Influence of Frequent Corticosteroid Local Injections on the Expression of Genes and Proteins Related to Fatty Infiltration, Muscle Atrophy, Inflammation, and Fibrosis in Patients With Chronic Rotator Cuff Tears

A Pilot Study

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Background: The effect of local corticosteroid (CS) injections on rotator cuff muscles remains poorly defined, despite the significance of muscle quality as a crucial prognostic factor for patients with rotator cuff tears (RCTs).

Purpose: To compare alterations in gene and protein expression patterns in the rotator cuff muscles of patients with RCTs who received frequent joint CS injections with alterations in those without a history of CS injections.

Study Design: Controlled laboratory study.

Methods: A total of 24 rotator cuff muscle samples with medium-sized tears from 12 patients with a frequent joint CS injection history (steroid group; 7 men and 5 women who had received $\geq$5 injections with at least 1 within the previous 3 months; mean age, 63.0 $\pm$ 7.2 years) and 12 age- and sex-matched control patients without a history of CS injections (no-steroid group) were acquired. Alterations in the expression of genes and proteins associated with adipogenesis, myogenesis, inflammation, and muscle fibrosis were compared between the groups using quantitative reverse transcription–polymerase chain reaction, Western blotting, and immunohistochemistry. Statistical analysis included comparison of group means using the Mann-Whitney *U* test, chi-square test, or Fisher exact test and logistic regression for multivariate analysis.

Results: In the steroid group, the mRNA expression levels of adipogenic CCAAT/enhancer-binding protein alpha (C/EBPα; P = .008) and muscle atrophy–related genes (atrogin; P = .019) were significantly higher, and those of myogenic differentiation 1 (MyoD; P = .035), inflammatory interleukin 6 (IL-6; P = .035), and high mobility group box 1 (P = .003) were significantly lower compared with the no-steroid group. In addition, MyoD (P = .041) and IL-6 (P = .026) expression were decreased in the steroid versus no-steroid group. Immunohistochemistry revealed increased expression of C/EBPα and atrogin and decreased expression of MyoD and IL-6 in the steroid versus no-steroid group.

Conclusion: Patients with RCTs and a history of frequent CS injections exhibited an upregulation of adipogenic and muscle atrophy–related genes and proteins within the rotator cuff muscles and a downregulation in the expression of myogenic and inflammatory genes and proteins in the same muscles.

Clinical Relevance: These altered gene and protein expressions by frequent local CS injections may cause poor outcomes in patients with RCTs.

Keywords: steroid injection; rotator cuff tear; fatty infiltration; muscle atrophy; inflammation; gene expression; protein expression

Corticosteroids (CSs) are potent anti-inflammatory agents used to treat various shoulder conditions.[1,4,10,19] In particular, local CS injections can be a highly effective approach for pain relief and rehabilitation in patients with rotator

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cuff tears (RCTs), even in cases where other nonoperative treatments have shown limited efficacy.[5]

The utilization of local CS injections is on the rise, coinciding with the increasing incidence of RCTs related to aging and sports participation.[23] Furthermore, it is frequently used after shoulder surgery, with approximately 20% of patients receiving these injections within 6 months, to manage pain and stiffness.[31,32] However, repeated CS injections may result in adverse effects, such as hyperglycemia, facial flushing, and localized arthropathy or weakness in tendons and muscles.[13]

In particular, several studies have reported that local CS injections could adversely affect the rotator cuff tendons, underpinning this limited use of CS injections in patients with RCTs.[11,26,36,37] Ramirez et al[28] reported that 12 weeks after subacromial local CS injection, 17% of patients developed full-thickness RCTs, with 66.6% of these occurring in patients with previous partial-thickness RCTs. In addition, Weber et al[36] noted a correlation between preoperative local CS injections and revision rotator cuff repair. Furthermore, several preclinical studies have noted the adverse effect of local CS injections on the rotator cuff tendon at the histological and biomolecular levels, suggesting poor outcomes for RCTs.[22,26]

Despite the possible negative effect of local CS injection on RCTs, molecule-level preclinical studies are rare, and all studies dealing with the effect of local CS were confined only to the rotator cuff tendon. To our knowledge, no study has investigated the effect of the local CS injection on rotator cuff muscles, although the quality of the rotator cuff muscles is a very important prognostic factor for the outcomes in patients with RCTs,[6] and frequent CS injections are highly likely to adversely affect the rotator cuff muscles and tendons.[2]

In this study, we aimed to evaluate the altered gene and protein expression patterns related to rotator cuff muscle quality in patients with RCTs who received frequent joint CS injections compared with patterns in those without a history of CS injections. We hypothesized that frequent CS injections in patients with RCT would lead to the upregulation of adipogenic and muscle atrophy–related genes and proteins, along with the downregulation of myogenic and inflammatory genes and proteins in the rotator cuff muscles.

