Characterization of a rat osteotomy model of impaired healing

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Introduction
To investigate new treatment options for delayed union or non-union the use of adequate animal model is inalienable. Several animal models for the investigation of bone healing are described and differ in the surgical approach (open, vs. close, fracture vs. osteotomy) and the healing outcome. Clinically relevant animal models of impaired bone healing – without further manipulation - are rare. Moreover, specific descriptions are limited although understanding of the biological course of pathogenesis of fracture non-union is essential for therapeutic approaches. Aim of the study was to establish, characterize and compare the time course of two different models varying in the healing outcome.

Material & Method
Adult female Sprague-Dawley rats weighing 250-280 g were used. The medullary cavity of the tibia was opened at the level of the proximal metaphysis and reamed with a 1 mm Kirschner steel wire.

For the osteotomy model the tibia was osteotomized in an open approach using a diamond disk (Horico). The fibula was fractured manually. Osteotomy was stabilized intramedullary with a titanium Kirschner wire coated with PDLLA [Schmidmaier JBiomedMaterRes 2001], a prospective carrier for therapeutics. After stabilization the wound was closed by a vicryl suture and gentamycin ointment was applied locally. The closed fracture of the tibia and fibula was produced in a standardized manner as published previously [Schmidmaier EJT 2004]. Except of the fracture/osteotomy both models were identical.

Radiology: X-ray examinations (p.a. and lat.) were performed throughout the whole experimental period.

Biomechanical Testing: After sacrifice both tibiae were dissected for biomechanical torsional testing using the Zwick 1455 material testing machine (Ulm, Germany).

Histomorphometry: 5µm longitudinal sections were cut and stained with Safranin O/Lightgreen and von Kossa. Histological parameters of the fracture callus were measured using the Zeiss KS 400 image analysis system. Vessels were visualized with an immunohistochemical staining against anti smooth muscle actin (SMA).

Statistics: Mann-Whitney U test

Results
The surgical intervention was tolerated by all animals. In contrast to the fracture model radiological screening of osteotomized animals revealed neither complete consolidation nor original remodelling of cortices until 84 d after surgery. The majority of animals showed hypertrophic bone fragments. In a minority of animals osteolytic zones could be observed.

The biomechanical testing revealed a significantly lower torsional stiffness and maximum load in the osteotomy group compared to the fractured group at all investigated time points (Fig. 1). After 84 days the osteotomized tibia showed stiffness below 50% compared to the intact contralateral tibia.

Histological examination at 28, 42 and 84d post surgery revealed microscopically a non-union of osteotomized tibiae except in 3 out of 6 examined animals at 84 d. These bone samples revealed incomplete union. Histological slices of fractured tibiae revealed bony bridging and status of remodelling until 84 d. Investigating the early healing phase differences were also detectable. At day 10 after surgery, the osteotomized animals revealed a larger periosteal callus with a higher vascularization compared to the fractured animals (Fig. 2).

Discussion
The described two healing models are identical for the used animals (breed, age, gender), the stabilization method (intramedullary nail, 1mm), the used methods to investigate the healing but differ in two aspects: open approach with osteotomy vs. closed approach with fracture.

Comparing both models clear differences in the healing process could be observed. The osteotomized tibiae showed significant lower values for maximum torque and torsional stiffness after 42 and 84 days as compared to the fractured tibiae. Even though sample preparation for biomechanical testing at an earlier time point (28 d) was not possible for all osteotomized bone samples, mainly due to the very instable callus, the results were in accordance with those in a study from Shefelbine et al. [Shefelbine JOR 2005] demonstrating also very low biomechanical data 21 d after rat tibial osteotomy. The biomechanical results are supported by the histological examination showing a delayed healing and less bridging in the osteotomy model. In the early phase a larger callus with a high vascularization was detectable in the osteotomy group at day 10.

These results clearly demonstrate that the open osteotomy by itself results in an impaired healing process without further manipulation and might therefore serve as a clinically relevant model for the investigation of delayed healing.

This model can be used for the screening of new drugs for the stimulation of bone healing. The intramedullary nails used in this study were already coated with a local drug delivery system based on poly(D,L-lactide). As known from previous studies, PDLLA coating of the titanium implant has no negative effect on bone healing [Schmidmaier Bone 2001, 2004]. Thus, further investigations with therapeutic application of pharmaceutical agents (e.g. growth factors) via the PDLLA-coated implant should allow evaluation of the potency of locoregionally administered drugs for non-union therapy.

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