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The biologic effect of selective serotonin reuptake inhibitor for rotator cuff tear healing in rat model

동물모델에서 선택적 세로토닌 재흡수 억제제인 Sertaline이 회전근 개 건골 봉합부위의 조직 재생에 미치는 영향분석

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The biologic effect of selective serotonin reuptake inhibitor for rotator cuff tear healing in rat model

by

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Abstract

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BACKGROUND Selective serotonin reuptake inhibitors (SSRIs) are believed to accelerate wound healing and are thus expected to have a positive effect on rotator cuff repair. We hypothesized that SSRI has a positive effect on the healing of the bone–tendon interface (BTI). Improved rotator cuff tear healing was confirmed via mechanical strength measurements and histological assessment of the restored tendon.

METHODS Forty male adult Sprague–Dawley wild-type rats were used in this study. The animals were divided into two groups: group-SSRI (supraspinatus [SSP] repair with SSRI injection) and group-C (conventional SSP repair only without SSRI injection). Biomechanical and histological analyses were performed 8 weeks after the index rotator cuff surgery.

RESULTS The ultimate load (N) was significantly higher in group-SSRI than in group-C (54.8 ± 56.9 vs 25.1 ± 11.1, P = 0.031). In the histological evaluation, the Bonar score confirmed significant differences in collagen fiber density (group-C: 0.1 ± 0.6, group-SSRI: 0.6 ± 0.5, P = 0.024), vascularity (group-C: 0.1 ± 0.2, group-SSRI: 0.3 ± 0.4, P = 0.024), and cellularity (group-C: 1.7 ± 0.4, group-SSRI: 2.0 ± 0.0, P = 0.023) between the groups. Based on the total score, group-SSRI was found significantly better than group-C (6.3 ± 2.7 vs 4.3 ± 1.9, P = 0.019).

CONCLUSION Our model showed improved biomechanical and histological outcomes of healing in group-SSRI 8 weeks after rotator cuff repair, suggesting that SSRI improved healing and facilitate rotator cuff repair.

Keyword : Serotonin uptake inhibitors; Anti-inflammatory agents; Histology; Biomechanics; Rotator cuff healing

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I. Introduction

As the incidence of rotator cuff tears (RCTs) increases due to aging and sporting activities, it is hoped that the inflicted injury can be repaired through surgical treatment. However, despite the implementation of novel surgical interventions for rotator cuff repair, failure rates remain quite high [1]. In addition, the stress applied to the patient before and after surgery reduces clinical success.

Serotonin (5-hydroxytryptamine, 5-HT) is a well-known mood modulator that is strongly associated with the etiology of depression [2]. Based on the role of 5-HT, selective serotonin reuptake inhibitors (SSRIs) are used as first-line treatment for major depressive disorder (MDD) [3]. The major function of SSRIs is to increase the amount of serotonin in the synaptic cleft by downregulating the expression of post-synaptic beta and serotonin receptors in the brain [4, 5]. A study has suggested that innate immune activation during chronic medical illnesses (e.g., cancer, heart disease, type 2 diabetes mellitus [DM], and autoimmune disorders), which are characterized by elevated levels of inflammatory cytokines, may contribute to high rates of depression [6]. Another recent study has shown that adult patients with MDD have elevated levels of proinflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α) and decreased levels of anti-inflammatory cytokines (IL-4 and IL-10), leading to an imbalance in inflammatory factors [7]. In particular, antidepressant treatment can significantly reduce IL-6 and TNF-α levels [8]. Many studies

have proven the effectiveness of SSRIs in treating depression, and it is believed that a link exists between SSRIs and certain inflammatory cytokines that are involved in depression. SSRIs exhibit anti-inflammatory effects by significantly decreasing the levels of proinflammatory cytokines, including IL-1β, IL-6, and TNF-α, and by significantly increasing the levels of the anti-inflammatory cytokine IL-10 [9, 10]. However, no study has considered the administration of SSRIs for rotator cuff tendon-to-bone healing. This study aimed to investigate the effect of SSRI on bone–tendon interface (BTI) healing following rotator cuff repair in a rat model. We hypothesized that SSRIs have a positive effect on BTI healing. The mechanical strength and histological findings of the restored tendon support the anticipated beneficial effects.

II. Materials and methods

A. Animal model

All animal procedures were approved by the institutional animal commission at the author's institution. The G*Power software, version 3.01 (Franz Faul, Christian-Albrechts-Universität Kiel, Kiel, Germany) was used to calculate the sample size required for the comparison between two independent means in a 1:1 allocation ratio. Based on previous studies that used power analysis, it was determined that 20 specimens per group

(comparison between two groups, α error = 0.05, power = 0.95, dropout rate = 20%) were required for the detection of significant differences in the ultimate fracture load [11]. We used 12-week-old male Sprague–Dawley rats that were housed in a specific pathogen-free facility. Prior to the experiment, the rats were acclimated to a 12/12-h light/dark cycle at 22 ± 2 °C for 1 week and were allowed unlimited access to food and water. The right shoulder of the rats was used for subsequent biomechanical evaluation, including assessment of the ultimate failure load at 8 weeks, and the left shoulder was used for histological analyses. Forty rats were randomly allocated to two groups (n = 20 rats/group): group-C (repair only; control) and group-SSRI (SSRI treatment group).

B. Surgical procedure

All animals were anesthetized using zolazepam (0.05-0.3 ml/kg, Zoletil®, Virbac S.A., Carros Cedex, France) and xylazine hydrochloride (0.15 ml/kg, Rompun®, Bayer HealthCare, Germany). This offered pain relief and muscle relaxation, which helped the animals maintain their sleep state. The right shoulder of each rat was shaved and sterilized to maintain aseptic conditions. Consequently, rotator cuff incision was performed. Briefly, the scapular spine was palpated, and a 3-cm longitudinal incision was made. The deltoid muscle was divided bluntly, and the supraspinatus tendon was confirmed. The supraspinatus tendon was dissected using metzembaum and cut at the end of the tendon insertion site using a blade. The greater tuberosity was widely

exposed, and two parallel bone tunnels were made using a drill. The supraspinatus tendon was repaired using a single-row technique through the tunnel using 3-0 Ethibond (Ethicon, Somerville, NJ, USA) [11] (Fig. 1). Rats in the control group underwent surgical repair and had no other interventions. To the experimental group rats, sertraline was injected intraperitoneally after the repair. Sertraline is the most commonly used SSRI-class drug along with fluoxetine and paroxetine; it is known to have a greater dose effect than the other drugs. Sertraline (Hanmi Pharm. Co., Suwon-si, South Korea) was prepared at a dose of 10 mg/kg/day and intraperitoneally injected at 0.2 ml each time for a period of 2 weeks (five times/week). The drug dose and regimen were based on the existing rat model experiments using SSRI [12–14]. Eight weeks after repair, all rats were sacrificed, and tendon tissue was harvested.

C. Biomechanical evaluation

After sacrificing, supraspinatus tendons of the right shoulder were harvested. With the aid of a digital micrometer (MDC-25SB; Mitutoyo Co., Kanagawa, Japan), the cross-sectional area was evaluated at the mid-portion of the supraspinatus tendon using optical methods, and its width was calculated [15]. Load to failure, mode of failure (insertional tear or mid-substance tear), and ultimate stress were measured at a rate of 10 mm/min with a 20-kg load cell using a universal test machine (OTT-03; Oriental TM, Siheung-si, South Korea), and a custom fixture clamping system was included

for the mechanical evaluation parameters. An insertional tear suggests relatively weak tendon-bone healing, whereas a mid-substance tear suggests strong tendon-bone healing [16]. Allowing the supraspinatus tendon to be fixed to this system along its anatomic direction led to tensile loading and a tendon-to-bone interface, consequently forming a right angle. A personal computer-based data acquisition system was employed for automated mechanical data collection [11] (Fig. 2).