METHODS

Patient Selection

The study protocol received institutional review board approval, and written informed consent was obtained from all participants. This was a retrospective case-control study involving a laboratory trial. A total of 229 patients were surgically treated for full-thickness RCTs at our institution between March 2019 and May 2020. Biopsies were conducted on all 229 patients during the study period. Among these, we only included patients with medium-sized RCTs (n = 107; medium tears were defined as measuring 1-3 cm according to the rating system of DeOrio and Cofield[12]), which is the most common tear size corresponding to an operative indication, to minimize bias.[15,34] Tear size was measured intraoperatively using a calibrated probe after debridement of the degenerated tendon edges. The anterior-posterior dimension of the tear was measured at the lateral edge of the footprint, and medial retraction was estimated based on the distance from the apex of the tear to the lateral footprint. The exclusion criteria were as follows: traumatic onset of RCT (n = 3), preoperative stiff shoulder (n = 9), current heavy smoking with a smoking history of >20 pack-years (n = 5), uncontrolled diabetes (n = 2), systemic inflammatory disorder (n = 0), previous surgery on the same shoulder (n = 1), history of systemic CS injection (n = 0), and refusal to participate in the study (n = 3). Traumatic onset was defined as obvious high-energy trauma that could cause acute RCTs, such as a falling accident or acute shoulder dislocation. Frequent CS injections, regardless of being intra-articular or subacromial, were defined as a history of ≥5 injections, with the last injection being within the previous 3 months.

Of the remaining 84 patients, 12 patients had a frequent CS injection history and were included as the intervention group (steroid group). Among the remaining 72 patients, we retrospectively selected 12 age- and sex-matched patients without a history of CS injection as the control group (no-steroid group) using the 1:1 propensity score matching technique.

Outcome Assessment

In both groups, all patients underwent preoperative magnetic resonance imaging (3.0-T, Signa HDx; GE Healthcare), and the fatty infiltration of each rotator cuff muscle (supraspinatus, infraspinatus, and subscapularis) was graded according to the criteria established by Goutallier et al[14] by an orthopaedic surgeon (S.W.C.) with 18 years of experience who specialized in shoulders. All patients included in the study were regularly followed up at 3-month intervals during the first year after surgery and subsequently at 1-year intervals to assess their clinical outcomes.

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Ethical approval for this study was obtained from Konkuk University Medical Center (ref No. KUMC 2022-07-011).

TABLE 1
Primers Used for the qRT-PCR Analysis[a]

Gene Full Name	Gene Symbol (Human)	Main Role	Sequences
CCAAT/enhancer-binding protein alpha	C/EBPα	Adipogenic transcription factor	F: 5′-AAGAAGTCGGTGGACAAGAAC-3′ R: 5′-GTCATTGTCACTGGTCAGCTC-3′
Peroxisome proliferator-activated receptor gamma	PPARγ	Adipogenic transcription factor	F: 5′-CGGTTTCAGAAATGCCTTGC-3′ R: 5′-ATCTCCGCCAACAGCTTCTC-3′
Atrogin	Atrogin	Muscle atrophy	F: 5′-ATGCACACTGGTGCAGAGAG-3′ R: 5′-′TGTAAGCACACAGGCAGGTC-3′
Myogenic differentiation 1	MyoD	Myogenic transcription factor	F: 5′-CAAGCGCAAGACCACCAACG-3′ R: 5′-ATATAGCGGATGGCGTTGC-3′
Interleukin 6	IL-6	Inflammatory cytokine	F: 5′-ACTCACCTCTTCAGAACGAATTG-3′ R: 5′-CCATCTTTGGAAGGTTCAGGTTG-3′
High mobility group box 1	HMGB1	(Nucleus) transcription factor; (cytoplasm) inflammatory cytokine	F: 5′-GAAGTGCTCAGAGAGGTGGAA-3′ R: 5′-GGTTTTCATTTCTCTTTCATAACG-3′
Matrix metallopeptidase 9	MMP9	Degradation of the extracellular matrix, tissue remodeling	F: 5′-TTGACAGCGACAAGAAGTGG-3′ R: 5′-GCCATTCACGTCGTCCTTAT-3′
Actin, alpha 2, smooth muscle	α-SMA	Myofibroblast formation	F: 5′-CTGTTCCAGCCATCCTTCAT-3′ R: 5′-CCGTGATCTCCTTCTGCATT-3′
Collagen type 1 alpha 1 chain	COL1	Collagen fiber formation	F: 5′-AATCACCTGCGTACAGAACG-3′ R: 5′-CATAGCCATAAGACAGCTGG-3′
Collagen type 3 alpha 1 chain	COL3	Collagen fiber formation	F: 5′-CATCTTGGTCAGTCCTATGC-3′ R: 5′-TGGTTGTCCTGGAATACCAG-3′

[a]F, forward; qRT-PCR, quantitative reverse transcription–polymerase chain reaction; R, reverse.

The clinical outcomes were assessed at 1 year postoperatively by measuring forward flexion, external rotation, external rotation at 90° of abduction, and internal rotation. In addition, patients completed the American Shoulder and Elbow Surgeons score, Constant score, and visual analog scale (VAS) for pain.

Tissue Acquisition

All patients underwent arthroscopic surgery under general anesthesia in the beach-chair position. For each patient, 3 supraspinatus muscle tissue samples (3 × 3 mm) were acquired approximately 1 cm from the musculotendinous junction after repair using an arthroscopic punch through the lateral portal. Two samples were frozen immediately at −80°C for polymerase chain reaction (PCR) analysis and Western blot analysis. Another sample was fixed in fresh 10% buffered formalin for 16 to 24 hours at 4°C, dehydrated, and embedded in paraffin for immunohistochemical analysis.

