Effect of MMP-2 Inhibitor on Skeletal Muscle Atrophy after Tendon Rupture

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INTRODUCTION:
Skeletal muscle atrophy is a serious consequence of tendon rupture. In our previous work, we reported increased expression of an extracellular matrix degenerative enzyme—matrix metalloproteinase-2 (MMP-2) in gastrocnemius muscle atrophy after Achilles tendon transection in a mouse model [1]. We also reported that mice lack of the MMP-2 gene experienced significantly reduced muscle atrophy after Achilles tendon transection compared to wildtype mice [2]. These findings suggested that MMPs, especially MMP-2, may serve as a critical mediator in the pathobiology of skeletal muscle atrophy. Therefore, we hypothesized that blocking MMP-2 activity with exogenous inhibitors may be a novel therapeutic approach for treating skeletal muscle atrophy. In this study, we tested the efficacy of a commercially available MMP-2 inhibitor in treating muscle atrophy using a mouse Achilles tendon transection model.

METHODS:
Mouse muscle disuse model. 10 wildtype FVB/N mice (Charles River Laboratories Inc.) at 3 months old were used in this study. Mice underwent unilateral Achilles tendon transection as described previously [1] and were then randomly divided into 2 groups (treated group and control group, N=5 for each). 10 mg/kg MMP-2/3 inhibitor III (EMD bioscience Inc.) was administrated daily by intraperitoneal injection to mice in the treated group for 2 weeks. The first dose was given immediately after the surgery. Vehicle was administrated to mice in the control group. This procedure has been approved by local animal research committee.

Muscle harvesting. Mice were sacrificed 2 weeks after the surgery. Bilateral gastrocnemius muscles were harvested and weighted immediately. Muscles were then homogenized and total protein was extracted using T-per solution (Invitrogen Inc.) for zymography study to analyze MMP-2 activity.

Zymography. Zymography was conducted using 10% gelatin SDS-PAGE gel (Invitrogen) according to manufacturer’s instructions. Same amount of total protein from each sample was used in this experiment. The brightness of the bands of MMP-2 was analyzed using Scion Image™. Statistical significance will be determined by student T-test.

RESULTS SECTION:
The relative muscle weight loss in the control group was 29.7 ± 1.5 % (mean ± SE). In comparison, relative muscle weight loss in the MMP inhibitor treated group was 21.0 ± 3.1 % (mean ± SE). There was a significant difference between these two groups (P=0.04) (Figure 1). Zymography confirmed that the MMP inhibitor significantly reduced in vivo MMP-2 activity in the mice in treated group after 2 weeks of administration (Figure 2). The intensity of MMP-2 bands in the vehicle treated group was about 3 fold higher than in the MMP-2/3 inhibitor treated group (P<0.05).

DISCUSSION:
Increased expression of MMP-2 in muscle atrophy has been reported in multiple studies. Results from MMP-2 null mice in our previous work strongly suggested that MMP-2 plays a critical role in skeletal muscle atrophy [2]. In this study, we further demonstrated significantly attenuated muscle atrophy with the treatment of exogenous MMP-2 inhibitor. This result provided strong supporting evidence suggesting that MMP-2 is a critical mediator in skeletal muscle atrophy.

Due to the similar structure of MMPs, there is no absolutely specific MMP-2 inhibitor available at this time. MMP-2/3 inhibitor III is a relative selective MMP-2 inhibitor. Its affinities to MMP-2, -3 and -13 are about 100 times higher than to MMP-1, -7, -9 and -14. In this study, this inhibitor showed satisfying in vivo inhibitory effect of MMP-2 in skeletal muscle in our mouse model.

Morris and colleagues have reported the effect of a serine protease inhibitor called Bowman-Birk inhibitor (BBI) in treating disuse-induced muscle atrophy in a mouse hindlimb suspension model [3]. In this study, we further demonstrated the role of a MMP-2 inhibitor in reducing muscle atrophy in a mouse tendon transection model. All these data suggested that protease inhibitors may serve as a pharmacological strategy in treating skeletal muscle atrophy.

In summary, this study demonstrated the feasibility of treating muscle atrophy using specific inhibitors of MMP-2, which may serve as a novel strategy to treat skeletal muscle atrophy.

REFERENCES:

ACKNOWLEDGEMENTS:
This work was supported by Orthopedic Research & Education Foundation and Illinois Bone and Joint Institute.