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Progesterone Antagonist RU 486 Has Bone-Sparing Effects in Ovariectomized Rats

E. I. BARENGOLTS, P. V. LATHON, and F. G. LINDH

Section of Endocrinology and Metabolism, University of Illinois College of Medicine and the VA West Side Medical Center, Chicago, IL 60612, USA

We demonstrated previously that progesterone prevents ovariectomy-induced bone loss. RU 486 is a synthetic steroid with antiprogesterone activities in reproductive tissue. We used 12-week-old rats to evaluate effects of RU 486 in sham-operated rats and to compare medroxyprogesterone (MPA), RU 486, and medroxyprogesterone plus RU 486 combined treatment in ovariectomized rats. Ovariectomized rats were treated with MPA (60 mg/kg intramuscularly), RU 486 (10 mg/kg/day subcutaneously every 4 days), both, or vehicle. Sham-operated rats were treated with similar doses of RU 486 or vehicle. After 4 weeks of treatment the rats were sacrificed and bone histomorphometry was performed on proximal tibial metaphysis. Trabecular bone volume was lower (33.9 ± 1.5% vs. 46.3 ± 1.4%) and bone turnover was higher in ovariectomized than in sham-operated rats. The fraction of trabecular bone in OVX rats treated with MPA, RU 486, or both were 41.6 ± 2.5%, 44.8 ± 2.8%, and 38.4 ± 1.4%, respectively. Medroxyprogesterone treatment tended to preserve bone mass by inhibiting the increased resorption indices while maintaining higher formation rates seen in ovariectomized rats. The effects of RU 486 were similar to those of medroxyprogesterone, suggesting an agonist-like effect. Medroxyprogesterone effects were attenuated when it was combined with RU 486, suggesting that RU 486 acted as a partial antagonist in the presence of exogenous progesterone. Bone parameters were less affected in sham-operated RU 486-treated rats, and there was no significant change in bone volume (43.2 ± 1.7%). Thus, RU 486 mimicked the effects of progesterone on trabecular bone in ovariectomized rats when given alone, but acted as a partial antagonist when administered together with progesterone. (*Bone 17:21–25; 1995*)

Key Words: Osteoporosis; Ovariectomy; Histomorphometry; Rat bone.

Introduction

RU 486 (Mifepristone) is a potent antiprogesterone recently synthesized by Roussel–Uclaf, France.[7] In reproductive tissues RU 486 acts as a potent progesterone antagonist without detectable agonist effects, even at high doses. It suppresses proliferation of endometrium, folliculogenesis, and ovulation by competitive binding and blocking the progesterone receptor.[8,16,17,22] It is effective in inhibiting ovulation, inducing luteolysis, and in disrupting endometrial integrity when administered to normally cycling women.[13,21,33] RU 486 has found widespread use clinically as a terminator of pregnancy and has been shown to benefit patients with endometriosis,[20] uterine leiomyomata,[26] and hormone-dependent breast cancer.[27]

Recently, administration of RU 486 to intact and estrogen-treated adult rats was shown to produce no effects on histomorphometric parameters of bone formation. Thus, it was suggested that progesterone does not play a significant role in skeletal metabolism.[1] However, the results of other studies suggest that progesterone is involved in the regulation of mineral homeostasis.[2,3,25,30–32,34,35] The potential clinical uses of RU 486 raise questions of its safety for skeletal tissue. In the present study we evaluated the skeletal action of RU 486 alone and the interaction between RU 486 and progesterone to better understand the mechanisms of progesterone effects on bone cells. To do this we measured the effects of RU 486 on histomorphometric parameters in sham-operated (SH) and ovariectomized (OVX) adult rats. The effects of RU 486 in OVX rats also were compared with the effects of progesterone alone and the combination of RU 486 and progesterone.

Methods

Animals and Drug Treatments

Forty-eight 12-week-old rats, weighing 200–220 g, were obtained from Sprague–Dawley Breeding Laboratories, Indianapolis, IN. The rats were randomly divided into six groups: sham operated treated with vehicle (SH + V); sham operated treated with RU 486 (SH + RU); ovariectomized treated with vehicle (OVX + V); OVX treated with medroxyprogesterone acetate (OVX + MPA); OVX treated with RU 486 (OVX + RU); and OVX treated with MPA and RU 486 (OVX + MPA + RU). Ovariectomy was performed via abdominal approach under Ketamine/Xylazine anesthesia (80/10 mg/kg, i.p.). In sham-operated rats, abdomen was opened and ovaries were exposed. All rats were fed a standard pelleted rat diet (RNH 3000, Agway, Arlington Heights, IL).

Treatment was started a week after surgery (sham or OVX) and continued for four weeks. Medroxyprogesterone acetate was generously provided by Upjohn Co. (Kalamazoo, MI) and RU 486 [17β-hydroxy-11β-4-dimethylaminophenyl)-17α-(1-propynyl)-estra-4,9-dien-3-one] was generously supplied by Roussel–Uclaf (France). MPA suspension was administered once

Address for correspondence and reprints: Elena I. Barengolts, M.D., VA West Side Medical Center (MP 115), 820 South Damen Avenue, Chicago, IL 60612, USA.

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intramuscularly at the dose of 60 mg/kg, which was demonstrated to have bone-sparing effects in preliminary studies.[4] RU 486 powder was dissolved in corn oil by ultrasonication and administered subcutaneously every four days. The dose of RU 486, 10 mg/kg/day, was chosen because it had an antiprogesterone effect in reproductive tissue in vivo.[14] Corn oil served as the vehicle for both RU 486 and MPA. At the end of the experiment, the rats were euthanized with Ketamine/Xylazine and killed by exsanguination.

Bone Histomorphometry

For fluorescent labeling, all rats were injected with 7.5 mg/kg calcein intraperitoneally 6 and 1 day before sacrifice. The right and left tibias were dissected and fixed immediately in 10% cold phosphate-buffered formalin for 24 h. For evaluation of dynamic parameters, the undecalcified sections from right tibias were obtained as follows: the bones were dehydrated in acetone for 36 h, and then in xylene for 24 h before embedding in methyl methacrylate (Eastman Organic Chemicals, Rochester, NY). 10 μm frontal sections were cut on Reichert–Jung Microtome 2050 (Leica, Deerfield, IL). These unstained sections were evaluated by fluorescent microscopy for dynamic histomorphometry. For evaluation of static histomorphometric parameters, left tibias were decalcified in 10% EDTA (pH 7.4) for 1 week and then dehydrated in 70% and 95% ethanol for at least 24 h each. The bones were then infiltrated in JB4 glycol methacrylate (Polysciences Inc., Warrington, PA) for 7 days and embedded at 4°C. Midline frontal 5 μm sections were stained with tartrate-resistant acid phosphatase (Sigma Diagnostics, No. 386) and counterstained with hematoxylin.

All measurements were performed utilizing a digitizing morphometry system. The system consists of an epifluorescent microscope (Olympus, BH-2), a digitizing tablet coupled to an IBM computer, and the Bioquant System IV morphometry program (R & M Biometrics Inc., Nashville, TN). A standard sampling site was established in the secondary spongiosa of the cancellous bone located 1 mm distal to the growth plate-metaphyseal junction of the tibia. Dynamic measurements were completed on unstained sections evaluated by epifluorescent microscopy at either 200× magnification for cancellous perimeter, single- and double-labeled perimeters; or 400× magnification for interlabel width of double-fluorescent labels. Mineralized surface (MdS, double-labeled surface + ½ single-labeled surface), mineral apposition rate (MAR) and bone formation rate (BFR) were calculated from the primary data. Static evaluations were made in an area of approximately 4.32 mm^2 of secondary trabeculae at 200× magnification. Osteoclast parameters were assessed on cells lining trabecular bone and staining intensely red for tartrate-resistant acid phosphatase. Measurements consisted of the total tissue area, trabecular bone area and perimeter, and osteoclast perimeter and number. Calculated parameters were expressed in three dimensions and included fraction of trabecular bone volume determined as the trabecular bone area within 1 mm^2 total metaphyseal area multiplied by unit thickness (BV/TV), osteoclast number per 1 mm^2 of trabecular surface (Oc.No/BS), and trabecular surface lined by osteoclasts (OcS/BS).[5]

Statistics

Means and standard errors of the means (SEM) were calculated. The significance of differences among the means was determined by one-way analysis of variance with Bonferroni's test for multiple comparisons using SSPS software. The differences between sham-operated and OVX rats were analyzed by two-tailed *t*-tests. A p value of 0.05 or less was considered significant.

