

Metformin Lotion Application Blocks Degenerative Changes in Mouse Tendon Caused by Mechanical Overuse

Jianning Zhang¹, Derek Maloney¹, Vasy Pastukh¹, Soichi Hattori¹, James H-C. Wang^{1, 2, 3#}

¹MechanoBiology Laboratory, Departments of Orthopaedic Surgery, ²Bioengineering, ³Physical Medicine and Rehabilitation, University of Pittsburgh, Pittsburgh, PA. #wanghc@pitt.edu

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INTRODUCTION: Tendinopathy, a prevalent tendon disorder characterized by inflammation and/or degeneration, affects millions of Americans and costs billions of healthcare dollars every year. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most used medications for tendinopathy treatment. However, NSAIDs can cause serious side effects including bleeding, stomach ulcers, and liver and kidney issues. Current treatments of tendinopathy are ineffective in regaining tendon structure and function. Thus, there is an urgent need for safe and effective therapies with long term benefits that target the root cause of tendinopathy. High mobility group box-1 (HMGB1), an upstream potent inflammatory mediator, has been identified in high levels in early-stage tendinopathy patients [1]. Metformin (Met), an FDA approved oral drug used for the treatment of type-2 diabetes, specifically inhibits the activity of HMGB1 [2]. Our previous studies in our mouse tendinopathy model created by intensive treadmill running (ITR) have shown that HMGB1 released to extracellular matrix due to ITR can initiate tendon inflammation and degeneration, and IP injection of Met can inhibit tendinopathy development [3, 4, 5]. However, Met given as oral ingestion or IP injection has systemic side effects on the body, including nausea, diarrhea, etc. Therefore, we have initiated a study to develop Met lotion as a novel transdermal drug delivery system for targeted Met administration to the tendon. Presented below is a report detailing our study.

METHODS: Met lotion at 3% and 6% concentrations was developed at our lab and used for this study. A total of 30 mice were randomly divided equally into three groups: **Group 1:** ran on treadmill at 15 meters/min for 3 h/day, 5 days a week for 4 weeks (**ITR**); **Group 2:** smeared 3% Met lotion on the skin surface of the hind legs before ITR daily for 4 weeks (**ITR+3% Met-lotion**); and **Group 3:** smeared 6% Met lotion instead for 4 weeks (**ITR+6% Met-lotion**). All mice were sacrificed after 4 weeks of ITR. Achilles tendon tissues were harvested for histological analysis by Trichrome Masson staining, Picro Sirius Red staining, and immunostaining on collagen II and SOX-9 expression, two markers for cartilage tissues. This study was approved by IACUC.

RESULTS: The results obtained by Trichrome Masson staining showed that ITR induced tendon degenerative changes as evidenced by loose collagen fibers stained with blue in ITR tendons (white arrows in **Fig. 1C, D**). The Met lotion treatment inhibited these ITR induced degenerative changes as evidenced by dense collagen fibers being positively stained with red (yellow arrows in **Fig. 1G, H, K, L**). Picro Sirius Red staining showed that the collagen fibers in the tendons of ITR treated mice under bright light microscopy were red, loose, and disorganized, some damaged tissues were found in the enthesis areas of the mice treated with ITR. However, the tendons in Met lotion treated groups were dense with very good organization (data not shown). Under polarized light microscopy, those loose collagen fibers in the tendons of ITR mice were positively stained with collagen III (green birefringence in **Fig. 2A-D**). The tendons in Met lotion treated groups showed yellow or orange birefringence, indicating these collagen fibers were collagen type I (**Fig. 2E-H**). Very low levels of collagen III were positively stained in 6% Met lotion treated tendons (**Fig. 2I-L**). The inhibition effect of Met lotion on collagen III formation was in a concentration-dependent manner as evidenced by the expression of collagen III in the tendon matrix of all samples is ITR > ITR+3% Met-lotion > ITR+6% Met-lotion. Immunostaining results indicated that ITR increased collagen II (**Fig. 3A, D**) and SOX-9 (**Fig. 3G, J**) expression in mouse Achilles tendons, however, Met lotion application decreased collagen II and SOX-9 expression induced by ITR, and levels of both collagen II and SOX-9 in 6% Met-lotion treated tendons (**Fig. 3C, F, I, L**) were lower than 3% Met-lotion (**Fig. 3B, E, H, K**).