Quantitative Reverse Transcription–PCR Analysis

To assess the expression of various genes associated with inflammation, adipogenesis, myogenesis, fibrosis, and muscle structure, which may be related to frequent CS injection, quantitative reverse transcription (qRT)–PCR was performed on adipogenic genes (peroxisome proliferator-activated receptor gamma and CCAAT/enhancer-binding protein alpha [C/EBPα]), a fibrogenic gene (alpha-smooth muscle actin [α-SMA]), a protein degradation-related gene (atrogin 1), a transcription factor related to myogenesis (myogenic differentiation 1 [MyoD]), inflammation-related genes (interleukin 6 [IL-6] and high mobility group box 1 [HMGB1] protein), degradation of an extracellular matrix–related gene (matrix metalloproteinase 9), and collagen type 1 and 3 genes (COL1 and COL3, respectively). Total RNA was extracted from the isolated muscles using TRIzol (Invitrogen) according to the manufacturer's instructions, and cDNA was generated using the Maxime RT PreMix Kit (iNtRON Biotechnology). The qRT-PCR was conducted on a Light Cycler 480 System (Roche Diagnostics) with 2× qPCRBIO SyGreen Mix Lo-ROX (PCR Biosystems). All gene expression data were normalized against glyceraldehyde 3-phosphate dehydrogenase expression, and the measurements were quantitatively analyzed. The primer sequences are listed in Table 1.

Western Blot Analysis

To analyze the difference in protein expression patterns between the frequent CS injection and control groups, we performed Western blot analysis using supraspinatus muscle proteins. Whole-cell extracts from isolated muscle tissues were prepared using radioimmunoprecipitation assay buffer (Elpis-Biotech).[17] Proteins from whole-cell lysates were separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were probed with anti-C/EBPα (A0904; ABclonal Technology), anti-atrogin 1 (ab168372; Abcam), anti-MyoD (ab203383; Abcam), anti–IL-6 (A0286; ABclonal Technology), and HMGB1 (ab79823; Abcam) primary antibodies.

Immunoreactive proteins were visualized using an Amersham Enhanced Chemiluminescence kit (GE Healthcare) according to the manufacturer's instructions. Immunoreactive proteins were assessed using a LAS-3000 image analyzer (Fuji Film). Protein amounts were determined by densitometric analysis using the ImageJ software (US National Institutes of Health). All measurements were quantitatively analyzed.

Immunohistochemistry

For molecules that showed significant differences in the Western blot analyses, immunohistochemistry was further performed to validate the local expression of the target proteins. For immunohistochemical analysis, 5-μm paraffin-embedded tissue sections were prepared, deparaffinized in xylene, and rehydrated in an ethanol/water series. Antigen retrieval was performed using a citrate buffer (pH, 6.0). The slides were incubated with primary antibodies for 1 hour at room temperature, washed 3 times with phosphate-buffered saline, and incubated with the corresponding secondary antibody conjugated to horseradish peroxidase for 30 minutes at room temperature, followed by washing 3 times with phosphate-buffered saline. All slides were analyzed under an Eclipse Ni-U microscope (Nikon), and images were acquired with a Nikon DS-Ri1 and analyzed using NIS Elements F4.00.00 Version 4.0.

Statistical Analysis

Data were reported as mean ± standard error. Mean values were compared between the steroid and no-steroid groups using the Mann-Whitney *U* test for continuous variables and the chi-square or Fisher exact test for categorical variables. Differences were considered statistically significant at $P < .05$. Statistical analyses were performed using SPSS for Windows (Version 18.0; SPSS). In multivariate analysis, logistic regression with a forward stepwise technique was performed.

RESULTS

Participant and Clinical Data and Clinical Outcomes

The 12 patients in the steroid group received a mean of 7.6 ± 2.2 injections. The participant and clinical data of the steroid and no-steroid groups are presented in Table 2. There were no significant differences observed between the 2 groups regarding symptom duration; side of involvement; hypercholesterolemia; smoking status; tear size; fatty infiltration in the supraspinatus, infraspinatus, and subscapularis muscles; or initial VAS pain scores. In addition, there were no significant differences between the 2 groups in 1-year postoperative clinical outcomes (Table 3). There were no occurrences of retear in either group.

TABLE 2
Participant and Clinical Data[a]

Variable	No Steroid (n = 12)	Steroid (n = 12)	*P*
Age, y	62.83 ± 7.77	63.00 ± 7.23	.957
Sex, male/female	7/5	7/5	>.999
Symptom duration, mo	13.7 ± 20.9	12.1 ± 22.5	.711
Side involved, D/ND	9/3	8/4	.653
Hypercholesterolemia	3	4	.653
Smoking (<20 pack-years)	4	5	.673
Tear size, mm			
AP dimension	19.75 ± 5.36	21.08 ± 5.66	.56
Medial retraction	15.75 ± 9.19	18.58 ± 6.14	.384
Fatty infiltration[b]			
Supraspinatus muscle	1.91 ± 0.67	2.25 ± 0.75	.264
Infraspinatus muscle	1.50 ± 0.67	1.67 ± 0.49	.496
Subscapularis muscle	0.67 ± 0.65	1.08 ± 0.99	.238
Initial VAS pain score	5.50 ± 2.02	4.91 ± 1.62	.444

[a]Data are reported as mean ± SD or No. of patients. AP, anterior-posterior; D, dominant; ND, nondominant; VAS, visual analog scale.

[b]Graded according to the criteria established by Goutallier et al[14]: grade 0 represents normal muscle, grade 1 indicates the presence of some fatty streaks, grade 2 signifies <50% fatty muscle atrophy, grade 3 denotes 50% fatty muscle atrophy, and grade 4 indicates >50% fatty muscle atrophy.

