PTH is Efficacious in Two Bone Healing Models with T-Cell Deficient Rats
Sato, Masahiko; Adrian, M.D.; Harvey, Anita; Zeng, Q.Q.; Ma, Y.L.
Musculoskeletal Research, Lilly Research Laboratories, Indianapolis, IN, USA.
Sato_masahiko@lilly.com

Introduction: Previously, T lymphocytes were shown to mediate the skeletal efficacy of PTH in nude mice, because these animals exhibited a blunted skeletal response to PTH (M. Terauchi et al. 2008). In an effort to better understand efficacy of PTH in a bone healing situation with immune-compromised animals, a dose-response analysis of skeletal efficacy was conducted in cortical defect models (Komatsu et al. 2009), using NTac:NiH-Whn and RH-Foxn1rnu athymic nude rats. Longitudinal, site-specific analyses were conducted for both bone healing and non-fractured sites.

Methods: Male, 14 week old, NTac:NiH-Whn (Taconic) or Hsd:RH-Foxn1rnu (Harlan), athymic rats were individually housed and 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 for 2 weeks, before conducting bilateral cortical defect surgery through both anterior and posterior cortices (D. Komatsu et al. 2009). Animals were weighed and randomized into treatment groups by weight. Then, 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 hole 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 (NTac:NiH-Whn) or 2.5 mm (Hsd:RH-Foxn1rnu) orthopedic drill bit (Baxter, Warsaw, IN). The hole was flushed liberally with saline (Baxter #27F124) using a syringe and a luer stub needle to clean the skin and remove bone dust. The muscles were then manually reformed back 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.

Animal groups included vehicle controls, low dose and high dose PTH groups treated with 0, 5 or 30 ug/kg/d sc hPTH1-38, respectively. Animals received 7 weeks of treatment, starting 8 days post-surgery. Sera were collected at study termination for analyses of bone formation (PINP, osteocalcin) and resorption (CTX1) activity. Cortical defects were analyzed longitudinally by volumetric-QCT (GE Locus Ultra VCT scanner) after 1, 3, 5 and 8 weeks, post-surgery. Lumbar vertebrae were also excised and analyzed by QCT (Alokia), post-neocropy.

Biomechanical properties of the femoral diaphysis, femoral neck and L5 vertebral body 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 on the 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 5kN load cell (MTS, Inc., Eden Prairie, Minnesota) and controlled by the Testworks software package (Version 4.06A build 854, MTS, Inc., Eden Prairie, Minnesota), with force and displacement data acquired at 10Hz.

Results: Hsd:RH-Foxn1rnu animals were significantly larger (40%) heavier at the time of surgery than NTac:NiH-Whn animals. Both strains of athymic rats gained weight following surgery, but NTac:NiH-Whn gained more weight faster than Hsd:RH-Foxn1rnu animals. PTH had no effect on body weight following surgery in either strain.

Longitudinal QCT showed dose-dependent efficacy of PTH in bone healing in both athymic nude rat strains, Fig 1. Both doses were efficacious from 35 days post-surgery in NTac:NiH-Whn with the high dose enhancing mineralization of the cortical defect from day 21, which was the earliest measured time point following initiation of dosing. By comparison, both doses of PTH enhanced bone healing in Hsd:RH-Foxn1rnu from 21 days post-surgery. Site-specific analyses showed PTH efficacy in the intramedullary spaces of both strains; however PTH efficacy in the anterior and posterior cortices was observed only for Hsd:RH-Foxn1rnu. Therefore, PTH was more efficacious in bone healing in Hsd:RH-Foxn1rnu in terms of bone mass, despite the larger cortical defect. However, load to failure analyses of the femora showed that the cortical defect showed greater strength with both PTH doses in NTac:NiH-Whn, while only the high dose strengthened femora in Hsd:RH-Foxn1rnu, post-neocropy.

Analyses of lumbar vertebra post-neocropy showed dose-dependent systemic efficacy of PTH to increase BMD in both strains. Dose dependent efficacy of both doses was observed in NTac:NiH-Whn and only the high dose for Hsd:RH-Foxn1rnu; however vertebral BMD efficacy translated to stronger vertebra only for Hsd:RH-Foxn1rnu.

Discussion: PTH was shown to be highly efficacious in enhancing bone healing in 2 strains of immune-compromised athymic nude rats. As shown previously in the cortical defect model with osteopetrotic ovariectomized rats (Komatsu et al. 2009), the posterior cortex was observed to heal faster than the anterior cortex in this model, as well. Longitudinal QCT analyses showed site specific differences of PTH efficacy in the cortical defect of NTac:NiH-Whn and Hsd:RH-Foxn1rnu animals. PTH was generally more efficacious in Hsd:RH-Foxn1rnu than in NTac:NiH-Whn, but efficacy in terms of bone mass did not always translate to biomechanical efficacy. Analyses of vertebra confirmed PTH efficacy systemically on skeletal sites distinct from bone fractures. Previously, Terauchi et al. 2008, noted a diminution of PTH efficacy in T cell deficient mice relative to normal controls. Parallel studies in normal rats are in progress to clarify if the same is true for athymic rats, as some strain-dependent differences in PTH efficacy were observed. In addition, other mechanistic analyses are in progress to clarify the basis to these PTH effects in athymic nude rat strains. In conclusion, These data indicate that the T-cell deficient rat skeleton remains highly responsive to PTH treatment. The relevance of these rat data to immune-compromised patients is unknown and will require clarification with additional clinical trials.

References: