IL-1β Induces Expression of Stromal Derived Factor-1 (SDF-1α) in Subacromial Bursal Cells

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INTRODUCTION:
More than 6 million out-patient visits to the orthopaedic surgeon for shoulder pain are made per year, with subacromial impingement syndrome and rotator cuff tendinitis comprising a large percentage of these visits. Despite the common nature of impingement syndrome and rotator cuff disease, little is known about their pathophysiology. Previous reports have demonstrated that inflammatory cytokines, including stromal cell-derived factor 1 (SDF-1α; CXCL12) and human interleukin IL-1β (IL-1β), are increased in the subacromial bursa in patients with rotator cuff disease. SDF-1α is a potent chemokine involved in the recruitment of inflammatory mediators in response to mechanical or biochemical signals in patients with rotator cuff disease. IL-1β is also an important chemokine regulator of inflammation. Increased IL-1β production has been shown in subacromial bursal cells in patients with impingement syndrome and rotator cuff disease and is elevated in the subacromial bursa in rotator cuff disease. Further, IL-1β has been shown to stimulate SDF-1α expression in rheumatoid synovium.1-3 We therefore hypothesized that cultured bursal synoviocytes from patients with rotator cuff disease could be directly stimulated to express SDF-1α induced by cytokine stimulation using IL-1β.

METHODS:
Subacromial bursal tissue was obtained intra-operatively from seven patients undergoing shoulder surgery (Columbia University IRB #6012). Specimens were freshly minced with scissors in Dulbecco’s minimum essential medium (DMEM) producing a cell suspension with small fragments of tissue. The suspension was pelleted by centrifugation and the small fragments were enzymatically digested in phosphate buffered saline (PBS) containing 1 mg/ml collagenase, 0.15 mg/ml DNase, and 0.15 mg/ml hyaluronidase for 1 hour at 37°C. The suspension was then passed through sterile gauze to remove any undigested fragments and the cells were seeded in 75 cm² flasks with DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.1% fungizone (amphotericin B). Cells were grown at 37°C in a humidified atmosphere of 5% CO2 and 95% air.

Early passage cells were plated in 6 well plates and grown to 85% confluency. Wells were then treated with 10ng/mL of IL-1β for 0, 6, 24, and 48hrs. Cells were harvested by trypsinization and resuspended in lysis buffer. Total RNA was isolated from the cells using Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). First stranded cDNA was synthesized from 1 μg of total RNA using Superscript TM Firststrand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA) and the Perkin Elmer DNA thermal cycler. Real time RT-PCR was performed for SDF-1α (Eppendorf Real Plex 4 Mastercycler) and results were standardized to GAPDH housekeeping gene. Statistical analysis was performed using the student’s t-test.

RESULTS:
SDF-1α expression was increased in bursal cells treated with IL-1β compared to controls (p<0.05). An increase in expression was first observed after 6 hours and continued to increase thereafter. Maximal stimulation was seen after 48 hours (p<0.05) of induction with IL-1β, showing a nearly five-fold increase in SDF-1α expression (p<0.05). In order to control for variability, testing was performed on three different bursal cell lines, and each assay for SDF-1α expression (and control group) was performed in triplicate.

DISCUSSION:
While subacromial impingement syndrome and rotator cuff disease is not a life-threatening condition, it is the most common presenting diagnosis in shoulder and elbow surgery, and its non-surgical and surgical treatment accounts for millions of health care dollars. Furthermore, the non-selective agent currently used in its treatment may lead to significant local (impaired rotator cuff tendon healing) and systemic (cardiovascular and gastrointestidal) adverse events. As molecular medicine has led to a more targeted approach in other conditions, this study represents an important and more targeted approach for the treatment of subacromial bursitis and rotator cuff disease.

The induction and regulation of SDF-1α plays a central role in the pathophysiology of subacromial impingement. However, the mechanisms by which SDF-1α is induced and regulated are not completely understood. SDF-1α is believed to play a central role in the mediation of pain and inflammation in the subacromial bursa of patients with rotator cuff disease. Prior studies have demonstrated that SDF-1α is elevated in the subacromial bursa of patients with rotator cuff disease as compared to controls, and can be induced by mechanical stimuli.1-3,4 Using a novel in-vitro model of subacromial impingement, the present study demonstrates that IL-1β can also induce expression of SDF-1α in subacromial bursal cells. These data provide further information on the biochemical signals which may mediate the molecular pathophysiology of rotator cuff disease. The ability to induce SDF-1α expression by chemical stimuli in the subacromial bursa will allow us to further understand the important triggers in subacromial bursitis. Additional investigations into these chemical stimuli may identify important signaling mechanisms between the injured rotator cuff and subacromial bursa, and may lead to the development of targeted therapeutic agents to treat subacromial impingement.

REFERENCES: