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An orally active calcium-sensing receptor antagonist that transiently increases plasma concentrations of PTH and stimulates bone formation

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ABSTRACT

Daily subcutaneous administration of exogenous parathyroid hormone (PTH) promotes bone formation in patients with osteoporosis. Here we describe two novel, short-acting calcium-sensing receptor antagonists (SB-423562 and its orally bioavailable precursor, SB-423557) that elicit transient PTH release from the parathyroid gland in several preclinical species and in humans. In an ovariectomized rat model of bone loss, daily oral administration of SB-423557 promoted bone formation and improved parameters of bone strength at lumbar spine, proximal tibia and midshaft femur. Chronic administration of SB-423557 did not increase parathyroid cell proliferation in rats. In healthy human volunteers, single doses of intravenous SB-423562 and oral SB-423557 elicited transient elevations of endogenous PTH concentrations in a profile similar to that observed with subcutaneously administered PTH. Both agents were well tolerated in humans. Transient increases in serum calcium, an expected effect of increased parathyroid hormone concentrations, were observed post-dose at the higher doses of SB-423557 studied. These data constitute an early proof of principle in humans and provide the basis for further development of this class of compound as a novel, orally administered bone-forming treatment for osteoporosis.

Introduction

Osteoporosis is prevalent in postmenopausal women, with approximately one in three women aged over 50 years affected [1], and is increasing in incidence due, in part, to an aging population [2]. Furthermore, osteoporosis affects around 200 million people worldwide, and thus represents a substantial financial burden [1]. In 2002, the combined annual cost of osteoporosis-related fractures was estimated at US$20 billion in the US and US$30 billion in Europe [3]. The pathogenesis of osteoporosis results from an imbalance in bone turnover and a net increase in bone resorption leading to reduced bone mass and an increased risk of fracture. Current treatment options for osteoporosis either inhibit bone resorption through the use of antiresorptive agents (e.g., bisphosphonates, estradiol, calcitonin and raloxifene) and promote bone formation with anabolic agents (e.g., intact parathyroid hormone [PTH(1–84)] and the 34-amino acid peptide [PTH(1–34)]) or a combination of both approaches as suggested for strontium ranelate [4]. However, controversy surrounding the bone-forming effect of strontium ranelate has recently arisen [5].

Net bone formation is characterized by an increase in bone mineral density (BMD) and increased trabecular and cortical areas, resulting in a bone architecture with increased strength that is more resistant to fracture [6]. The bone-forming effects and increased bone strength following transient exposure to PTH through intermittent, subcutaneous administration of PTH(1–84) or PTH(1–34) in animal models and healthy human volunteers, as well as in patients with osteoporosis, have been well documented [7-13]. In contrast, continuous infusion of PTH leads to increased bone turnover, but without net formation, resulting in overall bone loss [14].

PTH plays a central role in calcium homeostasis, through its effects on renal calcium excretion, bone resorption, and, indirectly, intestinal calcium absorption. PTH secretion from the parathyroid gland is negatively regulated by ionized serum calcium (Ca^{2+}), mediated through the calcium-sensing receptor (CaR). The CaR is a G-protein-coupled receptor present on cells of the parathyroid gland, thyroid C-cells, bone, kidney and gastrointestinal tissues, among others, and is activated by increased concentrations of extracellular Ca^{2+} [15]. As the concentration of Ca^{2+} in the blood increases, activation of CaRs on parathyroid cells inhibits PTH secretion. Conversely, a decrease in Ca^{2+} triggers PTH release. Targeting this regulatory mechanism, we have previously shown that small-molecule CaR antagonists stimulate

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significant but sustained increases in PTH concentrations in animal models, resulting in an increase in both bone formation and bone resorption [16-19]. The lack of favorable effects on bone remodeling was circumvented by co-treatment with estradiol, which reduced bone resorption leading to a net increase in bone formation and BMD [16]. In this report, we describe the development of a short-acting CaR antagonist (SB-423562) and its orally active precursor (SB-423557), demonstrating bone-forming effects in animal models and transient increased concentrations of plasma PTH in humans as a clinical proof of principle.

Methods

Synthesis of compounds

NPS2143 was synthesized by NPS Pharmaceuticals [16]; SB-423562 and SB-423557 were synthesized by GSK (details to be published elsewhere).

PTH release in the rat, dog, and monkey following oral administration of SB-423557

All procedures were performed in accordance with protocols approved by the GlaxoSmithKline Institutional Animal Care and Use Committee, and met or exceeded the standards of the American Association for the Accreditation of Laboratory Animal Care (AAALAC), the US Department of Health and Human Services and all local and federal animal welfare laws.

To determine the PTH(1–84) release in the plasma of the various species, SB-423557 was orally administered in a formulation of 1% DMSO, 20% Cavitron (Cargill, Inc., Cedar Rapids, IA). Blood was collected just prior to dosing and at various time points post-dosing, placed into heparinized tubes and centrifuged, and the plasma was then removed and frozen. The concentration of PTH(1–84) in the plasma was determined using a immunoradiometric assay for the rat and an ELISA for both the dog and monkey (Immutopics International, San Clemente, CA).

Bone formation oral SB-423557 in ovariectomized rats

To examine the bone-forming effect of SB-423557, 6-month-old virgin Sprague–Dawley female rats underwent ovariectomy (OVX) or sham surgery and were left untreated for 6 weeks to allow bone loss to occur. Rats were randomized into groups ($n = 8$–12 per group) and were treated for an additional 12 weeks. The sham and OVX animals received daily oral treatment with vehicle or SB-423557 (50 mg/kg). A further group of animals was treated with subcutaneous (s.c.) rat PTH(1–34) (5 μg/kg) (Bachem Americas, Torrance, CA) as a positive control for comparison purposes. To measure dynamic histomorphometric changes of lumbar vertebra (L3) and distal tibia, rats from all groups were given fluorochrome labels (calcein 10 mg/kg) twice daily, 6 and 13 days prior to sacrifice.

In vivo BMD measurements of the lumbar vertebrae (L3 to L6) were made at baseline (week -1) and at weeks 6, 10, 14 and 18 using dual energy X-ray absorptiometry equipped with high-resolution scanning software (Hologic QDR-4500A, Hologic, Bedford, MA). Individual animals were anesthetized with isoflurane and placed prone on the table. BMD was determined using a region of interest of 55 lines wide.

Volumetric trabecular BMD (eight animals per group) of the left proximal tibia was determined at the same time points by peripheral quantitative computed tomography using the Stratec/Norland Research M (Norland Medical Systems, Fort Atkinson, WI). Individual animals were anesthetized with isoflurane and placed on their left side. The left leg was secured and a three-dimensional 0.5 mm slice was taken at a point distal to the growth plate that was 15% of the length between the growth plate and the tibia–fibula junction. Settings for the mask were as follows: object length, 200 mm; voxel size, 0.1 mm; diameter, 40 mm; speed, 3 mm/s; number of blocks, 2; scout view (SV) speed, 30 mm/s; and SV distance between lines, 0.5 mm.

Rats were housed in metabolic cages for 24 h for the collection of urine during weeks 5 and 12, to determine deoxypyridinoline excretion. Blood was also collected at weeks 5 and 12 and serum was stored frozen for analysis of osteocalcin.

The distal tibia and L3 vertebrae were fixed in ethanol and stained with Villanueva Bone Stain (Polysciences, Inc., Warrington, PA). After destaining and dehydration, the bones were embedded in 90% methyl methacrylate/10% dibutyl phthalate/benzoyl peroxide (all from Sigma-Aldrich, St. Louis, MO). A 30 μm tibia–fibula junction cross section of the distal tibia was collected with a SP1600 microtome (Leica Instruments GmbH, Nusseloch, Germany) for analysis of cortical bone. A 5 μm crown section of the L3 vertebrae was cut using a Leica SM2500S microtome for analysis of trabecular bone. All measurements were performed using standard histomorphometric methods [20]. Undecalcified sections were analyzed using Osteomeasure software version 3.02 (Osteometrics, Atlanta, GA). Bone measurements were assessed using bright field fluorescence and polarized light microscopy for the dynamic and static parameters, respectively. A single observer who was blinded to the specimen identity made all measurements.

Biomechanical testing was performed on the right midshaft femur and L5 vertebrae. For the compression test of the L5 vertebrae, the posterior pedicle arch, spinous process and the cranial and caudal ends of each vertebral body were removed to obtain a vertebral body specimen with parallel surfaces and a height of approximately 4 mm. The specimens were then placed between two plates of an Instron Mechanical Testing Machine (Instron 4465 retrofitted to 5500, Norwood, MA), and a load was applied at a constant displacement rate of 6 mm/min until failure. The locations for maximum load at failure, stiffness and energy absorbed were selected manually from the load and displacement curve and then calculated by Merlin II software (Instron). The intrinsic properties, stress, elastic modulus and toughness were calculated from maximum load, stiffness, energy absorbed, cross-sectional area and height.

For the three-point bending test of the midshaft femur, the whole femur was placed on the lower supports of a three-point bending fixture with the anterior side facing downward in an Instron Mechanical Testing Machine. The span between the two lower supports was set at 14 mm. The upper loading device was aligned to the center of the femoral shaft. The load was applied at a constant displacement rate of 6 mm/min until failure. The locations of maximal load, stiffness and energy absorbed were selected manually and values were calculated by Merlin II software.

Statistical analyses

All data are presented as mean ± the standard error of the mean (SEM). Statistical comparisons for PTH release in the different species utilized one-way analysis of variance (ANOVA) repeated measures analysis. Statistical comparisons between the OVX control and the different treatments were performed by one-way ANOVA followed by Tukey–Kramer multiple comparison test. The data analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). $p \leq 0.05$ was considered statistically significant.

Parathyroid cell proliferation

Parathyroid cell proliferation was examined in sham and OVX rats which received SB-423557 at 10, 30 or 100 μmol/kg (5, 15, and 50 mg/kg, respectively) by daily oral gavage for 12 weeks. In addition, one OVX group received rat PTH(1–34) (1 μg/kg) by daily s.c.