The Novel Non-steroidal Selective Androgen Receptor Modulator S-101479 Has Additive Effects with Bisphosphonate, Selective Estrogen Receptor Modulator, and Parathyroid Hormone on the Bones of Osteoporotic Female Rats

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Received January 16, 2012; accepted April 6, 2012.

We have studied non-steroidal selective androgen receptor modulators (SARMs) to develop anti-osteoporosis drugs for males and females. Many SARMs have been studied for their anabolic effects on bone or muscle with reduced virilizing effects in male animals. However, the tissue selectivities of these agents in female animals have not been fully evaluated. We evaluated the novel SARM S-101479 from tetrahydroquinoline libraries in ovariectomized (OVX) rats. S-101479 preferentially bound to the androgen receptor with nanomolar affinity among nuclear receptors. It increased the bone mineral density (BMD) of femurs and diminished the effects on the uterus and clitoral gland in OVX rats. We then compared the effect of S-101479 on bone with those of commercial anti-osteoporosis drugs such as alendronate, raloxifene, and teriparatide. Furthermore, we evaluated the effects of combination treatments with these agents in OVX rats. After 16-week treatment, all agents significantly increased BMD, but the magnitude of bone mineral content (BMC) and/or bone size (projected bone area) were different. Alendronate, raloxifene, and teriparatide maintained BMC and bone size in this experimental dose. Only S-101479 increased BMC with bone size on single treatments. In combination treatment, S-101479 significantly increased BMC and bone size compared with single treatments of other agents. S-101479, like natural androgen, may have showed periosteal bone formation of the cortical area and indicated additive effects with commercial anti-osteoporosis drugs. These results indicate that S-101479 may be a useful anti-osteoporosis drug, particularly for patients with established severe osteoporosis.

Key words selective androgen receptor modulator; bone anabolic; additive effect

Androgens are known to have beneficial anabolic effects on various tissues such as bone, muscle, and red blood cells (RBCs).1–4 However, the clinical use of androgens has been limited because of their undesirable virilizing and metabolic actions. Their effect on the prostate gland is an extremely serious side effect because the risk of prostate cancer or benign prostate hyperplasia may increase.5,6 The side effects of androgens are not lethal in women, but many virilizing effects (e.g., hirsutism, voice change, and acne) have been observed.7,8 Anabolic steroids were developed to reduce the side effects of androgens and treat osteoporosis. However, their actions were not sufficient to reduce virilizing effects, and they exhibited hepatotoxic actions.9–12 Recently, the actions of non-steroidal selective androgen receptor modulators (SARMs) have been investigated in many laboratories.13–17 Typically, they were observed to be more tissue-selective than anabolic steroids and were orally available in preclinical models. We have focused on bone anabolic SARMs to develop anti-osteoporosis agents without serious virilizing effects. Several categories of anti-osteoporosis drugs exist in the form of bisphosphonates, selective estrogen receptor modulators (SERMs), and parathyroid hormones (PTHs). Although these effective agents are currently available, fragility fractures remain a significant problem worldwide. Bisphosphonates and SERMs increase bone mass and reduce the risk of osteoporotic fracture,18,19 but they cannot enhance bone formation. PTHs are unique bone formation agents and strongly affect bone.20,21 However, subcutaneous injections are required, and their use is limited to 2 years. We believe that the anabolic effects of androgens on bone are useful not only for age-related osteoporosis in males but also for postmenopausal osteoporosis in females.

We previously reported S-40503 to be a non-steroidal SARM.22 On subcutaneous injection, this compound exhibited bone-selective activity and reduced effects on the prostate gland in orchidectomized (ORX) rats. As observed with 5α-dihydrotestosterone (DHT), S-40503 resulted in periosteal bone formation and increased bone strength. However, S-40503 was not available for oral administration, and tissue selectivities were not sufficient. In this report, during compound screening, we discovered S-101479 to be an orally available SARM with bone anabolic activities. Patients with established osteoporosis are targets for SARM treatment. Bisphosphonates, SERMs, or PTHs are widely used for patients with osteoporosis; these patients may have used them previously or may be receiving them concurrently. If our SARM reduces or inhibits the effects of such agents, it may be difficult to use. Androgens can increase periosteal bone formation without estrogen conversion. This is a unique effect of androgen, and it was observed in SARMs such as S-40503.

The authors declare no conflict of interest.

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or LGD-3303 (developed by Ligand Pharmaceuticals). Conversely, anti-bone-resorptive agents such as SERMs or bisphosphonates strongly affect high bone turnover area (cancellous bone), and PTHs are known to stimulate cancellous bone formation. Therefore, we hypothesized that S-101479 has additive effects not only with anti-bone-resorptive agents such as bisphosphonates or SERMs but also with bone formation agents such as PTHs.

In the present study, we reported that the novel orally available SARM S-101479 was bound to the androgen receptor (AR) and showed agonistic activity. It increased bone volume with diminished side effects in female animals. Furthermore, S-101479 had additive effects on bone with alendronate, raloxifene, or teriparatide.

**MATERIALS AND METHODS**

**Animals** Sprague-Dawley (SD) rats and Japanese white (JW) rabbits were obtained from Japan SLC (Shizuoka, Japan). They were used for experiments after 1 week of breeding in our animal facility. Animals were housed in a room maintained at a 12-h light–dark cycle under controlled temperature (20–26°C) and humidity (30–70%). Animals had free access to commercial standard rodent feed (MF; Oriental Yeast Co., Tokyo) and filtered water.

**Compounds** All reagents were obtained from Nacalai Tesque (Kyoto, Japan) unless stated otherwise. (3α,45,9h5)-Y-[2-(8-Cyano-1-formyl-2,3,4,5,9b-hexahydro-1H-pyrrolo[3,2-c]quinolin-4-yl)-2-methylpropyl]-4,6-difluorobenzofuran-2-carboxyamide (S-101479; Fig. 1) is an AR ligand synthesized at Kaken Pharmaceuticals. Bicalutamide was extracted from Casoex tablets (AstraZeneca K.K., Osaka, Japan). Raloxifene hydrochloride (RAL) was purchased from Toronto Research Chemicals (Brisbane, Canada). Teriparatide (recombinant PTH (1–34)) was purchased from Eli Lilly and Company (Indianapolis, IN, U.S.A.) as Forteo. Alendronate sodium (ALN) was supplied by Perkin-Elmer (Kanagawa, Japan). [3H]17β-Estradiol (E2), and [3H] Progesterone (Pro) were purchased from MSD K.K. (Tokyo, Japan) as Fosamac tablets. Dexamethasone (Dex) and aldosterone (Ald) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Dexamethasone (Dex) and aldosterone (Ald) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.) and Acros Organics (Geel, Belgium), respectively. [3H]DHT, [3H]β-Estradiol (E2), and [3H]Progesterone (Pro) were supplied by Perkin-Elmer (Kanagawa, Japan). [3H]Dex and [3H]Ald were obtained from Amersham Pharmacia (Tokyo, Japan). All steroids and S-101479 were dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer for nuclear receptor binding studies and reporter assays. S-101479, RAL, and ALN were suspended in 0.5% methyl cellulose #400 (MC) for in vivo oral treatment. DHT was dissolved in DMSO and diluted 10-fold with olive oil. Teriparatide was diluted using Forteo’s solution plasmid. TE-85 cells were stably transfected by the calcium phosphate precipitation procedure with a reporter construct, mouse androgen receptor (hAR) cDNA was kindly provided by Professor Kato (Tokyo University, Tokyo, Japan). The hAR expression plasmid was constructed by inserting hAR cDNA into the pBk-CMV (Clontech, Mountain View, CA, U.S.A.) multicloning site. TE-85 cells were stably transfected by the calcium phosphate coprecipitation procedure with a reporter construct, mouse mammary tumor virus (MMTV)-Luc, containing the MMTV long terminal repeat linked to luciferase and the hAR expression plasmid. TE-85 cells were cultured in DMEM supplemented with 10% charcoal-stripped FBS. Cells were seeded with or without test compounds and incubated for 48 h before measurement of luciferase activities. The luciferase activity induced by 1000 nM DHT was set as 100% and used as the reference for quantifying the activity of S-101479. Half-maximal effective concentration (EC50) values were determined from a nonlinear regression analysis (probit analysis) of dose–response curves using the SPSS software program regression models 11.0J (SPSS, Chicago, IL, U.S.A.). The IC50 value is defined as the concentration of the competing ligand required to reduce specific binding by 50%. The inhibition constant (Ki) of test compounds was determined using the Cheng–Prusoff equation: \[K_i = IC_{50}/(1 + [L]/[L'])\] where, L is the concentration of the radioligand, and [L'] is the dissociation constant of the radioligand determined from Scatchard plot analyses.

