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ORIGINAL ARTICLE

Increased expression of type 1 cannabinoid (CB1) receptor among patients with rotator cuff lesions and shoulder stiffness

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Background: Shoulder stiffness is a disease manifested by pain, limited range of motion, and functional disability. The inflammatory and fibrosis processes play a substantial role in the pathogenesis of shoulder stiffness. The CB1 receptor has been recognized to mediate the processes of pathologic fibrosis. This study investigated the role of the CB1 pathway in pathogenesis of rotator cuff lesions with shoulder stiffness.

Methods: All of the patients undergoing repair surgery for rotator cuff lesions were recruited and subcategorized into subjects with and without shoulder stiffness. Reverse transcription-polymerase chain reaction assay was used to evaluate the expression level of CB1 and interleukin 1β (IL-1β) in the subacromial bursae, and enzyme-linked immunosorbent assay was used to measure the concentration of CB1 and IL-1β in the subacromial fluid. Tenocytes treated with CB1 agonists and antagonists were also studied for the relationship of CB1 and the inflammatory cytokine IL-1β.

Results: The patients with shoulder stiffness had higher messenger RNA (mRNA) expression ($P = .040$) and immunohistochemistry staining ($P < .001$) of CB1 in the subacromial bursa and higher CB1 concentration in the subacromial fluid ($P = .008$). Tenocytes treated with the CB1 agonist WIN 55,212-2 and antagonist AM251 showed increased expression of IL-1β mRNA ($P = .049$) and suppressed expression of IL-1β mRNA ($P = .001$), respectively.

This study was approved by the Chang Gung Medical Foundation Institutional Review Board (IRB approval number: 101-1810A3). Informed consent was signed and obtained from all of the participants.

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Discussion: The CB1 pathway is involved in the pathogenesis of shoulder stiffness. It may be a promising target for the treatment of rotator cuff lesions with shoulder stiffness.
Level of evidence: Basic Science Study; Molecular and Cell Biology

Keywords: Adhesive capsulitis; shoulder stiffness; cannabinoid receptor 1 (CB1); interleukin 1β (IL-1β); rotator cuff lesions; tenocytes

Shoulder stiffness is a commonly encountered but poorly understood disease manifested by pain, limited range of motion (ROM), and functional disability. Some patients have shoulder stiffness secondarily after identifiable underlying diseases, such as rotator cuff lesions, and some cases are idiopathic.[9] The inflammatory and fibrosis processes play a substantial role in the pathogenesis of shoulder stiffness.[3,10]

Cannabinoid receptors are a group of G protein–coupled receptors with 2 known subtypes, CB1 and CB2.[4] The CB1 receptor mediates the processes of pathologic fibrogenesis and is involved in the pathogenesis of major visceral diseases, such as renal, cardiac, pulmonary, and hepatic fibrosis.[1,5-8]

On the basis of these findings, we hypothesized that the cannabinoid receptors may be involved in the pathogenesis of shoulder stiffness, a disease entity involving the process of inflammation and fibrosis.

Materials and methods

Recruitment

A prospective comparative study was performed from June 2013 to March 2014. All of the patients undergoing open repair for rotator cuff lesions by the senior author were recruited.

All of the patients were aged 18-80 years and had magnetic resonance imaging findings suggestive of a complete or incomplete tear of the rotator cuff; their symptoms had lasted for >3 months despite pain medication and physical therapy. Patients with malignant neoplasms, chronic hepatic or renal disease, and shoulder disease other than rotator cuff lesions and stiffness (previous operation, fracture, instability, osteoarthritis) were excluded. The enrolled patients were separated into 2 groups. Patients with shoulder stiffness were assigned to group I, whereas patients without shoulder stiffness were assigned to group II. The criterion for shoulder stiffness was 50% loss of passive ROM for at least 3 months, with normal ROM considered to be 180° forward flexion, 180° abduction, 90° external rotation, and 90° internal rotation. Preoperative ROM and functional scores were recorded on the day before surgery (by Y.-H.H.) with ROM measured by a goniometer.[1,5-8] These ROM measurements were then added together to determine the sum of ROM. Patients were defined as having shoulder stiffness if the sum of ROM was <270°. These criteria have been adopted in our previous studies.[3] Because of the pilot nature of the study, we planned to recruit at least 25 patients for each group after consultation with the statistician.

Surgical procedure

The surgical procedure comprised manipulation under anesthesia, anterior acromioplasty, lysis of adhesions, partial bursectomy, and rotator cuff repair.[2] The patients are positioned in a beach chair position under general anesthesia. Gentle manipulation is performed with abduction force applied first, followed by flexion, external rotation, and internal rotation. A saber-shaped incision is extended from a point just lateral to the acromioclavicular joint to a point just lateral to the coracoid. The deltoid muscle is detached for 1.5 cm from the acromion, then split downward for 2 cm. Under arm traction, a flat elevator is placed under the acromion, and a thin osteotome is used to remove the protruding part of the acromion anterior to the clavicle; 1 cm of the coracoacromial ligament is excised. Extensive adhesiolysis is performed by resecting the contracted coracohumeral ligament and lysing the pericuff adhesions. Intra-articular distention and irrigation of the glenohumeral joint are performed through the cleft on the rotator interval or through the ruptured tendon. The torn cuff is repaired with No. 2 braided nonabsorbable polyester sutures and a tendon-to-bone suturing technique. Revo screws (Conmed Linvatec, Edison, NJ, USA) are used if the tendon cannot be sutured directly through the bone. The partial-thickness deltoid flap is sutured to the trimmed edges of the torn cuff for massive irreparable tears. The detached deltoid is sutured back onto the acromion, and the skin wound is closed. A triangular bandage is applied for immobilization.

Specimen preparation

The subacromial fluids with (0.5 mL) and without (1 mL) shoulder stiffness were collected immediately after anterior acromioplasty by sterile syringes, centrifuged at 12,000 × *g* at 4°C for 30 minutes, then stored at −20°C for further analysis. Subacromial bursae from the greater tuberosity to the coracoid process were harvested as well. The reason for sampling of the bursal tissue is that inflammation of subacromial bursae is a pivotal part of the pathogenesis of shoulder stiffness; the analysis of subacromial bursae for the pathogenesis of shoulder stiffness has been described in our previous work.[3]

Immunoblotting

The subacromial bursae were immersed in liquid nitrogen, crushed, and homogenized. Protein extracts in the tissue homogenate were prepared by the Pro-Prep lysis buffer (iNtRON Biotechnology, Sangdaewon-Dong, Korea). Proteins in the lysates were separated, blotted, and probed by the primary antibodies against CB1 (Thermo Fisher Scientific, Waltham, MA, USA) and interleukin 1β (IL-1β)