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Generation of Closed Transverse Fractures in Small Animals

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Summary

The most common procedure that has been developed for use in rats and mice to model fracture healing is described. The nature of the regenerative processes that may be assessed and the types of research questions that may be addressed with this model are briefly outlined. The detailed surgical protocol to generate closed simple transverse fractures is presented and general considerations when setting up an experiment using this model are described.

Keywords

Fracture; Healing; Surgical; Model; Rodent

1. INTRODUCTION

1.1. General Information on the Closed Model of Fracture Healing

Models of fracture healing generally are developed to assess repair after fracture of appendicular bones and have mainly focused on the long bones of the hind limbs. These models primarily heal through an endochondral bone formation process and with the development of an external callus, although the extent of callus formation is dependent on the type of fixation and the degree to which the fixation method stabilizes the fractured bone and is greatly influenced by mechanical signals that the healing callus experiences (1–4).

The most common model of bone repair used in rats and mice is produced by externally applied blunt trauma to generate a closed, simple transverse fracture. The most widespread application of this model was first described in Bonnarens and Einhorn, 1984 (5), for use in rats and has been subsequently adapted in various forms for use in mouse by numerous investigators (6–9). The fracture is generated via three-point bending to a long bone (usually the femur or tibia). Stabilization of the fracture is achieved by inserting an intramedullary pin prior to generating the fracture. The use of this model is the closest in anatomical site, etiology and fixation method to the most common fractures seen clinically since these fractures tend to be closed injuries that are produced by a traumatic event such as falls and other accidents. The model is well suited for high-throughput screening, owing to the simplicity, speed (~15 minutes per animal), and reproducibility of the procedure (9). The model can also be used to assess basic molecular processes that affect endochondral bone formation, and can be extrapolated to both embryological development (10–14) and post-natal epiphyseal growth of long bones (13, 15). [See these reviews for discussion of the comparison between developmental and fracture endochondral bone formation (16–18)].

1.2.Applications and Limitations of the Closed Model of Fracture Healing

It has been widely applied to assess the safety and efficacy of systemic pharmaceuticals that might affect fracture healing (19–22). Due to its closed nature, it has a lesser degree of reproducibility for the local delivery of biological therapeutics and pharmaceuticals than an open procedure. This is due to the fact that delivery of the therapeutics is via percutaneous injection at the fracture site (23), and its actual anatomical delivery in the callus can only be approximated by palpitation. Similarly, placement of the site of the fracture is more subjective than in an open osteotomy procedure since control over placement is achieved only by visible inspection of the positioning of the leg and by palpitation of the bone through skin and muscle before fracture. Fractures generated in this model can also have some degree of comminution (9). Figure 1 shows a series of radiographs of fractures in the murine tibia and femur (Figure 1A), and compares these optimal fractures to cases that would be excluded from a study, due to the fractures being displaced, poorly positioned, or comminuted (Figure 1B).

2. MATERIALS

2.1. Animals

For rat studies, Sprague Dawley rats 350- to 450 grams in weight are typically used with no more than a 50g variation in group weights.

For mice, ages between ten and 18 weeks can be used although within a group of mice that is used for a study individual mice should be within two weeks of each other.

2.2. Instruments

The instruments and materials that are needed for carrying out the surgical procedure in either rats or mice is shown in Figure 2A. Simple schematic drawings for making a fracture device for generating controlled blunt trauma are provided in Bonnairens and Einhorn, 1984 (5). The size of the device can be scaled appropriately for rats or mice. A more recent modification of this device that provides for more accurate positioning of the animal and better control for release of the weight that drives the blunt striker to generate the fractures was reported by Marturano et al., 2008 (9), and is currently in use in our laboratory (Figure 2B).

2.3. Fixation devices

In the adult rat, the intramedullary fixation is facilitated by using a smooth, ~0.9mm stainless steel K-wire with a threaded tip.

In mice, a stainless steel 23- to 27-gauge spinal needle stylet is used. Figure 2C shows a display of the three types of fixation pins that we have used.

3. METHODS: Protocol of Closed Fracture

The surgical steps of the procedure are shown in Figure 3. The general protocol is as follows:

3.1. Preparation of an Animal Protocol

For all animal studies, a protocol approved by an Institutional Care and Use Committee should be generated to define the scientific rationale and goals for the study, number of animals needed, operative procedure, operative anesthesia, and postoperative analgesia and care. Detailed information on animal welfare, selection of methods for anesthesia, monitoring animals while anesthetized, sterile surgical techniques and post-operative monitoring are available at <https://www.aalaslearninglibrary.org/>.

