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Kinetic reconstruction reveals time-dependent effects of romosozumab on bone formation and osteoblast function in vertebral cancellous and cortical bone in cynomolgus monkeys☆

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ABSTRACT

Romosozumab, a humanized monoclonal sclerostin antibody under development for the treatment of osteoporosis, has a unique mechanism of action on bone—increasing bone formation and decreasing bone resorption. The effects on bone formation are transient, eliciting a rapid increase in bone formation that attenuates with continued treatment. Although bone formation attenuates, bone mineral density (BMD) continues to increase. To explore potential tissue-level mechanisms that could contribute to a progressive increase in spine BMD, we used kinetic reconstruction techniques to examine the effects of romosozumab on modeling and remodeling units in vertebral cancellous bone from adult cynomolgus monkeys administered romosozumab for 10 and 28 weeks.

The 10-week study duration captured a period of high modeling-based bone formation, and the 28-week study duration followed the self-regulation or attenuation of bone formation in cancellous bone that occurs with long-term treatment. Sequential fluorochrome labels applied for the kinetic reconstruction were also used to evaluate treatment effects on osteoblast function as early as 3 weeks, and on bone formation and bone accrual in the vertebral cortex over 28 weeks.

Kinetic reconstruction of remodeling and modeling formation sites in vertebral cancellous bone revealed that romosozumab effected significant transient increases in mineral apposition rate in remodeling sites at week 3 that was not sustained with continued treatment. However, romosozumab treatment caused sustained improvement in fractional labeling of osteoid, an index of osteoblast efficiency, at remodeling formative sites at both weeks 10 and 28 that was the major contributor to significant increases in final wall thickness (W.Th) of remodeling packets. Remodeling W.Th matched the final W.Th of modeling packets at week 10. At both weeks 10 and 28, romosozumab significantly decreased eroded surface (ES/BS). At week 28, romosozumab also significantly reduced resorption period (Rs.P) and final resorption depth (Rs.De). The reduced final Rs.De combined with the increased W.Th resulted in a significant increase in bone balance (BB) at the level of the remodeling unit. Assessment of bone formation on the vertebral periosteal and endocortical surfaces following 28 weeks of treatment revealed that romosozumab significantly increased bone formation on these surfaces, which had attenuated by week 28, resulting in significant increases in new periosteal and endocortical bone by week 28.

These data suggest that multiple factors potentially contribute to the increase in spine BMD with romosozumab treatment. In the early period of treatment, increased modeling-based bone formation, increased W.Th at remodeling sites, a decrease in remodeling space secondary to decreased ES/BS in vertebral cancellous bone, and increased periosteal and endocortical bone formation in the vertebral cortex contribute to the early increase in

Abbreviations: 2QM, twice per month; Ac.F, activation frequency; Aj.AR, adjusted apposition rate; BB, bone balance; Ct.B.Ar, cortical bone area; Ec.L.Pm, percent endocortical labeled perimeter; ES/BS, eroded surface; FP, formation period; ILD, interlabel distance; MAR, mineral apposition rate; MLT, mineralization lag time; MS/BS, modeling- and remodeling-based mineralizing surface; MS/OS, fractional labeling of osteoid; MS/OS, osteoid surface; New Ec.B.Ar, new endocortical bone area; New Ps.B.Ar, new periosteal bone area; O.Wi, osteoid width; Ps.L.Pm, percent periosteal labeled perimeter; Rs.De, resorption depth; Rs.P, resorption period; W.Th, wall thickness; W.Wi, mineralized wall width.

☆ Conflicts of interest: RWB is an employee of Amgen Inc. and holds Amgen Inc. stock and/or stock options. QN and MSO are former Amgen employees and hold Amgen stock.

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spine BMD. Following the self-regulation of bone formation when modeling-based bone formation has attenuated, a decrease in remodeling space secondary to reduced ES/BS and a positive BB secondary to decreased final Rs.De and increased W.Th contribute to the progressive increase in spine BMD with long-term treatment.

1. Introduction

Romosozumab, a humanized monoclonal sclerostin antibody, increases bone formation and decreases bone resorption in animals and humans, thus improving bone mass and strength [1–4]. Following treatment with romosozumab, or a surrogate sclerostin antibody with the same complementarity-determining regions as romosozumab, there is an initial rapid increase in bone formation. In animal models, this increase has been associated with activation of lining cells and stimulation of modeling-based bone formation [5–7]. In both animals and humans, the stimulation of bone formation is transient. Detailed studies in rats and cynomolgus monkeys have demonstrated that maximal bone formation at the tissue level occurs within the first 3 months of sclerostin antibody treatment in cancellous bone with subsequent progressive attenuation to levels observed in control animals by 6 to 12 months, followed by a continued increase in spine bone mineral density (BMD) [1,8–10]. Similarly, results from clinical trials showed a continued progressive increase in spine BMD after bone formation markers decreased to baseline levels [2,3].