Results

Body weight was significantly increased in OVX (254.9 ± 4.5 g) and OVX MPA-treated (252.9 ± 6.6 g) compared with sham-operated (218.8 ± 3.2 g) rats. Treatment of OVX rats with RU 486 and the combination of MPA plus RU 486 was associated with slightly higher body weight (234.3 ± 3.2 g and 236.2 ± 7.3 g, respectively) than in sham-operated rats. There was no differences in body weight between vehicle and RU 486 (217.4 ± 6.4 g) treatment in sham-operated rats.

The effects of OVX, MPA, and RU on trabecular bone are shown in **Figure 1.** Ovariectomy resulted in a significant decrease in the fraction of trabecular bone from 46.3 ± 1.4% in sham-operated rats to 33.9% ± 1.5% in ovariectomized rats. Treatment of OVX rats with MPA, RU, or a combination of both tended to prevent the decrease in BV/TV observed in OVX rats, but the differences reached statistical significance only for OVX + RU rats. The fractions of trabecular bone in OVX rats treated with MPA, RU, and MPA plus RU were 41.6 ± 2.5%, 44.8 ± 2.8%, and 38.4 ± 1.4%, respectively. Treatment of sham-operated rats with RU had no effect on bone volume (43.2 ± 1.7%).

The osteoclast surface was significantly higher in OVX than in sham-operated rats. The increase in osteoclast surface was prevented by treatment of OVX rats with MPA, RU, and the combination of MPA and RU. Treatment of sham-operated rats with RU did not affect osteoclast surface **(Figure 2A).** The effects of OVX, MPA, and RU on osteoclast number were similar to those on osteoclast surface (Figure 2B).

The effects of OVX, MPA, and RU on mineralized perimeter, mineral apposition rate, and bone formation rate are shown in **Figure 3.** Ovariectomy resulted in increased mineralized surface, MAR, and BFR compared with sham-operated rats. In ovariectomized rats, mineralized surface was lower in RU and MPA + RU treated than in vehicle-treated group. In sham-operated rats mineralized surface was decreased in RU-treated compared with vehicle-treated rats (Figure 3A). Treatment of OVX rats with MPA, RU, and their combination was associated with higher MAR than in ovariectomized rats, but the differences reached statistical significance only for the OVX + RU group. RU 486 treatment of sham rats resulted in increased MAR compared with sham vehicle-treated rats (Figure 3B). BFR was slightly higher in OVX + MPA and OVX + RU than in ovariectomized rats but the differences did not reach statistical sig-

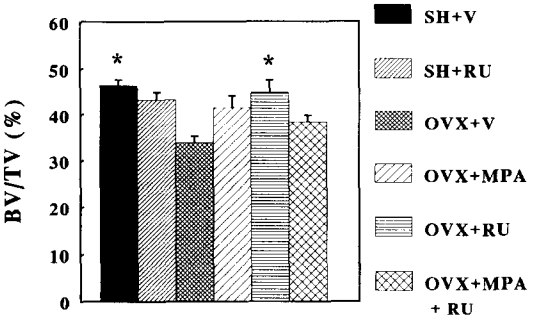

Figure 1. Effects of vehicle (V), medroxyprogesterone acetate (MPA), RU 486 (RU), and MPA + RU combination on the fraction of trabecular bone (BV/TV) at the proximal tibial metaphysis in sham-operated (SH) and ovariectomized (OVX) rats. Bar represents mean ± SEM. *$p < 0.05$ vs. OVX + V.

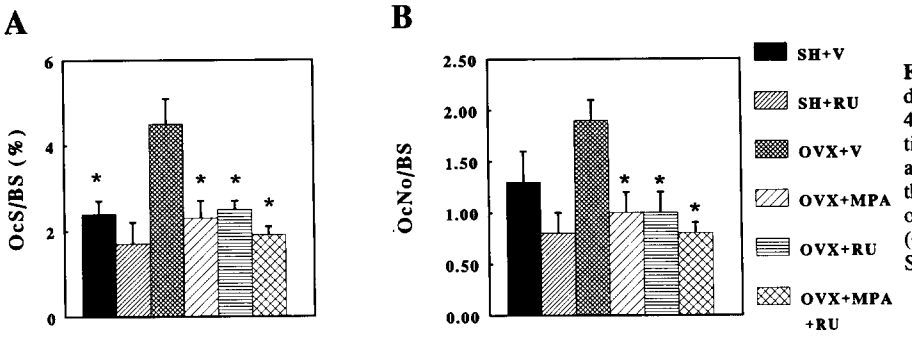

Figure 2. Effects of vehicle (V), medroxyprogesterone acetate (MPA), RU 486 (RU), and MPA + RU combination on osteoclast surface (OcS/BS) and osteoclast number (OcNo/BS) at the proximal tibial metaphysis in sham-operated (SH) and ovariectomized (OVX) rats. Bar represents mean ± SEM. *$p < 0.05$ vs. OVX + V.

nificance. There was no effect of RU treatment on BFR in sham-operated rats (Figure 3C).

Discussion

Ovariectomy, as expected, resulted in net trabecular bone loss, suggesting that bone resorption predominated over formation. Parameters of bone resorption (osteoclast number and surface) and bone formation (MAR and BFR) were higher in ovariectomized than in sham-operated rats, indicating increased bone turnover. These data are in agreement with the results from Wronski et al.,[38] demonstrating that in OVX rats bone turnover was increased and bone volume was decreased in the proximal tibial metaphysis at 30 days post-OVX.

Medroxyprogesterone treatment partly prevented the reduction in bone mass induced by ovariectomy. Osteoclast number and surface were lower in OVX + MPA than in OVX + V rats, suggesting that suppressed bone resorption was the mechanism underlying the bone-sparing effect of MPA. Bone formation rate was higher in OVX + MPA than in SH + V rats and was slightly, but not significantly, higher than in OVX + V rats, suggesting possible stimulation of bone formation. Previously, Snow and Anderson[34] demonstrated an increase in bone formation rate on OVX beagle dogs treated with MPA for six months. Consistent with these data are our earlier observations in OVX progesterone[2] and MPA[4] treated rats. Progesterone preserved bone mass in OVX rats by inhibiting bone resorption while formation was maintained at an elevated level seen in OVX rats.[2] In the study by Kalu et al.,[19] lower doses of progesterone did not prevent a decrease in trabecular bone volume observed in OVX rats. However, serum osteocalcin, a biochemical marker of bone formation, was higher in progesterone-treated than in vehicle-treated OVX rats, supporting the evidence that progesterone may stimulate bone formation.[19]

The present study demonstrated that the effects of RU 486 on bone in OVX rats were similar to those of MPA, i.e., trabecular bone volume was preserved, bone resorption was suppressed, and formation was maintained at a level slightly higher than in OVX rats. These results suggest an agonist-like effect of RU 486 on bone in the absence of progesterone. Simultaneous administration of MPA and RU resulted in attenuation of the bone-sparing effects of MPA. Trabecular volume (BV/TV) and bone formation rate were slightly lower in OVX + MPA + RU than in OVX + MPA rats. Bone resorption was suppressed in ovariectomized MPA plus RU-treated rats to the same extent as in ovariectomized MPA-treated rats. These results indicated that RU acted as a partial antagonist in the presence of exogenous progesterone in OVX rats. In sham-operated rats, no differences in bone volume, osteoclast surface, and number or bone formation rate were observed between vehicle-treated and RU-treated rats. These findings are consistent with our previous observations in sham-operated progesterone-treated rats[3] and with the results of Abe et al.,[1] who reported no effects of RU 486 on bone formation in intact rats. However, mineralized surface was decreased while mineral apposition rate was increased, and there was a trend toward lower osteoclast surface and number in RU compared to vehicle-treated sham-operated rats. These effects may be explained by a different interaction with the progesterone receptor in the presence of endogenous progesterone and estrogen or, alternatively, by antiglucocorticoid activity of RU 486.[18] Also, only one dose of RU 486 was tested in the present study, and it is possible that higher or lower doses of RU 486 would have had different skeletal effects.

The results of the present study suggest a possible dual mode of action for RU 486 on bone: an agonist-like effect on resorption and formation when given alone to estrogen-depleted rats, and a partial antagonistic effect when administered with progesterone. A similar dual mode of action, depending on the presence or

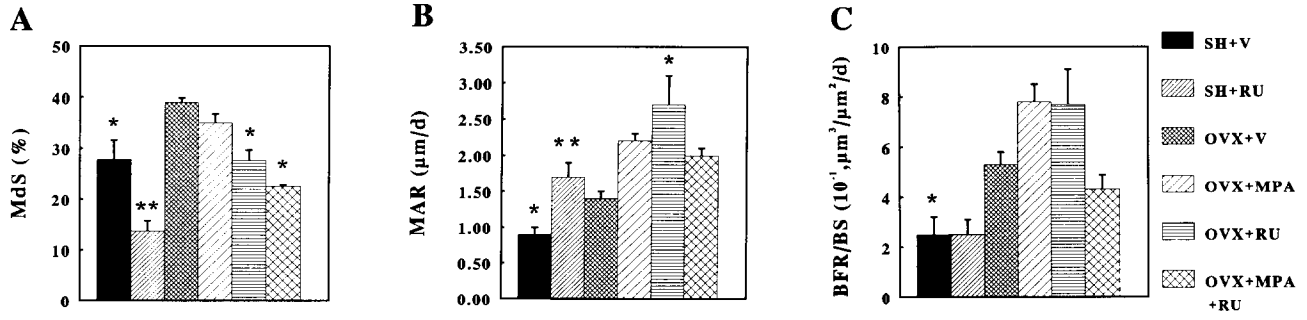

Figure 3. Effects of vehicle (V), medroxyprogesterone acetate (MPA), RU 486 (RU), and MPA + RU combination on mineralized surface (MdS), mineral apposition rate (MAR), and bone formation rate (BFR) at the proximal tibial metaphysis in sham-operated (SH) and ovariectomized (OVX) rats. Bar represents mean ± SEM. *$p < 0.05$ vs. OVX + V, **$p < 0.05$ vs. SH + V.

absence of progesterone, was shown for RU 486 effects on the sexual behavior of rats.[29] The interactions of RU 486 and progesterone appear to be similar to the interactions of tamoxifen and estrogen. Tamoxifen is a well-recognized estrogen antagonist in reproductive tissue,[12] but acts as a partial estrogen agonist on bone.[24,36] Tamoxifen, similar to estrogen, prevented bone loss in OVX rats. However, when estrogen and tamoxifen were administered together, tamoxifen attenuated the effect of estrogen on trabecular bone, acting as a partial antagonist.[24]

The present data suggest that RU 486 treatment inhibited increased bone resorption in the presence or absence of exogenous progesterone in OVX rats but attenuated the actions of administered progesterone on bone formation. The reason for this difference is not clear, but may be related to the different type and/or number of progesterone receptors on osteoclasts[28] and osteoblasts.[11] Alternatively, the observed effects may be explained by antiglucocorticoid activity of RU 486 and MPA,[15,18] mediated through the glucocorticoid receptor, which is known to be present in osteoblasts.[10] There may be an interaction between antiprogesterone and antiglucocorticoid activity and modulation of progesterone receptor via glucocorticoid receptor similar to the effects demonstrated in breast cells.[37] In addition, local mediators of bone resorption and formation[9,23] may influence progesterone effects on bone. We recently demonstrated that steady-state level of IGF-I mRNA in bone and calvarial periosteum as well as IGF-I and IGFBP-3 levels in serum changed in the same direction as BFR in OVX and OVX progesterone-treated rats,[6] suggesting that IGF-I may be a mediator of progesterone action on bone.

In summary, RU 486 mimicked the effects of progesterone on trabecular bone in OVX rats when given alone, but acted as a partial antagonist when administered together with progesterone. The results of our study showed that RU 486 may serve as a valuable tool for studying mechanisms of progesterone effects on bone, and suggested that progesterone analogs may be designed with site-specific beneficial effects on skeletal tissue.

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