Deficiency of Macrophage Migration Inhibitory Factor Gene Delays Bone Tendon Healing: A Biomechanical and Biological Study

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Introduction: The biochemical and biomechanical characteristics of ligament-substitution procedures in the knee have been widely investigated. Some studies demonstrated that tendon-to-bone healing proceeds by formation of a fibrovascular interface tissue between the tendon and bone [1,2]. The cell types that initiate and regulate the process of healing at the tendon-to-bone junction and the temporal sequence of this process are still unknown. The earliest cellular response following surgical implantation of a tendon graft in a bone tunnel most likely involves accumulation of inflammatory cells. It is likely that macrophages play an important role in tendon healing [3] and stimulate fibroblasts or other stem cells to proliferate [4]. On the other hand, Macrophage migration inhibitory factor (MIF) was initially identified as a soluble factor in culture medium of activated T cells [5,6]. MIF is released as a proinflammatory cytokine and a glucocorticoid-induced immune modulator in response to a variety of inflammatory stimuli [7]. Recently, we revealed that MIF gene-deficient mice (MIFKO) mice exhibited delayed ligament healing of the MCL after injury [8]. Therefore, there is a strong possibility that MIF plays a significant role in bone tendon healing process. The purpose of this study was to test the hypothesis that MIF contributes to bone tendon healing.

We have used a bone tendon healing model of mice to identify the pattern of cell proliferation at the healing tendon-to-bone interface, using immunohistochemical techniques. Also, we used biomechanically evaluating system the difference of bone tendon healing process between wild-type mice (WT) and MIFKO mice at the early phase of healing.

Methods: Thirty-six female MIFKO mice [9] and 36 female WT mice, both at 10 weeks of age, were used in this study. In each 12 animals, both the Achilles tendon-calcaneeus complexes were harvested for the graft, using a dissecting microscope. In the remaining each 24 animal, a bone tunnel was made in the right proximal tibia using 0.7 mm-diameter C-wire. Then, the harvested Achilles tendon was placed in a bone tunnel from the lateral side to the medial side (Fig. 1) according to previous study [1]. All mice were allowed unrestricted cage activity after surgery. In MIFKO and WT groups, 12 animals were used for biomechanical and histological evaluations. For biomechanical evaluation, each 6 mice were sacrificed at 7, and 14 days after surgery. For histological evaluation, each 4 mice were sacrificed at 4, 7, and 14 days after surgery. Biomechanical testing was performed using a micro-tensile tester. As a method for fixation of a sample to the tensile testing machine [10], the tibia-tendon-calcaneeus complex was embedded using resin in aluminum tubes. After preconditioning, the specimen was stretched to failure with a cross-head speed of 5 mm/min. The structural properties of the tibia-Achilles tendon-calcaneeus-complex were determined on the load-elongation curves. For histological analysis, the samples were fixed in formalin, embedded in paraffin, sections 4 micrometers thick, and made in the frontal and sagittal directions. The sections were stained with HE and immunohistochemistry (procollagen type 1, BMP-2, VEGF). Statistical analyses were made using the Student’s t-test. The significance level was set at p=0.05.

Results: At 7 days after surgery, all the grafts were pulled out from the tibial tunnel in the remaining grafts in both groups, while the grafts mid-substance were torn in all specimens in both groups at 14 days after surgery. Only the specimens pulled out from the tibial tunnel were used to determine the structural properties of the tibia-Achilles tendon-calcaneeus complex. Concerning the structural properties of the complex, the maximum load and stiffness of MIFKO were significantly lower than those of WT at 7 days after surgery (p = 0.028, p = 0.048, respectively, Fig.2). Histologically, healing between the tendon and bone occurred by an initial formation of a fibrous granulation tissue between the tendon and the bone tunnel either both groups at 4 days after surgery. There was a rapid accumulation of inflammatory cells and fibrous tissue in the tendon-to-bone interface by 4 days after surgery in WT, followed by a significant drop by 7 days after surgery. However, MIFKO had poor cell accumulation and fibrous tissue both 4 and 7 days (Fig.3). Fourteen days after surgery, the space between the tendon and the bone (tendon-bone gap) was filled with granulation tissue rich in fibroblasts and vessels in both the groups. In the granulation tissue, the perpendiccular collagen fibers connecting the tendon to the bone, which resembled Sharpey’s fibers, appeared in each group. Positive staining for procollagen type 1 was seen in interface of WT tendon at 4 days after surgery, while in same area of MIFKO tendon at 7 days after surgery (Fig. 3). There were no statistical differences in positive stain for BMP-2 and VEGF between WT and MIFKO at 4 and 7 days after surgery.

Discussion: This study demonstrated that the structural properties of the tibia-Achilles tendon complex were significantly lower in MIFKO at 7 days after surgery. This result suggested that the important role played by MIF in improving mechanical properties of healing tissues in the repair process after ligament reconstruction. We believed that these biomechanical differences with statistical significance were enough to show the differences in bone tendon healing process between WT and MIFKO. It is already reported that procollagen type 1 level could be considered a remarkable marker of osteoblastic activity [11]. Kobayashi reported that delayed fracture healing in MIFKO was mainly attributable to a delay in osteoid mineralization [12]. Histological
finding suggested that the bone tendon healing was significantly delayed in MIFKO compared with WT. As to clinical relevance from the present study, though further studies are needed to verify, a possible effect of localized enhance of MIF expression on the bone tendon healing.

**Significance:**

**Acknowledgments:**

**References:**


![Schema of a bone tendon healing model of mice.](image)
Fig. 2 Structural properties of the tibia-tendon-calcaneus complex at 7 days after surgery. The actual values of maximum load (A) and stiffness (B).

Fig. 3 HE (x10) and procollagen type 1 immunohistochemical stain (x20) of the tendon-to-bone surface in WT and MIFKO. Black arrows shows that positive staining for procollagen type 1.

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