D. Histological evaluation

All specimens from each group were histologically analyzed to determine the extent of regeneration. The left shoulder of each rat was harvested. Specimens were fixed in neutral-buffered 10% formalin (pH 7.4), and paraffin blocks were prepared. These were cut into 4-μm thick sections and then deparaffinized and rehydrated [17]. Sample slides were randomly selected and stained with hematoxylin and eosin (H&E), Picro Sirius red, and Safranin-O. The analyses were performed by two investigators who were blinded to the animal groups. Bonar scoring with an H&E-stained slide was used for histological assessment [11, 18]. The score was set from 0–3, with 0 representing the worst result; the greater the healing observed, the higher the score. The slides were interpreted using a scoring scale, and various features of the tendon tissue were evaluated. The whole slide was used to assess areas of increased cellularity and vascularity, the proportion of collagen fibers, and the level of maturation of the BTI structure. The scored items included (1)

continuity of collagen fibers, (2) orientation of collagen fibers, (3) density of collagen fibers, (4) maturation of the tendon-to-bone interface structure, (5) vascularity, and (6) cellularity. The four-point scoring system was applied again. The histological findings for each of these items were graded semi-quantitatively into four stages (grade [G] 0, 1, 2, and 3), where G0 indicated the poorest ruptured tendon appearance, G1 indicated a poorer appearance, G2 indicated a better appearance, and G3 indicated a noticeably regenerated appearance. Overall, the total score varied between 0 (ruptured tendon) and 18 (the greatest degree of regeneration).

With regard to the collagen fiber continuity and parallel collagen fiber orientation, the stages were divided using a percentage value: < 1/4 proportion (grade 0), 1/4–1/2 proportion (grade 1), 1/2–3/4 proportion (grade 2), and > 3/4 proportion (grade 3). The collagen fiber density was graded as very loose (grade 0), loose (grade 1), dense (grade 2), or very dense (grade 3). Each tissue slide was photographed under a microscope equipped with a LAS V4.8 software imaging system (Leica DM IL LED; Leica Microsystems, Wetzlar, Germany). Three observations were made at the rotator cuff tissue at the same location and of the same area at 40× magnification [19]. In addition, the area of discoloration with Safranin-O staining was measured and quantified (in mm^2) using ImageJ (National Institutes of Health, Bethesda, MD, USA) [20]. All images were obtained using the same illumination and magnification parameters. After a photomicrograph was captured, 8-bit digitization was performed using ImageJ. Images were converted to black and white and

processed using the same thresholds set for areas of metachromasia. The areas of metachromasia within the standardized field were measured automatically [20].

E. Statistical analysis

All statistical analyses were performed using the SPSS software (version 12.0; SPSS Inc., Chicago, IL, USA), and statistical significance was set at *P*-value < 0.05. The chi-square test, followed by Student's t-test, was used to evaluate biomechanical and histological differences between the groups. Data are presented as mean and standard deviation.

III. Results

A. Gross inspection and biomechanical evaluation

In total, we evaluated 20 rats per group. No evidence of re-tear was observed in any of the experimental groups. A comparison of failure modes after supraspinatus repair, bone-to-tendon failure, and mid-substance failure did not indicate any significant differences between the groups ($P = 0.736$) (Table 1). Biomechanical evaluation showed that the ultimate load (N) was significantly higher in group-SSRI (54.8 ± 56.9) than in group-C (25.1 ± 11.1) ($P = 0.031$). However, there were no significant differences in the cross-sectional area (mm^2) and ultimate stress between the groups ($P = 0.932$ and

0.249, respectively) (Table 2).

