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The effect of myofibroblasts and corticosteroid injections in adhesive capsulitis

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Hypothesis: Adhesive capsulitis is a condition that results in restricted glenohumeral motion. Fibroblasts have been implicated in the disease process; however, their role as a contractile element in the development of fibrosis and capsular contracture is not well understood. We hypothesized (1) that myofibroblast prevalence in capsular biopsy specimens from patients with adhesive capsulitis would be increased compared with controls and (2) that patients treated with an intra-articular injection of corticosteroid would have fewer myofibroblasts.

Methods: The study prospectively enrolled 20 consecutive patients with adhesive capsulitis scheduled for capsular release and matched controls. Tissue samples were collected from the posterior and anterior capsule for histomorphologic and immunohistologic analyses. Identical sectioning and preparation was performed in 14 additional adhesive capsulitis specimens from patients who had not received corticosteroid injections.

Results: Patients with adhesive capsulitis not treated with preoperative corticosteroid demonstrated more histologic evidence of fibromatosis, synovial hyperplasia, and an increase in positive staining for α-smooth muscle actin than patients who had received intra-articular injections of steroid. No specimens obtained from control patients demonstrated positive staining for α-smooth muscle actin.

Discussion: There was a higher prevalence of myofibroblast staining in patients with adhesive capsulitis, implicating activation of the myofibroblast in the pathophysiology of capsular contracture. Intra-articular steroid injection decreases the presence and amount of fibromatosis, vascular hyperplasia, fibrosis, and the presence of fibroblasts staining for α-smooth muscle actin. This supports the use of steroid injections to alter the disease process by decreasing the pathologic changes found in the capsular tissue.

Level of evidence: Basic Science Study; Histology

Keywords: Adhesive capsulitis; corticosteroid injection; myofibroblast; frozen shoulder; synovial hyperplasia; fibrosis

Adhesive capsulitis is a debilitating condition that results in restricted active and passive glenohumeral range of motion (ROM) with a prevalence of 2% to 5%.[3,5] The etiology of the disease is unknown but is associated with multiple factors, including female gender,[1] diabetes,[3,12] thyroid disease,[2] trauma, stroke or myocardial infarction[12] or history of autoimmune

The Hospital for Special Surgery Institutional Review Board approved this study (IRB#: 27071).

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diseases.[4] The pathogenesis of adhesive capsulitis is poorly understood, which has limited the development of standard treatment protocols for the different stages of the disease.

Inflammation and fibrosis have been implicated in the pathogenesis of the disease. The early inflammation is characterized by synovial hyperplasia and hypervascularity with increased lymphocytic perivascular infiltration.[6,18,20] Rodeo et al[18] reported an increase in transforming growth factor-β (TGF-β), platelet-derived growth factor, interleukin-1, tumor necrosis factor, and hepatocyte growth factor. They proposed that these cytokines were involved in the inflammatory and fibrotic components of adhesive capsulitis and postulated that there was an alteration of the normal homeostasis between fibroblast proliferation and apoptosis.[18] Capsular fibroblasts are responsive to specific growth factors. Suzuki et al[20] demonstrated that platelet-derived growth factor, hepatocyte growth factor, and insulin-like growth factor 1 increased the migration of fibroblasts in a dose-dependent fashion from 130% to 700%.[20]

Perivascular and capsular fibrosis predominate in later stages of the disease.[8,16] Fibroblastic proliferation is accompanied by differentiation of fibroblasts into myofibroblasts.[6] The extent of myofibroblast proliferation and their role as a contractile component in the development of fibrotic changes in adhesive capsulitis has not been studied.

We hypothesized (1) that there would an increase in myofibroblasts in capsular biopsy specimens obtained from patients with adhesive capsulitis as compared to controls and (2) that patients treated with intra-articular corticosteroid injections before surgical treatment and capsular biopsy would have fewer capsular myofibroblasts than patients not treated with corticosteroid.

Materials and methods

The study prospectively enrolled 20 sequential patients with a clinical diagnosis of idiopathic adhesive capsulitis scheduled for capsular release along with controls matched for age and sex undergoing shoulder surgery for other diagnoses. A preoperative history and physical examination were performed. ROM was recorded preoperatively, after regional anesthesia, after release, and at postoperative appointments.