**Reporter Assays** Phenol red-free Dulbecco’s modified Eagle’s medium (DMEM) was obtained from Sigma-Aldrich. Fetal bovine serum (FBS) was purchased from Gibco (Carlsbad, CA, U.S.A.). Human osteosarcoma cell line TE-85 cells were obtained from American Type Culture Collection (Manassas, VA, U.S.A.). Human androgen receptor (hAR) cDNA was kindly provided by Professor Kato (Tokyo University, Tokyo, Japan). The hAR expression plasmid was constructed by inserting hAR cDNA into the pBk-CMV (Clontech, Mountain View, CA, U.S.A.) multicloning site. TE-85 cells were stably transfected by the calcium phosphate coprecipitation procedure with a reporter construct, mouse mammary tumor virus (MMTV)-Luc, containing the MMTV long terminal repeat linked to luciferase and the hAR expression plasmid. TE-85 cells were cultured in DMEM supplemented with 10% charcoal-stripped FBS. Cells were seeded with or without test compounds and incubated for 48 h before measurement of luciferase activities. The luciferase activity induced by 1000 nM DHT was set as 100% and used as the reference for quantifying the activity of S-101479. Half-maximal effective concentration (EC50) values were determined from a nonlinear regression analysis (probit analysis) of dose–response curves using the SPSS software program Regression Models 11.0J (SPSS).

**Animal Studies. Ovariectomized (OVX) Study 1: Dose–Response Study of S-101479** In the experiments described below, 12-week-old female SD rats were used. These rats were bilaterally OVX or sham-operated under ether anesthesia. After surgery, all rats were maintained without treatment for 2 weeks before. Crude progesterone receptor (PR) and estrogen receptor (ER) protein fractions were prepared from the uteruses of young JW rabbits (body weight (BW), 1.6 kg). Crude glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) protein fractions were prepared from the livers and kidneys of 8-week-old male SD rats, respectively, which were adrenalec-tomized 4 d before. Specific binding was defined as the difference between the binding of radioligands in the presence (non-specific binding) and absence (total binding) of an excess of unlabeled ligands. Steroid standards were included in each assay. Data were plotted as percentage inhibition of the change in radioactivity between the specific binding of the radioligand and that of the test compound at various concentrations. Half-maximal inhibitory concentration (IC50) values were determined from a nonlinear regression analysis (probit analysis) of dose–response curves using the SPSS software program regression models 11.0J (SPSS, Chicago, IL, U.S.A.). The IC50 value is defined as the concentration of the competing ligand required to reduce specific binding by 50%. The inhibition constant (Ki) of test compounds was determined using the Cheng–Prusoff equation: \[K_i = IC_{50}/(1 + [L]/[L'])\] where, L is the concentration of the radioligand, and [L'] is the dissociation constant of the radioligand determined from Scatchard plot analyses.
4 weeks to permit bone loss before the beginning of bone anabolic therapy (as in established osteoporosis). During this period, standard chow was replaced with a modified American Institute of Nutrition diet containing 0.4% calcium and 0.5% phosphate (Oriental Yeast Co.) to continue bone loss more progressively. Thereafter, OVX animals were regrouped into 8 experimental groups such as vehicle, DHT (20 mg/kg), RAL (1 mg/kg), and S-101479 (0.1, 0.3, 1, 3, 10 mg/kg) before the start of 12-week treatments. Each group comprised 12 animals. Initial body weights were equalized among treatment groups. The vehicle (0.5% MC) was administered to sham rats. S-101479 and vehicle were orally administered once daily in a volume of 5 mL/kg BW. DHT was injected subcutaneously 3 times a week in a volume of 1 mL/kg BW. At the end of the experiment, the rats were euthanized by exsanguination from the abdominal aorta. After 12-week treatments, the uteruses and clitoral glands were excised and weighed immediately. Both femurs were dissected free of soft tissue. Right femurs were fixed in 100% ethanol to be used for the measurement of bone mineral density (BMD), bone mineral content (BMC), and projected bone area (AREA). BMC and AREA were measured by dual-energy X-ray absorptiometry utilizing a bone mineral analyzer (DCS-600EX-IIIR; Aloka, Tokyo, Japan). BMD equaled BMC divided by AREA. Femurs were divided into 20 equal regions from distal (region 1) to proximal (region 20), and BMD of each region was measured. Left femurs were frozen in saline for measuring bone strength. The uteruses were fixed in 10% neutral formalin and then used for histopathology. Statistical analyses were done using the SPSS Base 11.0J software program (SPSS). All data are presented as the mean and standard deviation (S.D.). The difference was evaluated by the Student’s t-test when comparing two groups or by one-way analysis of variance followed by the Dunnett’s test for multiple groups. p < 0.05 was considered to be statistically significant. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Kaken Pharmaceutical Co., Ltd.

Histopathology Histopathological evaluations were conducted for the fixed uterus. Fixed samples were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined under a light microscope using standard procedures.

Biomechanical Analyses To examine the biomechanical strength of the left femurs of OVX rats, three-point bending was performed in the femoral midshaft region using a load torsion tester (EZ-L; Shimadzu, Kyoto, Japan). Femurs were placed in a testing jig resting on two lower supports located 14 mm apart, with their midpoint centered between the two lower supports; load was applied at a constant deformation rate of 10 mm/min at the midpoint of the femur. The maximum load (N) was interpreted and calculated from the load–deflection curve, which was continuously recorded in a computerized monitor linked to the tester.

**RESULTS**

**NR Binding Assays** Competitive binding assays were performed to determine the affinity of S-101479 for AR and other steroid receptors. S-101479 had a high affinity for AR (Kᵢ, 5.14 nm) with extremely low affinity for PR, ER, GR, and MR (Kᵢ, >5090; >7790; 40300; and >39300 nm, respectively). This affinity was as strong as that of the non-steroidal antiandrogen bicalutamide (Kᵢ, 1.81 nm). DHT, a non-aromatizable AR ligand used as the positive control, showed an approximately 10-fold higher affinity for AR (Kᵢ, 0.613 nm) than S-101479; it showed more than 30-fold higher affinity for PR, GR, and MR (Kᵢ, 20.3; 1240; and 510 nm, respectively) than S-101479. The affinity of DHT for ER was the same as that of S-101479 (Kᵢ, >7790 nm) (Table 1).

**Reporter Assays** Reporter assays were used to identify the agonistic activity of S-101479 for AR. S-101479 showed a dose–response increase in luciferase activity. Its maximal transactivation relative to 1000 nm DHT reached an agonistic activity of 83.0 ± 2.7%. The EC₅₀ of S-101479 (EC₅₀, 13.4 nm) was 2.4-fold that of DHT (EC₅₀, 5.7 nm) (Fig. 2). The androgenic activity of S-101479 was a slightly weaker than that of DHT, but it clearly showed agonistic activity.

**OVX Study 1: Dose–Response Study of S-101479** To demonstrate the difference between the androgenic activities of DHT and S-101479, we orally administered these agents to OVX rats daily. We compared the effects of S-101479 with SERM RAL. BMD of total femurs decreased significantly by OVX (sham vehicle, 129.8 ± 5.45 and OVX vehicle, 117.2 ±

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**Table 1. Nuclear Receptor Binding Affinity of S-101479**

<table>
<thead>
<tr>
<th>Kᵢ (nm)</th>
<th>AR</th>
<th>PR</th>
<th>ER</th>
<th>GR</th>
<th>MR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHT</td>
<td>0.613</td>
<td>20.3</td>
<td>&gt;7790</td>
<td>1240</td>
<td>510</td>
</tr>
<tr>
<td>Natural ligand</td>
<td>0.38</td>
<td>0.453</td>
<td>6.74</td>
<td>10.4</td>
<td></td>
</tr>
<tr>
<td>Bicalutamide</td>
<td>1.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-101479</td>
<td>5.14</td>
<td>&gt;5090</td>
<td>&gt;7790</td>
<td>40300</td>
<td>&gt;39300</td>
</tr>
</tbody>
</table>

Specifc binding was defined as the difference between the binding of radioligands in the presence (non-specific binding) and absence (total binding) of an excess of unlabeled ligands. Each binding assay was conducted in duplicate, and Kᵢ values were calculated as described in the Materials and Methods section. The natural steroid ligand in each assay was as follows: PR, progesterone; ER, 17 β-estradiol; GR, dexamethasone; MR, aldosterone; AR, androgen receptor; PR, progesterone receptor; ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; DHT, 5α-dihydrotestosterone.
S-101479 increased BMD compared with the OVX vehicle in a dose-dependent manner (0.1 mg/kg, 118.6 ± 4.40; 0.3 mg/kg, 122.0 ± 5.61; 1 mg/kg, 123.4 ± 5.16; 3 mg/kg, 126.3 ± 6.44; and 10 mg/kg, 125.7 ± 5.14); its effect reached a plateau at 3 mg/kg and was the same as that of 20 mg/kg DHT (125.7 ± 5.53). RAL (1 mg/kg) did not significantly increase BMD (121.8 ± 6.21) compared with the OVX vehicle; BMD was equal to that of 0.3 mg/kg S-101479 (122.0 ± 5.61). At the distal metaphysis (regions 1–5), which was rich in cancellous bone, all compounds significantly increased BMD to the same degree (10 mg/kg S-101479, 135.3 ± 6.46; 1 mg/kg RAL, 136.5 ± 6.46; and 20 mg/kg DHT, 135.7 ± 6.61) compared with the OVX vehicle (128.2 ± 5.13) (Fig. 3a). At the diaphysis (regions 8–12), S-101479 (3 mg/kg, 135.3 ± 6.46; 1 mg/kg RAL, 136.5 ± 6.46; and 20 mg/kg DHT, 135.7 ± 6.61) significantly increased BMD compared with the OVX vehicle (112.2 ± 4.54) and that of 3 and 10 mg/kg S-101479 were over the sham vehicle group (116.0 ± 4.90). RAL (1 mg/kg) had no effects on diaphysial BMD (111.3 ± 7.42) (Fig. 3c). Bone strength of the mid-femur was not altered by OVX (sham vehicle, 149.6 ± 13.96 and OVX vehicle, 142.0 ± 12.40). S-101479 increased the maximum load dose-dependently (0.1 mg/kg, 143.3 ± 13.36; 0.3 mg/kg, 150.7 ± 14.85; 1 mg/kg, 159.3 ± 14.35; 3 mg/kg, 168.6 ± 23.82; and 10 mg/kg, 165.6 ± 14.46) compared with the OVX vehicle. S-101479 (3 mg and 10 mg/kg) and 20 mg/kg DHT (165.8 ± 6.44) increased bone strength compared with the OVX vehicle (142.0 ± 12.40).
significantly increased bone strength over the sham vehicle group. RAL (1 mg/kg) did not show any effect (133.7 ± 18.49) (Fig. 4).

The weight of clitoral gland decreased by OVX (sham vehicle, 0.14 ± 0.027 and OVX vehicle, 0.09 ± 0.020) and recovered by 10 mg/kg S-101479 (0.15 ± 0.124). However, they were not different from those of the sham vehicle group at 10 mg/kg of S-101479. Conversely, DHT increased the weight exceptionally (0.41 ± 0.096). The effect of 10 mg/kg S-101479 on the clitoral gland was very different from that of DHT (change in weights from OVX vehicle, 0.06 and 0.32, respectively) (Fig. 5a). OVX caused a significant decrease in uterus weight (sham vehicle, 0.55 ± 0.117 and OVX vehicle, 0.09 ± 0.006). S-101479 (3 mg/kg, 0.12 ± 0.021 and 10 mg/kg, 0.16 ± 0.026) or 20 mg/kg DHT (0.57 ± 0.058) significantly increased uterus weight, but their effects were extremely different (change in weight from OVX vehicle, 0.07 and 0.49, respectively). The effect of 1 mg/kg RAL (0.12 ± 0.013) was slightly stronger than the same dose of S-101479 (0.09 ± 0.010) (Fig. 5b). On histopathological analyses, OVX reduced the thicknesses of the endometrium and myometrium; the thickness of the endometrium was considerably reduced. DHT (20 mg/kg) considerably increased the thickness of the circular and longitudinal muscles of the myometrium. The effects on the endometrium were not as strong, but a wavy structure was observed in the epithelial layer. 10 mg/kg S-101479 and 1 mg/kg RAL increased uterus weight, but histological changes were not obvious (Fig. 6). S-101479 and DHT significantly increased BMD and bone strength of the femur. However, the effects on the clitoral gland and uterus differed largely between these agents.

**OVX Study 2: Combination Treatment**

To differentiate between the effects of S-101479 and commercial anti-osteoporosis drugs on bone, we administered S-101479, RAL, ALN, and teriparatide in OVX rats daily. In addition, we evaluated the possibility of combination therapy of S-101479 and these agents. BMD of total femurs was significantly decreased by OVX (sham vehicle, 130.3 ± 4.28 and OVX vehicle, 118.9 ± 4.60). All compounds significantly increased BMD (1 mg/kg S-101479, 124.0 ± 4.21; 1 mg/kg ALN, 126.2 ± 4.82; 1 mg/kg RAL, 124.3 ± 3.70 and 1 µg/kg teriparatide, 125.8 ± 4.69) of the femur compared with the OVX vehicle by single treatment. Combination treatment of S-101479 and ALN or RAL
significantly increased BMD (133.6±6.73 and 129.8±4.23, respectively) compared with a single treatment of ALN or RAL. The combination treatment of S-101479 and teriparatide did not significantly increase BMD (128.7±4.59) compared with a single treatment of teriparatide. All combination treatment groups had significantly increased BMD compared with the single treatment group, which was administered 1 mg/kg S-101479 (Fig. 7a).

We analyzed not only BMD but also BMC and AREA. We used AREA as an index of bone size. BMC was significantly decreased by OVX (sham vehicle, 315.1±14.75 and OVX vehicle, 290.6±16.07). One milligram per kilogram ALN, 1 mg/kg RAL, and 1 µg/kg teriparatide did not affect BMC (305.3±23.01, 294.5±14.54, and 309.2±12.61, respectively). S-101479 (1 mg/kg) significantly increased BMC (319.1±18.26) compared with the OVX vehicle. Combination treatment of S-101479 and ALN, RAL, or teriparatide significantly increased BMC (345.1±28.90, 327.5±18.19, and 337.5±22.38, respectively) compared with a single treatment of ALN, RAL, or teriparatide. Combination treatment of ALN and S-101479 significantly increased BMC compared with a single treatment of 1 mg/kg S-101479 (Fig. 7b). AREA of the femur was increased by OVX (sham vehicle, 158.6±11.00 and OVX vehicle, 139.5±7.69). 1 mg/kg S-101479, 1 mg/kg ALN, and 1 µg/kg teriparatide significantly increased bone strength (159.4±12.39, 155.8±18.26, and 160.9±10.88, respectively) compared with the OVX vehicle. RAL (1 mg/kg) did not affect the bone strength (148.6±12.23). Combination treatment of S-101479 and ALN or RAL significantly increased bone strength (185.7±22.31 and 170±12.14, respectively) compared with a single treatment of ALN or RAL. Combination treatment of S-101479 and teriparatide did not significantly increase bone strength (173.9±17.71) compared with a single treatment of teriparatide. Combination treatment of ALN or teriparatide and S-101479 significantly increased bone strength compared with a single treatment of 1 mg/kg S-101479 (Fig. 7d).

ALN, RAL, and teriparatide had no effects on the weight of the uterus or the clitoral gland in single treatment groups compared with the OVX vehicle group or in combination treatment groups compared with the single treatment group, which was administered 1 mg/kg S-101479 (data not shown).

