In Vivo Model to Measure Bone Repair Efficacy of Nanoparticle-based Drug Delivery

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Abstract

Bone repair required for successful arthroplasty can be compromised in patients with comorbid conditions, such as osteoporosis, diabetes mellitus, and chronic kidney disease. Biological compounds have been proposed to promote bone health and repair. The authors have designed a new animal model for testing bone promoting compounds in the in vivo environment. For initial validation of this model, they used a synthetic agonist of a nuclear receptor, liver X receptor, which has been postulated to play a regulatory role in modulating bone growth. A distal femoral unicortical osteotomy was surgically created on skeletally mature C57Bl/6 male and female mice. A nanoparticle carrier delivery system was used to directly introduce N,N-dimethyl-3β-hydroxycholenamide into the osteotomy. At 35 days post-procedure, the femora were harvested and specimens were obtained for histologic processing and qualitative analysis. The results indicate that the carrier nanoparticles entered the osteotomy defect. Results also indicate that bone repair occurred, although significant differences between groups were not detected in the current study. This study validates the mouse model for testing bone repair promoting compounds. This model can be combined with transgenic or other mouse models to simulate problematic bone repair environments, can be used with a variety of drug carriers, and can test many types of interventional compounds to evaluate potential orthopedic therapeutic applications.

Figure: Microscopic image of a thin section of a femur specimen harvested 1 day post-procedure demonstrating the unicortical defect (original magnification, ×4). An unstained, paraffin-embedded 5-µm-thick section.
Total joint arthroplasty has evolved over the preceding decades, and currently there are multiple options for the orthopedic surgeon, including whether to use cemented or uncemented components. Recent randomized control trials have demonstrated that little difference exists in clinical results between the use of cemented and uncemented components.\textsuperscript{1-3} The cement acts as a grout by filling the space between the implant and bone. However, the bone-cement interface is vulnerable to loosening of the components or even fracture of the cement mantle. In contrast, the lynchpin of uncemented implants relies on bony ingrowth. Essentially, the bone grows into microscopic surface irregularities to achieve a solid fixation. Unlike cemented arthroplasty components, bony ingrowth provides fixation that can evolve dynamically in response to biological signals, including differential forces at the bone-implant interface. An advantage of the uncemented component is the opportunity for adding bone growth promoting treatments at implantation.

The enhancement of bone growth could be particularly useful in cases with poor bone quality due to aging or comorbid conditions (eg, osteoporosis, diabetes mellitus, chronic kidney disease) that reduce bone repair success. To test the level of bone growth enhancement provided by a potential treatment, a translational in vivo testing system is needed. Two additional requirements are to simulate the injury/healing pattern following arthroplasty and to allow the administration of the test material.

In this study, the authors developed a bone repair model in a mouse specimen by drilling an osteotomy in the distal femur. The authors then filled the defect with a synthetic oxysterol agonist of the nuclear receptor: liver X receptor (LXR). The agonist was delivered in a slow-release nanoparticle system as a bone growth agonist was delivered in a slow-release nanoparticle system. The authors then determined whether the treatment remained in the defect and whether it affected bone repair.

Materials and Methods

All animal procedures were conducted in accordance with the University of Colorado Institutional Animal Care and Use Committee regulations. Thirty-four (17 male and 17 female) skeletally mature C57Bl/6 mice (older than 4 months) were used as the investigational model. Anesthesia by isoflurane gas was introduced and buprenorphine (0.2 mg/kg body weight) was injected subcutaneously for postoperative pain control. The hindquarter was shaved and sterilized and a 5-mm longitudinal incision was made over the distal femur. The iliotibial band was incised in line with the skin incision, and the vastus lateralis muscle was retracted anteriorly. The distal femur was exposed, and a 0.9-mm diameter Kirschner wire was used to create a unicortical defect in the lateral distal femoral metaphysis (Figure 1). A 5-µL volume solution containing nanoparticle carrier,\textsuperscript{4} either alone or impregnated with the LXR agonist N,N-dimethyl-3β-hydroxycholenediamide (DMHCA), was then injected into the cortical defect under direct visualization (DMHCA was synthesized and provided by Heidi Kratzer, PhD, University of Colorado School of Medicine). The soft tissue was then returned to its anatomical position overlaying the femoral defect, and the skin was closed with 5.0 nylon sutures. The mouse then recovered in a clean cage in isolation and was closely monitored until ambulating normally (approximately 10 minutes). All animals received subcutaneous buprenorphine every 12 hours for 72 hours for post-procedure analgesia.

