INTERLEUKIN-6 INDUCED ACTIVATION OF SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3) IN RUPTURED ROTATOR CUFF TENDON

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ABSTRACT INTRODUCTION:
Rotator cuff disease frequently causes severe shoulder pain in the middle-aged and elderly. The pathology of the disease consists of a ruptured rotator cuff tendon and coexisting inflammatory subacromial synovium (synovitis) (1). The subacromial synovitis has been observed in a site associated with shoulder pain in patients with rotator cuff disease. In contrast, the underlying molecular pathology of the ruptured rotator cuff tendon has not been precisely clarified yet (2).

Interleukin-6 (IL-6) is considered to be an important reactive cytokine during inflammation. IL-6 is also known to be an exercise-induced cytokine. IL-6 concentration in skeletal muscle and connective tissue has been shown to increase with prolonged exercise. In vitro human tendon fibroblasts can secrete IL-6 by cyclic mechanical stretching. These observations are presumably implicated in the adaptive response of tendons, although excessive stimulation of tendon may conceivably have a role in the development of tendon rupture as a result of overuse. The rotator cuff tendon constantly undergoes repetitive microtrauma and overloading by muscle force, leading to degenerative changes and finally to rupture itself. These findings, therefore, raised the possibility that IL-6 may be closely associated with the pathology of ruptured rotator cuff tendon. In this study, we hypothesized that IL-6 could be upregulated in ruptured rotator cuff tendon.

METHODS:
Specimens of ruptured rotator cuff tendons were obtained during surgery from 30 patients (20 males and 10 females, with an average age of 56 years, ranging from 35 to 80 years). The ruptured tendons consisted of 10 partial-thickness and 20 full-thickness tears. The average duration from onset of the disease to the surgery was 8 months, ranging from 2 to 13 months. We harvested rotator cuff tendon around the ruptured site, and subacromial synovium as near the ruptured site as possible. Care was taken to discriminate between the tendon and subacromial synovium. The defect after the resection of the ruptured site of the tendon was completely repairable. All specimens were obtained with informed consent and with permission from the Kurume University Ethics Committee.

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was used to measure IL-6 mRNA expression levels. Western blot analysis was employed to assess the activation of STAT3 (3) (confirmation of phosphorylation). Immunohistochemistry was performed to detect the cells producing IL-6, IL-6 receptor, and phosphorylated STAT3. These parameters in ruptured rotator cuff tendon were compared with those in coexisting inflammatory subacromial synovium (3).

The Mann-Whitney U-test was used for comparison of parameters between the ruptured rotator cuff tendon and the subacromial synovium. A p-value less than 0.05 was considered significant.

RESULTS SECTION:
Comparison of IL-6-mRNA expression levels between ruptured rotator cuff tendon and the subacromial synovium in rotator cuff disease The mRNA expression levels of IL-6 in ruptured rotator cuff tendons and subacromial synovia were measured using real-time RT-PCR. Expression levels of IL-6-mRNA were not significantly different between ruptured rotator cuff tendon and the subacromial synovium. Real-time RT-PCR results showed that mRNA expression levels of IL-6 were not significantly different between ruptured rotator cuff tendon and subacromial synovium. Next, we examined activation of STAT3 (phosphorylation of STAT3). Western blot analysis identified that phosphorylated STAT3 was constitutively and comparably expressed in ruptured rotator cuff tendon and subacromial synovium.

Real-time RT-PCR and Western blot analysis confirmed increased mRNA expression of IL-6 and STAT3 activation in ruptured rotator cuff tendon. By immunohistochemistry, we examined the cells producing IL-6, IL-6 receptor, and phosphorylated STAT3. In ruptured rotator cuff tendon, positive immunoreactivities for IL-6, IL-6 receptor, and phosphorylated STAT3 were mainly detected in the proliferative vessels and, to a lesser extent, in the fibroblasts around the vessels, and in the proliferative vessels and synovial lining cells in the subacromial synovium.

Immunohistochemical examination detected the cells producing IL-6, IL-6 receptor, and phosphorylated STAT3 mainly in proliferative vessels in ruptured rotator cuff tendon. In the cell typing analysis, CD34 positive immunoreactivities were consistent with the cells producing IL-6, IL-6 receptor, and phosphorylated STAT3. CD68 positive cells were sparsely noted around the vessels. CD20 and CD45 positive cells were not found throughout the specimens.

DISCUSSION:
Neovascularization in ruptured rotator cuff tendon is thought to be a secondary reaction as a consequence of ischemic changes (4). The current study detected STAT3-activated proliferative vessels in ruptured rotator cuff tendon. IL-6 promotes vessel proliferation by vascular endothelial growth factor (VEGF)-dependent angiogenesis via the STAT3 pathway. In addition, protection from ischemic injury, STAT3 is required for myocardial capillary growth, enhancing VEGF expression. In view of these points, STAT3 activation may play a critical role in tendon adaptation to ischemic stress by maintaining intratendinous vasculature.

Swelling/hyperemia in ruptured rotator cuff tendon was observed by ultrasound and MRI studies in rotator cuff disease, although its molecular pathomechanics has not been clarified. In this study, we demonstrated the presence of IL-6-expressing proliferative vessels in ruptured rotator cuff tendon. IL-6 can increase vascular permeability, leading to edema. Thus, our results may explain one of the reasons why swelling/edema occurs in ruptured rotator cuff tendon.

Several studies showed increased free nerve endings, substance-P positive and calcitonin gene-related peptide (CGRP) positive nerves in and around vessels in damaged tendons. Substance-P can induce inflammatory cytokines, such as IL-6, causing neurogenic inflammation and generation of pain. Proliferation of vessels secondary to ischemic change may be a contributory factor to pain in damaged tendon. The present study showed proliferation of vessels expressing IL-6 in ruptured rotator cuff tendon. These results suggest that proliferation of vessels in the ruptured rotator cuff tendon may be involved in the generation of shoulder pain in rotator cuff disease, together with the coexisting subacromial synovitis. The limitation of the present study was not using age-matched normal cuff as a control. Unfortunately, we could not employ either age-matched normal cadaveric cuff or normal cuff remote from the ruptured site as controls because of ethical reasons in our institution.

In conclusion, we discovered that even with the paucity of evidence of rotator cuff disease, activation of STAT3 induced by IL-6 is promoted mainly by proliferative vessels in the ruptured rotator cuff tendon. To our knowledge, this is the first report that has confirmed the activation of STAT3 induced by IL-6 in ruptured rotator cuff tendon. Our data provide new insight into the understanding of rotator cuff pathology.

REFERENCES: