A New Impaired Healing Rat Model to Test the Efficacy of Stimulating Factors

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Introduction
Stimulation of bone healing is important especially in the case of impaired bone healing. Rat models are well established to investigate bone healing, but normally they show a fast and uneventful healing. We recently established a new rat osteotomy model that showed an impaired healing. After 84 days the osteotomy was not bridged and cartilage was still detectable in the callus. This animal model seems to be appropriate to investigate the effect of biological stimulation on impaired bone healing comparable to delayed healing in human. The aim of the present study was to analyze if the impaired healing in this model could be stimulated by the local application of a growth factor.

Material & Method
The medullary cavity of the right tibiae of female adult Sprague-Dawley rats (n=96, Harlan-Winkelmann) was reamed using a 1 mm Kirschner steel wire. The tibia was osteotomized at the midshaft level using a diamond disk (Horico) and the fibula was fractured manually. The osteotomy was stabilized intramedullary with a titanium Kirschner wire coated with PDLLA [1], or with PDLLA plus BMP-2 (5% w/w of PDLLA). Animals were scarified after 5, 10 (IHC), or 28, 42, or 84 days (Biomech & Histology).

X-ray examinations (p.a. and lat.) were performed over the experimental period.

Biochemical Testing: Torsional testing using the Zwick 1455 material testing machine (Ulm, Germany). n= 6 per group and time point

Histomorphometry: Longitudinal sections from PMMA embedded tibiae were stained with Safranin O/Lightgreen and von Kossa. Histological parameters of the fracture callus were measured using the Zeiss KS 400 image analysis system. n= 6 per group and time point

Biomechanical Testing 28, 42, and 84 days after osteotomy. Data are shown in percentage to intact contra lateral tibia

Due to the local BMP-2 treatment the mineralized area in the callus region was significantly enhanced at day 42 compared to the control group (59.6% vs. 38.9%). The cartilage area in the periosteal callus decreased over time in the BMP-2 group (3.5% at day 28 to 0.4% at day 84). In the control group, however, an increase in the amount of cartilage was measured (5.2% at day 28 to 8.1% at day 84), although due to a high standard deviation this difference was not significant.

Investigating the early healing time points, a higher vascularisation was detected in the control group compared to the BMP-2 treated animals. At 10 days post surgery significantly less SMA-positive vessels were measurable in the BMP-2 animals (20.2 vessels/mm² vs. 42.6 vessels/mm²).

Discussion
The efficacy of BMP-2 to stimulate bone healing has been demonstrated in several animal studies resulting in its clinical approval for the treatment of tibial fractures. Most experimental studies investigating the efficacy of stimulating factors use normal healing or defect healing models, but only a few models are available for impaired bone healing.

The aim of the present study was the validation of a new rat model in respect to the biological stimulation of the impaired healing. The impaired healing was demonstrated in a previous study comparing the healing process of a fracture and an osteotomy model [2]. The impaired healing was shown by significantly lower biomechanical stability after 42 and 84 days compared to the normal rat fracture healing. The histological examination revealed a delayed healing, less mineralized bridging and a hypervascularization in the early phase of the osteotomy healing.

To test the feasibility of the biological stimulation, BMP-2 was applied locally and a rescue of the impaired healing was found. The radiological, biomechanical and histological data showed an enhanced healing due to the BMP-2 application. The hypervascularisation seen in the early phase of impaired healing was diminished due to the BMP-application. Taken together, the results of the different methods demonstrated a rescue of the impaired healing due to the biological stimulation. This model can be used in the future to preclinically test active substances concerning their efficacy to stimulate impaired bone healing.

1. Schmidmaier G et al. 2001 Journal Biomedical Material Research
2. Kratzel C et al. 2008 BMC Musculoskeletal Disorders

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