3.2. Preparation of the Surgical Site

To prepare the surgical site, it is wiped down with sterile 100% isopropyl alcohol wipes and then shaved with a small animal shaver. The area is then wiped down with surgical gauze that has been dipped in a solution of povidone-iodine. Surgery should be performed on a warming pad under sterile conditions.

3.3. Induction of Anesthesia

An isoflurane anesthesia machine may be used or a mixture of Ketamine and xylazine may also be used. For isoflurane induction, the animal is induced in a closed chamber with a 4% isoflurane/oxygen mixture. Once induced the animal is maintained on a 2% isoflurane/oxygen mixture.

For Ketamine and xylazine induction, the following dosage is used for mice (Ketamine 80–200 mg/kg and Xylazine 7–20 mg/kg) and the dosage for rats (Ketamine 80–100 mg/kg and Xylazine 5–10 mg/kg). Prior to incision, the animals are also given a dose of buprenorphine (Buprenex) at .01mg/kg to ensure that there will be immediate postoperative pain management.

3.4. Insertion of the fixation pin

Pin insertion is carried out prior to fracture by making an anterior longitudinal midline incision centered over the knee joint. This incision is followed by identifying the extensor mechanism, which consists of the quadriceps, patellar tendon, patella, and patellar ligament. Careful attention is made to not disrupt this mechanism in order to allow immediate ambulation of either the rat or mouse following surgery. A subsequent incision is made just medial to the patella and extensor mechanism, which is followed by elevating and displacing the quadriceps and extensor mechanism in a lateral fashion. After subluxating the patella and extensor mechanism laterally, the distal end of the femur as well as the proximal end of the tibia will be exposed. From this exposure, an entry hole is created in the center of the groove on either the femoral or tibia condyle for pin insertion.

For rats the hole is mechanically drilled. Rechargeable carpenter's drills, a Dremel Tool® or dental handpiece with a drill attachment are all suitable. For mice the hole can be generated by using the beveled end of a 23-gauge syringe needle with manual rotation. For rat surgeries, pins are precut to an approximate length of the femur plus about 5mm by sizing the length though palpitation of the bone through the muscle and visible inspection of the leg. For the mouse, pins are cut at the time of surgery when they are inserted. The pin is inserted down the length of the medullary canal in either a retrograde manner for the femur

or an antigrade manner for the tibia. The depth of insertion can be manually felt since the insertion into the canal encounters minimal resistance until meeting the cortical bone of the greater trochanter of the proximal femur or the distal epiphysis of the tibia.

For C57 B6 mice, the stylet of a 25-gauge spinal needle is used as the intramedullary pin. The pin is buried by twisting it either manually (mouse) or mechanically driving the pin into the underlying bone by affixing the pin to a drill (rat). The tip of the pin is then buried under the surface of the condyle. The length of the pin may be further trimmed using wire cutters if it is too long. The incision is then closed with 5-0 absorbable gut suture.

3.5. Generation of the Fracture

Following the surgical procedure, the fracture is then generated by dropping a weight onto the operated extremity. This weight is set at a defined initial height that will generate a large enough bending moment upon impact to fracture the bone. The combination of weight and initial height should be empirically determined for the specific strain, age, and sex of the animals.

3.6. Intra-operative Assessment of Quality of the Fracture

Immediately after fracture and before the animal revives from anesthesia, an X-ray should be taken (such as with a mobile dental X-ray unit) to check that placement of the intramedullary pin is adequate and that the fracture is mid-diaphyseal without comminution.

3.7. Post-operative management

Animals should be monitored until awaking and should be observed for their ability to freely ambulate over a 48 hour period. Analgesia is maintained with buprenorphine for 48 hours at 12-hour increments. Animals should be able to regain free mobility in 48 hours, and if not they should be euthanized.

Use of non-steroidal anti-inflammatory drugs (NSAIDs) should not be used as postoperative analgesics since they have been shown to inhibit bone healing after surgery (19, 24).

Occasional pin retraction is observed in cases in which the pin has not been fully buried and is tightly in place in the bone. In such cases the pin will be seen protruding through the skin at the knee. Such cases should be immediately euthanized since the fracture fixation will not be stable.

4. NOTES

4.1. Pin Size Recommendations and Non Stabilized Fractures

The exact size of the pin should be empirically determined for each experiment by considering the ratio between pin diameter and intramedullary diameter. This ratio, along with the purchase of the pin in the proximal or distal metaphysis, and the stiffness of the pin material determine the rigidity of the fixation, which greatly affects the extent of the external callus formation. We have found that using a fully threaded K-wire in the rat model produces a very rigid construct which will change the extent to which endochondral bone formation takes place due to the much greater stabilization of the fixation (Unpublished).

Using pins of different materials has been used experimentally to model the effects of varying amounts of micromotion on bone healing (25). In a more extreme case, not including a pin for fixation has also been used to increase the induction of periosteal endochondral bone formation. However, in the absence of any stabilization, the model can only be used for qualitative study of healing (11) because of the large degree of variability in the timing and quantities of new bone formation.

4.2. Selecting age and sex

In general, unless an experiment is specifically designed to examine fracture healing in the context of juvenile development or aging, skeletally mature animals at the end of their juvenile growth period are used. In a prior study in which we examined the effects of Denosumab, male mice ranging in age from 11 to 18 weeks were used. For this study, we saw no differences in callus structure, composition, or mechanical properties associated for mice of varying ages within a test group. It should be noted however that in aged mice (26, 27) and rats (28, 29) that there are definable differences in the rate of healing that are affected by changes in the observable molecular mechanism that effect healing. Finally it is important to note that there is considerable sexual dimorphism in bone structure and strength (30, 31) such that only one sex should be used in a given study or comparisons between sexes should be planned as part of the experimental design.

4.3. Defining the time-period of assessment of bone repair

Studies focusing on developmental processes related to endochondral bone formation may be purposely restricted to the early and intermediate periods of endochondral bone formation. On the other hand, if a study is directed at examining coupled remodeling then later periods will need to be examined. In healthy rats, the periods of endochondral formation through cartilage resorption can last until 28 days, while the period of coupled remodeling initiates around day 21 and lasts up until 12 weeks. In mice, the period of endochondral bone formation through resorption is 21 days, with the period of coupled remodeling initiating around day 14 days and lasting up until 8 weeks. Since specific experimental conditions can greatly alter the time-course of healing, pilot studies using several animals per group should be carried out for each new set of experimental conditions. For these studies, series of X-rays over a defined time-period can help determine the timeframe that should be experimentally examined. In studies examining therapeutics, end points should be chosen to appropriately assess regain of mechanical function and if a study is assessing therapeutic efficacy in the context of promoting healing, multiple time-points are needed to determine the rate of regain of mechanical strength. Because of the time-evolving nature of fracture healing, it is optimal to examine multiple time-points to capture times when key biological processes are taking place and to relate these processes to the regain in the functional properties of the callus.

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A Femur Fracture

Tibia Fracture

B Displaced

Distal

Comminuted

Figure 1.

Radiographic Examples of Closed Simple Transverse Fracture Model

A) Examples of the Rod Placement and Fracture of mouse femur (upper) and mouse tibia (lower) panels. Images were generated using dental x-ray device. B) Three examples of fractures that would be excluded from study. The exclusion criteria are denoted in the figure with the arrow indicating the position of the fracture on the radiograph. Images were generated using a Faxitron® device.

Figure 2.

Materials for carrying out closed fracture procedure

A) The instruments and materials that are needed for carrying out the surgical procedure in either rats or mice. B) Fracture devise for the generation of closed transverse fractures by controlled blunt trauma and three point bending. 1) Devise as generated from the schematic drawings courtesy of Dr. Kristen Billiar, and as described in Marturano et al., 2008 (9). 2) Drop weight and electromagnet striker release assembly. 3) Calibration scale to adjust distance of drop. 4) Blunt striking blade and anvil for positioning of femur and generation of

three point bending. C) Three types of fixation pins used to stabilize closed fractures in rats and mice.

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Figure 3.

The surgical steps of the closed fracture procedure

1) Manual palpitation and positioning to localize line for incision over the central patellar groove. 2) Exposure of the center of the groove on the femoral and tibia condyle for pin insertion. 3) Lateral subluxation of the patella and extensor mechanism. 4) Creation of the entry hole for pin insertion. 5) Pin insertion. 6) Position of the femur for fracture. 7) Positioning of the mouse for postoperative x-ray and immediate postoperative x-ray assessment showing a successful mid-diaphyseal fracture.