Intercellular Adhesion Molecule-1 (ICAM-1, CD54) Is Increased In the Frozen Shoulder

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Introduction
Frozen shoulder (adhesive capsulitis) is a very common cause of shoulder disability and pain. This disorder is characterized by dense fibrosis of the glenohumeral capsule, which restricts the shoulder motion. But the pathophysiology of this disorder is poorly understood and there are very limited reports regarding the cytokines and their expression in the frozen shoulder. Inflammation is thought to have a central role in the pathophysiology of the frozen shoulder. Recent studies have suggested a crucial role for intercellular adhesion molecule (ICAM-1) in inflammatory process because ICAM-1 mediated the leukocyte’s adhesion and migration to the endothelial cell at the inflammation site. In this study, we compared the expression of ICAM-1 at joint capsule, joint fluid, blood stream of patients with frozen shoulder to normal controls. And we also investigated whether the expression of this molecule can be reduced with treatment of steroid that is frequently used at the clinic.

Materials and Methods
Human tissues were obtained with the approval of the Catholic University School of Medicine Institutional Review Board.

RNA Extraction and Oligo-Array Analysis for Gene Expression in capsule tissue
20 patients were candidates for this study. They were divided into two groups: 15 patients with adhesive capsulitis and 5 normal controls from the patients with proximal humerus fracture. Capsule tissues were obtained during the surgery and stored in the RNAlater® (Ambion, CA) at -20 °C. Total RNA from the capsule tissues were extracted using TRIZOL (Invitrogen, Rockville, MD) according to the manufacturer's instructions. GEArray® Series (Bioscience Corporation, Fredrick, MD) for Human Extracellular Matrix & Adhesions Molecules were used.

Gene expression in capsule tissue by real-time reverse transcription-polymerase chain reaction (RT-PCR)
We can confirm the difference of gene expression between the capsule tissue from frozen shoulder (15 patients) and the normal capsule tissue (5 patients) from the fracture and instability patients. Real time PCR was performed up to 40 cycles using the SMART Cycler (Cepheid, Sunnyvale, CA) and Syber Green dye. Each sample were tested in duplicate and 18s gene was used as reference gene.

ICAM-1 detection in the joint capsule by immunohistochemical staining.
Samples were fixed with 10% buffered formalin overnight, washed, and dehydrated through a graded series of alcohol and were embedded in paraffin. Primary antibody mouse monoclonal anti-human ICAM-1 was incubated for overnight at 4 °C. Slides were counterstained with hematoxylin, and examined by light microscopy to determine ICAM-1 distribution. For a negative control, the primary antibody was omitted.

ICAM-1 detection in the joint fluid by western blotting
Joint fluid was extracted from the patients with frozen shoulder (7 patients) and anterior instability (2 patients). Anti-human ICAM monoclonal antibody was used. Staining was detected with an chemiluminescence kit, and quantified by densitometry with Image Analyzer LAS-3000 Multi Gauge software.

sICAM-1 (soluble ICAM-1) detection in the blood serum by enzyme-linked immunosorbent assay (ELISA).
Total sICAM-1 levels were quantified in blood serum from 32 patients with frozen shoulder, 20 patients with diabetes mellitus and 14 normal candidates without any disease. Serum samples were diluted 1:100 in 1 X assay buffer and added into wells with an enzyme conjugate of horseradish peroxidase-anti-sICAM-1 antibody.

Joint capsular cell culture
The capsular cells from three patients with frozen shoulder and two patients with anterior inferior instability as control were cultured in DMEM containing 10% FBS. Only the third cell passages were used in this experiment.

ICAM-1 mRNA expression in cultured capsular cells after steroid treatment by real-time RT-PCR.
One day after seeding, all cultured cells from frozen shoulder and controls were replaced with SFM and treated with a 10 nM of dexamethasone and the medium were exchanged every 24 hours. ICAM-1 mRNA in each sample cells was detected by real-time RT-PCR and compared the expression with control samples before steroid treatment, 1 day and 3 day of culture following steroid treatment.

Results

RNA Extraction, Oligo-Array Analysis for Gene Expression and immunohistochemical staining for ICAM-1 in joint capsule tissue
The gene expression of ICAM-1 was significantly increased in frozen shoulder compared to control in array (p<0.05). The average relative intensity of expression of frozen shoulder group was 14666.44 unit and those of control was 5881.22 unit. The Immunoreactivity for ICAM-1 showed that there was increased expression of the ICAM-1 molecule in the capsule of the patient of frozen shoulder compared to that of control.

Gene detection in capsule tissue by real time reverse transcription-polymerase chain reaction (RT-PCR)
Expression levels of ICAM-1 mRNA were significantly higher in the patient with frozen shoulder compared to control (p<0.05). Average expression level was 1.698±0.186 in frozen shoulder group and 0.999±0.236 in control.

ICAM-1 detection in the joint fluid by western blotting
The expression of ICAM-1 protein in the glenohumeral joint fluid of the patients with frozen shoulder (7 patients) was increased definitely compared to control (2 patients).

sICAM-1 expression in the blood serum by ELISA.
The s-ICAM-1 concentration was significantly increased in serum of the patient with frozen shoulder (633.219 ± 59.144 ng/ml) and diabetes mellitus (671.253 ± 27.080 ng/ml) compared to control (359.864 ± 44.286 ng/ml, P < 0.05).

Downregulated expression of ICAM-1 in the cultured capsular cells after treatment with steroid.
The expression level of ICAM-1 mRNA was significantly increased in frozen shoulder capsule cells (1.6364 ± 0.2509) than control cells (0.7004 ± 0.21165, P <0.05) before steroid treatment. After 3 days of culture following steroid treatment, ICAM-1 mRNA levels were significantly decreased in cultured cells from frozen shoulder (0.435 ± 0.056), while that of the control cells is little changed (1.0 ± 0.554). In contrast to ICMA-1 expression in dexamethasone treated cells from control for 3 days has tend to increase gradually.

Discussion & Conclusion
This study demonstrated that ICAM-1 expression is significantly increased in capsule tissue and joint fluid of the shoulder joint and blood serum of the patients with frozen shoulder compared to those of control. And we also demonstrated that the expression of ICAM-1 increased in cultured capsular cells and reduced after steroid treatment, which provides biological evidence for the injection of corticosteroid into glenohumeral joint as a treatment of frozen shoulder. The results imply this specific molecule might play an important role in immune-mediated inflammatory response and causing the stiffness in frozen shoulder. Therefore, regulation of ICAM-1 expression might be a key of anti-adhesion agent for treatment of frozen shoulder.

Keywords: Frozen shoulder, ICAM-1, Inflammation,