Effects of CP-336,156, a New, Nonsteroidal Estrogen Agonist/Antagonist, on Bone, Serum Cholesterol, Uterus, and Body Composition in Rat Models


ABSTRACT

We have discovered a new, nonsteroidal, potent estrogen agonist/antagonist, CP-336,156. CP-336,156 binds selectively and with high affinity to the human estrogen receptor-α with a half-inhibition concentration of 1.5 nM, which is similar to that seen with estradiol (4.8 nM). When given orally to immature (3-week-old) female Sprague-Dawley rats for 3 days at doses of 0.1, 1.0, 10, or 100 μg/kg/day, unlike 17α-ethynyl estradiol, CP-336,156 had no effect on uterine wet or dry weight. Similarly, no uterine hypertrophy was observed in aged (17-month-old) female rats treated (po) with CP-336,156 at 10 or 100 μg/kg/day for 28 days. We also found that CP-336,156 decreased total serum cholesterol and fat body mass and had no effect on lean body mass in these aged female rats. In 5-month-old ovariectomized (OVX) Sprague-Dawley female rats, CP-336,156 completely prevented OVX-induced increases in body weight gain, total serum cholesterol, and serum osteocalcin at doses between 10 and 1000 μg/kg/day after 4 weeks. At these doses, CP-336,156 completely prevented OVX-induced bone loss and inhibited the increased bone turnover associated with estrogen deficiency in lumbar vertebrae, proximal tibiae, and distal femora. Similar to estrogen, CP-336,156 induced apoptosis and p53 expression with a concomitant decrease in the number of tartrate-resistant acid phosphatase-positive multinuclear cells in rat bone marrow cell cultures in vitro, suggesting that the induction of apoptosis may be a mechanism for the estrogenic activities of CP-336,156 in bone. In summary, CP-336,156 is a new, orally active, nonsteroidal, potent estrogen agonist/antagonist that has similar effects in bone as estradiol but without the uterine-stimulating effects associated with estradiol in rats. (Endocrinology 139: 2068–2076, 1998)

Tissue-selective estrogen agonist/antagonists are currently being investigated as alternatives to estrogen for the prevention and treatment of postmenopausal osteoporosis (1–4). Estrogen, though having numerous beneficial effects on the skeleton and cardiovascular systems, has undesirable side effects that lead to poor compliance among postmenopausal women (5–7). A desirable estrogen agonist/antagonist would maintain estrogen’s positive bone and cardiovascular effects, while being devoid of several of the objectionable side effects of estrogen (such as breakthrough bleeding and increasing risk for breast and uterine cancer) (8). In addition to an improved side effect profile, compared with estrogen, it is also expected that tissue-selective estrogen agonist/antagonists may be useful in the treatment and prevention of breast and uterine cancer, because of their antiestrogen effects in these tissues (9, 10).

Tissue-selective estrogen agonist/antagonists currently undergoing clinical trials include tamoxifen, raloxifene, droloxifene, idoxifene, and levomeloxifene. Tamoxifen is the first estrogen agonist/antagonist that has been shown to inhibit bone loss in postmenopausal women with breast cancer (11, 12) and in the estrogen-deficient, ovariectomized (OVX) rat model (8, 13). However, the partial estrogenic effects of tamoxifen on uteri in women (14) and strong hepatocarcinogenic effects noted in rats (15) raise issues of safety of tamoxifen as an agent for chronic use in prevention and treatment of postmenopausal osteoporosis. Though the efficacy of other estrogen agonist/antagonists, such as raloxifene (3, 16, 17) and droloxifene (4, 18–21), in preventing bone loss in postmenopausal women requires further investigation, the preclinical reports suggest that these estrogen receptor agonists are considerably less potent than estrogen. Increased understanding of the action of these estrogen agonist/antagonists may lead to the discovery of compounds that act upon target tissues with potencies similar to that of estradiol.

We have discovered a new, nonsteroidal estrogen agonist/antagonist, CP-336,156, that is equal to estrogen in potency in selective target tissues. The purposes of this report were to describe: 1) binding affinity of CP-336,156 to human estrogen receptor-α (ER-α), compared with estradiol; 2) the estrogenic effects of CP-336,156 on uterine growth in an
immature female rat model, that is widely accepted as the model for studying estrogenic effects (9); 3) the effects of CP-336,156 on uterine weight, uterine histology, total serum cholesterol, and body composition in an aged female rat model, a model that most closely mimics the endocrine status of postmenopausal women (see Ref. 23); 4) the estrogenic effects of CP-336,156 on bone and total serum cholesterol in an OVX rat model of postmenopausal bone loss (22–24); and 5) a potential mechanism of CP-336,156's estrogen agonistic effects in bone using the rat bone marrow culture system, as described previously (25).

Materials and Methods

Human ER-α binding

Recombinant baculovirus-generated human ER-α and 6,7-3H-estradiol used for competitive binding analysis were purchased from PanVera Corporation (Madison, WI) and DuPont NEN (Boston, MA), respectively. 17β-estradiol (E2) was purchased from Sigma Chemical Co. (St. Louis, MO), and CP-336,156 [chemical name:cis-1R-[4'-pyrroldino-ethoxyphenyl]-2S-phenyl-6-hydroxy-1,2,3,4-tetrahydronaphthalene p(-)-tartrate salt, molecular formula: C28H31NO2.(-)-tartrate salt, Fig. 1A] was synthesized at Pfizer Central Research. E2 and CP-336,156 were initially dissolved in 100% ethanol before subsequent dilution in binding buffer (see below). ER-α binding analysis was performed essentially as previously described (26) as communicated by the ER-α vendor. Briefly, ER-α was diluted 1:100 in binding buffer [10 mM Tris (pH 7.4), 10% glycerol, 1 mM dithiothreitol, 1 mg/ml BSA] before use. Then, 100 μL binding reactions, containing a final concentration of 2 nM 3H-estradiol and increasing concentrations of unlabeled competitor estradiol (as noted in Fig. 1) in a mixture of 5 μL diluted ER-α and 95 μL binding buffer, were equilibrated at room temperature for 3 h. Subsequently, 100 μL of 50% hydroxylapatite slurry [equilibrated in 50 mM Tris (pH 7.4), 1 mM EDTA] was added to each reaction and vortexed three times over 15 min. One milliliter of wash buffer [40 mM Tris (pH 7.4), 1 mM EDTA, 1 mM EGTA, 100 mM KCl] was added to each reaction and was centrifuged at 10,000 × g for 5 min, and the supernatant was aspirated. This wash step was repeated two additional times. The hydroxylapatite pellet was resuspended in 400 μL ethanol, completely transferred to a scintillation vial with 4 ml scintillant, and counted. Data are expressed as the mean (n = 3) percent binding, relative to maximum binding (100%, zero unlabeled estradiol added).

Animals

Sprague-Dawley female rats, used in all studies, were supplied by Charles River (Wilmington, MA). All animals were allowed free access to water and a pelleted commercial diet (Agway ProLab 3000, Agway County Food, Inc., Syracuse, NY) containing 0.97% calcium, 0.85% phosphorus, and 1.05 IU/g Vit.D. The experiments were conducted according to Pfizer Animal Care approved protocols, and animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Immature female rat study

Seventy-two 3-week-old Sprague-Dawley female rats, weighing approximately 55 g, were treated by oral gavage with either vehicle (10% ethanol and 90% saline, 1 ml/rat); CP-336,156 at doses of 0.1, 1, 10, or 100 μg/kg/day, or 17α-ethynyl estradiol (EE, Sigma) at 30 μg/kg/day for 3 days. The rats were autopsied 24 h after the final dose. At autopsy, the uterine wet weight was determined. Next, the uteri were placed in an oven at 60 C for 12 h, and the uterine dry weight was determined.

Aged female rat study

Twenty-eight (n = 7 per group) 17-month-old Sprague-Dawley female rats, weighing approximately 580 g, were treated by oral gavage with either vehicle (10% ethanol and 90% saline, 1 ml/rat); CP-336,156 at doses of 10 or 100 μg/kg/day, or EE at 30 μg/kg/day for 28 days. One day before autopsy, all rats under ketamine/xylazine anesthesia underwent dual-energy X-ray absorptiometry (DXA, QDR-1000/W, Hologic Inc., Waltham, MA) using Rat Whole Body Scan software for lean and fat body mass determination. The rats were then autopsied, and blood was obtained by cardiac puncture. Total serum cholesterol was determined using a high-performance cholesterol colorometric assay (Boehringer Mannheim Biochemicals, Indianapolis, IN). The body weight gain was calculated as body weight at autopsy minus body weight at day zero. The uterine wet weight was determined immediately at autopsy. Five-micron, paraffin embedded, hematoxylin and eosin-stained uterine sections were used to determine the uterine luminal epithelial thickness (the average thickness at 0.2-mm intervals along the uterine luminal epithelial layer) using a Bioquant Image Analysis System (R&M Biometrics, Inc., Nashville, TN).

OVX rat study

Seventy-five 5-month-old Sprague-Dawley female rats, weighing approximately 280 g, were used in this study. Ten rats were sham-operated (sham) and treated by daily oral gavage with vehicle (10% ethanol and 90% saline, 1 ml/rat), while the remaining rats (n = 10/group) were bilaterally OVX and treated by oral gavage with either vehicle, or CP-336,156 at doses of 1, 10, 100, or 1000 μg/kg/day, or EE at 30 μg/kg/day for 28 days beginning 1 day post surgery. All rats were given sc injections of 10 mg/kg calcine (Sigma), a fluorochrome bone marker, at 12 and 2 days before death, to determine dynamic changes in bone tissues (27).
After 4 weeks of treatment, the rats were weighed, and body weight gain was obtained. Next the rats were euthanized by cardiac puncture under ketamine/xylazine anesthesia, and uterine wet weight and total serum cholesterol were determined as described above. Serum osteocalcin was determined by RIA (28).

Bone mineral measurements. The lumbar vertebrae (first through sixth) and the right femur from each rat were removed during autopsy, scanned ex vivo using DXA equipped with Regional High Resolution Scan software. Bone mineral density (BMD) of total lumbar vertebrae and distal femoral metaphysis were determined as described previously (18, 21).

Cancellous bone histomorphometry. Undecalcified, methyl methacrylate embedded sagittal sections of fifth-lumbar vertebral bodies and longitudinal sections of proximal tibial metaphysis (PTM) at 4- and 10-μm thickness were prepared for histomorphometry as described previously (18, 19). The 4-μm sections were stained with modified Masson’s Trichrome stain, whereas the 10-μm section remained unstained (20). A video image analysis system (KS6 Scientific Consultants, Magna, UT) was used for the static and dynamic histomorphometric measurements of cancellous bone of the lumbar vertebral body and PTM. The indices of bone mass (percent trabecular bone area), bone resorption (percent eroded perimeter, osteoclast number per mm trabecular surface, percent osteoclast perimeter), and bone formation and turnover (labeling perimeter and bone formation rate/bone volume referent [BFR/BV]) were determined as described previously (30, 31).

In vitro rat bone marrow culture study

The rat bone marrow osteoclast differentiation culture system was established as previously described (25). In brief, bone marrow was collected from 5-month-old OVX or sham rats 2 weeks post surgery. Bone marrow cells were plated at a density of 5 × 10⁶/cm² and treated with either vehicle, E₂, or CP-336,156. The cell cultures were maintained for 6 days with 50% of the media containing fresh compounds being replaced on the third day, followed by osteoclast staining and quantitative observation on the sixth day. To visualize osteoclasts formed in culture, histochemical staining for tartrate-resistant acid phosphatase (TRAP) was performed. To identify apoptotic bone marrow cells, fragmented nuclear DNA was labeled by terminal deoxy nucleotidyl-mediated deoxyuridine 5-triphosphate nick end labeling (TUNEL), according to the manufacturer’s directions (Boehringer-Mannheim). On day 3 of culture, p53 and CD61 colocalization was done, as previously described (25), using p53 and CD61-specific monoclonal antibodies (Oncogene Sciences, Cambridge, MA; and Pharmingen, San Diego, CA, respectively). Labeled cells were viewed and counted under an Olympus BH-2 microscope.

Statistics

Data are expressed as mean ± SEM. Statistics were calculated using StatView 4.0 package (Abacus Concepts, Inc., Berkeley, CA). The ANOVA test, followed by Fisher’s PLSD, were used to compare the differences between groups (32).

Results

Human ER-α binding

As shown in Fig. 1B, the binding affinity of CP-336,156 to human ER-α is similar to that of E₂. Binding IC₅₀ determined for E₂ and CP-336,156 are 4.8 nM and 1.5 nM, respectively.

Immature female rat study

In immature female rats, treatment with EE at 30 μg/kg-day for 3 days significantly increased uterine wet weight (+36%) in rats treated with 10 μg/kg-day (Fig. 2). Similarly, EE treatment significantly increased uterine dry weight (+57%), whereas CP-336,156 had no effect on uterine dry weight, regardless of dose, compared with vehicle-treated controls (Fig. 2).

Aged female rat study

All groups of aged female rats decreased body weight over the duration of the study (–28 to –80 g). Compared with vehicle-treated rats, CP-336,156 at 100 μg/kg-day significantly decreased body weight, whereas EE or CP-336,156 at 10 μg/kg-day had no significant effect on body weight (data not shown). The fat body mass in rats treated with CP-336,156 at 100 μg/kg-day decreased nonsignificantly by 25% (net decrease of 64 g), compared with vehicle-treated rats (Fig. 3). There was no difference in lean body mass among groups (data not shown). Compared with vehicle-treated controls, total serum cholesterol was significantly decreased by 48%, 54%, and 73% in rats treated with EE or CP-336,156 at 10 or 100 μg/kg-day, respectively (Fig. 3). EE treatment significantly increased uterine wet weight by 58% and uterine luminal epithelial thickness by 95% (Figs. 3 and 4). In contrast, CP-336,156 at 10 or 100 μg/kg-day had no significant effect on uterine wet weight or uterine luminal epithelial thickness in these aged female rats (Figs. 3 and 4).

OVX rat study

Four weeks post surgery, body weight gain in OVX controls was significantly greater than that in sham controls (62.6 ± 5.4 g vs. 0.66 ± 2.72 g, a 144% increase). Body weight gain in EE-treated OVX rats was significantly lower than in
OVX controls, but it did not differ from sham controls (Fig. 5A). CP-336,156 dose-dependently inhibited body weight gain, compared with OVX controls. At 10 μg/kg-day, CP-336,156 completely inhibited the weight gain induced by OVX. Body weight gain was significantly decreased in OVX rats treated with CP-336,156 at 1000 μg/kg-day compared with both sham and OVX controls (Fig. 5A).

Uterine weight in OVX control rats was significantly decreased compared with sham controls (−66%), and EE treatment maintained uterine weight in OVX rats to a level that did not differ from sham controls (Fig. 5B). At 1 μg/kg-day, CP-336,156 had no effect on uterine weight, compared with OVX controls. In OVX rats treated with CP-336,156 at 10–1000 μg/kg-day, uterine weight was significantly lower than both sham controls and EE-treated OVX rats, whereas it increased significantly, compared with OVX controls (Fig. 5B).

A significant increase (120%) in total serum cholesterol was found in OVX controls, compared with sham controls. CP-336,156 (at doses of 10 and 100 μg/kg) had no effect on this parameter. Hematoxylin and eosin staining sections. Original magnification: ×260.

**Fig. 3.** Fat body mass (A), total serum cholesterol (B), uterine weight (C), and uterine epithelial thickness (D) of 17-month-old female rats treated with vehicle (VEH), EE at 30 μg/kg-day, or CP-336,156 at 10 or 100 μg/kg-day for 28 days (mean ± SEM). *, P < 0.05 vs. VEH.

**Fig. 4.** Photomicrographs of uterine luminal epithelial and stromal layers from aged (17-month-old) female rats treated with vehicle, EE at 30 μg/kg-day, or CP-336,156 at 10 or 100 μg/kg-day for 28 days. Arrows point to the luminal epithelial layer. EE increased luminal epithelial thickness, whereas CP-336,156 (at doses of 10 and 100 μg/kg) had no effect on this parameter. Hematoxylin and eosin staining sections. Original magnification: ×260.
dependently decreased total serum cholesterol in OVX rats (Fig. 5C).

Four weeks post surgery, serum osteocalcin (a biochemical marker of bone turnover) was significantly increased (+85%) in OVX controls, compared with sham controls (Fig. 5D). This increase was completely prevented by treatment with CP-336,156 (at doses between 10–1000 μg/kg/day) or by treatment with EE. Therefore, 10 μg/kg/day of CP-336,156 was approximately equivalent to 30 μg/kg/day of EE in preventing the increase in osteocalcin seen in OVX rats (Fig. 5D).

Significant decreases in BMD of total lumbar vertebrae and distal femoral metaphysis were found in OVX controls, compared with sham controls (Fig. 5E and F). CP-336,156 (at doses of 100 or 1000 μg/kg/day) or EE completely prevented the decrease in BMD of total lumbar vertebrae and distal femoral metaphysis (Fig. 5, E and F). At doses of 1 or 10 μg/kg/day, CP-336,156 prevented the decrease in BMD of total lumbar vertebrae but not in distal femoral metaphysis. Furthermore, BMD of total lumbar vertebrae in OVX rats treated with 100 μg/kg-day of CP-336,156 increased significantly, compared with sham controls (Fig. 5, E and F).

The decrease in percent trabecular bone area induced by OVX was prevented by treatment with CP-336,156 at doses of 1–1000 μg/kg-day in the fifth-lumbar vertebral bodies (LV5) and at doses of 10–1000 μg/kg-day in the PTM (Table 1). In LV5, CP-336,156 (at all dose levels) completely prevented the OVX-induced increases in percent eroded perimeter, labeling perimeter, and BFR/BV (Table 1). Similarly, the OVX-induced increases in osteoclast number, osteoclast perimeter, labeling perimeter, and BFR/BV in the PTM were completely prevented by treatment with CP-336,156 at doses of 10–1000 μg/kg-day (Table 1). These bone-protective effects of CP-336,156 were identical to those observed with EE.

**In vitro rat bone marrow culture study**

Compared with sham controls, OVX significantly increased the number of TRAP-positive multinuclear cells...
EFFECTS OF CP-336,156 ON RAT MODELS

TABLE 1. Selective histomorphometric parameters of fifth lumbar vertebral body and proximal tibial metaphysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham + Vehicle</th>
<th>E2 10 µg</th>
<th>CP-336,156 1000 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular bone area (%)</td>
<td>34.6 ± 0.77 %</td>
<td>35.6 ± 0.77 %</td>
<td>35.8 ± 0.77 %</td>
</tr>
<tr>
<td>Eroded perimeter (%)</td>
<td>0.67 ± 0.06 %</td>
<td>0.62 ± 0.06 %</td>
<td>0.62 ± 0.06 %</td>
</tr>
<tr>
<td>Osteoclast number/mum (mean)</td>
<td>15.4 ± 1.5 µm</td>
<td>16.0 ± 1.5 µm</td>
<td>16.2 ± 1.5 µm</td>
</tr>
<tr>
<td>Osteoclast perimeter/µm (mean)</td>
<td>5.15 ± 0.73 µm</td>
<td>5.74 ± 0.73 µm</td>
<td>6.25 ± 0.73 µm</td>
</tr>
<tr>
<td>Bone formation rate BV/mum/year</td>
<td>0.49 ± 0.07 µg</td>
<td>0.49 ± 0.07 µg</td>
<td>0.49 ± 0.07 µg</td>
</tr>
<tr>
<td>Mean ± SEM, doses expressed as µg/kg-day, po</td>
<td>0.60 ± 0.07 µg</td>
<td>0.60 ± 0.07 µg</td>
<td>0.60 ± 0.07 µg</td>
</tr>
</tbody>
</table>

Fig. 6. Inhibition of osteoclast differentiation by CP-336,156. Number of TRAP (+) MNC in bone marrow cultures from sham rats (Sham), and O VX rats treated with vehicle (OVX), E2 at 10^-11, 10^-10, and 10^-8 M, or CP-336,156 at 10^-12, 10^-10, and 10^-8 M. Cells were stained for TRAP after 6 days of culture (mean ± SEM). *, P < 0.05 vs. OVX; #, P < 0.05 vs. sham.

Discussion

Estrogen replacement therapy, or commonly prescribed hormone replacement therapy (HRT), is the most common therapy for postmenopausal osteoporosis. The recent clinical investigations by Cauley et al. (33) and Maxim et al. (34) found that long-term HRT prevented fractures of all types, including wrist, vertebrae, and hip. Thus, it was recommended that HRT should be initiated soon after menopause and continued indefinitely. However, the results from other clinical studies (7, 35) indicated that HRT significantly increased the risk for breast and endometrial cancers in postmenopausal women. The increased risk for breast and endometrial cancers and some other undesirable side effects of estrogen, including break through bleeding, reduce the compliance of...
HRT in postmenopausal women. It would be of great benefit to postmenopausal women if one could develop an estrogen agonist that is as potent and efficacious as estrogen in preventing bone loss and reducing fracture risks but without the major side effects associated with HRT.

CP-336,156, a new nonsteroidal estrogen agonist/antagonist, binds selectively and with high affinity to the human ER-α, and it prevents decreases in bone mass in vertebrae, proximal tibiae, and distal femur in the estrogen-deficient, OVX rat model. The inhibition of bone turnover and bone loss observed in OVX rats treated with CP-336,156, a third-generation estrogen receptor agonist, were comparable with

Fig. 7. Induction of apoptosis by CP-336,156. TUNEL-labeled 3-day bone marrow cells seen upon CP-336,156 (10⁻⁸ nM) or estrogen (10⁻⁸ nM) treatment, respectively (A and B). Colocalization of p53 expression in apoptotic cells is shown in A' and B'. The apoptotic cell (C), upon CP-336,156 treatment, expresses CD61 (C'). Apoptotic cells (TUNEL- and p53-positive) significantly increased by treatment with 10⁻⁸ M estrogen or CP-336,156 (D). *, P < 0.05 vs. vehicle.
those achieved with EE at 30 μg/kg-day, indicating that CP-336,156 is a full estrogen agonist on bone. However, in contrast to EE, no uterine hypertrophy was found in immature, aged, or OVX female rats treated with CP-336,156 at doses equal to or greater than that required for full bone protection.

In OVX rats treated with EE, BMD of vertebrae and distal femoral metaphysis, and trabecular bone area and bone turnover indices of vertebral body and PTM were maintained at the levels of sham controls, suggesting that estrogen levels in OVX rats, after EE treatment, produced effects comparable with those observed in rats with intact ovaries.

Potency of CP-336,156 is at least equal to that of estradiol in preventing bone loss and inhibiting bone turnover in OVX rats. Four weeks post surgery, BMD of total lumbar vertebrae and distal femoral metaphysis significantly decreased in OVX rats, compared with sham controls. CP-336,156 was able to prevent the decreases in BMD of lumbar vertebrae at doses of 1–1000 μg/kg-day, and of distal femoral metaphysis at doses of 100–1000 μg/kg-day. Trabecular bone histomorphometric analyses showed that CP-336,156 completely prevented the OVX-induced decrease in trabecular bone area and the increase in bone resorption indices (percent eroded perimeter, percent osteoclast perimeter, and osteoclast number) and bone formation indices (labeling perimeter, BFR/BV) in lumbar vertebrae at doses as low as 1 μg/kg-day, and in proximal tibiae at doses of 10–1000 μg/kg-day. Taken together, these results indicated that CP-336,156 maintained bone mass and bone turnover indices at levels comparable with those seen with EE at 30 μg/kg-day in OVX rats, as well as those observed in rats with intact ovaries. Therefore, the oral ED50 for CP-336,156 in preventing OVX-induced bone loss in rats was estimated to be less than 1 μg/kg-day in lumbar vertebrae and less than 10 μg/kg-day in PTM. It has been reported that ED50 ranges from 0.1–1 mg/kg-day for both raloxifene (3) and droloxifene (21) and is between 3–30 μg/kg-day for EE (21) in preventing bone loss in OVX rats. Thus, CP-336,156 is approximately 100- to 1000-fold more potent than raloxifene and droloxifene and at least as potent as estradiol in preventing bone loss in OVX rats.

The reasons for the increased potency of CP-336,156 in vivo, relative to other estrogen agonist/antagonists, are not completely understood. However, it may be partially explained by the higher binding affinity to the estrogen receptor and the improved pharmacokinetic properties of this agent. The IC50 for binding affinity of CP-336,156 to the human estrogen receptor is 1.5 nM (Fig. 1B), which is at least 10-fold higher than those reported for other agents in this class, such as raloxifene (3), tamoxifen (9), and droloxifene (9). Furthermore, the oral bioavailability of CP-336,156 in rats is approximately 60%, which is a significant improvement over other agents in this class, including tamoxifen, raloxifene, and droloxifene (5–27%).

In the OVX rats study, significant effects of CP-336,156 were found in body weight, uterine weight, serum cholesterol, and serum osteocalcin at 10 μg/kg-day or higher doses (100 and 1000), compared with OVX controls (Fig. 5). Thus, in these four different parameters, the minimum dose that produced statistically significant effects was 10 μg/kg-day. However, the minimum dose that produced statistically significant effects in BMD of lumbar vertebrae was 1 μg/kg-day; and in BMD of distal femoral metaphysis, it was at 100 μg/kg-day (Fig. 5). These results may suggest that CP-336,156 is more potent in preserving bone mass in the axial skeleton (lumbar vertebrae) than in the long-bone (femur) metaphysis.

The cellular and molecular mechanisms for the bone-protective effect of CP-336,156 and estrogen may be similar. Both CP-336,156 and estrogen reduced the number of TRAP(+)MNC in in vitro cultures of rat bone marrow cells. This decrease parallels the in vivo effect of both CP-336,156 and estrogen on osteoclast number. Treatment of rat bone marrow cells, with either CP-336,156 or estrogen, resulted in an increase in cells undergoing apoptosis. As seen previously with estrogen and droloxifene (25), all the apoptotic bone marrow cells expressed p53. These results indicate that the decrease in the number of osteoclasts seen both in vivo and in vitro upon CP-336,156 treatment could be caused by the ability of CP-336,156 to induce apoptosis in osteoclast precursor cells. It is possible, however, that the apoptotic cells themselves are not osteoclast precursors but are cells required for the differentiation of precursor cells into mature osteoclasts. It should be noted that at least 20% of the apoptotic cells express the cell surface marker CD61, which has been previously shown to be present on the cell surface of osteoclasts and osteoclast precursors (36), suggesting that cells of the osteoclastic lineage do undergo apoptosis upon CP-336,156 treatment.

The immature female rat model is widely accepted as a desirable animal model of estrogen action on reproductive tissues (9). The uterine wet and dry weights did not increase in immature rats receiving oral doses of CP-336,156 at 1 and 100 μg/kg-day for 3 days, indicating that this compound did not influence uterine growth in these rats. Although a slight, but significant, increase in uterine wet weight was found in immature rats treated with 10 μg/kg-day of CP-336,156, the uterine dry weight in this group did not differ from vehicle-treated controls. Thus, it seems that CP-336,156 at 10 μg/kg-day may have some effects on rehydration of the uterine tissues. These results differed from what has been reported for tamoxifen (9), another estrogen receptor agonist, which has been reported to dose-dependently stimulate uterine growth in this model (9). We also examined the estrogenic properties of CP-336,156 in uterine tissue in the aged female rat model, which closely mimics the endocrine status of postmenopausal women (23). The results from the aged female rat study indicate that no uterine hypertrophic effects were observed in 17-month-old female rats treated with CP-336,156 at 10 or 100 μg/kg-day for 28 days. Thus, CP-336,156 had very little effect on uterine tissue of immature and aged female rats. However, we found that CP-336,156 (at doses of 10–1000 μg/kg-day) significantly increased uterine wet weight in OVX rats, although it was much less pronounced than estrogen. The slight discrepancy in uterine response to CP-336,156 among immature rats, aged female rats, and OVX rats requires further investigation.

The body composition analysis by DXA showed that the weight loss in aged rats induced by higher doses of CP-336,156 was caused by a loss of fat body mass, with no change in lean body mass. Food intake was not determined in these
studies. The loss in body weight and fat body mass may be caused, in part, by a reduction in food intake, given that our previous findings for estrogen and droloxifene (4) indicated that these compounds induced weight loss, fat loss, and decreased food intake in OVX rats.

In both intact aged female rats and OVX rats, CP-336,156 significantly decreased total serum cholesterol, at doses equal to or greater than 10 µg/kg/day, in a dose-dependent manner. These effects were similar to those observed with EE, indicating that CP-336,156 acts as an estrogen receptor agonist on serum cholesterol in rat models of postmenopausal osteoporosis.

In conclusion, CP-336,156 is an estrogen agonist/antagonist that is at least equally potent as estradiol in preventing bone loss and lowering total serum cholesterol but without the hypertrophic effects on uterine tissue in rat models. The higher potency of CP-336,156 suggests that this agent may be superior to estrogen and other estrogen agonist/antagonists for prevention and treatment of postmenopausal osteoporosis.

References
32. Nishimura, Niigata City, Japan, pp 101–122