Multiple tissue-based mechanisms could contribute to the continued increase in spine BMD with romosozumab treatment, following the self-regulation of bone formation. In addition to secondary mineralization of newly formed bone, there may be effects at the remodeling unit at the resorptive and/or formative site that would result in a net positive bone balance (BB) and continued accrual of bone volume. Because romosozumab reduces bone resorption markers as well as the surface extent of resorption in animal models [10], inhibitory effects on resorption may extend to the individual remodeling unit and result in a reduction in final resorption depth (Rs.De). In addition, the effects on osteoblast function may extend to the remodeling unit, where romosozumab may affect the formative site, enhancing osteoblastic activity resulting in increased wall thickness (W.Th). To explore the effects of romosozumab on the remodeling unit at the time of peak modeling-based bone formation and following self-regulation, we performed kinetic reconstruction of the formative site in vertebral cancellous bone from adult cynomolgus monkeys treated with romosozumab for either 10 or 28 weeks. This analysis, pioneered by Eriksen [11] and later simplified by Steiniche [12], reconstructs the matrix and mineralized bone growth curves at formative sites of the remodeling unit and allows the determination of final W.Th in contemporaneous remodeling sites. This method avoids biasing the W.Th estimates, as it does not include measurements from remodeling sites formed prior to treatment. Because the majority of bone formation is modeling-based early in the course of treatment with romosozumab in monkeys [7], we also reconstructed modeling formative sites in adult monkeys after 10 weeks of treatment to compare modeling and remodeling final W.Th. The thickness of modeling-based formation packets in response to romosozumab has not previously been characterized. The temporal effects of romosozumab on osteoblast function indices were examined, including fractional labeling of osteoid (MS/OS) and mineral apposition rate (MAR), which would contribute to the kinetics of the formative site. We also evaluated the effects of romosozumab on resorptive cell activity by determining the final Rs.De at the resorptive site using the methods of Eriksen [13].

In addition, the potential contribution of changes in vertebral cortical bone formation and mass to increases in spine BMD observed with romosozumab was also examined by exploiting sequential fluorochrome labeling performed in the 28-week study. Time-dependent effects of romosozumab on dynamic indices of bone formation were assessed on the vertebral cortical periosteal and endocortical surfaces, and their contribution to cortical area was determined.

2. Methods

2.1. Animals and experimental design

2.1.1. 10-week study

Four- to five-year-old male cynomolgus monkeys that were part of a fracture repair study and underwent bilateral fibular osteotomy with internal stabilization were used for this study. Monkeys received either vehicle (n = 10) or 30 mg/kg romosozumab (n = 10) subcutaneously (SC) twice per month (2QM) for 10 weeks beginning the day after surgery. Tetracycline (25 mg/kg) was infused intravenously (IV) on days 14 and 24, and calcein (8 mg/kg) was infused IV on days 56 and 66. After 10 weeks of treatment, lumbar vertebrae (L1) were collected for analyses.

The study was conducted at Charles River Laboratories, Montreal, Canada. The study was conducted in accordance with the testing facility's standard operating procedures (SOPs), the study plan, and study plan amendments. All research study plans were approved by the Institutional Animal Care and Use Committee. Additional details regarding the design, conduct, and endpoints for this study have been reported [7,14,15].

2.1.2. 28-week study

We used 10- to 12-year-old male cynomolgus monkeys that underwent ulnar osteotomy stabilized by plating to create a critical gap defect from a fracture repair study for our analyses. Monkeys received either vehicle (n = 8) or 30 mg/kg romosozumab (n = 8) SC 2QM for 28 weeks beginning the day after surgery. Lumbar vertebrae (L2 and L3) were collected from these monkeys after 28 weeks of treatment. Sequential fluorochrome labeling was performed at week 4 (tetracycline 25 mg/kg IV slow infusion), week 7 (alizarin complexone 25 mg/kg SC), week 10 (tetracycline 25 mg/kg IV slow infusion), week 16 (alizarin complexone 25 mg/kg SC), week 26 (calcein 8 mg/kg SC), and week 27.5 (calcein 8 mg/kg SC).

The study was conducted at Guangxi Weimei Bio-tech Co. (Guangxi, China), where all in-life procedures were performed. The study was approved by the Institutional Animal Care and Use Committee, and was conducted according to the study protocol and the current Guangxi Weimei Bio-tech Co. guidance documents and SOPs.

2.1.3. Animal care

Monkeys were maintained in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) internationally accredited facilities and were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*. Additional details of animal care are provided in the Supplementary Materials.

2.2. Histomorphometry

For cancellous bone analyses, lumbar vertebrae (L1 for 10-week study, L2 for 28-week study) were bisected longitudinally at necropsy, fixed in 10% neutral buffered formalin for 72 h, and then transferred to 70% ethanol. Samples were stained with Villanueva osteochrome bone stain (Polysciences, Inc., Warrington, PA) en bloc, and were