DISCUSSION: This study has demonstrated the preventive effect of Met lotion on an ITR-induced tendinopathy model in mice. Our results have shown that mechanical overloading by ITR induced typical degenerative changes of tendinopathy in mouse tendons characterized by the presence of chondrocyte-like cells, increased levels of collagen II and SOX-9, and scar-like tissues, as indicated by disorganized tendon structure and increased collagen III levels. However, Met lotion application at 3% and 6% doses reduced these tendinopathic changes with 6% lotion more effective than 3% lotion.

SIGNIFICANCE: The results of this study indicate the promising therapeutic potential of Met Lotion in preventing tendinopathy. In contrast to symptom-modifying drugs like NSAIDs, Met Lotion offers several advantages. It directly addresses the underlying cause of tendinopathy and provides ease of use, eliminating the common side effects associated with oral administration.

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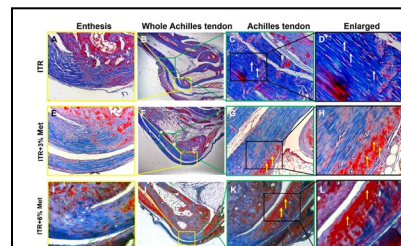


Fig. 1 Met lotion decreases loose collagen formation and enhances dense collagen formation in mouse Achilles tendons: Histological analysis by Trichrome Masson staining. A-D: ITR only images; E-H: ITR+3% Met-lotion images; I-L: ITR+6% Met-lotion images. The images of A, E, and I are enlarged images of the yellow box areas in B, F, J. The images of C, G, K are enlarged images of the green box areas in B, F, J. The images of D, H, L are enlarged images of the black box areas in the images of C, G, K.

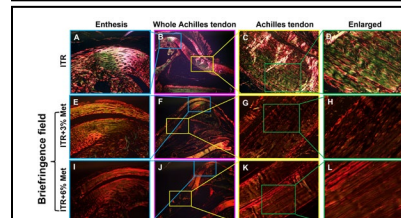


Fig. 2 Met lotion decreases collagen III formation and enhances dense collagen I formation in mouse Achilles tendons: Histology analysis by Picrosirius red staining. A-L: Images obtained from polarized light microscopy; A-D: ITR only group; E-H: ITR+3% Met-lotion group; I-L: ITR+6% Met-lotion group; A, E, I: Enlarged images of the blue box areas in B, F, J; C, G, K: Enlarged images of the yellow box areas in B, F, J; D, H, L: Enlarged images of the green box areas in C, G, K.

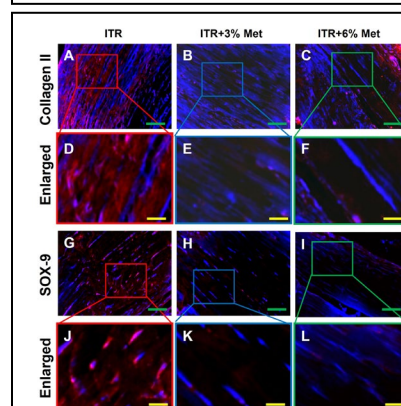


Fig. 3 Met lotion decreases the expression of two chondrocyte markers collagen II and SOX-9 in mouse Achilles tendon. A-F: Collagen II staining; G-L: SOX-9 staining; A, D, G, J: ITR only group; B, E, H, K: ITR+3% Met-lotion group; C, F, I, L: ITR+6% Met-lotion group; D, E, F: Enlarged images of the green box areas in A, B, C; J, K, L: Enlarged images of the yellow box areas in G, H, I. Green bars: 50 μ m; yellow bars: 12.5 μ m.