kg</td>
<td>706 ± 25</td>
<td>17.8 ± 0.6</td>
<td>21.1 ± 0.8</td>
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ANOVA <.0001 .0036 .0449

TABLE 2

Prevention model (experiment 1): Rats were ovariectomized (except for Sham) and orally treated for 5 weeks with vehicle (Ovx), 0.0001–10 mg/kg/day LY353381.HCl, 0.1 mg/kg/day 17α ethynyl estradiol (EE2), or 1 mg/kg/day raloxifene (RA), as indicated. QCT was used to analyze the volumetric bone mineral density (BMD, mg/cc), bone mineral content (BMC, mg), and cross-sectional area (X-Area, mm²) for the proximal tibia metaphysis. Data are mean ± S.E.M. for n = 6–7 for each group. Comparisons were made to Sham and OVX controls; significant differences (P < .05) from Ovx are designated “o”, while significant differences from Sham are designated “s” (Fisher’s PLSD). Dose-dependent effects of LY353381.HCl were observed for the BMD and BMC with half-maximal efficacy of about ED₅₀ = 0.01 mg/kg.

Cortical bone effects were assessed by 3-point bending analyses of the femoral diaphysis, table 2. Ovariectomy lowered the ultimate load (Fₚₐₐₚ), the proximal femur compared to Sham. This decrease was prevented by 0.1 mg/kg EE2, but not 1 mg/kg RA. 0.1 mg/kg LY353381.HCl partially prevented this decline, as Fₚₐₐₚ were intermediate but not different from either Sham or OVX.

Body weight, serum cholesterol, uterine weight, and bones were shown previously to be sensitive to estrogen or raloxifene levels in ovariectomized rats (Black et al., 1983; 1994; Sato et al., 1994, 1995, 1996a, 1996b).

LY353381.HCl effects on these tissues were evaluated in animals treated orally after ovariectomy for 5 weeks. In these studies, 17α ethynyl estradiol was used in place of 17β estradiol because the latter was not observed to lower serum cholesterol levels in our hands. LY353381.HCl prevented the ovariectomy stimulated gain in body weight with ED₅₀ = 0.001 mg/kg, with efficacy similar to that of estrogen. LY353381.HCl inhibited the ovariectomy stimulated increase in serum cholesterol levels with ED₅₀ between 0.001–0.01 mg/kg. LY353381.HCl lowered cholesterol levels significantly below Sham, with efficacy comparable to estrogen. Previous dose response curves showed ED₅₀ of 0.3 mg/kg and 0.003 for raloxifene and 17α ethynyl estradiol, respectively (Sato et al., 1996b). Therefore, LY353381.HCl appears to be 100 times more potent than raloxifene and comparable in potency to 17α ethynyl estradiol in preventing weight gain and reducing cholesterol in ovariectomized rats (Black et al., 1994; Sato et al., 1994, 1996b).

LY353381.HCl was observed to marginally affect the uterine weight or the epithelial cell height of the uterine endometrium, as compared to OVX controls. LY353381.HCl differed substantially from estrogen, tamoxifen, and nafinoxide in the rat uterus as detailed previously (Sato et al., 1996b), but resembled raloxifene in having minimal stimulatory effects on the uterus in aged and immature rats (Black et al., 1983, 1994; Sato et al., 1994, 1996b; Ashby et al., 1997). Interestingly, in clinical studies with postmenopausal women, raloxifene had no stimulatory effects on the uterus after 2 years of treatment (Delmas et al., 1997), suggesting that these marginal effects in rat uteri may not be clinically relevant.

In the immature rat dosed with estrogen, LY353381.HCl functioned primarily as an estrogen antagonist in reducing uterine weight down to the level of vehicle treated controls, like raloxifene (Black et al., 1983). By comparison, previous studies showed that tamoxifen only partially blocked estrogen-induced uterine weight gain in the immature rat model (Jordan et al., 1980; Bryant et al., 1996; Willson et al., 1997; Ashby et al., 1997). As with the hypocholesterolemic and body weight effects, LY353381.HCl was considerably more potent than raloxifene in antagonizing estrogen stimulation of the uterus.

In the tibial metaphysis, LY353381.HCl prevented the ovariectomy induced loss of bone, with an ED₅₀ of about 0.01 mg/kg/day. Maximal efficacy comparable to estrogen, tamoxifen levels in ovariectomized rats (Black et al., 1983; 1994; Sato et al., 1994, 1995, 1996a, 1996b).
ifen, or raloxifene was observed between 0.1–1 mg/kg/day (Sato et al., 1996b). Raloxifene was shown previously in this model to have ED₅₀ = 0.3 mg/kg with maximal efficacy at 1 mg/kg in the axial and appendicular skeleton (Sato et al., 1994, 1995, 1996b). Therefore, LY353381.HCl is 30 times more potent in bone than raloxifene and compares favorably with estrogen in preventing the ovariectomy induced loss of trabecular bone. Additional studies are in progress to clarify possible kinetic or mechanistic differences between LY353381.HCl and other agents.

Biomechanical analyses showed that LY353381.HCl compared favorably with estrogen in preserving the strength of the femoral neck. Interestingly, raloxifene failed to preserve strength at this site, contrary to previous reports (Turner et al., 1994). This discrepancy may be explained by the lower dose of raloxifene (1 mg/kg) and the shorter duration (5 weeks vs 6 months) utilized in these studies (Turner et al., 1994). Interestingly, LY353381.HCl preserved the strength of cortical bone and improved the Young’s modulus to beyond estrogen or raloxifene treatment. Taken together, these data show that LY353381.HCl compares favorably in potency and efficacy with other pharmacological agents including calcitriol, bisphosphonates, other SERMs, and estrogens as evaluated in ovariectomized rat models (Wronski et al., 1991; Toolan et al., 1992; Sato et al., 1996; Ke et al., 1997; Willson et al., 1997).

The distinct tissue-specific pattern of pharmacological effects fits the SERM profile, described previously for raloxifene (Bryant et al., 1996; Kaufman and Bryant, 1996; Sato and Bryant, 1996). The pharmacology of SERMs may be best understood in terms of the different chemical structures and resulting consequences of the estrogen receptor/ligand complex (McDonnell et al., 1995). The core structures of these estrogen receptor ligands include estrogens, triphenylethylenes (tamoxifen, drolaxifene), naphthalenes (nafodixifene), benzopyrans (ormeloxifene, Bain et al., 1995) and benzothiophenes (raloxifene, LY353381.HCl). The minimal uterine stimulation and cortical bone data suggest a potential therapeutic advantage to LY353381.HCl over estrogen or raloxifene in the treatment of postmenopausal women. However, additional studies are required to elucidate the possible clinical advantages of LY353381.HCl over presently available treatment modalities.

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