An internal locking plate to study intramembranous bone healing in a mouse femur fracture model

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INTRODUCTION:
Fractures heal by two different mechanisms. The process of healing may be dominated by endochondral or intramembranous ossification. This is thought to be dependent on the stability of the fracture fixation. A highly stabilized fracture heals intramembranously without a prolonged cartilaginous intermediate. The mesenchymal cells differentiate directly into osteoblasts. In case of less stability, fracture healing is associated with a greater amount of callus tissue by endochondral bone formation.

Animal models are essential to analyze these highly differentiated processes during bone repair. The mouse is an excellent experimental animal model to study the molecular and genetic basis of fracture healing, given the availability of a large number of inbred and mutant strains and the ability to create targeted mutations in its genome. Because of these properties, the mouse is becoming more commonly used for bone healing research.

In the present study, we hypothesized that a highly rigid fixation of a femur fracture in the mouse results in a predominantly intramembranous bone healing, and that the predominant intramembranous healing accelerates osseous bridging of the fracture gap. To test these hypotheses, we established a novel locking plate to rigidly stabilize femur fractures in mice. By this we studied the healing process after locking plate fixation and compared it to that after the less rigid intramedullary screw fixation using radiographical, histological and biomechanical techniques.

METHODS:
For the study a total of 36 SKH-1Ha mice (12 to 14 weeks old) were used. All animal procedures were performed according to the National Institute of Health guidelines for the use of experimental animals and were approved by the German legislation on the protection of animals. After creating an open osteotomy of the diaphyseal part of the femur, a titanium locking plate with a cylindrical undersurface (length 7.75 mm, width 1.5 mm, thickness 0.7 mm) was used for highly rigid fixation of the femur fractures. An intramedullary titanium screw (length 18 mm, diameter 0.5 mm) was used. After 2 and 5 weeks bone repair was analyzed by radiographical, biomechanical and histological methods.

RESULTS:
Radiological analyses 2 weeks after fracture stabilization using the intramedullary screw showed that only 25% of the femora healed according to the Goldberg score. After 5 weeks, 50% of the femora indicated radiological healing. In contrast, stabilization with the locking plate showed after two weeks radiologically healing in 75%. After five weeks, all fractures were found healed radiologically. At 2 weeks after osteotomy and stabilization with the locking plate, all bones showed a significantly higher bending stiffness when compared with that after the intramedullary screw fixation. Analysis at 5 weeks revealed also a significantly higher bending stiffness after stabilization with the locking plate.

 Femora that were stabilized by the locking plate demonstrated exclusive intramembranous callus formation. In contrast, femora that were stabilized by the intramedullary screw showed fracture healing predominantly by endochondral ossification. In bones stabilized by the locking plate the histological analysis demonstrated at 2 weeks after osteotomy a significantly smaller callus diameter/area without cartilage formation when compared to bones, which were stabilized by the intramedullary screw. In addition, bones which were stabilized with the intramedullary screw showed a distribution of callus tissue typical for endochondral healing comprising ~22% cartilaginous tissue. In contrast, bones stabilized with the locking plate showed complete lack of cartilaginous and fibrous tissue. Accordingly, screw fixation revealed significantly less bone formation when compared to that observed after locking plate stabilization. In both groups bone formation included woven but not lamellar bone. After 5 weeks remodeling was observed without significant differences between the two groups. Abundant bone formation was found in both groups.

DISCUSSION:
We hypothesized that a highly rigid fixation of a femur fracture in the mouse results in a predominantly intramembranous bone healing, and that the predominant intramembranous healing accelerates osseous bridging of the fracture gap. Our experiments corroborated our hypothesis. After rigid stabilization with the newly developed internal locking plate femur fractures healed intramembranously. In addition, we found accelerated bone healing compared to bones, which were stabilized by the intramedullary screw fixation. This was most probably due to the anatomical reduction and the highly stable fixation. The callus of fractures stabilized by the intramedullary screw showed a considerable amount of cartilaginous tissue, indicating secondary bone healing through simultaneous endochondral and intramembranous ossification. In contrast, the bones stabilized by the locking plate healed without fibrous and cartilaginous tissue. This confirms bone healing by exclusive intramembranous ossification.

Of interest, fractures stabilized by the locking plate did not show periosteal callus formation. Although this is characteristic for intramembranous ossification during secondary bone healing, lack of callus formation may also be observed in primary bone healing. However, in contrast to secondary bone healing which involves woven bone formation, primary bone healing involves exclusively lamellar bone formation through the Haversian system in men or resorption cavities in mice. The fact that we could observe only woven bone formation but not any lamellar bone formation during the early healing period excludes the presence of primary bone healing after locking plate fixation.

Our in vivo study demonstrates a bending stiffness of 80% at 5 weeks after locking plate fracture healing compared with the non-fractured contralateral femur. This indicates an almost normalized loading capacity. The bending stiffness of the fractured bones, which were stabilized with the intramedullary screw was only 35% of that of the contralateral femur. These results indicate an accelerated process of bone healing due to exclusive intramembranous ossification after stabilization with the locking plate.

In patients, fracture treatment aims at stable fixation. Accordingly, fracture studies in mice should also consider a stable osteosynthesis, aiming at mimicking the human fracture healing process as close as possible.

We are aware that the open surgical approach which is required to insert the implant, i.e. the internal locking plate or the external fixator, induces soft tissue injury, which may affect fracture healing. However, the use of 3-point bending devices to create closed fractures also induces soft tissue trauma and additional healing and increases the variability of fracture configurations, which further affects the course of fracture healing. In contrast to this, the open osteotomy creates a standardized and highly reproducible fracture configuration. Thus, the use of the internal locking plate may represent an interesting alternative to the external fixator to study molecular and genetic aspects of intramembranous fracture healing in mice.

REFERENCES: