A Non-secosteroidal Vitamin D Receptor Ligand is Efficacious in a Rat Cortical Defect Model
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Introduction: An orally bioavailable, tissue selective, non-secosteroidal, VDR ligand (Sato et al. 2009) was ascertained to be efficacious in a rat bone healing model. Previous studies showed this compound to be an agonist. A longitudinal, region-specific analysis of this synthetic vitamin D receptor agonist (VDRM2) was conducted in a cortical defect model with aged, osteopenic, ovariectomized rats and compared to PTH as a positive control.

Methods: Six-month old, virgin, virus antibody free, female, Sprague Dawley rats (Harlan, Indianapolis) were maintained on a 12hr light/dark cycle at 22°C with ad lib access to food (TD 89222 with 0.5% Ca and 0.4% P; Teklad, Madison, WI) and water. Bilateral ovariectomies were conducted on sixty animals and they were allowed to lose bone for 2 months. Then, animals were weighed and randomized into treatment groups by weight. For cortical defect surgery as detailed previously (Komatsu et al. 2009), animals were anesthetized using 2-5% Isoflurane gas in oxygen, and body temperature was monitored. Once properly anesthetized, artificial tears were applied to the eyes, and the skin surrounding the pelvis and both hind limbs was shaved and surgically scrubbed. Placing the animal on the left or right side (alternating from rat to rat), the knee joint was secured with the index finger and thumb, and then an incision was made on the lateral surface of the quadriceps muscle using a surgical blade. The quadriceps muscle was bluntly dissected through to expose the anterior surface of the distal femur. Diaphysis, using retractors. Then, a drill was drilled completely through both cortices of the femur diaphysis (anterior to posterior position; flat surface to flat surface), using a Dremel power drill and a 2.0 mm orthopedic drill bit (Zimmer, Warsaw, IN). The hole was flushed liberally with saline (Baxter #2F7124) using a syringe and a luor stub needle to clean the site and remove bone dust. The muscles were then manually repositioned into the original position, closed with sutures, and the skin incision was closed with surgical adhesive. Rats were administered analgesic (buprenorphine) sc at 0.01 to 0.05 mg/kg prior to surgery and again that evening. The following morning, rats were given an additional dose of analgesic (ketoprofen) sc at 3-5 mg/kg.

Dosing was initiated the day following surgery. Treatment groups included ovariectomized vehicle controls with cortical defects, PTH(1-38) 10 μg/kg/d sc, and VDRM2 groups dosed with 0.1, 0.5 or 2.5 μg/kg/d po. Animals were weighed weekly and doses were adjusted accordingly for 5 weeks of dosing. There were 7-8 animals per group. Sera were collected at baseline, in the middle and at study termination for analyses of Ca and other markers. Cortical defects were analyzed longitudinally by volumetric-QCT (GE Locus Ultra VCT scanner) on days 7, 28 and 35, post-surgery.

Biomechanical properties of the femora were ascertained at study termination. Femora were excised, cleaned of soft tissue, and placed in phosphate buffered saline (Gibco D-PBS, Invitrogen Corp., Carlsbad, California) and equilibrated to room temperature. The femora were then positioned, anterior surface up, in the center of a custom designed stainless steel 3-point loading jig. A monotonic load to failure was then applied to the anterior surface at a cross-head speed of 20 mm/min, under displacement control, using an MTS Alliance RT15 equipped with a 25kN load cell (MTS, Inc., Eden Prairie, Minnesota) and controlled by the Testworks software package (Version 4.08A build 854, MTS, Inc., Eden Prairie, Minnesota), with force and displacement data acquired at 10Hz.

Results: Reductions in body weight were observed by 7 days post-surgery for all groups; however VDRM2 was observed to further reduce body weight by 15% relative to baseline in a dose dependent manner. As shown in Figure 1, slice-by-slice analyses showed dose dependent stimulation of mineralization of the posterior cortex, followed by the anterior cortex, with little mineral of the intramedullary spaces for VDRM2, relative to vehicle controls. PTH stimulated mineralization of the intramedullary spaces, followed by the posterior cortex, and then posterior cortex, relative to vehicle controls.

Discussion: As shown by Komatsu et al. 2009, the posterior cortex was observed to heal faster than the anterior cortex in this model. The positive control PTH enhanced bone regeneration in the posterior cortex followed by the anterior cortex, but had the greatest effect in the intramedullary spaces, relative to vehicle controls. PTH did not stimulate periosteal mineral apposition. The orally bioavailable VDRM2 also significantly increased mineralization of the posterior cortex, anterior cortex, whole bone and intramedullary spaces; however the magnitude of efficacy in the marrow was less than 1/3 of PTH. VDRM2 did appear to enhance periosteal mineralization. All doses of VDRM2 were efficacious relative to vehicle controls, but the magnitude was less than 1/3 of PTH. PTH had no effect on the periosteal surface and increased cortical area by stimulating mineral apposition unto the endocortical surfaces. VDRM2 also increased cortical area but also increased total area, suggesting some stimulation of periosteal mineral apposition. Load to failure analyses in 3-point bending showed no differences in strength (ultimate load, N) between groups; however, the mid and high doses of VDRM2 and PTH increased stiffness (N/mm) relative to vehicle controls.

References: