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Journal of Orthopaedic Research 23 (2005) 1029–1034

Journal of Orthopaedic Research

www.elsevier.com/locate/orthres

A single systemic dose of pamidronate improves bone mineral content and accelerates restoration of strength in a rat model of fracture repair

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Abstract

Complications in fracture repair that lead to a delay in union remain clinically problematic. We believe that unwanted pre-mature catabolism of the healing callus, for example, in stress shielded situations, diminishes the rate at which strength is restored in bone repair and possibly leads to delayed union. We hypothesized that a single systemic dose of a nitrogen-containing bisphosphonate (N-BP) would increase bone mineral content (BMC), volume, and mechanical strength of union in fracture repair. We also set out to investigate local delivery to assess whether systemic exposure could be eliminated, due to concerns of bisphosphonate dosing of non-target organs. After an open osteotomy fixed with a K wire, 40 12-week old Wistar male rats were divided into four groups of 10: saline control, bolus systemic subcutaneous injection of pamidronate (3 mg/kg), local low dose of pamidronate (0.1 mg), and a local high dose of pamidronate (1.0 mg). Rats were sacrificed 6 weeks post-operatively. Operated and non-operated femora underwent radiographic evaluation, quantitative computer tomography, and biomechanical testing in torsion. The growth plates and metaphyses of the tibia of the non-operated side were assessed for evidence of systemic exposure in the local groups. Significant increases in callus BMC and volume of the bolus systemic dose group were found compared to the saline control ($p \leqslant 0.05$). Further, the strength of the systemic dose callus was increased by 60% from 0.35 Nm (±0.11) for the saline control callus to 0.56 Nm (±0.25) for the systemic group ($p = 0.05$). Local treatment did not result in increased strength. The contralateral tibial growth plates of the local groups showed evidence of systemic exposure by the presence of retained primary spongiosa. This study confirms that a single perioperative systemic dose of pamidronate leads to significant increases in the BMC, volume, and strength of healing fractures in rats, making single dose N-BP therapy an appealing candidate for further examination in fracture repair.

Keywords: Bisphosphonates; Fracture healing; Biomechanical testing; Osteotomy; Local delivery

Introduction

Delay in fracture healing or non-union can be a devastating complication after an already long recovery time. Fractures heal in response to the initial anabolic stimulus driven by the inflammatory response and the resultant cytokine and growth factor mediated recruitment and differentiation of cartilage and bone forming

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doi:10.1016/j.orthres.2005.03.004

cells. This response is also heavily modulated by the mechanical environment [9]. The initial callus that forms is remodeled into lamellar bone by the combined action of osteoclasts resorbing the initial matrix coupled with further deposition of bone by osteoblasts.

Bone turnover in early callus formation is rapid. In the presence of stress-shielding or disuse, pre-mature remodeling of unloaded callus may result in removal of callus prior to bridging of the fracture fragments. Such pre-mature catabolism in bone repair may be undesirable in some circumstances, especially where callus formation is already scant. We have hypothesized that by delaying the removal of this early callus we can increase the size and strength of initial repair. Our studies in distraction osteogenesis in a rabbit model documented that one or two perioperative bolus doses of nitrogen-containing bisphosphonates (N-BPs) increased callus size and strength [15,16]. The results of these studies led us to question whether bisphosphonates could be beneficial in fracture healing.

Bisphosphonates are calcium avid molecules widely used in osteoporosis, metabolic bone disease, and cancer. Bisphosphonates have no defined role in orthopaedic surgery to date, although investigations into their use in wear debris osteolysis is ongoing [22]. Bisphosphonate treatment reduces the fracture risk in osteoporosis [20]. However, as these patients still sustain fractures, investigations have been conducted to assess fracture healing in the climate of continuous bisphosphonate dosing. Such studies usually showed an increase in callus size due to inhibition of remodeling, but not always an increase in callus strength [1,8,11,18,19,23]. Current opinion is that it is probably safe to continue bisphosphonate dosing during fracture repair [6].

We wanted to ask the question: "Can nitrogen-containing bisphosphonates be utilized to have a positive effect in fracture repair?" To our knowledge, no previous attempts have been made to assess the efficacy of a single bolus administration protocol in fracture repair. Although bisphosphonates are selectively taken up at the site of skeletal injury, some concern exists regarding possible effects on non-target organs such as the kidneys and growth plates in children [2,4,5]. We thus chose to evaluate local delivery of bisphosphonates and compare this to a systemic administration. In this experiment, we explore the hypothesis that a temporary delay in remodeling using single systemic dose of N-BP can increase bone mineral content and accelerate the restoration of strength in an open fracture model in rats at the time of initial union.

