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Prevention of bone loss by EM-800 and raloxifene in the ovariectomized rat

Céline Martel, Sylvain Picard, Virgile Richard, Alain Bélanger, Claude Labrie *, Fernand Labrie

MRC Group in Molecular Endocrinology, Laboratory of Molecular Endocrinology, CHUL Research Center and Laval University, 2705 Laurier Boulevard, Québec, Canada G1V 4G2

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Abstract

Some undesirable effects are associated with chronic estrogen and progestin administration used to prevent bone loss in postmenopausal women, thus leading to poor compliance and the need for improved therapeutic and preventive agents. We have thus studied the ability of the new antiestrogen EM-800 (SCH 57050) to prevent bone loss and lower serum cholesterol levels and compared its effects with those of raloxifene. Ovariectomized (OVX) female rats were treated by oral gavage for 37 weeks with increasing daily doses (0.01, 0.03, 0.1, 0.3 or 1 mg/kg) of EM-800 or raloxifene. At 35 weeks after OVX, lumbar spine bone mineral density (BMD) was 19% lower than in intact animals ($P < 0.01$), while the OVX animals given EM-800 or raloxifene had 90–93 and 85–90%, respectively, of the BMD values observed in intact rats. Similar effects were observed on femoral BMD. Bone histomorphometry measurements were performed on proximal tibia. At the 0.01 mg/kg dose, EM-800 prevented the effect of OVX on TBV by 34% ($P < 0.01$), while raloxifene had no detectable effect. Treatment with 1 mg/kg EM-800 and raloxifene resulted in, respectively, 68% ($P < 0.01$) and 64% ($P < 0.01$) prevention of the OVX-induced decrease in TBV. In addition, the administration of 0.01 and 0.03 mg/kg EM-800 caused, respectively, 54% ($P < 0.01$) and 56% ($P < 0.01$) inhibitions of serum cholesterol levels, while raloxifene administered at the same doses caused, respectively, 24% ($P < 0.01$) and 41% ($P < 0.01$) decreases of the value of the same parameter. At the highest doses used (0.1–1 mg/kg), both compounds lowered serum cholesterol levels by approximately 65% ($P < 0.01$). No stimulatory effect of EM-800 was observed on the endometrial epithelial cells at doses up to 1 mg/kg, while hypertrophy of uterine epithelium was observed with raloxifene. EM-800 and raloxifene achieve the same degree of effectiveness on bone and serum cholesterol at higher doses, but EM-800 is at least three to ten times more potent than raloxifene at lower concentrations and has no stimulatory effect on uterine epithelium. The present data show the potent effect of EM-800 preventing bone loss and lower serum cholesterol levels without the negative effect on the endometrium, thus suggesting the particular interest of this new fully tissue-specific selective estrogen receptor modulator.

Keywords: SERM; EM-800; Raloxifene; Osteoporosis; Cholesterol

1. Introduction

Osteoporosis is a disease characterized by a generalized loss of bone mass with the associated increased risk of fracture [1]. The reduction in circulating ovarian estrogen levels at menopause is thought to be largely responsible for the accelerated bone loss in women [2], and is also associated with the higher risk of coronary heart disease that is, at least partially, related to an increase in serum lipids [3,4]. The estrogen replacement therapy, commonly used to prevent or treat osteoporosis, reduce hot flushes and decrease the risk of coronary heart disease in postmenopausal women [5], requires the addition of progestins to counteract the endometrial proliferation induced by estrogens. However, some undesirable effects are associated with chronic estrogen and progestin administration, including a perceived increased risk for uterine and/or breast cancer [6–9]. Consequently, compliance with estrogen replacement therapy is low, thus indicating the need to develop novel approaches free of such risk.

* Corresponding author. Tel.: +1-418-6542704; fax: +1-418-6542735.

E-mail address: fernand.labrie@crchul.ulaval.ca (C. Labrie).

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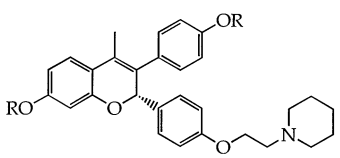
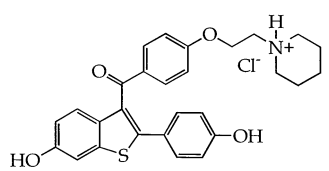
Tamoxifen, an antiestrogen with partial agonistic properties, has been shown to maintain bone mass and lower serum cholesterol levels in postmenopausal women [10,11]. The uterotrophic activity of tamoxifen, however, is well documented, thus limiting its acceptability for the prevention and treatment of osteoporosis. Raloxifene, a selective estrogen receptor modulator (SERM) synthesized a few years ago, was found to protect against bone loss and to reduce serum cholesterol levels [12–14]. Clinical studies with raloxifene in post-menopausal women have confirmed the protective effect of raloxifene on bone and the reduction in the risk of breast cancer [14–17]. However, raloxifene has a weak but statistically significant stimulatory effect on the uterus [18–20].

Recently, we reported the synthesis of the new nonsteroidal antiestrogen EM-800 [21], and have demonstrated the pure and highly potent antiestrogenic activity of this compound in the most representative in vitro and in vivo model systems [22–30]. In a recent study in rats, we observed that the addition of EM-800 to dehydroepiandrosterone treatment showed an additive effect on many parameters of bone physiology, thus suggesting a positive action of EM-800 in bone [31]. The present report describes the ability of EM-800 to prevent bone loss and lower serum cholesterol levels in ovariectomized (OVX) rats and compares its effects with those of raloxifene [12].

The OVX rat is a well-recognized animal model that mimics the development of estrogen deficiency-induced osteopenia in humans. It is also a useful model to study the lipid profile of compounds [32], a close parallelism being found between the effect of SERMs as inhibitors of serum cholesterol and prevention of bone resorption [12]. Increasing doses of EM-800 and raloxifene were thus administered orally for 37 weeks to OVX animals and the effect of these two compounds, as well as that of 17β-estradiol (E_2), were examined on parameters of bone physiology, serum lipids and uterine histology.



EM-652 R=H
EM-800 R=$COC(CH_3)_3$



Raloxifene

Fig. 1. Chemical structure of the compounds.

2. Materials and methods

2.1. Chemicals

EM-800 ((+)-7-pivaloyloxy-3-(4′-pivaloyloxyphenyl)-4-methyl-2-(4″-(2‴-piperidinoethoxy)phenyl)-2H-benzopyran) and raloxifene (Fig. 1) were synthesized in the medicinal chemistry division of our laboratory [21]. These compounds, analyzed under GLP conditions, were >98% pure. 17β-Estradiol was purchased from Steraloids (Wilton, NH).

2.2. Animals and treatment

Ten- to 12-week-old female Sprague–Dawley rats (Crl:CD(SD)Br) (Charles River Laboratory, St. Constant, Canada) weighing approximately 225–250 g at the start of treatment were used. The animals were acclimatized to the environmental conditions (temperature, 22 ± 3°C; humidity, 50 ± 20%; 12 h light:12 h dark cycles; lights on at 07:15 h) for 1 week before starting the experiment. The animals were housed three per cage and were allowed free access to tap water and a pelleted certified rodent feed (Lab Diet 5002; Ralston Purina, St. Louis, MO). The experiment was conducted in a Canadian Council on Animal Care (CCAC) approved facility in accordance with the CCAC Guide for Care and Use of Experimental Animals.

One hundred and fifty-six rats were randomly distributed between 13 groups of 12 animals each as follows: group 1, intact control; group 2, OVX control; groups 3–7, OVX + EM-800 (0.01, 0.03, 0.1, 0.3 or 1 mg/kg); groups 8–12, OVX + raloxifene (0.01, 0.03, 0.1, 0.3 or 1 mg/kg); group 13, OVX + estradiol (E_2, implant). On day 1 of the study, the animals of the appropriate groups were bilaterally ovariectomized (OVX) under isoflurane anesthesia. One Silastic implant of estradiol (E_2) was inserted subcutaneously in the dorsal area of each animal of group 13. Implants, chosen in preliminary experiments to give low physiological levels of E_2, had the following steroid concentration and size: E_2:cholesterol (1:100, w:w); 0.5 cm length of diluted steroid in silastic tubing; 0.125 inch outer diameter of silastic tubing; and 0.062 inch inner diameter of silastic tubing. During the course of the experiment, the E_2 implants were replaced monthly. Treatment with EM-800, raloxifene or vehicle (4% ethanol, 4% polyethylene glycol-600, 1% gelatin and 0.9% NaCl) was initiated on day 2 of the study. The appropriate compound or vehicle alone was given once daily by oral gavage in 0.5 ml/rat for 37 weeks. Approximately 24 h after the last dosing, overnight fasted

animals were killed by exsanguination at the abdominal aorta under isoflurane anesthesia. The uteri were removed, stripped of remaining fat, weighed and kept in 10% buffered formalin for histologic examination. The right tibia from each animal was removed and fixed in 10% buffered formalin for subsequent measurement of bone mineral density by dual-energy X-ray absorptiometry (DEXA).

2.3. Bone mineral content and density measurements

After 35 weeks of treatment, individual rats under isoflurane anesthesia had their lumbar spine as well as their right femur scanned using DEXA (QDR 4500A; Hologic, Waltham, MA) and REGIONAL HIGH RESOLUTION SCAN software. The bone mineral content (BMC), and the bone mineral density (BMD) of the lumbar spine (vertebrae L1–L4) and femur were determined. The BMC and BMD of the proximal metaphysis of the excised tibia collected at necropsy were also measured by DEXA. The fibula was removed before scanning the tibia.

2.4. Bone histomorphometry

At necropsy, the left tibia of five animals per group was collected for histomorphometric analysis. The bone specimens were fixed in ice-cold 70% ethanol for a 48-h period. Before processing, soft tissues were gently removed and each tibia was placed in a Tissus-Tek mega unicassette (Miles Inc., Elkhart, IN, USA). All specimens were then dehydrated in a graded series of ethanol, cleared in xylene, infiltrated and embedded in pure methyl methacrylate at low temperature as described [33–35].

Four groups of three serial sections were cut on each tibia using a Jung K microtome equipped with HK3 tungsten-carbide knives. Four nonserial 5 μm thick sections were stained with modified Golder's trichrome and used for histomorphometry analysis.

Bone histomorphometric analysis was performed using a semi-automated image analysis system (Bioquant Meg IV System; R & M Biometrics, Inc. Nashville) and a SummaSketch II (Summagraphics) digitizing tablet in conjunction with a Leitz Aristoplan microscope (Leica, Canada). All results were derived from two-dimensional primary measurements: tissue volume or area, bone volume or area and bone surface. The measurements were performed between 1 and 5 mm from the growth plate metaphyseal junction to restrict measurement to the secondary spongiosa. All the following abbreviations used are as proposed by the ASBMR histomorphometry nomenclature committee [36].

1. Bone volume corresponds to the percentage of trabecular cancellous bone within the spongy space: $TBV = (BV/TV) \times 100$ [36].
2. Trabecular number and trabecular separation were calculated according to the parallel plate model: $Tb.N = (BV/TV)/(10/Tb.Th)$ [36].

2.5. Histology

The uteri were immersed in a solution of 10% buffered formalin for 24–48 h, routinely processed in a tissue processor and embedded in paraffin as described elsewhere [37]. Sections of 4–5 μm were cut and stained with hematoxylin–eosin. Histopathologic examination of tissue slides was performed by light microscopy.

2.6. Serum cholesterol and triglyceride assays

Blood samples were collected from overnight fasted animals (under isoflurane anesthesia) by exsanguination at the abdominal aorta. The samples were centrifuged for 10 min at 3000 rpm and the serum was removed, transferred to new tubes and frozen at -20°C until assay. Total serum cholesterol and triglyceride levels were determined using the Boehringer Mannheim Diagnostic Hitachi 911 Analyzer (Boehringer Mannheim Diagnostic Laboratory Systems).

2.7. Urinary deoxypyridinoline assay

Twenty-four hour urine was collected from nonfasted animals for measurement of urinary deoxypyridinoline (Dpd) and creatinine levels. Dpd was measured by enzyme immunoassay on frozen urinary samples (diluted 1/5) using the Pyrilinks-D kit obtained from Metra Biosystems, Inc. (Mountain View, USA), while creatinine levels were determined using the Boehringer Mannheim Diagnostic Hitachi 911 Analyzer (Boehringer Mannheim Diagnostic Laboratory Systems).

2.8. Statistical analyses

Data are expressed as the means $\pm$ S.E.M. Statistical significance was determined according to the multiple-range test of Duncan–Kramer [38].

3. Results

The mean pretreatment values of BMD measured in vivo by DEXA during the acclimation period at the lumbar spine and femoral site were 0.148 ± 0.003 and 0.245 ± 0.004 g/cm^2, respectively. The effect of 35 weeks of treatment with increasing daily oral doses of EM-800 or raloxifene on lumbar spine BMD is illustrated in Fig. 2A. BMD of the lumbar spine was 19% lower in OVX control rats than in intact controls ($P < 0.01$). The animals given EM-800 or raloxifene at

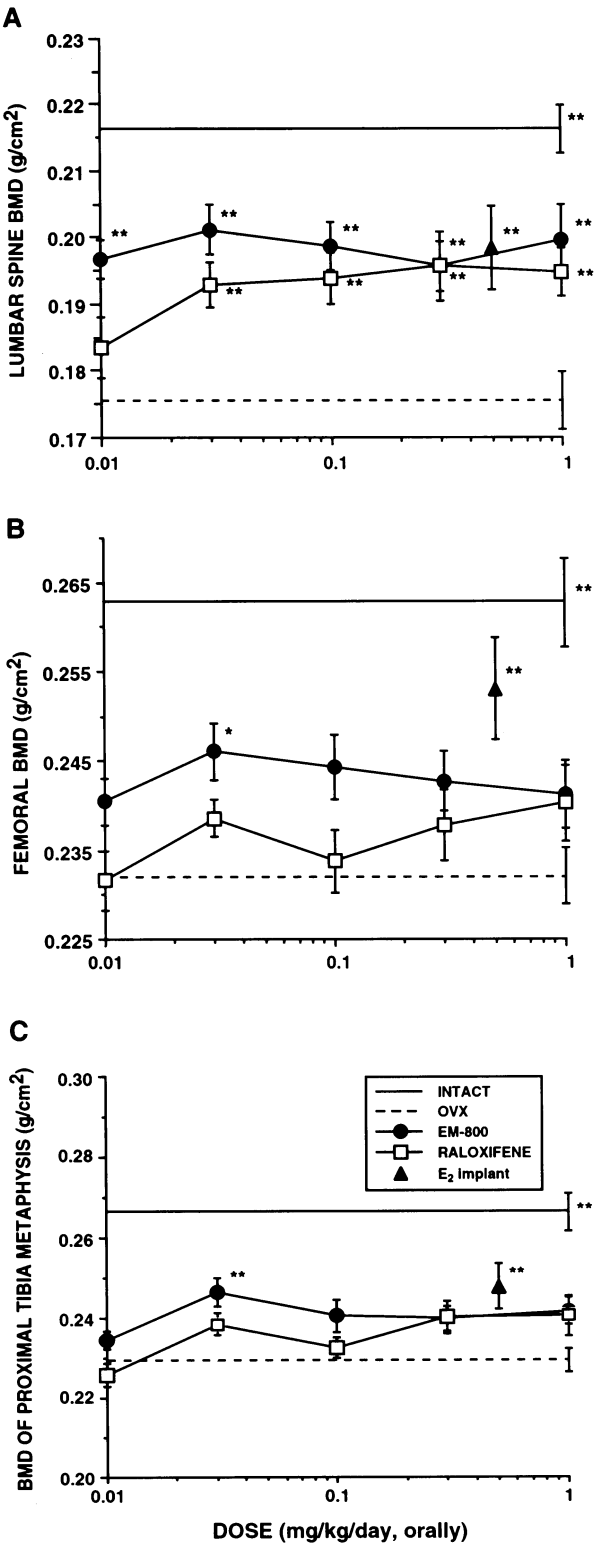


Fig. 2. Effect of 35-week treatment with increasing daily oral doses of EM-800 or raloxifene on lumbar spine BMD (A), femoral BMD (B) and proximal tibia metaphysis BMD (C) (37-week treatment) in OVX rats. Intact control, OVX control and OVX animals bearing an implant of 17β-estradiol (E_2) were included as control groups. * $P < 0.05$, ** $P < 0.01$, experimental versus OVX control rats.

doses of 0.01–1 mg/kg had 90–93 and 85–90, respectively, of the BMD observed in intact rats, the BMD values being significantly higher than those of OVX control rats ($P < 0.01$), with the exception of the lowest dose of raloxifene (0.01 mg/kg), which did not have a statistically significant effect on this parameter. Lumbar spine BMD of rats supplemented with a low physiological dose of E_2 was 92% ($P < 0.01$ versus OVX control group) of that observed in the intact controls. This stimulatory effect of E_2 is not statistically different from that of EM-800 at all doses studied. It is of interest to mention that EM-800 already had a maximal stimulatory effect on lumbar spine BMD at the lowest dose used (0.01 mg/kg body weight (BW); $P < 0.01$), while a statistically significant effect of raloxifene was first observed at the 0.03 mg/kg BW ($P < 0.01$). These results suggest that, at lower doses, EM-800 is approximately three times more potent than raloxifene on lumbar spine BMD, although both compounds achieve the same preventive effect with high doses.

The effect of EM-800 and raloxifene was then evaluated on femoral BMD (Fig. 2B). Thirty-five weeks after ovariectomy (OVX), femoral BMD had decreased by 12% ($P < 0.01$ versus intact controls). The animals given EM-800 at doses of 0.01–1 mg/kg had 92–94% of the BMD observed in intact rats, while rats given raloxifene at the same doses had 88–91% of the BMD of intact animals. Comparative with OVX control animals, the only statistically significant difference was observed with the 0.03 mg/kg daily dose of EM-800 ($P < 0.05$). Estradiol (E_2), on the other hand, preserved femoral BMD at 96% ($P < 0.01$) of the value of intact animals.

BMD of the proximal tibia metaphysis was also measured by DEXA on excised bone specimens. As shown in Fig. 2C, 37 weeks after ovariectomy, BMD of the proximal tibia was 14% lower in the OVX control rats compared with animals of the intact group ($P < 0.01$). The animals given EM-800 at doses of 0.01–1 mg/kg had 88–93% of the BMD of intact rats, while rats given raloxifene at the same doses had 85–90% of the BMD of intact rats. The only statistically significant difference was observed with the 0.03 mg/kg daily dose of EM-800 ($P < 0.01$ versus OVX controls). Estradiol, on the other hand, preserved the proximal tibia BMD at 93% ($P < 0.01$ versus OVX controls) of the value of intact animals. This effect of E_2 was not statistically different from the effect of EM-800 observed at doses ranging from 0.03 to 1 mg/kg.

Bone histomorphometry measurements were performed on proximal tibia. Thirty-seven weeks after ovariectomy, marked decreases of 73% ($P < 0.01$ versus intact controls) and 77% ($P < 0.01$ versus intact controls) in trabecular bone volume (Fig. 3A) and trabecular bone number (Fig. 3B), respectively, were observed at 1–5 mm of the growth plate metaphyseal junction of

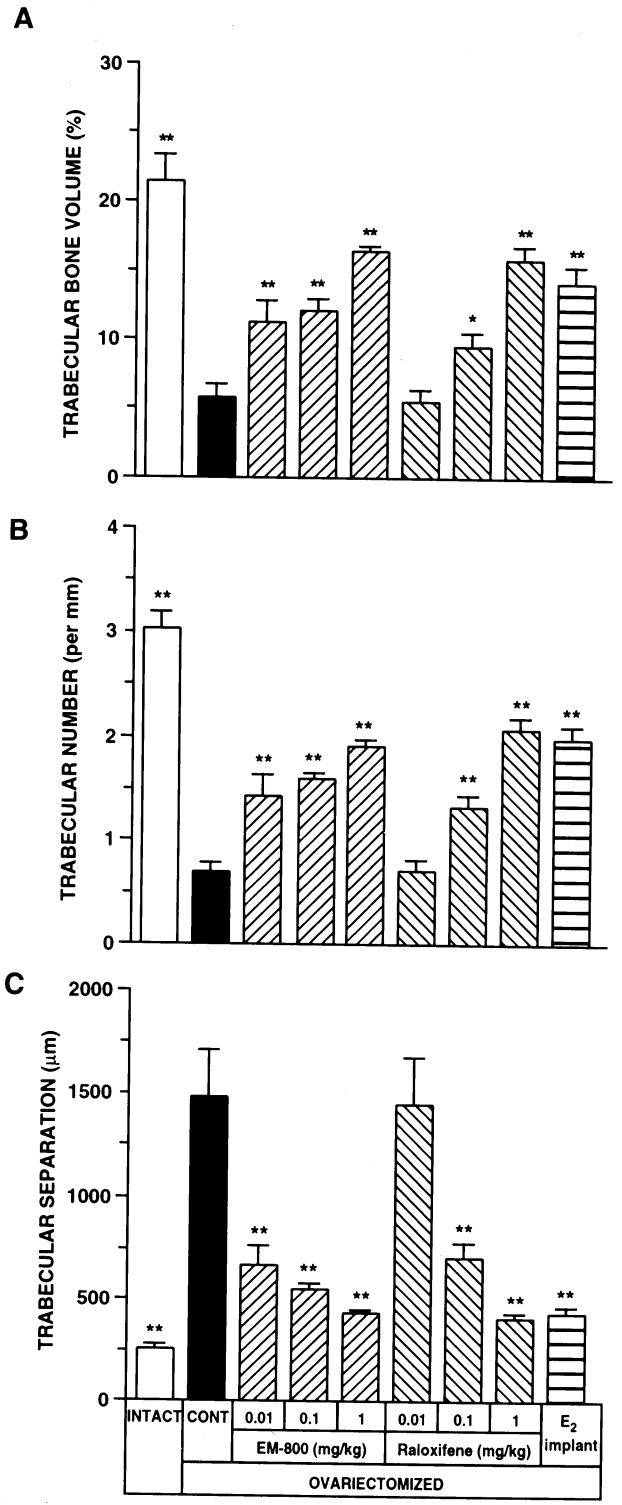


Fig. 3. Effect of 37-week treatment with increasing daily oral doses of EM-800 or raloxifene on trabecular bone volume (A), trabecular bone number (B) and trabecular bone separation (C) in OVX rats. Intact control, OVX control and OVX animals bearing an implant of 17β-estradiol (E_2) were included as control groups. * $P < 0.05$, ** $P < 0.01$, experimental versus OVX control rats.

the proximal tibia. Simultaneously, a marked increase in trabecular bone separation was observed (Fig. 3C) from a control value in intact rats of 262 ± 19 to 1486 ± 236 μm in OVX animals ($P < 0.01$). Treatment with 1 mg/kg EM-800 and raloxifene prevented, by 68% ($P < 0.01$) and 64% ($P < 0.01$), respectively, the decrease in trabecular bone volume (TBV) caused by ovariectomy. In fact, after treatment with 1 mg/kg EM-800 or raloxifene, the trabecular bone volume of the proximal tibia was 16.4 ± 0.4 and 15.8 ± 1.0%, respectively, comparative with 5.8 ± 0.9% in OVX control animals and 21.5 ± 2.0% in intact control rats. The preventive effects of EM-800 and raloxifene on TBV are not statistically different from that achieved with E_2. At the lowest dose used (0.01 mg/kg), EM-800 already prevented, by 34% ($P < 0.01$), the OVX-induced decrease of TBV, while raloxifene had no detectable effect. The administration of 0.1 mg/kg EM-800 and raloxifene, on the other hand, resulted in 40% ($P < 0.01$ versus OVX controls) and 24% ($P < 0.05$ versus OVX controls) prevention of the OVX-induced decrease in TBV (Fig. 3A), respectively.

A partial prevention of the marked decrease in trabecular bone number (Fig. 3B) associated with ovariectomy was achieved after treatment with 1 mg/kg EM-800 or raloxifene, thus resulting in 175% (from 0.69 ± 0.09 to 1.90 ± 0.07/mm; $P < 0.01$) and 200% (from 0.69 ± 0.09 to 2.07 ± 0.11/mm; $P < 0.01$) higher values in trabecular bone number compared with OVX controls, respectively. At the 0.01 and 0.1 mg/kg dose, EM-800 increased trabecular number from 0.69 ± 0.09/mm in OVX control animals to 1.43 ± 0.21/mm ($P < 0.01$) and 1.59 ± 0.06/mm ($P < 0.01$), respectively. Raloxifene had no detectable effect at the lowest dose used (0.01 mg/kg), while it increased trabecular number to 1.32 ± 0.11/mm ($P < 0.01$ versus OVX controls) at the 0.1 mg/kg daily dose. The preventive effect of E_2 was comparable with the effect observed with the 1 mg/kg dose of EM-800 or raloxifene.

It can be seen in Fig. 3C that the 0.01 mg/kg dose of EM-800 already prevented, by 66% ($P < 0.01$ versus OVX controls), the effect of OVX on trabecular separation, while a 76% ($P < 0.01$ versus OVX controls) preventive effect was observed at the 0.1 mg/kg dose. Raloxifene, on the other hand, comparative with OVX control animals, had no detectable effect on trabecular separation at the lowest dose used (0.01 mg/kg), while it prevented the effect of OVX by 63% ($P < 0.01$ versus OVX controls) at the 0.1 mg/kg dose. At the 1 mg/kg dose, EM-800 and raloxifene prevented, by 85% ($P < 0.01$ versus OVX controls) and 88% ($P < 0.01$), respectively, the OVX-induced decrease of trabecular bone separation. E_2, on the other hand, prevented the effect of OVX by 85% ($P < 0.01$), a value similar to that

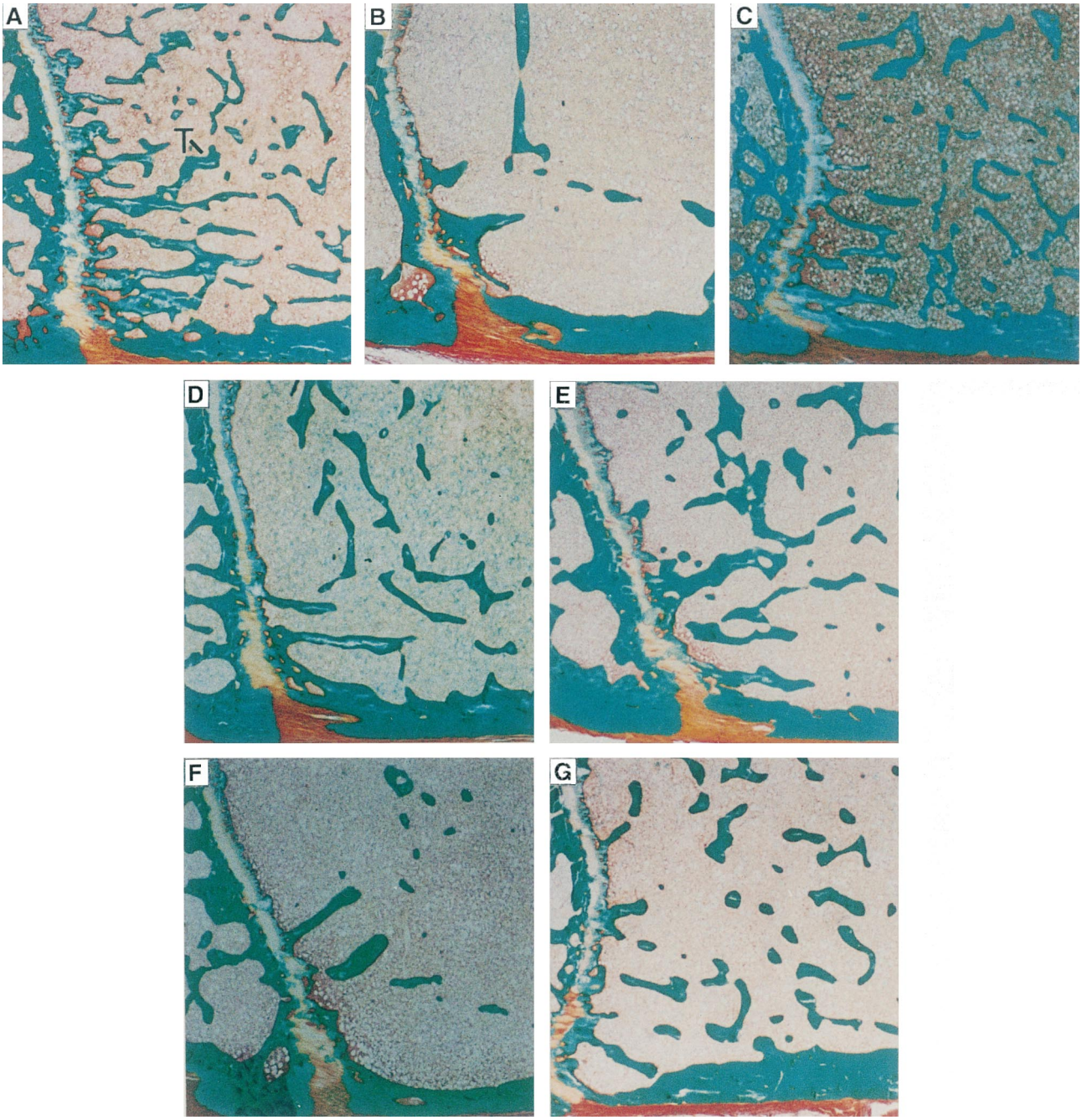


Fig. 4. Proximal tibia metaphysis from intact control (A), OVX control (B), OVX animals bearing an implant of 17β-estradiol (C), and OVX rats treated with 0.01 mg/kg (D) and 1 mg/kg (E) EM-800 or 0.01 mg/kg (F) and 1 mg/kg (G) raloxifene. Note the reduced amount of trabecular bone in OVX control animals comparative with intact control rats, and the significant prevention of OVX-induced loss of trabecular bone volume following treatment with EM-800 (0.01 and 1 mg/kg) or with the highest dose of raloxifene (1 mg/kg). Modified trichrome Masson-Goldner (magnification, × 100). T, Trabeculae.

achieved with the 1 mg/kg dose of EM-800 or raloxifene.

Fig. 4 illustrates the trabecular bone volume in the proximal tibial metaphysis of intact controls (Fig. 4A), OVX controls (Fig. 4B), OVX animals supplemented with E_2 (Fig. 4C) as well as OVX animals treated with EM-800 (Fig. 4D,E) or raloxifene (Fig. 4F,G). The administration of 0.01 mg/kg EM-800 (Fig. 4D) already prevented, by 34%, the OVX-induced loss of TBV, while raloxifene had no detectable effect at the same dose (Fig. 4F). Treatment with 1 mg/kg EM-800 (Fig. 4E) or raloxifene (Fig. 4G) resulted in an approximately 65% prevention of the OVX-induced loss of TBV.

The preventive effects on bone loss of EM-800 and raloxifene were also correlated with the urinary Dpd

Table 1
Effect of 37-week treatment with increasing daily oral doses of EM-800 or raloxifene on body weight, uterine wet weight, serum triglyceride levels and urinary Dpd excretion in ovariectomized rats[a]

Group	Body weight at necropsy (g)	Uterine weight (mg)	Triglycerides (mmol/l)	Deoxypyridinoline/creatinine (nM/mM)
Intact control	356 ± 16**	813 ± 42**	1.2 ± 0.1	16 ± 3**
OVX control	491 ± 16	108 ± 3	1.4 ± 0.2	40 ± 3
OVX + E_2 (implant)	442 ± 18*	594 ± 43**	0.7 ± 0.1	22 ± 2**
OVX + EM-800 (0.01 mg/kg)	385 ± 17**	162 ± 4	1.2 ± 0.3	28 ± 2**
OVX + EM-800 (0.03 mg/kg)	364 ± 10**	151 ± 6	1.2 ± 0.2	ND
OVX + EM-800 (0.1 mg/kg)	369 ± 17**	151 ± 8	1.2 ± 0.2	27 ± 2**
OVX + EM-800 (0.3 mg/kg)	359 ± 7**	137 ± 5	1.3 ± 0.2	ND
OVX + EM-800 (1 mg/kg)	368 ± 6**	156 ± 8	1.8 ± 0.5	30 ± 2**
OVX + raloxifene (0.01 mg/kg)	439 ± 13*	131 ± 9	1.3 ± 0.2	33 ± 4
OVX + raloxifene (0.03 mg/kg)	451 ± 14*	169 ± 9	2.0 ± 0.4	ND
OVX + raloxifene (0.1 mg/kg)	389 ± 14**	176 ± 9*	1.4 ± 0.1	29 ± 2**
OVX + raloxifene (0.3 mg/kg)	367 ± 9**	207 ± 11**	1.3 ± 0.2	ND
OVX + raloxifene (1 mg/kg)	342 ± 12**	209 ± 14**	0.9 ± 0.1	22 ± 3**

[a] Comparison is made with intact rats and ovariectomized animals bearing an implant of 17β-estradiol (E_2). * $P<0.05$, ** $P<0.01$, experimental versus OVX control group. ND, Not determined.

excretion, a marker of bone resorption. In the present experiment, the urinary Dpd/creatinine ratio excretion was increased from 16 ± 3 nM/mM in intact animals to 40 ± 3 nM/mM ($P<0.01$) in ovariectomized animals (Table 1), thus suggesting an excessive bone resorption. The administration of 0.01, 0.1 and 1 mg/kg EM-800 prevents, by 50, 54 and 42% (all $P<0.01$ versus OVX controls), respectively, the OVX-induced increase of urinary Dpd/creatinine ratio. Raloxifene, on the other hand, had no significant preventive effect on this parameter at the lowest dose used, while the administration of 0.1 and 1 mg/kg prevents, by 46% ($P<0.01$) and 75% ($P<0.01$), respectively, the OVX-induced increase of urinary Dpd/creatinine ratio.

We then compared the effect of increasing doses of EM-800 and raloxifene on serum cholesterol levels (Fig. 5). Thirty-seven weeks after ovariectomy, a 35% increase in serum cholesterol was observed in OVX control rats compared with intact controls ($P<0.01$). The daily oral administration of 0.01 and 0.03 mg/kg EM-800 to OVX animals already caused, respectively, 54% ($P<0.01$) and 56% ($P<0.01$) inhibitions of serum cholesterol levels, while raloxifene administered at the same doses caused, respectively, 24% ($P<0.01$) and 41% ($P<0.01$) decreases of the value of the same parameter. When administered at the daily doses of 0.1, 0.3 and 1 mg/kg, EM-800 caused, respectively, 58, 58 and 66% (all $P<0.01$ versus OVX controls) inhibitions of serum cholesterol levels, while raloxifene caused, respectively, 60, 62 and 65% decreases of this parameter at the same doses (all $P<0.01$ versus OVX controls). The estradiol implant (E_2), on the other hand, only reduced serum cholesterol by 20% compared with OVX control rats ($P<0.01$). No significant change was observed on serum triglyceride levels with any of the treatments used (Table 1).

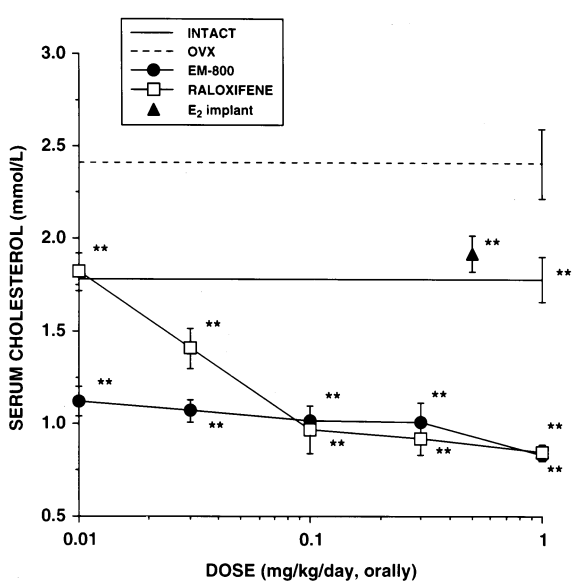


Fig. 5. Effect of 37-week treatment with increasing daily oral doses of EM-800 or raloxifene on total serum cholesterol levels in OVX rats. Comparison is made with intact rats and ovariectomized animals bearing an implant of 17β-estradiol (E_2). ** $P<0.01$, experimental versus OVX control rats.

The effects of EM-800 and raloxifene on uterine wet weight in OVX rats are presented in Table 1. Thirty-seven weeks after ovariectomy, an approximately 95% decrease in uterine wet weight was observed ($P < 0.01$ versus intact control rats). Uterine wet weight in rats receiving 0.01–1 mg/kg EM-800 was nonsignificantly increased at any dose used (Table 1). Raloxifene, on the other hand, when administered at daily doses of 0.1, 0.3 and 1 mg/kg, caused, respectively, 63% ($P < 0.05$), 92% ($P < 0.01$) and 93% ($P < 0.01$) stimulation of uterine wet weight, compared with the OVX control group. Uterine histology, shown in Fig. 6, clearly illustrated the absence of stimulatory effect of long-term treatment (37 weeks) with 1 mg/kg EM-800 on the epithelial cells of the endometrium (Fig. 6D) comparative with the OVX control group (Fig. 6B). However, hypertrophy of uterine epithelium was observed after treatment with 1 mg/kg raloxifene (Fig. 6E), this stimulatory effect being correlated with the noted increase in uterine wet weight. On the other hand, the 17β-estradiol implant caused a 450% increase in uterine weight compared with the OVX control group ($P < 0.01$) (Table 1 and Fig. 6C). Uterine weight of E_2-treated rats reached 73% of the weight measured in intact animals, which was correlated with the seric estradiol levels measured by gas chromatography mass spectrometry. In fact, estradiol levels were decreased from 22 ± 6 pg/ml in intact animals to below the limit of quantification (< 5 pg/ml) in OVX rats, while E_2 levels of OVX rats bearing the estradiol implant were 6 ± 2 pg/ml. Finally, both EM-800 and raloxifene prevented the body weight increase observed after OVX (Table 1), although EM-800 had a maximal effect at a dose ten times lower than raloxifene (0.03 mg/kg for EM-800 versus 0.3 mg/kg for raloxifene).

4. Discussion

The best recognized fact concerning menopause is that there is a progressive decrease and, finally, an arrest of estrogen secretion by the ovaries. The cessation of ovarian estrogen secretion is illustrated by the marked decline in circulating E_2 levels. This easily measurable change in circulating E_2 levels coupled with the demonstrated beneficial effects of estrogens on menopausal symptoms and bone resorption [39] has concentrated most of the efforts of hormone replacement therapy on various forms of estrogens as well as to combinations of estrogen and progestin in order to avoid the potentially harmful stimulatory effects of estrogens used alone on the endometrium, which can result in endometrial hyperplasia and cancer. It should be mentioned that recent data suggest that not only estrogens but also progestins can have a negative impact on breast cancer [8,9,40,41].

Despite the well-known beneficial effects of estrogen therapy on menopausal symptoms [42,43] and their role in reducing bone loss, hot flushes and coronary heart disease [44–47], compliance is low. Women decide not to take estrogens and stop treatment early because of the fear of breast and uterine cancer [43] and of symptoms associated with estrogen therapy, namely uterine bleeding, breast tenderness, and water retention.

The bone loss observed at menopause in women is partially related to an increase in the rate of bone resorption, which is not fully compensated by the secondary increase in bone formation. In fact, the parameters of both bone formation and bone resorption are increased in osteoporosis, and both bone resorption and formation are suppressed by estrogen replacement therapy. The inhibitory effect of estrogen replacement on bone formation is thus believed to result from a coupled mechanism between bone resorption and bone formation, such that the primary estrogen-induced reduction in bone resorption entrains a reduction in bone formation [48].

Bone loss in the OVX rat parallels the early skeletal changes observed in postmenopausal women: rapid decrease in bone mass, preferential loss of trabecular bone, and greater responsiveness to estrogen replacement therapy [49]. In the present study, 35 weeks of ovariectomy resulted in a significant osteopenic response in the femur, tibia and lumbar spine as measured by DEXA. 17β-Estradiol, on the other hand, prevented OVX-induced bone loss as previously described [49,50].

Recently, raloxifene, a compound derived from a benzothiophene series of antiestrogens [12], has been found to exert a protective effect on bone loss and have beneficial effects on serum cholesterol [12,16] without the important stimulatory effect of estrogens in the mammary gland and uterus [51,52]. EM-800 is a benzopyran derivative first developed, like raloxifene, as an antiestrogen for the treatment of breast cancer [21–23,26,27,29,30,53–57]. This orally active compound shows pure antiestrogenic activity in the mammary gland and endometrial epithelium in the rat, monkey, and mouse [22,28] (Sourla et al., unpublished data), as well as in human breast carcinoma cells in vitro [21,26,27] and in vivo as xenografts in nude mice [56]. It should be mentioned that EM-652 is the active compound derived from the inactive precursor EM-800 [21]. EM-652 is thus responsible for the effects already described on bone and serum lipids, as well as for the previously described antiproliferative effects in models of human breast and uterine cancer in vitro [26,27] and in vivo [56].

Menopause is associated with a greater increase in the rate of bone turnover and a more rapid bone loss in sites rich in trabecular bone. The effects of EM-800 and raloxifene on lumbar spine and the proximal tibia are

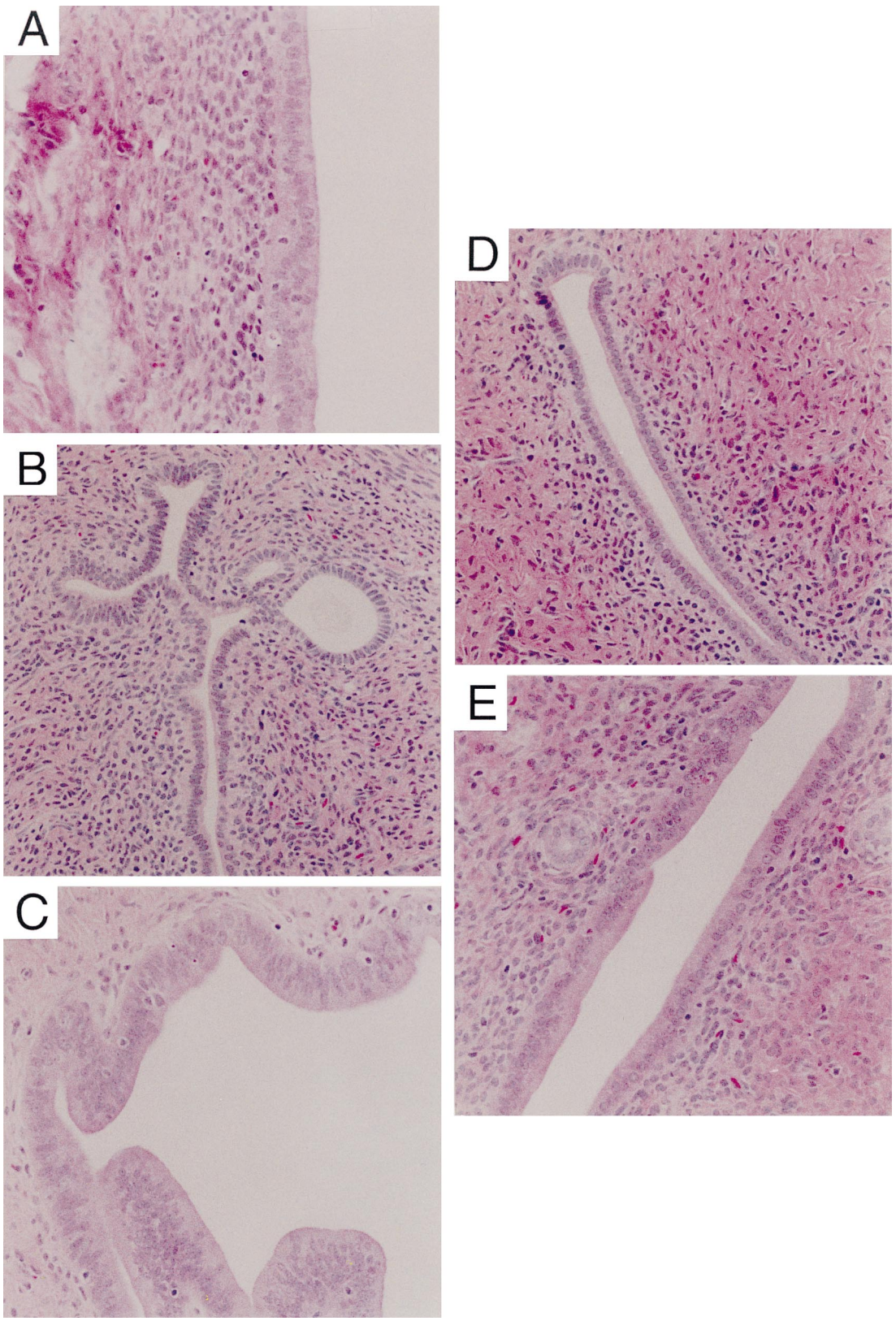


Fig. 6. Hematoxylin and eosin-stained sections of rat uteri illustrating epithelial lining cells obtained from intact control (A), OVX control (B), OVX animals bearing an implant of 17β-estradiol (C) and OVX rats treated for 37 weeks with 1 mg/kg EM-800 (D) or raloxifene (E). Note the absence of stimulatory effect of EM-800 on the endometrial epithelial cells comparative with the OVX control group while, at the same dose (1 mg/kg), a hypertrophic effect of raloxifene on uterine epithelial cells was observed (magnification, $\times 220$).

thus more easily detected since these regions have high trabecular bone content. On the other hand, the distal femur is characterized by the highest content of trabecular bone, while the other regions of the femur are composed almost entirely of cortical bone, which is slower to respond to estrogen deficiency [58]. This fact could explain the smaller response to EM-800 and raloxifene observed on total femoral BMD.

In the present study, EM-800 is shown to prevent OVX-induced bone loss in the rat, a maximal effect being already achieved on most parameters at 0.01 mg/kg, compared with 0.03 mg/kg for raloxifene. In a previous short-term study (6-week treatment), a dose–response effect of EM-800 on the prevention of bone loss in OVX female rats was observed at doses ranging from 0.001 to 0.01 mg/kg (Luo et al., unpublished data). A high potency of EM-800 as inhibitor of serum cholesterol was also observed in this study. The rat model is useful for detecting the pharmacological effects of estrogens and antiestrogens on total serum cholesterol [12,59].

The mechanisms responsible for the hypocholesterolemic effects of these agents in the rat are somewhat different from those involved in humans. In both humans and rodents, however, estrogen lowers cholesterol by upregulating the hepatic low-density lipoprotein (LDL) receptor, thus resulting in an increased removal of serum cholesterol from the circulation [60]. This effect results in a preferential reduction of LDL cholesterol in humans. However, in the rat, both high-density lipoprotein (HDL) cholesterol and LDL cholesterol are reduced, because rat HDL contains apoprotein E (not found in human HDL), which also binds to the hepatic LDL receptor [61]. Thus, in the rat, as opposed to humans, HDL cholesterol is the predominant form of circulating cholesterol, and estrogen therapy lowers both HDL cholesterol and LDL cholesterol.

The data obtained in this study suggest that EM-800 is approximately tenfold more potent than raloxifene in reducing serum cholesterol levels in the rat. In fact, the administration of 0.01 mg/kg EM-800 for 37 weeks caused a 54% decrease in serum cholesterol levels, while raloxifene caused a similar decrease in serum cholesterol levels at the dose of 0.1 mg/kg. However, at high doses, EM-800 and raloxifene exerted similar maximal inhibitory effects on cholesterol levels. In postmenopausal women, 2 weeks of treatment with daily oral dose of 5, 10, 20 and 40 mg EM-800 caused a 10% inhibition of total serum cholesterol, while a 15% inhibition of serum triglyceride levels was already observed after 1 week (Labrie et al., unpublished data).

An ideal therapy at menopause should prevent bone loss and, simultaneously, reduce cardiovascular risks without producing significant estrogenic effects on the endometrium and mammary gland that seriously limit the acceptance of the current estrogen replacement therapy. The nonsteroidal compounds EM-800 and raloxifene produce effects similar to estrogen on bone mineral density and serum cholesterol while acting as antiestrogens in the mammary gland and endometrium. EM-800 completely lacks a stimulatory effect on the endometrium, as shown at histopathological examination in this study as well as in previous ones [28,62]. Similarly, EM-800 shows pure antiestrogenic activity in human endometrial Ishikawa carcinoma cells [27]. Raloxifene, on the other hand, has been shown to have no stimulatory effect on the endometrium on short-term studies in rat [12,63]. However, in the present long-term study, hypertrophy of uterine endometrium was observed after treatment with raloxifene.

Although the stimulatory effect of raloxifene on the endometrial epithelium is not as marked as the hypertrophic effect of estradiol or tamoxifen, it is observed at doses of raloxifene that are effective to prevent bone loss (0.1–1 mg/kg). It should be mentioned also that raloxifene, droloxifene, and tamoxifen stimulate, to various degrees, the estrogen-sensitive parameter alkaline phosphatase in human endometrial Ishikawa carcinoma cells, the stimulatory effect of these compounds being fully reversible by EM-800 [21,27].

Taking into account the potent effect of EM-800 on bone and lipids, and its pure antiestrogenic activity in the mammary gland and endometrium, EM-800 should be classified as a fully tissue-specific SERM, a property not so far found for any other compound. Recently, Schafer et al. [64] have classified EM-800 as a raloxifene-like compound based on some aspects of molecular pharmacology, although the models used do not represent intact biological systems as shown in the present study.

The present data clearly demonstrate that, in the rat, low doses of EM-800 prevent bone loss and lower serum cholesterol levels without stimulatory effect on the endometrium, while previous studies have described the pure antiestrogenic activity of this compound in the mammary gland. Although comparable data in humans remain to be obtained, such data are encouraging and suggest that the active metabolite EM-652 and its precursor EM-800 could have the potential of exerting simultaneous beneficial effects on many important aspects of woman's health, namely prevention and/or treatment of breast cancer, osteoporosis and coronary heart disease.

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References

[1] B.L. Riggs, Overview of osteoporosis, West. J. Med. 154 (1991) 63–77.
[2] J.C. Stevenson, B. Lees, M. Devenport, M.P. Cust, K.F. Ganger, Determinants of bone density in normal women: risk factors for future osteoporosis?, Br. Med. J. 298 (1989) 924–928.
[3] K.A. Matthews, E. Meilahn, L.H. Kuller, S.F. Kelsey, A.W. Caggiula, R.R. Wing, Menopause and risk factors for coronary heart disease, N. Engl. J. Med. 321 (1989) 641–646.
[4] T. Gordon, W.B. Kannel, M.C. Hjortland, P.M. McNamara, Menopause and coronary heart disease: the Framingham Study, Ann. Int. Med. 89 (1978) 157–161.
[5] S.R. Cummings, Evaluating the benefits and risks of postmenopausal hormonal therapy, Am. J. Med. 91 (Suppl. 5B) (1991) 14S–18S.
[6] H.L. Judd, D.R. Meldrum, L.J. Deftos, B.E. Henderson, Estrogen replacement: indications and complications, Ann. Int. Med. 98 (1983) 195–205.
[7] D.C. Smith, R. Prentice, D.J. Thompson, W.L. Herrmann, Association of exogenous estrogen and endometrial carcinoma, N. Engl. J. Med. 293 (1975) 1164–1167.
[8] L. Bergkvist, H.O. Adami, I. Persson, R. Hoover, L. Schairer, The risk of breast cancer after estrogen and estrogen-progestin replacement, N. Engl. J. Med. 321 (1989) 293–297.
[9] G.A. Colditz, S.E. Hankinson, D.J. Hunter, W.C. Willett, J.E. Manson, M.J. Stampfer, C. Hennekens, B. Rosner, F.E. Speizer, The use of estrogens and progestins and the risk of breast cancer in postmenopausal women, N. Engl. J. Med. 332 (1995) 1589–1593.
[10] R.R. Love, D.A. Wiebe, P.A. Newcomb, L. Cameron, H. Leventhal, V.C. Jordan, J. Feyzi, D.L. DeMets, Effects of tamoxifen on cardiovascular risk factors in postmenopausal women, Ann. Intern. Med. 115 (1991) 860–864.
[11] R.R. Love, R.B. Mazees, H.S. Barden, S. Epstein, P.A. Newcomb, V.C. Jordan, P.P. Carbone, D.L. DeMets, Effects of Tamoxifen on bone mineral density in postmenopausal women with breast cancer, N. Engl. J. Med. 326 (1992) 852–856.
[12] L.J. Black, M. Sato, E.R. Bowley, D.E. Magee, A. Bekele, D.C. Williams, G.J. Cullinan, R. Bendele, R.F. Kaufman, 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.
[13] R.F. Kauffman, W.R. Bensch, R.E. Roudebush, H.W. Cole, J.S. Bean, D.L. 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, J. Pharmacol. Exp. Ther. 280 (1997) 146–153.
[14] B. Ettinger, M. Dennis, H. Bruce, K. Ronald, N. Thomas, K. Harry, C. Glaus, D. Pierre, R. Jose, S. Jacob, C. Claus, K. Kathryn, J. Frederic, E. Stephen, E. Kristine, V. Louis, L. Paul, R. Steven, Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with Raolxifene (results from a 3-year randomized clinical trial), J. Am. Med. Assoc. 282 (1999) 637–645.
[15] 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, N. Engl. J. Med. 337 (1997) 1641–1647 (see comments).
[16] M.W. Draper, D.E. Flowers, W.J. Huster, J.A. Neild, K.D. Harper, C. Arnaud, A controlled trial of raloxifene (LY139481) HCl: impact on bone turnover and serum lipid profile in healthy postmenopausal women, J. Bone Miner. Res. 11 (1996) 835–842.
[17] S.R. Cummings, S. Eckert, K.A. Krueger, D. Grady, T.J. Powles, J.A. Cauley, L. Norton, T. Nickelsen, N.H. Bjarnason, M. Morrow, M.E. Lippman, D. Black, J.E. Glusman, A. Costa, V.C. Jordan, The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation, J. Am. Med. Assoc. 281 (1999) 2189–2197 (see comments).
[18] J. Ashby, J. Odum, J.R. Foster, Activity of raloxifene in immature and ovariectomized rat uterotrophic assays, Regul. Toxicol. Pharmacol. 25 (1997) 226–231.
[19] 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.
[20] M. Sato, M.K. Rippy, H.U. Bryant, Raloxifene, tamoxifen, nafoxidine, or estrogen effects on reproductive and nonreproductive tissues in ovariectomized rats, FASEB J. 10 (1996) 905–912.
[21] S. Gauthier, B. Caron, J. Cloutier, Y.L. Dory, A. Favre, D. Larouche, J. Mailhot, C. Ouellet, A. Schwerdtfeger, G. Leblanc, C. Martel, J. Simard, Y. Mérand, A. Bélanger, C. Labrie, F. Labrie, (*S*)-(+)-4-[7-(2,2-dimethyl-1-oxopropoxy)-4-methyl-2-[4-[2-(1-piperidinyl)-ethoxy]phenyl]-2H-1-benzopyran-3-yl]-phenyl 2,2-dimethylpropanoate (EM-800): a highly potent, specific, and orally active nonsteroidal antiestrogen, J. Med. Chem. 40 (1997) 2117–2122.
[22] S. Luo, C. Martel, A. Sourla, S. Gauthier, Y. Mérand, A. Bélanger, C. Labrie, F. Labrie, Comparative effects of 28-day treatment with the new antiestrogen EM-800 and tamoxifen on estrogen-sensitive parameters in the intact mouse, Int. J. Cancer 73 (1997) 381–391.
[23] S. Luo, C. Martel, S. Gauthier, Y. Mérand, A. Bélanger, C. Labrie, F. Labrie, Long term inhibitory effects of a novel antiestrogen on the growth of ZR-75-1 and MCF-7 human breast cancer tumors in nude mice, Int. J. Cancer 73 (1997) 735–739.
[24] C. Martel, L. Provencher, X. Li, A. St-Pierre, G. Leblanc, S. Gauthier, Y. Mérand, F. Labrie, Binding characteristics of novel nonsteroidal antiestrogens to the rat uterine estrogen receptors, J. Steroid Biochem. Mol. Biol. 64 (1998) 199–205.
[25] C. Martel, C. Labrie, A. Bélanger, S. Gauthier, Y. Mérand, L. Provencher, X. Li, F. Labrie, Comparison of the effects of the new orally antiestrogen EM-800 with ICI 182 780 and toremifene on estrogen-sensitive parameters in the ovariectomized mouse, Endocrinology 139 (1998) 2486–2492.
[26] J. Simard, C. Labrie, A. Bélanger, S. Gauthier, S.M. Singh, Y. Mérand, F. Labrie, Characterization of the effects of the novel non-steroidal antiestrogen EM-800 on basal and estrogen-induced proliferation of T-47D, ZR-75-1 and MCF-7 human breast cancer cells in vitro, Int. J. Cancer 73 (1997) 104–112.
[27] J. Simard, R. Sanchez, D. Poirier, S. Gauthier, S.M. Singh, Y. Mérand, A. Bélanger, C. Labrie, F. Labrie, Blockade of the stimulatory effect of estrogens, OH-Tamoxifen, OH-Toremifene, Droloxifene and Raloxifene on alkaline phosphatase activity by the antiestrogen EM-800 in human endometrial adenocarcinoma Ishikawa cells, Cancer Res. 57 (1997) 3494–3497.
[28] A. Sourla, S. Luo, C. Labrie, A. Bélanger, F. Labrie, Morphological changes induced by six-month treatment of intact and ovariectomized mice with tamoxifen and the pure antiestrogen EM-800, Endocrinology 138 (1997) 5605–5617.
[29] A. Tremblay, G.B. Tremblay, C. Labrie, F. Labrie, V. Giguère, EM-800, a novel antiestrogen, acts as a pure antagonist of the transcriptional functions of estrogen receptors α and β, Endocrinology 139 (1998) 111–118.

