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JTT-305, an orally active calcium-sensing receptor antagonist, stimulates transient parathyroid hormone release and bone formation in ovariectomized rats

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ABSTRACT

Intermittent administration of parathyroid hormone (PTH) has a potent anabolic effect on bone in humans and animals. Calcium-sensing receptor (CaSR) antagonists stimulate endogenous PTH secretion through CaSR on the surface of parathyroid cells and thereby may be anabolic agents for osteoporosis. JTT-305 is a potent oral short-acting CaSR antagonist and transiently stimulates endogenous PTH secretion. The objective of the present study was to investigate the effects of JTT-305 on PTH secretion and bone in ovariectomized rats. Female rats, immediately after ovariectomy (OVX), were orally administered vehicle or JTT-305 (0.3, 1, or 3 mg/kg) for 12 weeks. The serum PTH concentrations were transiently elevated with increasing doses of JTT-305. In the proximal tibia, JTT-305 prevented OVX-induced decreases in both the cancellous and total bone mineral density (BMD) except for the 0.3 mg/kg dose. At the 3 mg/kg dose, JTT-305 increased the mineralizing surface and bone formation rate in histomorphometry. The efficacy of JTT-305 at the 3 mg/kg dose on the BMD corresponded to that of exogenous rat PTH1–84 injection at doses between 3 and 10 μg/kg. In conclusion, JTT-305 stimulated endogenous transient PTH secretion and bone formation, and consequently prevented bone loss in OVX rats. These results suggest that JTT-305 is orally active and has the potential to be an anabolic agent for the treatment of osteoporosis.

1. Introduction

Parathyroid hormone (PTH) is an attractive agent for the treatment of osteoporosis. Intermittent PTH injection stimulates new bone formation and remarkably restores ovariectomy (OVX)-induced bone loss in rats (Fox et al., 2006; Meng et al., 1996; Mitlak et al., 1996; Sato et al., 2002) and primates (Brommage et al., 1999; Fox et al., 2007; Jerome et al., 2001). In a study in humans, teriparatide (recombinant human PTH1–34) increased the vertebral, femoral, and total-body bone mineral density (BMD), and decreased the risk of vertebral and nonvertebral fractures in postmenopausal osteoporosis (Neer et al., 2001). Furthermore, teriparatide increased the vertebral and hip BMD, and decreased new vertebral fractures in glucocorticoid-induced osteoporosis (Saag et al., 2007). Preos (recombinant full-length human PTH1–84) increased the vertebral and hip BMD, and decreased new or worsening vertebral fractures in postmenopausal osteoporosis (Greenspan et al., 2007). However, PTH must be administered subcutaneously and is very expensive owing to its peptide formulation.

The calcium-sensing receptor (CaSR), which was cloned from the bovine parathyroid gland in 1993, is a member of the class C family of G protein-coupled receptors (Brown et al., 1993). CaSR is functionally expressed in the parathyroid gland and kidney, and plays a key role in calcium homeostasis (Brown and MacLeod, 2001). The function of CaSR on the parathyroid gland is to regulate endogenous PTH secretion in response to blood calcium concentrations (Portale et al., 1997; Udén et al., 1992). Several pharmacological approaches to regulate PTH secretion through CaSR have been reported. CaSR agonists, which are also called calcimimetics, suppressed endogenous PTH secretion in humans (Goodman et al., 2002; Silverberg et al., 1997) and rats (Fox et al., 1999; Nemeth et al., 2004), whereas CaSR antagonists, which are also called calcilytics, stimulated endogenous PTH secretion in rats (Arey et al., 2005; Marquis et al., 2009; Nemeth et al., 2001; Shinagawa et al., 2010). Therefore, orally active CaSR antagonists that can mimic the pharmacokinetics of intermittently injected PTH may be appropriate anabolic agents for osteoporosis. Several CaSR antagonists have been advanced to clinical trials (Fitzpatrick et al., 2008; John et al., 2011; Kumar et al., 2010; Widler et al., 2008).

JTT-305 (Fig. 1) was discovered as a potent oral short-acting CaSR antagonist that stimulates endogenous pulsatile PTH secretion (Shinagawa et al., 2011), and is currently undergoing clinical trials for the treatment of postmenopausal osteoporosis (Fukumoto et al., 2009). The objective of the present study was to investigate the effects of oral administration of JTT-305 on PTH secretion and bone in ovariectomized rats. To confirm whether the efficacy of JTT-305 on BMD was caused by endogenous PTH secretion, the effect of rat PTH1–84 on OVX-induced bone loss was also evaluated.

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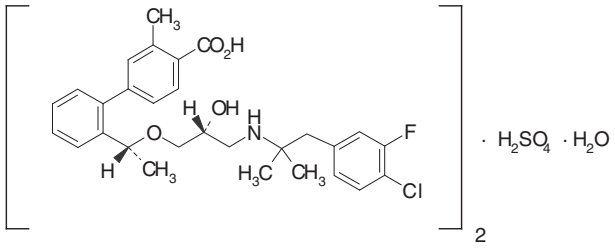

Fig. 1. Chemical structure of JTT-305.