TABLE 3
Clinical Outcomes at 1 Year Postoperatively[a]

Variable	No Steroid (n = 12)	Steroid (n = 12)	*P*
Forward flexion, deg	167.91 ± 10.96	164.16 ± 11.83	.429
External rotation, deg	62.91 ± 22.40	58.75 ± 17.59	.617
ER 90, deg	75.83 ± 16.35	70.00 ± 19.42	.435
Internal rotation, deg	9.08 ± 3.11	10.08 ± 2.57	.401
ASES score	91.66 ± 5.34	90.16 ± 4.89	.481
Constant score	89.66 ± 6.22	87.83 ± 8.15	.542
VAS pain score	0.75 ± 0.86	0.83 ± 0.93	.823

[a]Data are reported as mean ± SD. ASES, American Shoulder and Elbow Surgeons; ER 90, external rotation at 90° of abduction; VAS, visual analog scale.

Gene Expression Patterns Between the Study Groups

As shown in Figure 1, the mRNA expression of C/EBPα, an adipogenesis-related transcription factor, was significantly higher in the steroid group compared with the no-steroid group ($P = .008$). In addition, atrogin, the muscle atrophy–related gene, showed significantly higher expression in the steroid group than in the no-steroid group ($P = .019$). On the contrary, the mRNA expression of MyoD, a myogenesis-related gene, was significantly lower in the steroid group than in the no-steroid group ($P = .035$), and the expression of inflammation-related genes IL-6 and

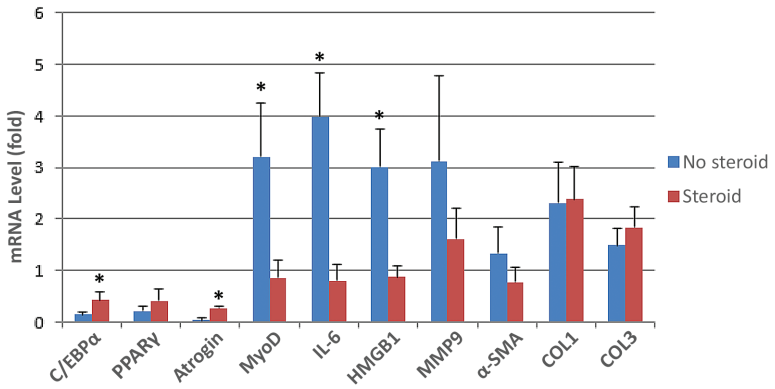

Figure 1. Comparison of the relative mRNA expression levels (target/GAPDH) in supraspinatus muscles between the steroid group and no-steroid group by real-time quantitative reverse transcription–polymerase chain reaction analysis. *Significant difference between groups ($P < .05$). α-SMA, alpha-smooth muscle actin; C/EBPα, CCAAT/enhancer-binding protein alpha; COL, collagen; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HMGB1, high mobility group box 1; IL-6, interleukin 6; MMP9, matrix metalloproteinase 9; MyoD, myogenic differentiation 1; PPARγ, peroxisome proliferator-activated receptor gamma.

TABLE 4
Results of Multivariate Logistic Regression Analysis (Stepwise Forward: Condition)[a]

Dependent Variable	Independent Variable	*P*	Exp(β)	95% CI
C/EBPα	Frequent steroid injection	.037	0.271	0.018 to 0.524
Atrogin	Frequent steroid injection	.028	0.238	0.028 to 0.447
IL-6	Frequent steroid injection	.005	−3.193	−5.266 to −1.119
MyoD	Frequent steroid injection	.045	−1.909	−3.768 to −0.050
MyoD	Tear size in AP dimension	.027	−0.211	−0.395 to −0.027

[a]AP, anterior-posterior; C/EBPα, CCAAT/enhancer-binding protein alpha; IL-6, interleukin 6; MyoD, myogenic differentiation 1.

HMGB1 was also significantly lower in the steroid group ($P = .035$ and .003, respectively). There were no differences in the mRNA expression of the fibrogenesis-related genes (α-SMA, COL1, and COL3) between the groups ($P > .05$ for all).

To assess factors affecting the gene expression levels of C/EBPα, atrogin, MyoD, and IL-6, we performed stepwise multivariate regression analysis. The analysis included frequent local CS injection status, tear size in the anterior-posterior dimension, tear size in medial retraction, and fatty infiltration in the supraspinatus, infraspinatus, and subscapularis muscles as independent variables and gene expression levels of C/EBPα, atrogin, MyoD, and IL-6 as dependent variables. The results revealed that frequent local CS injections had an independent impact on C/EBPα, atrogin, and IL-6 but not MyoD (Table 4).

Protein Expression Patterns Between the Frequent CS Injection and Control Groups

As shown in Figure 2, MyoD and IL-6 protein expression levels were significantly lower in the steroid group than in the no-steroid group ($P = .041$ and .026, respectively). In addition, although the differences were not significant, the protein expression levels of C/EBPα and atrogin were almost 2-fold higher in the steroid group than in the no-steroid group ($P = .132$ and .240, respectively).

Immunohistochemical Analysis

In accordance with Western blot analysis, immunohistochemical staining, confirming relative differences, revealed increased expression of C/EBPα and atrogin and decreased expression of MyoD and IL-6 in the steroid group compared with the no-steroid group (Figure 3).

DISCUSSION

The most important finding of this study, the first to our knowledge to investigate the effects of local CS injections on the rotator cuff muscles in humans, was that frequent local CS injections significantly altered fatty infiltration-, atrophy-, and inflammation-related gene expressions (C/EBPα, $P = .008$; atrogin, $P = .019$; MyoD, $P = .035$; IL-6, $P = .035$; HMGB1, $P = .003$) and protein expressions (MyoD, $P = .041$; IL-6, $P = .026$).