Methods

An open femoral rat osteotomy model with stabilization via Kirschner wires (K-wires) was used. Forty 12-week old male Wistar rats (average weight = 417 g) were randomly assigned to one of four treatment groups (Table 1). One group received 0.9% saline (control group), and a second group received 3 mg/kg pamidronate s.c. at the time of surgery. The latter is a commonly used therapeutic dose in humans [7]. Of the remaining two groups, one received a low dose of 0.1 mg of pamidronate and the other a high dose of 1.0 mg of pamidronate locally delivered on a coated K-wire. The low and high local doses were determined by approximating the proportion of a therapeutic dose that would reach the femur, based on preliminary work utilizing Tc^{99} tagged pamidronate [10].

The drug delivery system was incorporated in the K-wires used for stabilizing the fracture, using a similar coating technique previously reported for delivery of growth factors from a poly(D,L-lactide) (PDLA) coating [21]. The drug carrier chosen for the present application was poly(l-lactide) (PLLA) (Fluka, Sydney, Australia) due to its superior mechanical properties compared to PDLA. Threaded K-wires (Zimmer, Warsaw, IN) with a diameter of 1.6 mm were modified to include a smaller central neck of 1.1 mm diameter with a neck length of 15 mm (Fig. 1). The coating solution was prepared by dissolving PLLA in chloroform solvent ($CHCl_3$, Sigma, Sydney, Australia). Pamidronate (disodium pamidronate, Cipla Ltd, Bombay, India) was added at a 1:1 ratio of pamidronate to PLLA by weight. Total coating mass (TCM) was determined by weighing the K-wire before and after coating. The required TCM for the local treatment groups is listed in Table 1.

Ethical approval for the protocol was granted by the local Animal Ethics Committee. Rats were allowed one week acclimatization prior to surgery, and throughout the experimental period were housed in cages in groups of 4 and allowed free weight bearing and free access to standard rat chow and water. Surgery was performed under general anesthetic (ketamine 75 mg/kg and xylazine 10 mg/kg i.p.). A lateral approach was made to the right femur, and a midshaft osteotomy was made using an oscillating saw. The PLLA coated K-wire was driven antegrade into the medullary canal of the distal femur, the femur was reduced, and the wire was driven retrograde with the coating in line with the stabilized fracture site. Rats were sacrificed 6 weeks post-operatively, and both femora harvested. Plain radiographs were made of anteroposterior and lateral orientations of left and right femur.

Right and left femora were scanned using a pQCT scanner and analysis software (Stratec XCT-960A, Stratec Medizintechnik Gmbh, Pforzheim, Germany). Nine 1 mm thick slices at 1 mm intervals were taken in the right (operated) femur, centered on the callus. Five 1 mm thick slices at 2 mm intervals were taken in the left (non-operated) femur, centered on the mid-diaphysis. A further 1 mm slice was measured through the left, non-operated distal femur for evaluation of systemic effects. The scanner software calculated BMC (mg/mm) and cross-sectional area (mm^2) for each slice. The volume for each slice was calculated by multiplying the calculated cross-section by the slice thickness (1 mm). The BMC and volume for the entire 9 mm length of the right callus and left femoral mid-diaphysis were calculated by summation of the individual slice values, and BMD was calculated as BMC/Volume.

The proximal halves of the left non-operated tibia were harvested to evaluate the systemic effect of bisphosphonate. Systemic exposure was evaluated by the presence of white bands of unresorbed primary spongiosa, retained due to the inhibitory effects of pamidronate on remodeling. The tibiae were decalcified in standard EDTA solution for 2 weeks, bisected longitudinally, and scanned photographically using an optical scanner (EPSON Perfection Scanner 1200U). The length of the white band was measured for all tibial cross-sections using Adobe Illustrator (Adobe, Version 10) to compare thicknesses between treatment groups.

Left and right femora were tested in torsion. Bones were thawed and kept moist until testing with 0.9% saline. Bone ends were embedded in the center of hexagonal nuts using two-part 5 min epoxy resin (Araldite, Selleys Pty Ltd, Sydney, Australia). After the resin set, a materials tester (ELF 3400, EnduraTEC, Minnetonka, MN) was used to test right and left femora to failure in external rotation at an angular displacement rate of 6°/s. Torque and angular displacement were recorded. Maximum failure torque was compared between groups. The stiffness of the bones was calculated by the slope of the torque–angular displacement curve. The energy absorbed to fracture was calculated by the area under the curve using the trapezoidal rule.