DISCUSSION

We have developed bone anabolic SARMs for the treatment of osteoporosis. It is extremely important to reduce the androgenic activities of SARMs because a large percentage of patients with osteoporosis are women. We previously reported that S-40503 reduced the effect on the prostate gland and increased BMD in ORX rats but did not have sufficient selectivity in OVX rats. The purpose of our study is to identify a useful drug candidate for osteoporosis in post-menopausal women.

We discovered S-101479 from the tetrahydroquinoline libraries from which S-40503 was discovered, and it appeared to bind to AR as strongly as S-40503 or bicalutamide with high NR selectivity. In this experiment, PR and ER were extracted from rabbits; AR, GR, and MR were extracted from...
analyses revealed that remarkable ch
observed for DHT or the sham-operated group. Histological
physiology of the uterus is regulated by estrogens. Long-term
dose of RAL (which is used clinically and has been shown to
anabolic effect on the myometrium could induce or worsen
uterine wavy structure of the epithelial layer indicated that DHT also
increase in endometrial weight is a serious side effect that
can be safer for the uterus). At 10 mg/kg, S-101479 increased uterus
weight, but the degree of increase was very different from that
of RAL, particularly for diaphyseal BMD and bone strength. In osteoporotic patients,
bone volume was decreased, but bone size was unchanged. Anti-osteoporosis drugs restore or inhibit the decrease in bone
volume but do not affect bone size. Because of such bone
changes, BMD has until date been the best tool to evaluate osteoporo
tic bone. In case of androgens, it stimulated periosteal bone formation of the cortical area (i.e., bone size increased). A bone mineral analyzer directly measured BMC and AREA, and BMD was calculated (BMC divided by AREA). If BMC increased with increasing AREA, BMD would not increase. S-101479 significantly increased BMC, but BMD was same as in the other drug treatment groups. It indicated that BMC is a more important parameter than BMD when evaluating SARMs.
Aged rats were not used in the present study; therefore, the results obtained were not sufficient evidence for the effects of the drugs in post-menopausal women. However, the skeletal dynamics of 90-day-old OVX rats are representative of that of post-menopausal women.\footnote{40} In addition, OVX rats of about the same age were used in evaluating commercial anti-osteoporosis drugs, and they showed positive effects.\footnote{37,41,42} Androgenic compounds such as anabolic steroids have positive effects on osteoporosis,\footnote{43,44} and long-term treatment with anti-androgen bicalutamide decreased BMD in prostate cancer patients.\footnote{45} S-101479 could be beneficial in treating osteoporosis.
In addition, therapeutic gain may be stronger in combi-
nation treatments. ALN and RAL are anti-bone-resorptive agents, and therefore, they rarely enhance bone formation. The bone anabolic effects of S-101479 did not conflict with the anti-bone-resorptive effects of ALN and RAL. Teriparatide has a very strong bone anabolic effect, but its high dose and long-
term administration induced osteosarcoma in animal models.
S-101479 also showed additive effects with teriparatide, i.e., it reduced the teriparatide dose or the duration of teriparatide administration. This is the first report showing that SARM has additive effects with other bone formative agents such as teriparatide.

We did not show the other effects that S-101479 has on androgenic activities. The effects of S-101479 on lipid metabolism, luteinizing hormone secretion, liver or kidney weight, and RBC number were reduced compared with those of DHT. Whether these effects are sufficient will be discussed in another study. Furthermore, other effects such as the one on libido were not evaluated and must be assessed. Nevertheless, we have shown that non-steroidal compounds will have androgenic activity for a particular organ. We investigated bone selectivity, but SARMs that are selective for muscle cells, RBGs, or the prostate gland could be synthesized. We will attempt to discover the mechanisms of the tissue selectivities of S-101479 and will report the results in the near future.

Acknowledgments The authors gratefully acknowledge Dr. Shigeaki Kato (Institute of Molecular and Cellular Biosciences, the University of Tokyo) for kindly preparing full-length wild-type human AR and Dr. Tsutomu Nakamura for his advice on this manuscript.

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