Clinical staging was assigned.[8,15,17] For stage 1 (S1), symptoms were present for less than 3 months, and there was pain with active and passive ROM. After injection with local anesthetic or with examination under anesthesia (EUA), ROM approached symmetry with the contralateral shoulder. In stage 2 (S2), symptoms were present for 3 to 9 months with chronic pain with active and passive ROM, and rest and night pain. After injection with local anesthetic or with EUA, limitation in ROM persisted. In stage 3 (S3), the symptoms were present for 9 to 15 months, the shoulder was free of pain at rest but was increasingly stiff with pain at end ROM. There were no stage 4 (S4) patients in this study, with minimal pain and improving ROM.

The patients who underwent intra-articular injections before surgery received an injection containing 80 mg methylprednisolone, 3 mL of 0.25% bupivacaine, and 5 mL of 1% lidocaine injected by a posterior approach to the glenohumeral joint. Intra-articular injection was confirmed by resolution of pain 15 to 20 minutes after injection.

At the time of shoulder arthroscopy, and before capsular release, tissue samples were collected from the posterior and anterior capsule. These specimens and the arthroscopic shavings were sent to the pathology department. All tissues were placed in formalin and prepared for standard histologic assessment using paraffin embedding. Sections (5 μm thick) were stained with hematoxylin and eosin. Immunohistochemical staining for α-smooth muscle actin (α-SMA) and CD-31 were performed on adjacent sections. α-SMA labels smooth muscle cells, myofibroblasts, and myoepithelial cells. CD-31 is an immunohistochemical stain for endothelial cells and was used to differentiate the α-SMA staining cells positive for fibroblasts (myofibroblasts) from myoendothelial cells.

Immunohistochemical analysis

The specimens for α-SMA were fixed in 10% formalin, washed, dehydrated, embedded, and sectioned. Slides were treated with 3% hydrogen peroxide to eliminate endogenous peroxidase activity and placed in 0.1% trypsin (pH 8.0) for 15 minutes at 37°C to optimize antigen retrieval. Slides were incubated with a monoclonal mouse anti-human α-SMA antibody. The secondary antibody was horse anti-mouse antibody, and the tertiary antibody was Strept ABComplex/HRP. All antibodies were obtained from Dako North America (Carpinteria, CA, USA). A positive signal was developed with 3,3′-diaminobenzidine-tetrahydrochloride-dihydrate chromogen, and hematoxylin was used as a counterstain.

CD-31 specimens were fixed in 10% formalin, washed, dehydrated, embedded, and sectioned. Slides were treated with 3% hydrogen peroxide to eliminate endogenous peroxidase activity and pretreated with citrate buffer (pH 6.0) for 10 minutes. Slides were incubated with a monoclonal mouse anti-human CD-31 antibody. The secondary antibody was horse anti-mouse antibody, and the tertiary antibody was Strept ABComplex/HRP. Positive signal was developed with 3,3′-diaminobenzidine-tetrahydrochloride-dihydrate chromogen, and hematoxylin was used as a counterstain.

Identical sectioning and preparation was performed in 14 additional adhesive capsulitis capsule specimens from patients not treated with corticosteroid injections. These specimens had been collected for a prior study. All slides were concurrently examined, using a multiheaded microscope, by a musculoskeletal pathologist (E.F.D.) and an orthopedic surgeon (J.A.H.) who were blinded to the clinical stage of adhesive capsulitis and the treatment before surgery. Specimens were graded for synovial hyperplasia, perivascular inflammation, interstitial fibrosis, and the presence of myofibroblasts positive for α-SMA.

Slides of specimens that stained positive for α-SMA were digitized at a resolution of 3.22×10^{-4} mm/pixel through a ×20 objective on an Olympus VS110 scanning microscope (Olympus, Center Valley, PA, USA). Cells staining positive and negative were automatically counted in the digitized images using VisioMorph software (Visiopharm A/S, Hørsholm, Denmark). Brown (positive) and blue (negative) cells were isolated from the tissue area using a linear Bayesian classifier for a color-based segmentation.

Brown cells associated with blood vessel perimeters were then distinguished from independent brown cells. This distinction was made by performing a dilation function on all identified brown pixels, which effectively drew together cells associated with blood vessel perimeters and created an area larger than the area associated with