[30] G.B. Tremblay, A. Tremblay, F. Labrie, V. Giguere, Ligand-independent activation of the estrogen receptor α and β by mutations of a conserved tyrosine can be abolished by antiestrogens, Cancer Res. 58 (1998) 877–881.
[31] C. Martel, A. Sourla, G. Pelletier, C. Labrie, M. Fournier, S. Picard, S. Li, M. Stojanovic, F. Labrie, Predominant androgenic component in the stimulatory effect of dehydroepiandrosterone on bone mineral density in the rat, J. Endocrinol. 157 (1998) 433–442.
[32] S.G. Lundeen, J.M. Carver, M.L. McKeen, R.C. Winneker, Characterization of the ovariectomized rat model for the evaluation of estrogen effects on plasma cholesterol levels, Endocrinology 138 (1997) 1552–1558.
[33] D. Chappard, C. Alexandre, M. Camps, J.P. Montheard, G. Riffat, Embedding iliac bone biopsies at low temperature using glycol and methyl methacrylates, Stain Technol. 58 (1983) 299–308.
[34] D. Chappard, S. Palle, C. Alexandre, L. Vico, G. Riffat, Bone embedding in pure methyl methacrylate at low temperature preserves enzyme activities, Acta Histochem. 81 (1987) 183190.
[35] R.K. Shenk, A.J. Olah, W. Herrmann, Preparation of calcified tissues for light microscopy, in G.R. Dickson (Ed.), Methods of Calcified Tissue Preparation, Elsevier, New York, 1984, pp. 1–57.
[36] A.M. Parfitt, M.K. Drezner, F.H. Glorieux, J.A. Kanis, H. Malluche, P.J. Meunier, S.M. Ott, R.R. Recker, Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee, J. Bone Miner. Res. 2 (1987) 595–610.
[37] L.G. Luna, Histopathologic Methods and Color Atlas of Special Stains and Tissue Artefacts. American Histolabs Inc., Publication Division, Johnston Printers, Maryland 1992.
[38] C.Y. Kramer, Extension of multiple range tests to group means with unique numbers of replications, Biometrics 12 (1956) 307–310.
[39] C. Christiansen, M.S. Christensen, N.E. Larsen, I.B. Transbol, Pathophysiological mechanisms of estrogen effect on bone metabolism. Dose–response relationships in early postmenopausal women, J. Clin. Endocrinol. Metab. 55 (1982) 1124–1130.
[40] K.B. Horwitz, The molecular biology of RU486. Is there a role for antiprogestins in the treatment of breast cancer?, Endocr. Rev. 13 (1992) 146–163.
[41] C.L. Clarke, R.L. Sutherland, Progestin regulation of cellular proliferation, Endocr. Rev. 11 (1990) 266–301.
[42] G.A. Greendale, H.L. Judd, The menopause: health implications and clinical management, J. Am. Geriatr. Soc. 41 (1993) 426–436.
[43] D. Grady, S.M. Rubin, D.B. Petitti, C.S. Fox, D. Black, B. Ettinger, V.L. Ernster, S.R. Cummings, Hormone therapy to prevent disease and prolong life in postmenopausal women, Ann. Intern. Med. 117 (1992) 1016–1037.
[44] R.A. Lobo, Clinical review 27: effects of hormonal replacement on lipids and lipoproteins in postmenopausal women, J. Clin. Endocrinol. Metab. 73 (1991) 925–930.
[45] M.J. Stampfer, G.A. Colditz, W.C. Willett, J.E. Manson, B. Rosner, F.E. Speizer, C.H. Hennekens, Postmenopausal estrogen therapy and cardiovascular disease. Ten year follow up from the nurses' health study, N. Engl. J. Med. 325 (1991) 756–762 (see comments).
[46] R. Lindsay, Hormone replacement therapy for prevention and treatment of osteoporosis, Am. J. Med. 95 (1993) 37s–39s.
[47] C.S. Field, S.J. Ory, H.W. Wahner, R.R. Herrmann, H.L. Judd, B.L. Riggs, Preventive effects of transdermal 17 beta estradiol on osteoporotic changes after surgical menopause: a two year placebo controlled trial, Am. J. Obstet. Gynecol. 168 (1993) 114–121.
[48] A.M. Parfitt, The cellular basis of bone remodeling: the quantum concept reexamined in light of recent advances in the cell biology of bone, Calcif. Tissue Int. 36 (Suppl. 1) (1984) S37–S45.
[49] D.N. Kalu, E. Salerno, C.C. Liu, R. Echon, M. Ray, M. Garza Zapata, B.W. Hollis, A comparative study of the actions of tamoxifen, estrogen and progesterone in the ovariectomized rat, Bone Miner. 15 (1991) 109–123.
[50] T.J. Wronski, M. Cintron, A.L. Doherty, L.M. Dann, Estrogen treatment prevents osteopenia and depresses bone turnover in ovariectomized rats, Endocrinology 123 (1988) 681–686.
[51] L.J. Black, C.D. Jones, J.F. Falcone, Antagonism of estrogen action with a new benzothiophene derived antiestrogen, Life Sci. 32 (1983) 1031–1036.
[52] M.W. Draper, D.E. Flowers, J.A. Neild, W.J. Huster, R.L. Zerbe, Antiestrogenic properties of raloxifene, Pharmacology 50 (1995) 209–217.
[53] S. Luo, A. Sourla, C. Labrie, A. Bélanger, F. Labrie, Combined effects of dehydroepiandrosterone and EM-800 on bone mass, serum lipids, and the development of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat, Endocrinology 138 (1997) 4435–4444.
[54] S. Luo, M. Stojanovic, C. Labrie, F. Labrie, Inhibitory effect of the novel antiestrogen EM-800 and medroxyprogesterone acetate (MPA) on estrone-stimulated growth of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat, Int. J. Cancer 73 (1998) 580–586.
[55] S. Luo, C. Labrie, F. Labrie, Prevention of development of dimenthylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat by the new nonsteroidal antiestrogen EM-800 (SCH 57050), Breast Cancer Res. Treat. 49 (1998) 1–11.
[56] S. Couillard, M. Gutman, C. Labrie, A. Bélanger, B. Candas, F. Labrie, Comparison of the effects of the antiestrogens EM-800 and Tamoxifen on the growth of human breast ZR-75-1 cancer xenografts in nude mice, Cancer Res. 58 (1998) 60–64.
[57] S. Couillard, C. Labrie, A. Bélanger, B. Candas, F. Pouliot, F. Labrie, Effect of dehydroepiandrosterone and the antiestrogen EM-800 on the growth of human ZR-75-1 breast cancer xenografts, J. Natl. Cancer Inst. 90 (1998) 772–778.
[58] R.B. Mazess, H. Barden, M. Ettinger, E. Schultz, Bone density of the radius, spine, and proximal femur in osteoporosis, J. Bone Miser. Res. 3 (1988) 13–18.
[59] L.F. Ferreri, H.K. Naito, Effect of estrogens on rat serum cholesterol concentrations: consideration of dose, type of estrogen, and treatment duration, Endocrinology 102 (1978) 1621–1627.
[60] M.S. Brown, J.L. Goldstein, The estradiol stimulated lipoprotein receptor of rat liver, J. Biol. Chem. 254 (1980) 10454–10471.
[61] Y.S. Chao, E.E. Windler, G.C. Chen, R.J. Havel, Hepatic catabolism of rat and human lipoproteins in rats treated with 17α-ethinyl estradiol, J. Biol. Chem. 254 (1979) 11360–11366.
[62] S. Luo, A. Sourla, S. Gauthier, Y. Mérand, C. Labrie, A. Bélanger, F. Labrie, Effect of 24-week treatment with the antiestrogen EM-800 on estrogen-sensitive parameters in intact and ovariectomized mice, Endocrinology 139 (1998) 2645–2656.
[63] G.L. Evans, H.U. Bryant, D.E. Magee, R.T. Turner, Raloxifene inhibits bone turnover and prevents further cancellous bone loss in adult ovariectomized rats with established osteopenia, Endocrinology 137 (1996) 4139–4144.
[64] J.I. Schafer, H. Liu, D.A. Tonetti, V.C. Jordan, The interaction of raloxifene and the active metabolite of the antiestrogen EM-800 (SC 5705) with the human estrogen receptor, Cancer Res. 59 (1999) 4308–4313.