Promoting Tendon Regeneration by Activating AMPK and Decreasing α-SMA+ Cell Migration

Jianying Zhang1, Roshawn Brown1,2, Kengo Shimozaki1, MaCalus V. Hogan1,2, James H-C. Wang1,2,3#
1MechanoBiology Laboratory, Departments of Orthopaedic Surgery, Bioengineering, Physical Medicine and Rehabilitation, University of Pittsburgh, Pittsburgh, PA. #wanghc@pitt.edu


INTRODUCTION: Current strategies for enhancing tendon repair after injury consist mainly of applying stem cells, growth factors, natural and artificial biomaterials, and surgical treatments [1]. However, such treatments typically result in scar tissue formation or fibrosis, resulting in compromised tendon function. High levels of α-smooth muscle actin (α-SMA) and collagen III are two markers of scar tissue formation. High mobility group box 1 (HMGB1), a potent inflammatory mediator, plays a key role in hypertrophic scar formation in skin and scar-forming wound in fetal mice [2]. Adenosine monophosphate-activated protein kinase (AMPK) has been linked to the regulation of tissue inflammatory signaling. AMPK activation can inhibit scar tissue formation [3]. Metformin (Met), a drug commonly used for the treatment of type-2 diabetes, is known to inhibit HMGB1 and activate AMPK [4, 5]. Met has been shown to prevent lung fibrosis previously [6]. In this study, we investigated the effect of Met on wounded tendon healing using SMA-Ai9-Scx-GFP transgenic mice that express both Scx (a tendon marker) and α-SMA (progenitor cell marker) in tendon cells.

METHODS: The tamoxifen-inducible α-SMA-CreERT2 mice were crossed with Scx-GFP mice, and then crossed with Ai9 Cre reporter mice to generate triple transgenic α-SMA-Ai9-Scx-GFP mice (referred to as A9 mice hereafter). Five intraperitoneal (IP) injections of tamoxifen (100 mg/kg) were administered consecutively over five days to a total of 24 A9 mice before surgery. A window defect was created in the Achilles tendon of all mice by a 0.5 mm diameter biopsy puncher. The wounded animals were divided into four groups with 6 mice/group and treated with IP injections of saline and Met (160 mg/kg) daily for total 6 weeks as follows: Group 1: Saline for 6 weeks; Group 2: Met 2 weeks before surgery and saline 4 weeks after surgery (Met-B); Group 3: Saline 2 weeks before surgery and Met for 4 weeks after surgery (Met-A); and Group 4: Met 2 weeks before surgery and 4 weeks after surgery (Met-H&A). The animals were sacrificed at day-30 post-surgery. The Met effect on wounded tendon healing was investigated by ELISA for HMGB1 levels in mouse serum and histological analysis on mouse tendon tissue sections.

RESULTS: The wounded tendon treated with saline at 30 day post-surgery formed scar tissue with high density cells (red arrow in Fig. 1A) and poorly organized, loose collagen fibers (green box area in Fig. 1A) stained with blue (black arrow in Fig. 1E). In contrast, Met IP injection resulted in well-organized tendon structure with dense collagen fibers (white arrows in Fig. 1B-D) stained with red (green arrows in Fig. 1F-H). The healed tissue in saline treated tendon was formed by collagen III as evidenced by yellow fluorescence under polarized light microscopy (Fig. 1M). However, the healed tendon tissues in the wound areas of the mice treated with IP injection of Met were collagen I as evidenced by yellow fluorescence under bright field (Fig. 1L) and polarized light microscopy (Fig. 1N-P). The saline-treated mice serum had high levels of HMGB1 which was significantly decreased in Met-A and Met-B&A groups (Fig. 1Q). Under fluorescent microscope, the frozen tendon tissue sections of the mice showed that Met IP injection inhibited the migration of α-SMA+ cells (less red fluorescence) in the wounded mouse Achilles tendons (Fig. 2E-H). There was no significant difference of the Scx+ cells (green fluorescent cells) in the wound areas of four groups (Fig. 2A-D). Immunostaining showed increased number of α-SMA+ cells (brown in Fig. 3A), low level of AMPK staining (Fig. 3E), high level of TGF-β1 (Fig. 3I) and HMGB1 (Fig. 3M) in the wound area of saline injection mice. However, in Met treated groups, less α-SMA+ cells (Fig. 3B-D), and high level of AMPK staining (Fig. 3F-H) were found in the wounded tendons of mice compared to saline treated group. Met injection also inhibited the expression of TGF-β1 (Fig. 3J-L) and HMGB1 (Fig. 3N-P) in mouse tendons.

DISCUSSION: This study set up a useful animal model to investigate the wounded tendon healing using transgenic A9 mice which can track cell migration during healing. Using this model, we have demonstrated that Met injection reduces scar formation in wounded tendon by reducing HMGB1 levels, inhibiting α-SMA+ cell migration, decreasing collagen III but increasing collagen I production, activating AMPK, and decreasing TGF-β1 expression. With longer treatment of Met injections, scar tissue formation was reduced at a larger extent, as evidenced by the wound treated with Met-B&A healed better than Met-B and Met-A only. In response to injury, tissue remodeling and formation of fibrotic scars are initiated by TGF-β1, which stimulates anabolic metabolism in activated myofibroblasts. Our findings indicated that AMPK activation may inhibit myofibroblast differentiation by TGF-β1, supporting a preventive role of AMPK in the development of fibrosis.

SIGNIFICANCE/CLINICAL RELEVANCE: Metformin may be used to promote tendon regeneration by reducing scar tissue formation.

ACKNOWLEDGMENTS: This work was supported in part by a DOD Award (W81XWH-22-9-0016-014) and Pittsburgh Foundation Awards (AD2022-130408; AD2023-134256).


ORS 2024 Annual Meeting Paper No. 2310