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Differential temporal effects of sclerostin antibody and parathyroid hormone on cancellous and cortical bone and quantitative differences in effects on the osteoblast lineage in young intact rats☆

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ABSTRACT

Sclerostin antibody (Scl-Ab) and parathyroid hormone (PTH) are bone-forming agents that have different modes of action on bone, although a study directly comparing their effects has not been conducted. The present study investigated the comparative quantitative effects of these two bone-forming agents over time on bone at the organ, tissue, and cellular level; specifically, at the level of the osteoblast (Ob) lineage in adolescent male and female rats. Briefly, eight-week old male and female Sprague–Dawley rats were administered either vehicle, Scl-Ab (3 or 50 mg/kg/week subcutaneously), or human PTH (1–34) (75 μg/kg/day subcutaneously) for 4 or 26 weeks. The 50 mg/kg Scl-Ab and the PTH dose were those used in the respective rat lifetime pharmacology studies. Using robust stereological methods, we compared the effects of these agents specifically at the level of the Ob lineage in vertebrae from female rats. Using RUNX2 or nestin immunostaining, location, and morphology, the total number of osteoprogenitor subpopulations, Ob, and lining cells were estimated using the fractionator or proportionator estimators. Density estimates were also calculated referent to total bone surface, total Ob surface, or total marrow volume.

Scl-Ab generally effected greater increases in cancellous and cortical bone mass than PTH, correlating with higher bone formation rates (BFR) at 4 weeks in the spine and mid-femur without corresponding increases in bone resorption indices. The increases in vertebral BFR/BS at 4 weeks attenuated with continued treatment to a greater extent with Scl-Ab than with PTH. At 4 weeks, both Scl-Ab and PTH effected equivalent increases in total Ob number (Ob.N). Ob density on the formative surfaces (Ob.N/Ob.S) remained similar across groups while mineral apposition rate (MAR) was significantly higher with Scl-Ab at week 4, reflecting an increase in individual Ob vigor relative to vehicle and PTH. After 26 weeks, Scl-Ab maintained BFR/BS with fewer Ob and lower Ob.N/Ob.S by increasing the Ob footprint (bone surface area occupied by an Ob) and increasing MAR, compared with PTH. The lower Ob.N and Ob.N/Ob.S with Scl-Ab at 26 weeks were associated with decreased osteoprogenitor numbers compared with both vehicle and PTH, an effect not evident at week 4. Osteoprogenitor numbers were generally positively correlated with Ob.N across groups and timepoints, suggesting dynamic coordination between the progenitor and Ob populations. The time-dependent reductions in subpopulations of the Ob lineage with Scl-Ab may be integral to the greater attenuation or self-regulation of bone formation observed at the vertebra, as PTH required more Ob at the formative site with correlative increased numbers of progenitors compared with Scl-Ab indicating potentially greater stimulus for progenitor pool proliferation or differentiation.

1. Introduction

Sclerostin antibody (Scl-Ab) and parathyroid hormone (1–34) (PTH) are bone-forming agents that have different modes of action on bone. In animals, Scl-Ab increases cancellous and cortical bone mass predominately through increased modeling-based bone

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formation, with effects on bone resorption that are either neutral or inhibitory [1]. This increase in bone formation is effected initially by activation of bone lining cells (L.Ce) [2], with bone formation maintained presumably through recruitment of progenitors to the formative surface. In contrast, data in both animals and humans have demonstrated that intermittent PTH increases cancellous bone mass largely by increasing the remodeling rate with increased bone formation coupled to increased bone resorption and a net positive bone balance at the basic multicellular unit level [3,4]. Modeling-based formation has also been shown to contribute to the initial response to PTH by activation of L.Ce [5,6], similar to Scl-Ab, but the extent of modeling-based formation is substantially less than that with Scl-Ab [7,8]. Although both agents have been demonstrated to increase cortical bone mass, data in humans suggest that these effects are greater with Scl-Ab [9] at clinical doses. In addition, intracortical remodeling is not systemically increased with Scl-Ab in primates [10], unlike the effects of PTH [11,12].

With chronic administration, both agents display attenuation of the stimulation of bone formation biomarkers but with apparent differences in temporal patterns, most recently illustrated in a comparative clinical trial with the Scl-Ab, Romosozumab [9]. Romosozumab effected transient increases in bone formation markers maximal at 1 month, returning to or below baseline by 2–9 months, accompanied by a sustained moderate decrease in resorption marker. In contrast, PTH affected a sustained increase in both formation and resorption markers over the 12-month treatment period, with maximal increase in formation markers at 6 months. Although the human skeleton is composed primarily of cortical bone, cancellous bone contributes a larger surface area. With similar remodeling rates, cancellous bone would therefore have a greater contribution to circulating bone biomarkers [13]. Thus the temporal pattern of changes in bone biomarkers in the comparative clinical trial suggests that the attenuation of cancellous bone formation occurs more rapidly with Scl-Ab compared with PTH. The increase in bone formation occurred in the face of sustained suppression of bone resorption with Scl-Ab, in contrast to sustained increased resorption with PTH. Animal studies with Scl-Ab indicate that although bone formation rate attenuates on cancellous surfaces following 6 months of dosing, endocortical formation is maintained and resorption parameters remain lower on bone surfaces [14]. Although animal studies assessing the temporal changes associated with chronic PTH treatment are limited in number and scope, the increases in bone formation in humans also progressively decrease in cancellous bone following 18 months of dosing, with the bone-forming effects of PTH on the endocortex appearing to be temporally coupled with the attenuation in cancellous bone [15].

Although the comparative clinical trial illustrated differential effects between the Scl-Ab, romosozumab, and PTH on bone formation markers and bone mineral density (BMD), a study directly comparing their effects at the tissue level in cancellous and cortical bone has not been reported. To this end, we conducted a study comparing the temporal changes in bone biomarkers, mass, and histomorphometric indices of bone formation and resorption in cancellous and cortical bone in male and female rats administered Scl-Ab and PTH. Because these agents exploit different modes of bone formation to increase bone mass, we hypothesized that these differences are related to or reflected in differential effects on the osteoblast (Ob) and osteoprogenitor subpopulations. Therefore, unbiased stereological methods were employed to obtain estimates of number of Ob, L.Ce, and subpopulations of osteoprogenitors to explore the potential differential quantitative effects of these agents on the Ob lineage over time.

2. Methods

2.1. Study design

Male and female Sprague–Dawley (CD®IGS) rats (Charles River Laboratories, Hollister, CA), 148/sex and 8 weeks of age, were divided into four treatment groups: vehicle (Veh) (n = 20/sex), 3 mg/kg Scl-Ab (n = 70/sex), 50 mg/kg Scl-Ab (n = 38/sex), and 75 μg/kg human PTH (1–34) (n = 20). Because the Scl-Ab used in this study, romosozumab, is immunogenic in rats, Scl-Ab groups were powered to allow for removal of rats that developed anti-drug antibodies (ADA) from the study since ADAs can attenuate exposure and pharmacological response. Rats were assigned to treatment groups using a computerized blocking procedure (Pristima Version 6.1.0 Build 45, Xybion Corp., Morris Plains, NJ) designed to achieve body weight balance with respect to treatment groups. Scl-Ab (romosozumab, 34.97 mg/mL) and Veh (55 mM acetate, 13 mM calcium, 6% sucrose, 0.006% polysorbate 20, pH 5.2) were administered by subcutaneous (SC) injection weekly at a dose volume of 1.43 mL/kg. hPTH (1–34) (human parathyroid hormone H-483; Bachem, Torrence, CA) was administered daily by SC injection. PTH was reconstituted with sterile water, aliquoted in vials and stored at −60 °C until day of use, then diluted to 0.15 mg/mL in buffer (2% bovine serum albumin, 0.001 N HCl, 0.15 M NaCl) for injection at a dose volume of 0.5 ml/kg. All dosing was performed in the morning (prior to approximately noon) within the home animal room or at the home cage; Veh animals were dosed first, followed by the Scl-Ab and PTH-treated animals.

One cohort of rats was dosed for 4 weeks and the second cohort was dosed for 26 weeks. Serum was collected from Scl-Ab-treated rats on study day (D) 29 (week-4 necropsy), D85, and D183 (week-26 necropsy) and analyzed for ADA and serum drug concentrations. ADA assay and serum drug concentration results were used to confirm adequate drug exposure throughout the study. All ADA-positive animals were excluded from the study, with further reductions in ADA-negative animals when required to reduce the group size for subsequent analyses to 10/sex/timepoint, based on assigned animal number. Data from these animals were used for statistical analysis and study interpretation.

Serum was collected from the week 26 cohort on D7, D57, D120, and D183 (±2 days) for bone biomarker analyses that included N-terminal type I procollagen (P1NP), osteocalcin (OC), tartrate-resistant acid phosphatase-5b (TRACP-5b), and collagen type 1 cross-linked C-telopeptide (CTx). Rats were administered the fluorochrome calcein green (10 mg/kg SC; Sigma-Aldrich, St. Louis, MO) 10 and 3 days prior to necropsy for dynamic histomorphometry. Fluorochrome solution was prepared on each morning of administration in 2% sodium bicarbonate in saline at a concentration of 10 mg/mL and filter sterilized (0.22 μm syringe filters).

For euthanasia, animals were anesthetized with isoflurane/oxygen and then exsanguinated. At necropsy, vertebrae T13–L2, left and right tibia, and left humerus were collected for routine histopathological evaluation of bone marrow and Ob morphology. Vertebra L3 and left femur were collected and fixed in 70% ethanol for peripheral quantitative computed tomography (pQCT) analysis. Vertebra L4, right femur diaphysis, and right femur distal metaphysis were collected, fixed in 10% neutral buffered formalin (NBF) for 48 h, transferred to 70% ethanol and then processed in methyl methacrylate undecalcified for histomorphometric analyses. L6 vertebral body was collected, fixed in 4% paraformaldehyde (PFA) at 4 °C for 48 h, decalcified in 10% EDTA with 2% PFA at 4 °C until decalcified, and then processed in paraffin for stereological analyses.

All animals were cared for in accordance to the *Guide for the Care and Use of Laboratory Animals, 8th Edition*[16]. Animals were group-housed (two per cage) at an Association for Assessment and Accreditation of Laboratory Animal Care, international-accredited facility in nonsterile, ventilated, microisolator housing with corn cob bedding. All research protocols were approved by the Institutional Animal Care and Use Committee. Animals had ad libitum access to pelleted feed (Rodent Diet 2020X, Teklad Diets, Madison, WI) and water (reverse osmosis purified) via an automatic watering system. Animals were maintained on a 12:12 h light:dark cycle in rooms with controlled temperature (72 °F +/− 2) and humidity (30–70%) and had access to enrichment opportunities (Nylabones and Rat Retreats or Rat Tunnels).