Validation of a Fibula Osteotomy Model in the Sprague-Dawley Rat

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Refining animal models in fracture research should optimize both animal welfare and scientific validity for appropriate medical use. The rat fibula osteotomy model is simple with limited invasive procedures. The objective of the study was to validate the rat model of fibula osteotomy with positive and negative controls (PTH(+1.34), Alendronate and Diclofenac). Bone healing was evaluated in vivo by X-ray and ex vivo using Faxitron, pQCT, micro-CT and biomechanical testing. The optimal healing process was assessed.

Eighteen male rats were assigned to each of 4 groups and 5 female rats were assigned to assess any gender effects on the model. Males were divided into two subgroups, one with termination at Day 14 and one with termination at Day 28. All females were euthanized on day 28. Vehicle Control Group 1 animals received 0.5% methylcellulose and 0.025%c Tween 20 by oral gavage daily, Group 2 animals received PTH (+1.34) at 60 ug/kg/dose subcutaneously, three times a week, Group 3 animals received alendronate at 0.03 mg/kg/dose subcutaneously, twice a week, Group 4 animals received Diclofenac at 5 mg/kg/day by oral gavage daily. Each rat received a dose of antibiotic (Benzathine Penicillin G + Procaine Penicillin G) intramuscularly prior to surgery and two days following surgery (total of 2 injections). A topical antibiotic was administered to the incision site daily until deemed unnecessary. Buprenorphine 0.05mg/kg was administered subcutaneously prior to surgery. Anesthesia was induced directly with isoflurane gas and animals remained under anesthesia for the entire procedure. Animals were prepared for surgery, which included shaving of the right hindlimb. The right fibula was exposed through a lateral incision of the skin at the level of the calf and the gastrocnemius muscle was separated from the extensor muscles to expose the full length of the bone. An osteotomy was performed at approximately midshaft, using a bone cutter. The soft tissues were meticulously closed in layers. A dressing (bandage) was applied after surgery and an Elizabethan collar placed on the animal following recovery from anesthesia, if necessary. Once the procedure was completed, the animal recovered from anesthesia in its cage. Animals were evaluated for signs of pain following surgery. In addition to analgesia given prior to surgery, each animal received 3 other injections of analgesic (Buprenorphine 0.05 mg/kg) at 8-12 hour intervals post first injection (for a total of 4 injections). Individual body weights were measured once at randomization and twice weekly thereafter commencing on the day prior to surgery and throughout the treatment period. Food consumption was measured weekly. The study was approved by IACUC committee.

Digital radiographs were taken of the right fibula of all animals immediately following surgery while still under the effect of anesthesia to confirm the fracture and then after Weeks 1, 2, 3 and 4 (lateral view/animal/occasion). All animals were anesthetized (isoflurane) prior to radiography. Digital radiographs were obtained using the Onyx-Rad Qc Acquire (version 4.1.214.4) and Orex Scanner Interface (version 4.5.1.11 SP3). Radiographs were scored for radiographic union to qualitatively assess the healing of the defect. Radiographic images of the excised right fibula (lateral view) were taken using a Faxitron instrument (at the Day 14 necropsy and Day 28 necropsy time points). Ex Vivo Periosteal QCT was used to measure cortical bone mineral content, bone mineral density and geometric parameters (i.e., cortical area) of the osteotomy of the right fibula. Peripheral QCT scans were performed using a XCT Research SA or SA+ bone scanner with software version 5.50D. Three slices were obtained: one at the osteotomy site and the other two at below and above the osteotomy site (approximately 1 mm apart from the site) in order to evaluate the extent of the callus formation. Data were averaged. Micro-CT scans were obtained ex vivo and analyzed using a Scanco Medical scanner with software version 6.0. The right fibula of all males was used to assess callus parameters. The scans included the total callus volume (TV), mineralized callus volume (BV) and callus mineralized volume fraction (BV/TV). Immature callus and mature callus were segmented.

Fibulae were tested in four-point bending. Biomechanical testing was performed using a Bose ElectroForce 1230 Test Instrument. Load and displacement data was collected using WinTest® 4.1. At the conclusion of testing, each bone was examined to ascertain the site at which failure occurred and the fracture pattern was documented. Peak load was measured as the maximum height of the load-displacement curve and stiffness was measured as the slope of the linear portion of the load-displacement curve. Area under the load-displacement curve (AUC = work to failure) was measured as the area of the outline from the peak load and is the amount of energy required to break the bone.

The animals recovered quickly after the surgery and did not show any signs of pain. No effect on body weight or food consumption was noted during the days following the surgery. Radiological evaluation showed a good progression of the healing at the end of Week 2 up to Week 4 in controls and animals treated with PTH and alendronate. At the end of Week 4, a similar degree of healing was observed between these groups with an almost complete healing. For animals treated with Diclofenac, the degree of healing was low during the course of the study compared to vehicle controls, with less cortical remodeling and bridging at the end of Week 4. Callus size measured by pQCT showed a marked increase in the PTH (+13%) and alendronate (+17%) groups compared to vehicle controls and a marked decrease in the Diclofenac group (-27%) at Day 14. Total callus BMC showed a marked increased in the PTH (+13%) and alendronate (+31%) groups compared to controls and a marked decrease in Diclofenac group (-22%) at Day 14. BMD of the total callus was increased of 9% in the alendronate group and 6% for the Diclofenac group but was not increased in the PTH group compared to vehicle controls. Changes in mature callus were particularly marked in the alendronate group with a 51% increase in BMC vs. controls at Day 14. A similar decrease in mature callus was observed in the Diclofenac group relative to controls at Day 14. Changes in immature callus were particularly marked in the PTH group with a 17% increase in BMC relative to controls and vehicle controls. Changes in callus size were confirmed by the micro-CT evaluation with BMC increased by 16% in the PTH group and 41% at Day 28 in the alendronate group, and decreased by -17% in the Diclofenac group.

At Day 14, differences in biomechanical competency of the osteotomy site were noted in 4-point bending. Peak load was increased 5% in the PTH group and 41% in the alendronate group and decreased 35% in the Diclofenac group. Stiffness was increased 22% in the PTH group and 44% in the alendronate group and decreased 54% in the Diclofenac group. At Day 28, peak load was increased 8% in the PTH group and 4% in the Alendronate group and decreased 25% in the Diclofenac group. Stiffness was increased 16% in the PTH group and 13% in the Alendronate group and decreased 34% in the Diclofenac group. No meaningful difference was noted in bone healing between male and female controls at Day 28.

It was concluded that the surgical, radiographical, densitometry and biomechanical procedures were adequate to appropriately determine healing progression in this model and to discriminate drugs known to affect bone healing positively or negatively. This model was sufficiently sensitive to detect changes in fracture healing with PTH na alendronate and the detrimental effect of Diclofenac to slow the healing process. This model was considered to provide a simple, cost-effective assessment of long bone regeneration in response to modulating agents. The advantages of this model over other larger long bones include increased manageability, no need for limb immobilization, relatively small amounts of compound or small implant required, minimal limb stress, rapid healing period and more importantly with a limited invasiveness of the procedures and a minimal impact on animal welfare.