CXCR4 blockade (AMD 3100 and T140 analog) inhibits SDF-1α expression and cell migration in human subacromial bursa cells

INTRODUCTION: Peri-articular inflammation in the shoulder is mediated in part by stromal cell derived factor 1 (SDF-1α) expression in subacromial bursa synoviocytes. Corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) are potent anti-inflammatory agents which may reduce shoulder inflammation, and have been shown to inhibit SDF-1α expression in these cells. However, these agents have significant side effects, including local inhibition of rotator cuff tendon healing as well as gastrointestinal and cardiovascular adverse events. Direct inhibition of SDF-1α expression in the subacromial bursa offers an alternative approach to reducing shoulder inflammation without these deleterious side effects. A potential target for SDF-1α inhibition includes its receptor CXCR4, and CXCR4 inhibitors (AMD3100 and T140 analog) are currently under development for other indications. In this study, we hypothesized that SDF-1α expression and subacromial bursa cell activity (migration) could be inhibited with CXCR4 inhibitors, AMD 3100 and T140 analog.

METHODS: Subacromial bursal tissue was obtained intra-operatively from patients undergoing shoulder surgery (RIH IRB #4047-08). Specimens were minced in Dulbecco’s minimum essential medium (DMEM) producing a cell suspension with small fragments of tissue. The suspension was pelletted by centrifugation and the fragments were enzymatically digested in phosphate buffered saline containing 1mg/ml collagenase, 0.15mg/ml DNase, and 0.15mg/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 75cm² flasks with DMEM supplemented with 10% fetal bovine serum, 100U/ml penicillin, 100μg/ml streptomycin, and 0.1% fungizone. Cells were grown at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Cell seeding: Early passage cells were plated in 6 well plates and grown to 85% confluency. Wells were then treated with 20ng/mL of IL-1β. After 24hrs, media was changed and inhibitors were added. Supernatants were collected after 48hrs and assayed for SDF-1α expression using Quantikine® Human SDF-1α enzyme immunoassay kit (R & D System) according to the manufacturer’s instructions. Standard curves were reconstituted using SDF-1α protein. The optical density of each well was determined using a microplate reader set to 450nm wavelength. Statistical analysis was performed using the Student’s t-test. Statistical significance was present when p<0.05.

Gene Expression: Cells were harvested by trypsinization and resuspended in lysis buffer. Total RNA was isolated from the cells using Qiagen RNAeasy Mini Kit. First stranded cDNA was synthesized from 1μg of total RNA using Superscript TM First strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA). Real time RT-PCR was performed for SDF-1α (DNA Engine Opticon™2 Detection System (MJ Research, Hercules, CA) and results were standardized to GAPDH housekeeping gene. Statistical analysis was performed using the student’s t-test.

Cell Migration: Migration assays were performed using first-passage cells (1x10⁵ cells/well). Supplemented DMEM was added to tissue culture plates and membrane inserts (Millicell polyethylene terephthalate filters (5μm pore size, Millipore, Billerica, MA) were pre-soaked in wells for 5 min. Cells were pre-incubated with inhibitors for 10 min then seeded into the pre-soaked wells. SDF-1α was added to each well and plates were then incubated for 3.5hrs at 37°C. Cells on the underside of the filters were quantified and compared.

RESULTS: SDF-1α protein and mRNA expression were decreased in subacromial bursa cells treated with CXCR4 inhibitors (Figures 1-2). Although maximal inhibition (87%) occurred in bursa cells treated with dexamethasone, both CXCR4 inhibitors (T140 analog and AMD3100) significantly decreased both SDF-1α protein and mRNA expression in these cells. T140 analog was the more potent inhibitor, decreasing SDF-1α protein production by 60 percent. These results were comparable to those seen with COX-2 inhibition (64%). While T140 analog significantly decreased SDF-1α mRNA expression, AMD3100 decreased SDF-1α protein production by 40 percent but caused only a slight decrease in SDF-1α mRNA expression.

Figure 1: SDF-1α protein production in human subacromial bursa cell cultures stimulated with IL-1β and treated with inhibitors.

Figure 2: SDF-1α mRNA expression in cultured bursa cells stimulated with IL-1β and treated with inhibitors.

Bursal cell migration in response to SDF-1 stimulation was decreased in the presence of both CXCR4 inhibitors (T140 analog and AMD3100). These results were comparable to those seen with COX-2 inhibitors and dexamethasone (data not shown) and were seen consistently in bursa cells cultured from multiple patients (Figure 3).

DISCUSSION: These data demonstrate that SDF-1α protein and mRNA expression may be inhibited with CXCR4 inhibitors, AMD 3100 and T140 analog. Biologic activity, as measured by bursa cell migration, was also decreased in the presence of both CXCR4 inhibitors, and these results were comparable to those seen with COX-2 inhibitors and dexamethasone. SDF-1α/CXCR4 blockade may offer a novel approach to reducing shoulder inflammation without the deleterious side effects of NSAIDs and steroids. Further experiments to assess the effects of these inhibitors on rotator cuff healing are under investigation.