Bone Morphogenetic Proteins Signaling in Rotator Cuff Muscles Following Massive Tendon Tears

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Introduction: Rotator cuff tears (RCT) are one of the most common tendon injuries seen in orthopaedic patients. Clinically, both muscle atrophy and fatty infiltration are reported to be important factors contributing to poor functional outcomes after RC repairs. However, the molecular mechanism of rotator cuff muscle pathology following massive RCT remains unknown. Bone morphogenetic proteins (BMPs) are a group of growth factors belonging to the transforming growth factor beta (TGFβ) super family. Recent work indicates that BMPs play an important role in regulating muscle mass (1,2). However, their role in rotator cuff muscle pathophysiology following RCT remains unknown. The purpose of this study was to investigate the activity of BMP signaling in rotator cuff muscles following massive RCT and evaluate its role in muscle atrophy and fatty infiltration.

Methods: Twelve adult male Sprague-Dawley rats received a complete supraspinatus and infraspinatus tendon transection as well as a suprascapular nerve transection (TT+DN) to simulate massive rotator cuff tears as described previously (3). Rats were sacrificed at 2 and 6 weeks after surgery (N=6 at each time point). Supraspinatus muscles from both treated and control groups were harvested. Muscles were sectioned in half and used for histology and biochemical analysis, including real-time RT-PCR and Western-blot. For PCR analysis, Ct values of interested genes were normalized to that of the GAPDH. For Western-blot analysis, the intensity of target bands of interest molecules were quantified using ImageJ Software (NIH). In order to define the functional role of BMPs in rotator cuff muscle atrophy and fatty infiltration, we used another group of 12 rats with TT+DN surgery. Rats were then randomly divided into two groups. The treated group (N=6) received daily I.P. injection of LDN-193189 at a dose of 5 mg/kg. The control group (N=6) received daily I.P. injection of vehicle only. Rats were sacrificed at 6 weeks after surgery and supraspinatus muscles were harvested for histology and biochemistry analysis as described above. A T-test was used for data analysis. Significance was defined as a p value of less than 0.05. Data is presented as the mean ± standard error.

Results: The expression of BMP-14 gene significantly increased at 2 weeks after surgery and remained up-regulated at 6 weeks after surgery. BMP-7 gene expression was also significantly increased at 6 weeks after surgery (Figure 1). There was no change in expression of other BMP ligands as we tested. Phosphorylation of SMAD 1/5/8 was also significantly increased at both 2 and 6 weeks after surgery (Figure 2). The BMP-specific inhibitor LDN193189 significantly reduced the phosphorylation of SMAD1/5/8. BMP inhibition led to increased rotator cuff muscle atrophy with increased gene expression of MuRF1 (12.5 fold) and MAFbx (4.3 fold), two key components in muscle atrophy. Inhibition of BMP signaling also resulted in a significantly reduction of fatty infiltration with Oil-red O staining (Figure 3).
LDN193189 also significantly reduced the expression of fatty infiltration-related genes (CEBPα with 3.3 fold decrease, PPARγ with 5.2 fold decrease and SERBP1 with 3.5 fold decrease) in rotator cuff muscles following RCT. Finally, inhibition of BMP signaling led to significant reduction of phosphorylation of Akt (3.8 fold decrease) in Western-blot.

**Discussion:** BMPs are a subgroup of the TGFβ super family and have been extensively studied in their role in bone development and fracture healing. Recently, Winbanks et al. found that increased expression of BMP and activation of BMP receptors induced muscle hypertrophy that is dependent on SMAD 1/5/8 activation [1]. Similarly, Sartori et al determined that inhibition of BMP signaling causes muscle atrophy through the regulation of the ubiquitin ligase family[2]. In this study, we demonstrated the gene expression of BMP-14 significantly increased at both early (2 weeks) and later stage (6 weeks) following RCT. Additionally, the gene expression of BMP-7 increased at later stage (6 weeks) following RCT. Our western blot analysis showed that phosphorylated Smad1/5/8, the downstream effort of canonical BMP signal pathway significantly increased at both early and later stages after RCT. These data suggests that BMP signaling is significantly upregulated in rotator cuff muscle following massive RCT. In order to test the functional role of BMPs in rotator cuff muscle atrophy and fatty infiltration, we blocked BMP signaling using LDN193189, a small molecule BMP type I receptor inhibitor. We saw significantly reduced pSmad1/5/8 expression suggesting LDN193189 successfully inhibited canonical BMP signaling pathway in rotator cuff muscle after RCT. Inhibition of BMP signaling resulted in a small decrease in muscle atrophy. This suggests that BMP signaling has an anti-atrophic role in rotator cuff muscle atrophy. However, inhibiting BMP signaling for 6 weeks only resulted in a small change in muscle size. This suggests other compensative mechanisms for BMPs may exist in rotator cuff muscle atrophy. Inhibition of BMP signaling significantly reduced rotator cuff muscle fatty infiltration as proved by both histology and RT-PCR. Interestingly, inhibition of BMPs results in a significant reduction of Akt phosphorylation. In our previous work, we have reported that Akt/mTOR pathway plays a critical role in rotator cuff muscle fatty infiltration (4). This data suggests that BMP signaling may control rotator cuff muscle fatty infiltration through Akt/mTOR pathway.

In summary, we are reporting significantly increased BMP signaling in rotator cuff muscles after RCT. Inhibiting BMP signaling results in slightly increased muscle atrophy but significantly reduced muscle fatty infiltration after RCT.

**Significance:** This study suggests that BMP signaling plays a critical role in rotator cuff muscle atrophy and fatty infiltration. BMP pathway may serve as novel therapeutic target for treating rotator cuff muscle fatty infiltration.
Figure 1 RT-PCR showed that the expression of BMP-14 significantly increased at 2 weeks after TT+DN (left). Both BMP-7 and BMP-14 are significantly upregulated at 6 weeks (right) in rotator cuff muscles after RCT.

Figure 2 Western-blot showed that significantly increased level of active (phospho) Smad1/5/8 in rotator cuff muscle following TT+DN.
Figure 3. Treatment of LDN193189 slightly decreased muscle mass (upper panel) but significantly reduced the fatty infiltration as shown in Oil-red O staining in rotator cuff muscles following massive RCT (lower panel).