2. Materials and methods

2.1. Cell culture, transfection, and intracellular Ca^{2+} mobilization assay

COS-7 cells (Riken Gene Bank and Cell Bank, Tokyo, Japan) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 μg/ml) at 37 °C under 5% CO_2. The pME18S vector was kindly provided by Dr. Maruyama (Tokyo Medical and Dental University). Transient transfections of the pME18S plasmid containing a human CaSR cDNA into COS-7 cells were performed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA). At 24 h after the transfection, the cells were loaded with 3 μM Fura 2-AM (Wako Pure Chemicals, Osaka, Japan) in loading buffer (0.5 mM $CaCl_2$, 1 mg/ml D-glucose, 126 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES, pH 7.4) containing 0.02% Pluronic F-127 for 30 min at room temperature. The cells were washed and resuspended in loading buffer containing 0.1% bovine serum albumin. The cell suspensions were excited with dual wavelengths (340 and 380 nm), and the fluorescence emission ratio at 500 nm was recorded using an intracellular calcium analyzer (CAF-110; JASCO Corporation, Tokyo, Japan). JTT-305 was incubated with the cell suspensions for 1 min before the extracellular calcium concentration was increased from 0.5 to 2 mM. The IC_{50} values for JTT-305 were calculated using GraphPAD Prism 4.00 (GraphPad Prism Software, San Diego, CA) from three individual experiments performed in duplicate.

2.2. Animals

Virgin female Sprague–Dawley rats were purchased from Charles River Laboratories Japan (Yokohama, Japan). The rats were maintained at 23 ± 3 °C on a 12-h/12-h light–dark cycle with *ad libitum* access to a standard diet (AIN93G; Oriental Yeast, Tokyo, Japan) and water. During the period of the experiment, time-restricted feeding was used, with the feeding time being approximately 7 h after dosing. All the animal procedures and protocols complied with the guidelines for animal experimentation set by the Ethics Committee for Animal Use at Japan Tobacco Inc.

2.3. Experimental design for OVX model

2.3.1. Experiment 1

Virgin female 29-week-old Sprague–Dawley rats were used. The rats were divided into one group of sham-operated animals (n = 12) and four groups of OVX animals (n = 10–12 per group) based on the BMD and body weight. The rats were subjected to either bilateral OVX or a sham operation. After a 2-day recovery period, the OVX rats were given vehicle (0.5% methyl cellulose) or JTT-305 (0.3, 1, or 3 mg/kg) suspended in vehicle orally once daily for 12 weeks. The sham-operated rats were given vehicle orally once daily for 12 weeks. Blood samples were collected predose and at 15, 30, 60, 120, and 240 min after the administration of vehicle or JTT-305 on the first day of dosing for assays of the serum PTH concentrations and the plasma concentrations of JTT-305. For dynamic bone histomorphometry, the rats were injected subcutaneously with calcein (8 mg/kg) at 12 and 5 days before necropsy. On the day following the last administration, the rats were killed by exsanguination from the abdominal aorta under anesthesia by inhalation of diethyl ether. The left tibia was excised, cleaned of excess soft tissue, and fixed in 70% ethanol for histomorphometric analysis.

2.3.2. Experiment 2

Virgin female 36-week-old Sprague–Dawley rats were used. The rats were divided into one group of sham-operated animals (n = 8) and four groups of OVX animals (n = 7–8 per group) based on the BMD and body weight. The rats were subjected to either bilateral OVX or a sham operation. After a 3-day recovery period, the OVX rats were injected subcutaneously with either vehicle (0.1% rat serum albumin and 0.001 N HCl) or rat PTH1–84 (Bachem, Bubendorf, Switzerland) (3, 10, or 30 μg/kg) dissolved in vehicle once daily for 12 weeks. The sham-operated rats were injected subcutaneously with the vehicle once daily for 12 weeks. Blood samples were collected for assays of the serum PTH concentrations predose and at 15, 30, 60, 120, and 240 min after the injection of vehicle or rat PTH1–84 on the first day of dosing. The necropsy procedures were the same as those in Experiment 1, but a histomorphometric analysis was not performed.

2.4. Pharmacokinetics and blood chemistry

Blood samples were collected from the tail vein. The plasma concentrations of JTT-305 (free base) were measured by liquid chromatography tandem mass spectrometry (LC/MS/MS). The time to the maximum plasma concentration (T_{max}) was obtained directly from the data. The elimination rate constant (k_e) was determined by linear regression on the logarithm of the plasma concentration–time curve from T_{max} to 4 h. The elimination half-life ($T_{1/2}$) was calculated using the equation $T_{1/2} = \ln 2 / k_e$. Serum PTH was measured using a commercial ELISA kit (Rat Intact PTH ELISA; Immutopics, San Clemente, CA).

2.5. Bone mineral densitometry

The cancellous and total BMD of the right tibia was measured preoperatively and at 12 weeks postoperatively by quantitative computed tomography (QCT) using a LaTheta LCT-100A (Aloka, Tokyo, Japan) with a pixel size of 170 × 170 μm and a slice thickness of 1 mm. The tube voltage of the X-ray generator was 50 kV (1 mA). The scan area was positioned at 3 mm distal to the proximal epiphysis of the tibia (approximately 1 mm distal to the growth plate). The rats were anesthetized with pentobarbital (40 mg/kg) during the measurement.

2.6. Histomorphometry

The fixed left tibia was embedded in methyl methacrylate and sectioned into 3-μm slices. The sections were stained with toluidine blue. The sections were subjected to histomorphometric analyses under a light microscope using an image analyzer system (Measure6; System Supply, Nagano, Japan). The measurement area was 2.15 mm in length from 0.1 mm below the growth plate. The following parameters were measured: osteoblast surface (Ob.S/BS); mineralizing surface (MS/BS); mineral apposition rate (MAR); bone formation rate (BFR/BS); osteoclast surface (Oc.S/BS); and eroded surface (ES/BS). The measurement parameters complied with the standard nomenclature approved by the American Society of Bone and Mineral Research (Parfitt et al., 1987).

2.7. Statistical analysis

All data are presented as means ± S.E.M. Statistical analyses were performed using SAS System Version 8.2 and SAS Preclinical Package