

Transient Drug Induced Modulation of Progenitor Differentiation Concurrently Mitigates Myo-Fibro-Adipogenic Chronic Degeneration of Skeletal Muscle

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INTRODUCTION: Injury to the rotator cuff muscles, which can range from tendinitis to massive tears, are common orthopaedic complications that are associated with disability, pain, and economic burden. Rotator cuff (RC) tears result in massive muscle wasting, scarring, and fat accumulation. While anti-fibrotic drugs have been proven to inhibit scar formation, it is still unknown how they affect the overlapping phases of muscle atrophy and adipogenesis.

METHODS: We combined a mouse model of massive RC tears with matched reporter cultures of injury activated RC cells to evaluate the therapeutic effects of 423F, a gp130 modulating anti-fibrotic compound. The effect of 423F on adipogenic differentiation of RC-derived fibro-adipogenic progenitors (FAP) was compared in vitro to that of another anti-fibrotic drug, CWHM-12. To study the effects of 423F in vivo, osmotic pumps transiently releasing the drug were inserted subcutaneously near the RC at day 0 (non-injured and injured), 5, and 14 days after TTDN and were removed after 7 days. The supraspinatus and infraspinatus muscles were harvested at 6 weeks post-TTDN and sectioned for structural analyses via H&E staining for assessment of normal/necrotic adipose tissue and picrosirius red staining of collagen for assessment of fibrosis at each timepoint. The adipogenic differentiation of 5-day-, 2-week- and 7-week-injury activated FAP (iaFAP) was tested in cultures in the presence or absence of 1 μ M and 10 μ M of either 423F or CWHM-12 (n=at least 3 wells per treatment). Matched untreated cultures served as controls. Cultures were fixed and imaged after 6 days of drug treatment and adipogenic differentiation was quantified with Oil Red O staining of lipids. Myogenesis of non-injured RC cultures was evaluated in the presence of 1 μ M and 10 μ M 423F by measurements of myotube size, number of nuclei and myotube branching.

RESULTS: Tendon transection and denervation (TTDN) resulted in massive RC muscle atrophy, fibrosis, and fatty degeneration at 6-week post-TTDN. 423F treatment had no effect on non-injured muscle. In injured muscle however, 423F diminished fibrosis, fat accumulation, and muscle atrophy in the supraspinatus and infraspinatus at all tested timepoints. Histological examination of injured untreated muscle revealed greater development of adipocytes, fibrosis, and atrophied myofibers, while 423F-treated muscles were less fibrotic and contained necrotic fat and regenerating myofibers. Greater effect was seen when 423F was administered before the intermediate stage of fibro-adipogenesis (immediately after induction of injury and 5-day post-TTDN). 423F decreased adipogenic differentiation of non-injured FAP and all iaFAP stages (5-day, 2-week, and 7-week post-TTDN). The most potent reduction of adipocytes was seen in 5-day- and 2-week-iaFAP in the presence of 423F. However, CWHM-12 mediated adipogenic differentiation in a concentration and iaFAP state-dependent manner. Both 1 μ M and 10 μ M CWHM-12 induced a reduction in adipogenic differentiation in non-injured FAP and 7-week-iaFAP. Conversely, 1 μ M CWHM-12 decreased adipogenic differentiation of 5-day-iaFAP, while 10 μ M CWHM-12 increased their adipogenic differentiation. Furthermore, CWHM-12 mediated a significant increase in adipogenesis of 2-week-iaFAP compared to non-injured FAP and the highest adipocyte counts were obtained from 2-week-iaFAP cultures treated with 1 μ M and 10 μ M CWHM-12. There continued to be profound myogenesis in the presence of varying concentrations of CWHM-12 and 423F in injury activated RC cultures. Additionally, 423F diminished muscle atrophy in vivo and mediated dose-dependent increase in the size, nuclei number and branching of myotubes in RC cultures.

CONCLUSION: Altogether, our findings demonstrate that, in the context of pharmacotherapy, CWHM-12-mediated inhibition of fibrosis can shift the balance towards increased adipogenesis in injury activated FAP, while 423F compound is more suitable for prevention of fibrotic and fatty degradation of the RC. Thus, it seems essential to validate the disease modifying activity of a candidate drug when multiple degeneration pathways are activated, particularly when functional crosstalk between fibrogenic and adipogenic signaling regulates FAP fate.

CLINICAL RELEVANCE: We studied the effect of both drugs on the balance between fibrogenic and adipogenic differentiation of prospectively isolated, injury-activated cultured FAP of rotator cuff muscle to reveal their clinical relevance for treatment of fibro-adipogenic muscle. Accordingly, 423F compound prevented fibrotic, fatty degradation and muscle atrophy of injured mouse RC.

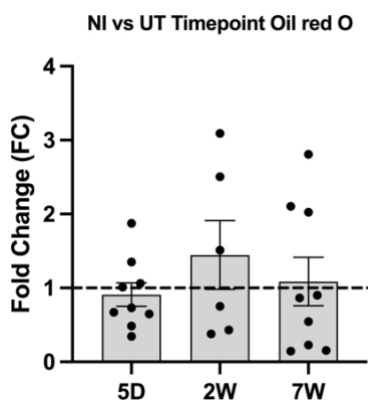


Figure 1. Adipogenic differentiation of injury activated fibro-adipogenic progenitors (FAP). Injury activated FAP were derived from RC at 5 days, 2- and 7-weeks post TTDN and cultured without further induction in 20%FBS/DMEM. Oil red O lipid quantification was normalized to that of FAP that were derived from non-injured RC (indicated by dashed line). Significant increase in adipogenesis was measured in cultures of 2w-TTDN activated FAP. * $p < 0.05$

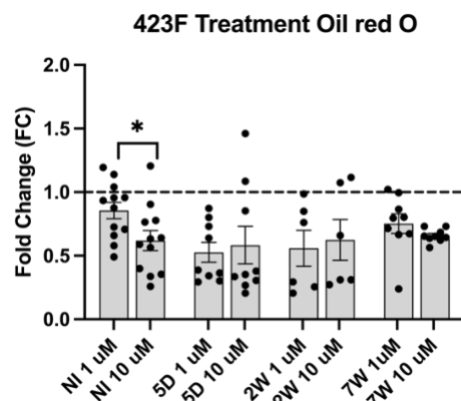


Figure 2. Treatment with the drug 423F alters the adipogenic differentiation of RC FAP in an injury stage-dependent manner. 423F mediated decreased adipogenesis in all tested conditions. Data are mean \pm SD. Data analyzed via unpaired t-test to compare differences in fold change from varying drug doses within their respective timepoints. * $p < 0.05$.

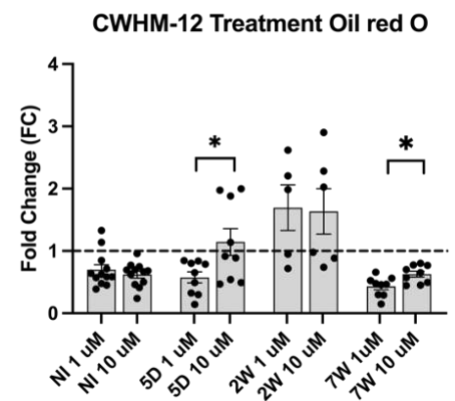


Figure 3. Treatment with the drug CWHM-12 alters the adipogenic differentiation of RC FAP in an injury stage-dependent manner. CWHM-12 induced increased adipogenesis in cultures of 2 weeks injury activated FAP and reduced adipogenesis in all other tested conditions. Data are mean \pm SD. Data analyzed via unpaired t-test to compare differences in fold change from varying drug doses within their respective timepoints. * $p < 0.05$.