Effect of Tamoxifen on Fatty Degeneration of Rotator Cuff Muscles In Chronic Rotator Cuff Tear: An Animal Model Study

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Introduction: Rotator cuff tear is a common condition affecting the elderly population. Following a cuff tear, the rotator cuff muscles undergo degenerative changes such as atrophy, fatty degeneration, and fibrosis. Fatty degeneration is irreversible and has been associated with poor clinical outcomes following rotator cuff repair. The exact pathophysiology underlying fatty degeneration is unknown, and no effective measures have been found to prevent or reverse these damaging, degenerative changes. Recently, Tamoxifen has been shown to significantly improve the overall muscle function and quality in mice with muscular dystrophy, decreasing fibrosis and degenerative changes.1 The goal of this study was to evaluate the effect of Tamoxifen on muscle degeneration, especially fatty degeneration, in mice with surgically created chronic rotator cuff tear. The ultimate goal was to potentially formulate a treatment that may reduce or prevent these degenerative changes.

Methods: Sixteen male C57BL/6J male mice aged 24 weeks were used for this study. The mice were randomly divided into two groups: Tamoxifen diet group (TAM), which were fed with 100 mg/kg Tamoxifen diet food, and regular control diet group (CTRL). The diets were started 3 weeks prior to the surgeries and continued for 16 weeks after surgery. The mice were pair fed to ensure no significant food intake different between TAM and CTRL groups. A massive rotator cuff tear was created surgically on the left shoulder by sharply detaching the supraspinatus and infraspinatus tendons and transecting the suprascapular nerve (surgical side) while the right shoulder did not receive surgery (nonsurgical side). Normal cage activities were allowed after surgery. Body mass, fat percentage, lean body weight, and water weight was monitored bi-weekly using Minispec body composition analysis. The mice were sacrificed at 16 weeks post-surgery, and the supraspinatus and infraspinatus muscles were harvested from bilateral shoulders. Four mice per group were allocated to histological analysis while the other four mice were allocated to RT-PCR analysis. The samples for histology were stained with H&E for morphological evaluation and Oil Red O for identification of intramuscular lipid. Histological analysis was performed by 3 independent observers, using a semi-quantitative scale.2 The mRNA levels of myogenic markers (Myf5, MyoG, and MyoD1), adipogenic markers (Leptin, PPARγ, and CEBPα), and myostatin were measured by Q-RT-PCR.

Results: The body mass was not significantly different between the groups, but the TAM group had a significantly increased lean body percentage and decreased fat percentage compared to the CTRL group (p<0.05). Histology showed a significantly increased amount of adipocyte accumulation, atrophy, inflammatory cell in the endomysium, and intramuscular lipid in the muscles of the surgical side compared to the nonsurgical side (Fig. 1 & 2). The degree of atrophy was significantly lower in the TAM group for both the supraspinatus and infraspinatus muscles on the surgical side compared to the CTRL
The amount of inflammatory cells in the endomysium was also significantly lower in the surgical side supraspinatus of the TAM group than in the CTRL group (p<0.05), but it was not significantly different in the infraspinatus. The TAM group showed only a slightly decreased amount of adipocytes compared with the CTRL group. The Q-RT-PCR data showed that the surgical side muscles had increased mRNA levels of MyoD1, Myogenin, Leptin, and PPARγ, and significantly decreased mRNA levels of myostatin (p<0.05) compared to the nonsurgical side (Fig. 3). However, no significant differences were found in any of the genes between the TAM and CTRL groups on the nonsurgical side.

**Discussion:** This study demonstrated that an oral diet of Tamoxifen led to decreased atrophy and inflammatory changes in the rotator cuff muscles following chronic cuff tear, but had no significant effect on fatty degeneration (adipogenesis). Interestingly, despite a significant decrease of whole body fat, we did not observe a significant decrease of adipocytes in the rotator cuff muscles of the surgical side in the TAM group. This finding suggests that atrophy and adipogenesis may be two separate phenomena occurring after rotator cuff tear, with factors affecting each separately and differently. Since Tamoxifen is a competitive inhibitor to estrogen receptors, estrogen likely plays a role in the development of muscle atrophy while only playing a minor role in the adipogenesis in the setting of chronic rotator cuff tear.

**Significance:** This study suggests a possible role of estrogen receptors in the mechanism of atrophy and fatty degeneration of the rotator cuff muscles following chronic cuff tear.