MOUSE FEMORAL INTRAMEDULLARY INJECTION MODEL: TECHNIQUE AND MICRO CT SCAN VALIDATION

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STATEMENT OF PURPOSE

Mice are used as research tools in bone biology in part because they are cost-effective, robust, easily bred and have a well-studied genome. Several authors have examined the effects of injected biomaterials or cancer cells in a murine intramedullary femoral model. The surgical techniques include a knee arthrotomy with patella dislocation for intramedullary access. The benefits of this procedure, however, have not been studied in comparison to a direct transpatellar tendon approach without dislocation which would have advantages including less disruption to the existing joint mechanics, less soft tissue damage and less need for anesthesia. In addition to the surgical approach, the optimum volume of biomaterials that may be injected into the murine femur has not been studied. A more precise and less invasive method for injecting materials into the murine femur would facilitate advanced studies on biocompatibility of materials used in orthopaedic surgery.

METHODS

Volumetric study

In order to quantify the volume of the murine femur, one mouse was euthanized in a CO2 chamber and underwent a microCTscanner (RS 150, GE Healthcare) with a 49 µm resolution. The femoral volume was calculated using the Amide software.

To precisely assess intramedullary volume and leakage, four different volumes (5, 10, 15 and 20 microliters) of iodine contrast were injected in the right femora of 4 euthanized mice via a transpatellar tendon approach. The patellar tendon was exposed through a 5 mm lateral skin incision. Then the lateral aspect of the femoral shaft was exposed by reflecting the lateral portion of the quadriceps. A 29-gauge needle on a 0.3 cc syringe was inserted 5 mm through the patellar tendon into the intramedullary canal. Radiographic contrast was then injected into the femur. MicroCTscanners were performed immediately post-operatively to identify leakage of the contrast outside of the femur.

In vivo study

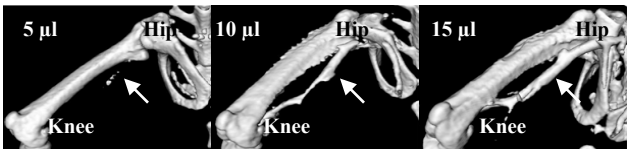
A 10µl solution containing 10% commercially available polymethylmethacrylate (PMMA) particles, 1 to 10 µm in diameter, was injected in the right femora of 12 mice. Mice were anesthetized with isoflurane gas. In half (six) of the mice, a medial arthrotomy with lateral patella dislocation was performed. An initial 5 mm longitudinal incision along the anteromedial aspect of the quadriceps-patellar complex was performed. The anterior portion of the medial condyle was visualized and the knee joint was assessed through a medial incision along the patella. The patella was dislocated laterally to expose the intercondylar groove. The PMMA injection was performed with a 29-gauge needle on a 0.3 cc syringe. The quadriceps-patellar complex was then repositioned and the medial arthrotomy was repaired. In the remaining (six) mice, a transpatellar tendon approach without disruption of the quadriceps-patellar complex was performed as described above. The intramedullary injection, in these mice, was performed directly through the patellar tendon. In both group, special attention was given to leakage during the procedure or bone breaking due to the needle insertion.

In the aim to define the PMMA particles behavior in the intramedullary canal, one mouse from each group was euthanized in a CO2 chamber each week. The right femur was dissected and transversally sectioned distal to the femoral neck. A 25-gauge needle was inserted into the intramedullary canal through the intercondylar groove. A histological "smear" of the contents of the femoral marrow space was then performed by connecting the needle to an empty 3 cc syringe and forcing the marrow through the proximal femur with an air flush. After staining, the PMMA particles were visualized with a microscope and 40X magnification.

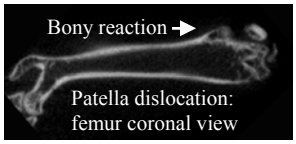
MicroCTscanners 49 µm resolution were performed after the first week for all the mice. Weekly scanners were performed for 6 weeks for the remaining mice.

RESULTS

The volume of the femur was estimated to be the same as a cylinder 12 mm long and 1 mm in diameter and represented a volume of 9.5 µl. Intramedullary injections of iodine contrast resulted in opacification of the femoral vasculature at volumes of 5, 10, 15 and 20 µl (white arrows on the next figure). The extent of vessel dilation was proportional to the volume of injected material and vessels appeared dilated at volumes of 15 and 20 µl. No leakage was observed, however artifacts around femora appeared at volumes of 10 µl and greater.



In both surgical groups, there was no extramedullary injection. Half of the mice having undergone a lateral patella dislocation with subsequent repair (3/6) had a patella dislocation post-operatively that produced a bony reaction with time. The six mice that had the transpatellar tendon approach demonstrated no patella dislocation. PMMA particles were observed on the histological smears of the femurs of both surgical groups during the six week study period.



DISCUSSION

The murine femoral model with medial parapatellar arthrotomy, lateral dislocation of the patella, and repair of the arthrotomy has been used to study biological reaction to biomaterials. Others researchers have sectioned the patellar tendon without repair. Arthrotomy of the knee increases the risk of infection and arthritic changes. Lateral dislocation of the patella increases the risk of chronic patella subluxation or dislocation. The transpatellar tendon method is a simple and reproducible surgical approach that reduces these potential problems. This approach does not require an arthrotomy, or ligament or tendon section, and the patella does not need to be dislocated. Analysis of the needle tracks and the histological smears suggest that injection of biomaterial can be easily accomplished using the transpatellar tendon approach. We choose to inject PMMA particles because of their clinical relevance and previous use in research, and the possibility to track their presence histologically.

Several studies have used injections ranging from in 10µl to 30µl of biomaterials. Our studies suggest that there is vascular uptake at volumes as little as 5µl and that vascular uptake is proportional to the volume of injected material. Volumes in excess of 10 µl may be of little value due to the limitations of the murine anatomy as well as the increased loss of materials due to leakage.

This murine model is a simple and effective tool for orthopaedic and oncologic research. The transpatellar tendon surgical approach may provide an alternate technique for intramedullary injections. It is efficient and less traumatic than the medial parapatellar arthrotomy and appears to reduce the post-operative incidence of patella dislocation. In addition, our data suggest that single injections of volumes in excess of 10µl should be avoided due to leakage. These modifications may help increase the applicability and precision of this murine intramedullary model.

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