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ORIGINAL ARTICLE

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

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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

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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 an independently identified brown cell. A maximum area constraint was applied to segregate the brown cells associated with blood vessels into a blood vessel category, which was omitted from counting. Similarly, blue cells immediately adjacent to an identified blood vessel were also placed in the blood vessel category and omitted from counting.

Resulting cell counts were expressed as the number of cells per tissue area, with tissue area defined as the total area of all cells, blood vessels, and noncellular collagenous tissue. This method was validated by having 1 author (C.M.H), an engineer (J.E.G.), and 2 masters-level engineering students perform manual counts on randomly selected samples, which were compared with automatic cell counts.

Results

The 20 specimens in the prospective arm of the study came from 13 women and 7 men with an average age of 51.2 ± 6.31 years (range, 42-65 years). Two patients had diabetes mellitus, 2 had hypothyroidism, and 2 had a history of breast cancer. All were nonsmokers. Preoperative steroid injections were administered to 15 of the 20 patients. The control group also comprised 13 women and 7 men, age matched within 5 years of the study patients. Of the 14 patients from the retrospective arm of the study, 12 were women, and the average age was 50.9 ± 10.4 years (range, 39-69 years).

Controls: None of the operated-on control specimens were positive for α-SMA or fibromatosus. Representative specimens are shown in Fig. 1.

S1: The S1 patient was a 54-year-old woman who had received a steroid injection. Specimens demonstrated slight synovial hyperplasia, no perivascular inflammation, and no interstitial fibrosis. The capsule was negative for α-SMA–positive myofibroblasts.

S2: We obtained 14 S2 specimens prospectively and 10 from a prior study. For the purpose of analysis, these groups are combined. The 24 total S2 specimens came from 16 women and 8 men, average age of 51.2 ± 6.9 years (range, 39-65 years), and 11 patients had received preoperative corticosteroid steroid injections. In the patients treated with preoperative corticosteroid, 3 demonstrated fibromatosus, 1 of which was very focal, 8 had synovial hyperplasia, and 5 had vascular hyperplasia. There was no perivascular fibrosis, and only 3 of 11 stained positive for α-SMA. Many of these patient biopsy specimens had only very focal areas of pathology surrounded by normal tissue. In the 13 patients who had not received preoperative corticosteroid, 10 had fibromatosus and synovial hyperplasia, 12 had vascular hyperplasia, 9 had perivascular inflammation, and 10 were positive for α-SMA–positive myofibroblasts (Table I and Fig. 2).

S3: There were 9 patients (8 women) with S3 adhesive capsulitis, 3 of whom had been injected. The average age was 50.3 ± 11.5 years (range, 39-69 years). In the 3 patients treated with preoperative corticosteroid, 1 had fibromatosus, 3 had synovial hyperplasia, none had vascular hyperplasia, 3 had slight interstitial fibrosis, and 2 had positive α-SMA myofibroblasts. There were areas of pathologic tissue scattered within normal appearing capsule. In the group not treated with corticosteroid, 5 of 6 had fibromatosus, 5 had synovial hyperplasia, 2 had interstitial fibrosis, and 5 were positive for α-SMA–positive myofibroblasts (Table II and Fig. 3).

The average myofibroblast count (per mm^2) for injected specimens was significantly less for noninjected

Figure 1 (**A**) Control capsule without fibromatosis-like sclerosis (original magnification ×20, hematoxlin and eosin). (**B**) Control capsule positive for smooth muscle actin (SMA) of vascular muscle but negative for fibroblasts (original magnification ×20, SMA). (**C**) Control synovium without fibrosis or inflammation (original magnification ×20, hematoxlin and eosin).

Table I Histologic data for stage 2 specimens

Variable	No.	Fibromatosus	Synovial hyperplasia	Vascular hyperplasia	Perivascular inflammation	Perivascular fibrosis	α-SMA+
		(No.)	(No.)	(No.)	(No.)	(No.)	(No.)
Injected	11	3 (1 focal)	8	5		0	3
Noninjected	13	10	10	12	9		10

SMA, smooth muscle actin.

Figure 2 (**A**) Stage 2 noninjected fibromatosis-like sclerosis of the capsular matrix with fibroblasts (original magnification ×20, hematoxlin and eosin). (**B**) Capsule with stage 2 positive smooth muscle actin (SMA) of vascular muscle (lower left) and fibroblasts (original magnification ×20, SMA. (**C**) Stage 2 synovium, with hyperplasia of the lining and vessels as well as perivascular chronic inflammation, original magnification, hematoxlin and eosin).

Table II Histologic data for stage 3 specimens

Variable	No.	Fibromatosus	Synovial hyperplasia	Vascular hyperplasia	Interstitial fibrosis	Perivascular fibrosis	α-SMA+
		(No.)	(No.)	(No.)	(No.)	(No.)	(No.)
Injected	3	1	3	0	3 (slight)		2
Noninjected	6	5	5		2		5

SMA, smooth muscle actin.

Figure 3 (**A**) Stage 3 injected specimens fibromatosis-like sclerosis of the capsular matrix with fibroblasts and hyperplasia of the synovium with fibrosis and residual chronic inflammation (original magnification ×20, hematoxlin and eosin). (**B**) Stage 3, smooth muscle actin (SMA) staining of capsular stromal fibroblasts and synovial vascular smooth muscle cells (original magnification ×20, SMA). (**C**) Stage 3 fibromatosis-like sclerosis of the capsular matrix with fibroblasts (original magnification ×20, hematoxlin and eosin).

Table III Range of motion data by stage of disease

Stage	No.	Preoperative AROM	Preoperative PROM	EUA	Postoperative AROM
Stage 1	1				
Forward flexion,°		160	165	170	170
Abduction,°		110	165	130	90
External rotation,°		35	45	70	
Internal rotation,°		0	5	40	
Stage 2	14				
Forward flexion,°		78	90	92	160
Abduction,°		62	68	73	56
External rotation,°		12	15	20	
Internal rotation,°		7	14	8	
Stage 3	5				
Forward flexion,°		78	87	91	158
Abduction,°		78	86	87	57
External rotation,°		18	24	28	
Internal rotation,°		0	0	5	

AROM, active range of motion; *EUA*, examination under anesthesia; *PROM*, passive range of motion.

specimens (104.2 ± 26.1 mm^2 vs. 442.5 ± 262.8 mm^2, $P = .01$).

ROM data for S1, S2, and S3 patients were evaluated prospectively and are reported in Table III. Postoperative ROM was recorded at an average of 11.7 weeks (range, 4.3-19.3 weeks).

Discussion

As hypothesized, none of the capsule specimens obtained from control patients stained positive for α-SMA. This finding in concert with the high prevalence of myofibroblast staining in the adhesive capsulitis patients implicates activation of the myofibroblast phenotype in the pathophysiology of capsular contracture and scar formation in adhesive capsulitis. Secondly, patients with adhesive capsulitis who were not treated with a preoperative corticosteroid injection demonstrated histologic evidence of fibromatosis, synovial hyperplasia, and a higher proportion of specimens with positive staining for α-SMA myofibroblasts than patients treated with intra-articular steroid.

Myofibroblasts play a key role in wound healing by promoting wound closure and matrix deposition. These cells normally disappear from granulation tissue by apoptosis once the wound has healed. In conditions such as adhesive capsulitis, myofibroblasts persist and contribute to pathologic stiffening and scarring of tissues.[19]

Adhesive capsulitis has previously been shown to involve both synovial hyperplasia and capsular fibrosis, and these processes may involve cytokines such as TGF-β and platelet-derived growth factor. In addition, matrix-bound TGF-β may act as a persistent stimulus resulting in capsular fibrosis.[18]

Mechanical stress and TGF-β1 are both essential factors required to convert fibroblasts to myofibroblasts.[10] Stretching myofibroblast-derived extracellular matrix activates latent TGF-β1 from stores in the extra cellular matrix.[22] This mechanical cue for myofibroblast differentiation via TGF-β1 then causes further connective tissue contracture and stiffening.[11] The presence of TGF-β1 in wounds produces contractures due to the persistence of myofibroblasts, and early elimination of TGF-β significantly reduces myofibroblast numbers, resulting in an increase in wound size.[14] In an in vivo study, an adenovirus was used to deliver and overexpress TGF-β1 in the knees of rats. A complete encasement of the knee in dense scar tissue was demonstrated at 5 and 10 days after injection, and a proliferation of synovial fibroblasts, followed by conversion to myofibroblasts, was observed on histologic examination.[21]

Mullett et al[13] aspirated synovial fluid from 15 patients with S2 adhesive capsulitis and 15 controls undergoing surgery for shoulder instability. Human fibroblast cultures were incubated with the aspirate, and fibroblast proliferation was significantly increased in the cultures incubated with the aspirate from the adhesive capsulitis patients. The fibroblast response was dose dependent to aspirate concentration and demonstrated that abnormal growth factor activity in adhesive capsulitis synovial fluid leads to abnormalities of fibroblast proliferation.

Hand et al[7] examined histologic specimens of 22 patients with adhesive capsulitis who had been injected but elected to undergo surgical treatment. Immunocytochemistry confirmed the presence of inflammatory cells. The CD34 antibody stained strongly positive, identifying high vascularity resulting from angiogenesis. α-SMA staining was negative in their samples, consistent with patients in our study who had been treated with corticosteroid injection.

Conclusion

This study demonstrates that intra-articular steroid injection decreases the presence and amount of fibromatosis, vascular hyperplasia, fibrosis, and myofibroblasts positive for α-SMA. These findings support adhesive capsulitis as both an inflammatory and fibrotic condition, depending on the stage of disease.[8,9] This treatment also supports inflammation in the pathogenesis of disease and supports the use of corticosteroid injections to shorten or reverse the disease process by decreasing the pathologic changes found in the capsular tissue. Injection may result in the decreased production of TGF-β1, decreased stimulation of the fibroblasts, and decreased transition from fibroblasts to myofibroblasts. Therapies designed to specifically inhibit fibrotic progression by blocking TGF-β1 may provide a target to inhibit extracellular matrix deposition and capsular scarring and contracture.

A limitation of the study is that specimens were only obtained from patients in whom conservative management had failed. The standard in our institution now is to treat patients with an intra-articular injection of corticosteroid in the conservative management of adhesive capsulitis. To attain significant numbers of patients who had not been injected, it was necessary to include patient specimens from a previous study.

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