Midkine Deficiency Significantly Impacts Fracture Healing in Mice

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Disclosures:

Introduction: One growth factor that potentially plays a role in fracture healing is midkine (Mdk). The 13kDa heparin-binding molecule has originally been indentified as a pivotal molecule in embryonic development [1]. Interestingly, its expression is strictly regulated in the adult organism [2]. It has been shown that Mdk is expressed during fracture repair [3]. Its expression is high in proliferative and hypertrophic chondrocytes during bone healing and a chondrogenic cell line overexpressing Mdk displays enhanced chondrogenesis. Furthermore, aged Mdk-deficient mice display a bone phenotype with increased trabecular bone formation and Mdk deficiency prevents mice from ovariectomy-induced bone loss [4]. In addition, Mdk-deficiency has an anabolic effect on mechanically induced cortical bone formation [5]. Treatment of osteoblasts with recombinant Mdk has a significant influence on Wnt-signaling and reduces the response of osteoblasts to mechanical loading. The aim of the present study was to evaluate the effects of Mdk-deficiency on bone repair in a standardized mouse femur osteotomy model

Methods: 18 female Mdk-deficient mice and 20 wildtype mice (C57BL/6) were used for the study. After induction of a standardized femur fracture stabilized with an external fixator, 13 mice were sacrificed at day 28 and 12 mice at day 21, respectively and fracture healing was assessed by 3-point-bending test, microCT based evaluation and histology without prior decalcification. 13 mice were sacrificed at day 10 and histomorphometrical data were assessed using decalcified histological slices. The data were analyzed towards their significance using a non-parametric Mann-Whitney-U test (n=6-7).

Results: Flexural rigidities of the intact left femurs were similar in both genotype groups, but animals lacking Mdk displayed a significantly decreased flexural rigidity of the fractured femur 21 days post-surgery. MicroCT data indicated that apparent bone mineral density in the periosteal callus in Mdk-deficient mice did not differ significantly from wildtype littermates. The moment of inertia in the bending axis (Ix) was significantly reduced in animals lacking Mdk indicating an altered callus geometry during fracture healing. After a healing period of 28 days, flexural rigidity of the fractured femurs reached approximately 50% of the intact femurs. There was no significant difference between both genotype groups concerning flexural rigidity and moment of inertia of the fractured femur at this time point. For further evaluation of the fracture healing progress, we analyzed the tissue composition of the fracture callus using histomorphometry. We observed that Mdk-deficient mice showed a significantly decreased amount of cartilage in the newly formed callus after 10 days, whereas the cartilage content was significantly increased after 21 days. There was no significant difference with regard to the amount of bone. 28 days after surgery, the quantity of fibrous tissue, cartilage and bone was similar in both animal groups.

Discussion: Mice lacking Mdk showed less cartilage in the early fracture callus, but increased amount of cartilage after 21 days. These results indicate a delayed chondrogenesis in Mdk-deficient mice. Together with the finding that a chondrogenic cell line overexpressing Mdk displays enhanced chondrogenesis [3], we hypothesize that Mdk plays a pivotal role in the development of cartilage tissue in the early fracture callus. The decreased moment of inertia and the worse mechanical competence of the fracture callus 21 days after fracture in Mdk-deficient mice could be due to delayed chondrogenesis. Since the progress of fracture healing was similar in both genotype groups 28 days after fracture, we conclude that fracture healing is altered in the absence of Mdk at an intermediate time point, but there is no delay in regaining the full mechanical competence of the fractured bone.

Significance: The findings of the present study indicate that a lack of Mdk significantly impacts fracture repair. Thus, Mdk seems to play an important role in bone healing and further analyses have to elucidate its exact mechanism to evaluate its potential as a therapeutic target.

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