The studies published to date have focused on the effects of CSs on rotator cuff tendons, reporting the adverse

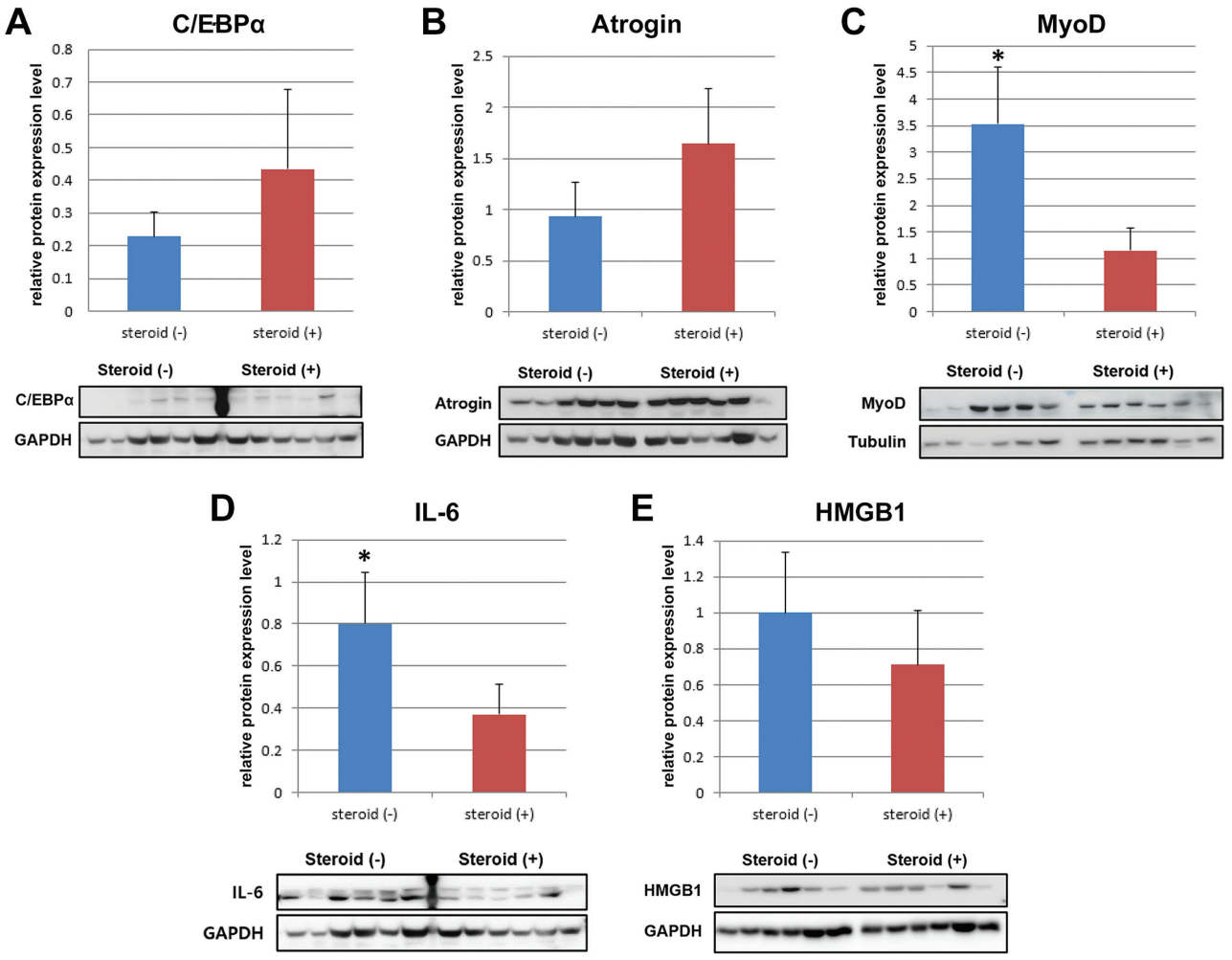

Figure 2. Relative protein expression levels (target/GAPDH or tubulin) in the supraspinatus muscle between the steroid group and no-steroid group by Western blot analyses. (A) C/EBPα, (B) atrogin, (C) MyoD, (D) IL-6, and (E) HMGB1. Higher C/EBPα and atrogin protein expressions and lower MyoD and IL-6 protein expressions were observed in the steroid group compared with those in the no-steroid group. *Significant difference between groups ($P < .05$) C/EBPα, CCAAT/enhancer-binding protein alpha; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HMGB1, high mobility group box 1; IL-6, interleukin 6; MyoD, myogenic differentiation 1.

effect of the CS injection on rotator cuff tendon quality or tendon tears.[27,28,36] However, the current study investigated the effect of CSs on the rotator cuff muscles and demonstrated that frequent local CS injections directly affect the supraspinatus muscle at the molecular level. Precisely, frequent local CS injections are suggested to aggravate fatty infiltration and muscle atrophy by upregulating fatty infiltration- and atrophy-related gene expression (C/EBPα and atrogin, respectively) and downregulating myogenesis-related gene expression (MyoD), which was confirmed in this study.

The transcription factor C/EBPα acts as a key regulatory factor in intramuscular preadipocytes, and it is known that increased C/EBPα expression increases muscle fatty infiltration.[35] Previously, it was proven that CSs could induce premature C/EBPδ expression, which is involved in the initial pathway of fibroblast differentiation into adipocytes, and the accumulated C/EBPδ is replaced by C/EBPα.[38] Thus, we theorize that frequent local CS injections may aggravate fatty infiltration in the rotator cuff muscles by inducing the differentiation of intramuscular fibroblasts into adipocytes via upregulation of the C/EBPα pathway.

In addition, CSs can increase forkhead/winged helix box (FOXO) gene expression, and it is known that FOXO overexpression can activate atrogin.[29] The activated atrogin expression, which is the representative gene for muscle atrophy, may aggravate rotator cuff muscle atrophy in patients who receive frequent local CS injections. This is supported by a study by Biedasek et al,[3] who demonstrated that CS increased proteolysis by increasing the expression of atrogin in a skeletal muscle.

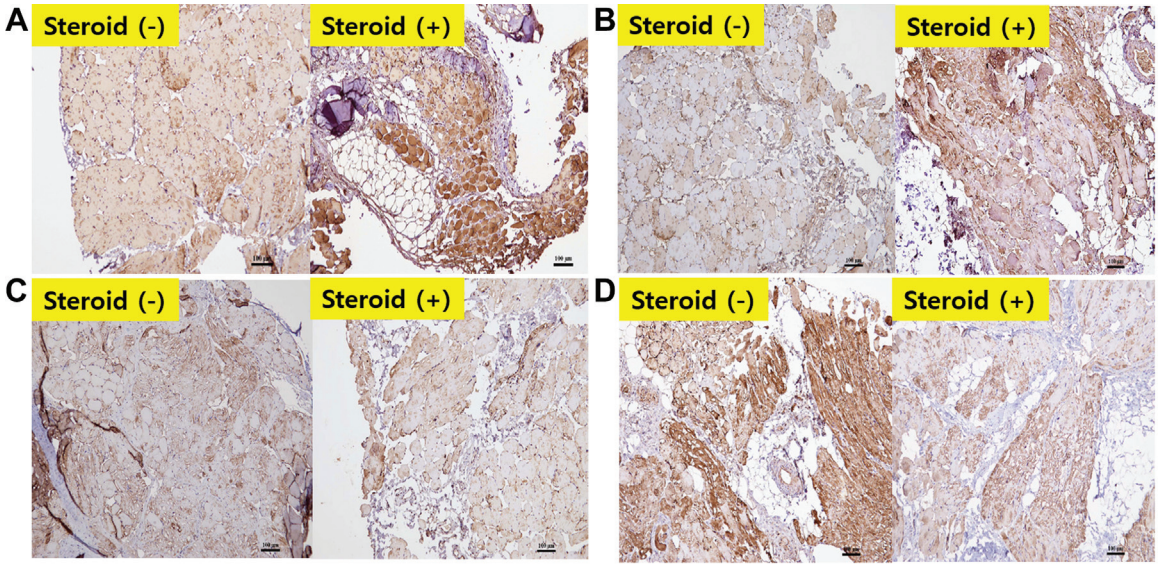

Figure 3. Protein expression in the supraspinatus muscle by immunohistochemistry. Scales bars = 100 μm. (A) C/EBPα, (B) atrogin, (C) MyoD, and (D) IL-6. Higher C/EBPα and atrogin expressions and lower MyoD and IL-6 expressions were observed in the steroid group compared with the no-steroid group. C/EBPα, CCAAT/enhancer-binding protein alpha; IL-6, interleukin 6; MyoD, myogenic differentiation 1.

For MyoD, which is a myogenesis-related transcription factor,[21] both the gene and protein expression levels decreased in the steroid group compared with the no-steroid group in this study. CSs are known to suppress MyoD expression through N-terminus ubiquitination and proteasome-dependent degradation of the transcription factor.[33] Moreover, the repression of MyoD expression by CSs could be exacerbated by the multiple actions of the insulin-like growth factor 1 pathway or mitogen-activated protein kinase kinase/extracellular signal-regulated kinase pathway, which is again affected by CSs.[8,24] As the inhibition of MyoD expression is related to skeletal muscle atrophy,[20] we can theorize that frequent local CS injections in the shoulder joint may induce rotator cuff muscle atrophy by downregulating MyoD expression.

The study results also demonstrated that the expression of inflammation-related mediators IL-6 and HMGB1 decreased in the steroid group compared with the no-steroid group. As CSs are well-known powerful anti-inflammatory agents,[9] the downregulation of IL-6 and HMGB1 seems natural. In addition to the proinflammatory action of IL-6 and HMGB1, IL-6 reportedly acts as an essential regulator of skeletal muscle hypertrophy, and HMGB1 is related to muscle function.[25,30] Thus, the altered downregulation of these inflammation-related genes may negatively affect the rotator cuff muscles.

The findings of the present study suggest that frequent local CS injections may influence the rotator cuff muscles in a multimodal manner. Specifically, local CS injections are associated with an increase in C/EBPα, which has the potential to exacerbate fatty infiltration; an increase in atrogin and a decrease in MyoD, which may contribute to muscle atrophy; and a decrease in IL-6 and HMGB1. These altered gene and protein expressions by frequent local CS injections may cause poor outcomes in patients with RCTs; thus, caution should be exercised when performing local CS injections in the shoulder joint, especially in patients who have a higher possibility of poor rotator cuff muscle quality, such as those with older age, larger tear size, or metabolic diseases.[7,16,18]