At 35 days post-procedure, the animals were euthanized by carbon dioxide inhalation for anesthesia followed by cervical dislocation. One animal was euthanized at 1 day post-procedure to verify surgical and histologic procedures. Femora were harvested, and the specimens were fixed in 10% neutral buffered formalin overnight followed by decalcification in RDO Rapid Decalcification Solution (APEX Engineering Products Corp, Aurora, Illinois) per manufacturer’s instructions. Samples were then processed for tissue histology and embedded in paraffin wax. Thin sections (5-µm thick) were produced in the coronal plane; stained with safranin O, hematoxylin, and fast green (Poly-Scientific R&D Corp, Bay Shore, New York); and then mounted with a coverslip.

A qualitative histological analysis was then performed on the region of the cortical defect using light microscopy. The nanoparticles were originally prepared with Nile Red to facilitate tracking of the distribution of the nanoparticle carrier medium following injection into the osteotomy site. Representative slides of the unicortical defect were examined to determine the ability to localize the bony defect and the presence of the nanoparticle carrier.

Results

The unicortical femoral defect was identified in thin sections of bone (Figure 2A). In addition, the nanoparticles were clearly visible in the bone marrow of the
defect area (Figure 2B). In most cases, nanoparticles were still visible in sections from the bone specimens obtained at 35 days. No qualitative differences were found between the control vehicle-treated group (Figure 3A) and the DMHCA-treated group (Figure 3B) at 35 days post-procedure. All animals exhibited full or near-full repair at 35 days post-procedure (Table). No detectable differences were found between sexes.

**DISCUSSION**

The objectives of this research are to determine the ability to create a unicortical bony defect that could then be examined at endpoint to assess interval bony ingrowth/repair and to qualitatively assess the efficacy of drug delivery using a unique nanoparticle carrier. This research study has developed and evaluated an experimental model for the testing of local delivery of therapeutics for enhancing bone repair. The developmental goal is to create an animal model with utility for improving osteogenesis in orthopedic surgery, specifically arthroplasty, although other applications, such as plate and screw fixation, may benefit. Other animal models are used for osteogenesis aimed at other surgery goals, such as fracture repair, and somewhat similar models are used for applying a drill to create a cylindrical defect. The authors’ study is unique in its testing of local delivery of nanoparticles in the mouse femur.

A limitation of the study was the variability of the exact trajectory and location of the unicortical defect. Although the procedure has been standardized and systematically executed for each animal, a minute alteration in the angle of the Kirschner wire with respect to the femur in the axial, coronal, and sagittal planes has presented challenges for histological specimen preparation.

An inherent weakness of all animal-based studies is the lack of evidence that efficacy (and safety) in an animal model will translate to humans. With all animal models, it is typically unclear whether it is possible to extrapolate results from a quadrupedal, murine model to a bipedal human patient. However, translational animal models allow mechanistic studies to determine how disease processes affect bone and further allow the testing of therapeutic agents in controlled physiological settings.

The advantage of developing this technology in a mouse as opposed to a larger animal is the potential for testing therapeutics in a controlled setting that simulates the physiological aspects of co-

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**Table**

<table>
<thead>
<tr>
<th>Animals, No.</th>
<th>Mouse Strain</th>
<th>Sex</th>
<th>Time, d</th>
<th>Treatment</th>
<th>Bone Repair, %</th>
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<tr>
<td>1</td>
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<td>M</td>
<td>1</td>
<td>DMHCA</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>C57Bl/6</td>
<td>M</td>
<td>35</td>
<td>DMHCA</td>
<td>80-100</td>
</tr>
<tr>
<td>8</td>
<td>C57Bl/6</td>
<td>M</td>
<td>35</td>
<td>Control</td>
<td>80-100</td>
</tr>
<tr>
<td>9</td>
<td>C57Bl/6</td>
<td>F</td>
<td>35</td>
<td>DMHCA</td>
<td>80-100</td>
</tr>
<tr>
<td>8</td>
<td>C57Bl/6</td>
<td>F</td>
<td>35</td>
<td>Control</td>
<td>80-100</td>
</tr>
</tbody>
</table>

*Time = number of days between osteotomy (initiation of treatment or control) and euthanasia.
DMHCA = nanoparticles filled with N,N-dimethyl-3ß-hydroxycholenamide.
Control = nanoparticles without DMHCA.
morbid conditions that affect orthopedic surgery. Multiple mouse models are used for conditions that are associated with poor bone repair. These models simulate aging,5,7 osteoporosis,5,9,7 obesity,10-12 diabetes mellitus,6,13,14 chronic kidney disease,15,16 and muscular dystrophy.17

An additional advantage of this model is the ability to use a drug carrier for local delivery of therapeutics. In this study, poly(lactic-co-glycolic) acid nanoparticles (PLGA)9 have been injected into the bone defect. The therapeutic agent is encapsulated within the PLGA particles and is slowly released over time as the particles degrade (up to 5 days in the current study). Currently, many classes of drug delivery systems are available that would be advantageous for bone repair therapeutics, including other polymer nano- and microparticles, in situ forming hydrogels, liposomes, and carbon nanotubes, among others (reviewed by Abolmaali et al18).

This study has taken the opportunity to incorporate an agonist of the LXRα into the PLGA drug delivery particles. The LXRs are part of the superfamily of ligand-activated nuclear receptors which perform a variety of functions in body tissues. These nonsteroidal nuclear receptors form heterodimers with retinoid X-receptors upon ligand activation, initiating transcription of downstream target genes (reviewed by Edwards et al19). Both cell culture and animal studies have demonstrated that other LXR agonists (eg, GW3965 and T0901317) can promote osteoblast function and inhibit osteoclast function suggesting that LXR activation enhances net bone increase.20-22 However, systemic activation of LXR using those agonists has undesirable side effects, namely induction of lipogenesis and fatty acid biosynthesis.23,24 Consequently, the objective of this research is to investigate the efficacy of local administration of the DMHCA LXR agonist. The exact mechanism remains to be elucidated, but preliminary evidence from the authors’ group suggests that local activation of LXR results in osteoblast differentiation (unpublished data). Published studies indicate that DMHCA has less severe side effects than other LXR agonists.25-27 The authors propose that, combined with a local delivery that avoids the lipogenic organs, DMHCA would be a therapeutic option with high potential for orthopedic surgery.

From an orthopedic surgery perspective, DMHCA presents intriguing possibilities as a therapeutic with many potential clinical applications ranging from total joint arthroplasty to fracture healing. Patient factors exist that present particular challenges for orthopedic surgeons. Nonmodifiable factors include patient age, bone mass, and genetic composition. Diabetes mellitus, malnutrition, and nicotine use have all been demonstrated to adversely affect bone healing. Although these factors are theoretically modifiable, in practice, patient compliance with therapeutic tactics varies across the spectrum. Thus, the addition of a biologically active compound that can improve bone ingrowth and healing would be particularly valuable, especially in patients with metabolic or endocrinologic impairment.

**CONCLUSION**

This study demonstrates the efficacy and reproducibility of the investigational model. The nanoparticle carrier provides a drug delivery system that can be used to distribute an investigational compound such as DMHCA into a bony defect. The murine osteotomy model provides a reproducible means of studying bone ingrowth and healing. Together, this study provides a foundation for orthopedic-relevant investigations into the modulation of bone healing with the use of any one of several drug delivery mechanisms.

**REFERENCES**