Limitations

This study has several limitations. First, it was limited by its small sample size, which restricted its statistical power. The lack of statistical significance may be attributed to the low power resulting from the relatively small number of cases, potentially leading to the reporting of false-negative results (type 2 error) in other genes and proteins. However, this was a pilot study, and we assume that the results of this study may serve as preliminary data. Second, the injection history was investigated based on the patient's statement for patients who underwent local CS injection at other hospitals. Although we reverified the local CS injection history by reviewing the medical records of the other hospitals, there could be an error in the history taking. In addition, we did not consider the exact dose of the CS injected or type of steroid given. However, we only confined the steroid group to those who received $\geq$5 injections; thus, we believe that the dose effect may not be enormous in this patient group with frequent steroid injections. In addition, as both intra-articular and subacromial injections can directly disseminate to the rotator cuff muscles in patients with RCTs, we do not anticipate the necessity of a separate analysis. Third, even though it was not statistically different and we confined the tear size to medium, there could be interindividual variations, and the

histopathologic status of muscle specimens collected from each group may have differed, which might have affected the outcomes. For example, the CS injection may have been performed in the more symptomatic patients who also had a bit more muscle degeneration. Nevertheless, we substantiated that frequent local CS injections exerted independent effects on the gene expression levels of C/EBPα, atrogin, and IL-6 through multivariate regression analysis. The study provided limited additional verification of this finding (Table 4).

As a fourth limitation, this study was a laboratory trial, and there may be a difference between statistical and clinical relevance. Fifth, to identify the potent molecule(s) associated with rotator cuff muscle physiology, we examined a limited number of molecules of interest. Various cytokines, transcription factors, and enzymes are involved in muscle physiology. We selected the most representative factors as candidate molecules to evaluate the deleterious effects of frequent CS injections. Sixth, we performed biopsies on the supraspinatus muscle approximately 1 cm from the musculotendinous junction; however, it is challenging to extrapolate that this specific region's analysis accurately represents the pathophysiology of all rotator cuff muscles. Finally, we did not investigate the molecular pathways involved in mediating the effect of frequent CS injections on myocytes. Future studies should address these limitations to identify other critical molecules affecting muscle physiology and elucidate the molecular mechanism underlying the frequent CS injection–induced process.

CONCLUSION

Patients with RCTs who had a history of frequent steroid injections exhibited an upregulation of adipogenic and muscle atrophy–related genes and proteins within the rotator cuff muscles. Conversely, there was a downregulation observed in the expression of myogenic and inflammatory genes and proteins in the same muscles.

REFERENCES

1. Arroll B, Goodyear-Smith F. Corticosteroid injections for painful shoulder: a meta-analysis. *Br J Gen Pract*. 2005;55(512):224-228.
2. Beiner JM, Jokl P, Cholewicki J, Panjabi MM. The effect of anabolic steroids and corticosteroids on healing of muscle contusion injury. *Am J Sports Med*. 1999;27(1):2-9.
3. Biedasek K, Andres J, Mai K, et al. Skeletal muscle 11beta-HSD1 controls glucocorticoid-induced proteolysis and expression of E3 ubiquitin ligases atrogin-1 and MuRF-1. *PLoS One*. 2011;6(1):e16674.
4. Blair B, Rokito AS, Cuomo F, Jarolem K, Zuckerman JD. Efficacy of injections of corticosteroids for subacromial impingement syndrome. *J Bone Joint Surg Am*. 1996;78(11):1685-1689.
5. Bytomski JR, Black D. Conservative treatment of rotator cuff injuries. *J Surg Orthop Adv*. 2006;15(3):126-131.
6. Chung SW, Kim JY, Kim MH, Kim SH, Oh JH. Arthroscopic repair of massive rotator cuff tears: outcome and analysis of factors associated with healing failure or poor postoperative function. *Am J Sports Med*. 2013;41(7):1674-1683.
7. Chung SW, Park H, Kwon J, Choe GY, Kim SH, Oh JH. Effect of hypercholesterolemia on fatty infiltration and quality of tendon-to-bone healing in a rabbit model of a chronic rotator cuff tear: electrophysiological, biomechanical, and histological analyses. *Am J Sports Med*. 2016;44(5):1153-1164.
8. Ciccarelli C, Marampon F, Scoglio A, et al. P21WAF1 expression induced by MEK/ERK pathway activation or inhibition correlates with growth arrest, myogenic differentiation and onco-phenotype reversal in rhabdomyosarcoma cells. *Mol Cancer*. 2005;4:41.
9. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol*. 2011;335(1):2-13.
10. Cunnington J, Marshall N, Hide G, et al. A randomized, double-blind, controlled study of ultrasound-guided corticosteroid injection into the joint of patients with inflammatory arthritis. *Arthritis Rheum*. 2010;62(7):1862-1869.
11. Dean BJ, Franklin SL, Carr AJ. A systematic review of the histological and molecular changes in rotator cuff disease. *Bone Joint Res*. 2012;1(7):158-166.
12. DeOrio JK, Cofield RH. Results of a second attempt at surgical repair of a failed initial rotator-cuff repair. *J Bone Joint Surg Am*. 1984;66(4):563-567.
13. Desai VS, Camp CL, Boddapati V, Dines JS, Brockmeier SF, Werner BC. Increasing numbers of shoulder corticosteroid injections within a year preoperatively may be associated with a higher rate of subsequent revision rotator cuff surgery. *Arthroscopy*. 2019;35(1):45-50.
14. Goutallier D, Postel JM, Bernageau J, Lavau L, Voisin MC. Fatty muscle degeneration in cuff ruptures. Pre- and postoperative evaluation by CT scan. *Clin Orthop Relat Res*. 1994;(304):78-83.
15. Kang JR, Gupta R. Mechanisms of fatty degeneration in massive rotator cuff tears. *J Shoulder Elbow Surg*. 2012;21(2):175-180.
16. Khazzam M, Sager B, Box HN, Wallace SB. The effect of age on risk of retear after rotator cuff repair: a systematic review and meta-analysis. *JSES Int*. 2020;4(3):625-631.
17. Kim D, Lee MS, Jo K, Lee KE, Hwang JK. Therapeutic potential of panduratin A, LKB1-dependent AMP-activated protein kinase stimulator, with activation of PPARα/δ for the treatment of obesity. *Diabetes Obes Metab*. 2011;13(7):584-593.
18. Kim HM, Dahiya N, Teefey SA, Keener JD, Galatz LM, Yamaguchi K. Relationship of tear size and location to fatty degeneration of the rotator cuff. *J Bone Joint Surg Am*. 2010;92(4):829-839.
19. Ladermann A, Piotton S, Abrassart S, Mazzolari A, Ibrahim M, Stirling P. Hydrodilatation with corticosteroids is the most effective conservative management for frozen shoulder. *Knee Surg Sports Traumatol Arthrosc*. 2021;29(8):2553-2563.
20. Lagirand-Cantaloube J, Cornille K, Csibi A, Batonnet-Pichon S, Leibovitch MP, Leibovitch SA. Inhibition of atrogin-1/MAFbx mediated MyoD proteolysis prevents skeletal muscle atrophy in vivo. *PLoS One*. 2009;4(3):e4973.
21. Montarras D, Chelly J, Bober E, et al. Developmental patterns in the expression of Myf5, MyoD, myogenin, and MRF4 during myogenesis. *New Biol*. 1991;3(6):592-600.
22. Nakamura H, Gotoh M, Kanazawa T, et al. Effects of corticosteroids and hyaluronic acid on torn rotator cuff tendons in vitro and in rats. *J Orthop Res*. 2015;33(10):1523-1530.
23. Naunton J, Harrison C, Britt H, Haines T, Malliaras P. General practice management of rotator cuff related shoulder pain: a reliance on ultrasound and injection guided care. *PLoS One*. 2020;15(1):e0227688.
24. Pansters NA, Langen RC, Wouters EF, Schols AM. Synergistic stimulation of myogenesis by glucocorticoid and IGF-I signaling. *J Appl Physiol (1985)*. 2013;114(9):1329-1339.
25. Pisetsky DS, Erlandsson-Harris H, Andersson U. High-mobility group box protein 1 (HMGB1): an alarmin mediating the pathogenesis of rheumatic disease. *Arthritis Res Ther*. 2008;10(3):209.
26. Puzzitiello RN, Patel BH, Forlenza EM, et al. Adverse impact of corticosteroids on rotator cuff tendon health and repair: a systematic review of basic science studies. *Arthrosc Sports Med Rehabil*. 2020;2(2):e161-e169.

27. Puzzitiello RN, Patel BH, Nwachukwu BU, et al. Adverse impact of corticosteroid injection on rotator cuff tendon health and repair: a systematic review. *Arthroscopy*. 2020;36(5):1468-1475.
28. Ramirez J, Pomés I, Cabrera S, Pomés J, Sanmartí R, Cañete JD. Incidence of full-thickness rotator cuff tear after subacromial corticosteroid injection: a 12-week prospective study. *Mod Rheumatol*. 2014;24(4):667-670.
29. Schakman O, Kalista S, Barbe C, Loumaye A, Thissen JP. Glucocorticoid-induced skeletal muscle atrophy. *Int J Biochem Cell Biol*. 2013;45(10):2163-2172.
30. Serrano AL, Baeza-Raja B, Perdiguero E, Jardi M, Munoz-Canoves P. Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab*. 2008;7(1):33-44.
31. Shin SJ, Do NH, Lee J, Ko YW. Efficacy of a subacromial corticosteroid injection for persistent pain after arthroscopic rotator cuff repair. *Am J Sports Med*. 2016;44(9):2231-2236.
32. Skedros JG, Adondakis MG, Knight AN, Pilkington MB. Frequency of shoulder corticosteroid injections for pain and stiffness after shoulder surgery and their potential to enhance outcomes with physiotherapy: a retrospective study. *Pain Ther*. 2017;6(1):45-60.
33. Sun L, Trausch-Azar JS, Muglia LJ, Schwartz AL. Glucocorticoids differentially regulate degradation of MyoD and Id1 by N-terminal ubiquitination to promote muscle protein catabolism. *Proc Natl Acad Sci U S A*. 2008;105(9):3339-3344.
34. Wagatsuma A. Upregulation of gene encoding adipogenic transcriptional factors C/EBPalpha and PPARgamma2 in denervated muscle. *Exp Physiol*. 2006;91(4):747-753.
35. Wang X, Khan R, Raza SHA, et al. Molecular characterization of ABHD5 gene promoter in intramuscular preadipocytes of Qinchuan cattle: roles of Evi1 and C/EBPalpha. *Gene*. 2019;690:38-47.
36. Weber AE, Trasolini NA, Mayer EN, et al. Injections prior to rotator cuff repair are associated with increased rotator cuff revision rates. *Arthroscopy*. 2019;35(3):717-724.
37. Wei AS, Callaci JJ, Juknelis D, et al. The effect of corticosteroid on collagen expression in injured rotator cuff tendon. *J Bone Joint Surg Am*. 2006;88(6):1331-1338.
38. Yeh WC, Cao Z, Classon M, McKnight SL. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev*. 1995;9(2):168-181.