B. Histological evaluation

Histologically, group-SSRI showed an organized BTI structure with higher collagen fiber density and vascularity than group-C. Furthermore, group-SSRI (0.1 ± 0.6) demonstrated a higher density of collagen fibers than group-C (0.6 ± 0.5; P = 0.024). However, there were no differences in collagen fiber continuity (P = 0.130) or collagen fiber orientation (P = 0.108) between the groups. Group-SSRI exhibited significantly increased vascularity and cellularity than group-C (P = 0.024 and 0.023, respectively). However, no differences were observed in tendon-to-bone maturation (P = 0.449). Group-SSRI (6.3 ± 2.7) had a significantly higher total score than group-C (4.3 ± 1.9) (P = 0.019) (Fig. 3; Table 3). We measured the areas of metachromasia using Safranin-O staining to confirm fibrocartilage at the tendon-to-bone junction. Metachromasia in group-SSRI (0.2 ± 0.1, P = 0.000) was stronger than in group-C (0.1 ± 0.0) (Table 4).

IV. Discussion

The primary finding was that the SSRI enhanced rotator cuff healing in the rat model 8 weeks postoperatively, as confirmed by biomechanical and histological examinations. The ultimate load was verified by biomechanical characteristics, and the group treated with SSRIs showed better healing than the control group. Furthermore, histological comparisons revealed that the collagen fiber density, vascularity, and cellularity were markedly better in group-SSRI than in group-C.

A nationwide, population-based, retrospective study revealed that patients with depression had a significantly higher risk of RCT, thus requiring subsequent repair surgery, compared with patients without depression [21]. However, the mechanism of action associated with depression in RCT remains unclear. We speculate that SSRIs play an essential role in the healing process of RCT through a mechanism that inhibits postsurgical, stress-mediated increase in the expression of proinflammatory cytokines and glucocorticoids. Identification of these mechanisms will be of assistance in improving postoperative outcomes in patients with emotional disorders, including depression.

SSRIs have anti-inflammatory effects; they act by significantly decreasing the levels of proinflammatory cytokines, including IL-1β, IL-2, IL-6, TNF-α, and IFN-γ, and by significantly increasing the levels of the anti-

inflammatory cytokines IL-4 and IL-10 [7, 8, 22]. However, IL-6 displays pleiotropic biological effects owing to its pro- and anti-inflammatory properties. Therefore, IL-6 appears to signal differently via different receptors. IL-6-knockout mouse experiments have demonstrated that IL-6 is involved in tendon healing; IL-6 also promotes angiogenesis through vascular endothelial growth factor (VEGF) expression [23, 24]. Studies have highlighted the role of different cytokines in the tendons. IL-1β and TNF-α strongly stimulate tenocytes to upregulate the expression of pro- and anti-inflammatory cytokines, such as IL-1β, TNF-α, IL-6, and IL-10, through auto/paracrine actions. They induce matrix metalloproteinases, which in turn promote the degradation of the tendon extracellular matrix, resulting in the loss of biomechanical properties of the tendon [25]. In the current study, SSRIs could regulate cytokine levels, affect collagen synthesis, and eventually influence the rotator cuff healing process.

Another possible mechanism for the improvement in rotator cuff healing may be related to the stress response to the surgery. SSRIs may help to limit the adverse effects of steroids by lowering the systemic steroid concentration and reducing the expression of proinflammatory cytokines. The stress response is related to the hypothalamic–pituitary–adrenal (HPA) axis. The endocrine response that is activated at the surgical site is transmitted to the hypothalamus via the spinal cord and medulla [26]. Corticotropin-releasing hormone (CRH) released from the hypothalamus stimulates adrenocorticotropic hormone (ACTH) secretion in the anterior pituitary.

Cortisol—a stress hormone—is produced by the adrenal glands in response to ACTH stimulation. The HPA axis is regulated by a negative feedback mechanism, through which cortisol inhibits CRH and ACTH release [27]. RCT surgery, including acromioplasty, is a painful procedure. In addition, the continuous wearing of braces after surgery, which limits daily life and social activities, causes great stress to patients. Chronic exposure to postoperative stress may cause HPA axis abnormalities, which may not adequately reduce elevated cortisol levels through negative feedback mechanisms [28]. Continuous stress after surgery is believed to influence the release of proinflammatory cytokines and the control of negative feedback, thus causing hypercortisolemia due to hyperactivity of the HPA axis. In addition, glucocorticoids prevent angiogenesis by suppressing VEGF, decreasing the production of factors necessary for healing, such as insulin-like growth factor and transforming growth factor, and suppressing collagen production [29-31]. However, SSRIs also improve microvascular circulation by affecting thrombocytes and increasing nitric oxide (NO) synthesis and angiogenesis [32]. The latter point is expected to have a positive effect on rotator cuff repair.

The injection site of SSRIs is an important issue. In this study, a systemic subcutaneous injection method was used; however, direct injection into the rotator cuff repair site for tissue regeneration may result in better biological healing results. Although this paracrine effect may lead to better mechanical results, further research is needed to determine whether it is better to relieve the postsurgical depressive state using systemic administration.

Although our study is the first to analyze the effect of SSRI on tendon-bone healing after rotator cuff repair, there are some limitations to the interpretation of the results. First, there are some limitations to the animal studies. The different anatomical features of humans and rats and the mechanisms of the healing response are limited in explaining the process in humans. In addition, we have not been able to directly evaluate the signaling factors using immunohistochemistry, which is necessary to test whether our hypothesis and suggested mechanisms are correct. Second, there are limitations in the study period and experimental design. The evaluation period of 8 weeks does not consider possible future events and may not be sufficient for the final prognosis. Although a common RCT is a chronic disease, our experimental design only reflected acute injury. Third, the histological evaluation was semiquantitative. We did not use a histological system specific to tendon-bone healing; we used a modified Bonar scoring system for the quantitative evaluation. In addition, we attempted to quantitatively evaluate the collagen fiber arrangement, continuity, and maturity. All slides were reviewed by a blinded pathologist and graded using a standard scale. There are several methods than can be used to evaluate the maturity of BTI. Metachromasia is a measure of the degree of proteoglycan in Safranin-O staining and is used as an indicator of maturity at the BTI [20]. We obtained good results by evaluating BTI maturity using metachromasia evaluation, but it has a limitation as this is not a quantifiable measure. Analyzing cytokines, such as FGF-2, VEGF, and BMP-2, using immunohistochemistry may be

helpful in evaluating the maturity of BTI. Finally, our study confirmed that there was no significant difference in failure mode. In recent animal biomechanical experiments, more precise measurements have been attempted using a strain gauge and optical tracker [33]. We used a conventional measurement system in the current study. Using recently developed precision measuring equipment may have yielded more accurate results.

V. Conclusion

Our study demonstrated that SSRIs can facilitate improved biomechanical and histological outcomes 8 weeks after rotator cuff repair in a rat model. Consequently, SSRIs may improve healing after rotator cuff repair.

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Table Legends

Table 1. Comparison of failure mode 8 weeks after supraspinatus repair in a rat model with and without systemic selective serotonin reuptake inhibitor (SSRI) administration at the time of surgery

	Group-C	Group-SSRI	*P*-value
Bone-to-tendon failure	14	13	0.736
Mid-substance failure	6	7	

*Statistically significant difference between groups ($P < 0.05$); group-SSRI, the supraspinatus (SSP) repair with systemic SSRI injection; group-C, conventional supraspinatus repair without systemic SSRI injection

Table 2. Comparison of the biomechanical characteristics of tendon repair 8 weeks after rotator cuff surgery in a rat model with and without systemic selective serotonin reuptake inhibitor (SSRI) administration at the time of surgery

	Group-C	Group-SSRI	*P*-value
Cross-section area (mm^2)	6.3 ± 2.0	6.4 ± 1.9	0.932
Ultimate load (N)	25.1 ± 11.1	54.8 ± 56.9	0.031*
Ultimate stress (MPa)	5.6 ± 6.7	8.4 ± 5.5	0.249

*Statistically significant difference between groups ($P < 0.05$); group-SSRI, the supraspinatus (SSP) repair with systemic SSRI injection; group-C, conventional supraspinatus repair without systemic SSRI injection

Table 3. Bonar score-based histological assessment of tendon repair 8 weeks after rotator cuff surgery in a rat model with and without systemic selective serotonin reuptake inhibitor (SSRI) administration at the time of surgery

	Group-C	Group-SSRI	*P*-value
Collagen fiber continuity	0.3 ± 0.4	0.6 ± 0.8	0.130
Collagen fiber orientation	0.4 ± 0.6	0.8 ± 0.8	0.108
Collagen fiber density	0.6 ± 0.5	1.1 ± 0.6	0.024*
Tendon-to-bone maturation	1.1 ± 0.5	1.3 ± 0.6	0.449
Vascularity	0.1 ± 0.2	0.3 ± 0.4	0.024*
Cellularity	1.7 ± 0.4	2.0 ± 0.0	0.023*
Total score	4.3 ± 1.9	6.3 ± 2.7	0.019*

*Statistically significant difference between groups ($P < 0.05$); group-SSRI, the supraspinatus (SSP) repair with systemic SSRI injection; group-C, conventional supraspinatus repair without systemic SSRI injection

Table 4 Comparison of the areas of metachromasia 8 weeks after rotator cuff surgery in a rat model with and without systemic selective serotonin reuptake inhibitor (SSRI) administration at the time of surgery

	Group-C	Group-SSRI	*P*-value
Area of metachromasia, mm^2/mm^2	0.1 ± 0.0	0.2 ± 0.1	0.000*

*Statistically significant difference between groups; group-SSRI, the supraspinatus (SSP) repair with systemic SSRI injection; group-C, conventional supraspinatus repair without systemic SSRI injection

Figure Legends

Fig. 1 Rotator cuff tear (RCT) surgical procedure in a rat model. **A–D** Establishment of the RCT model. **E–H** Reattachment of the supraspinatus tendon.

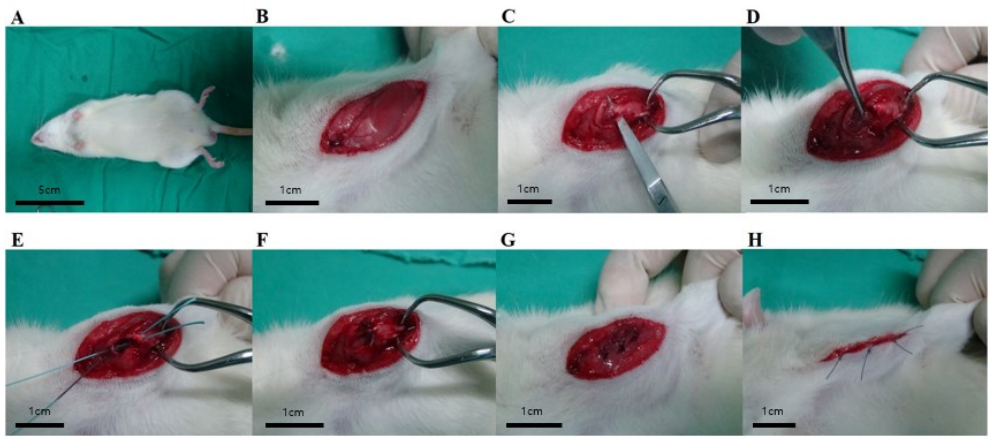


Fig. 2 Biomechanical evaluation. **A** Mode of failure, load to failure, and ultimate stress measurements. **B** Cross-sectional area measurements of the supraspinatus tendon.

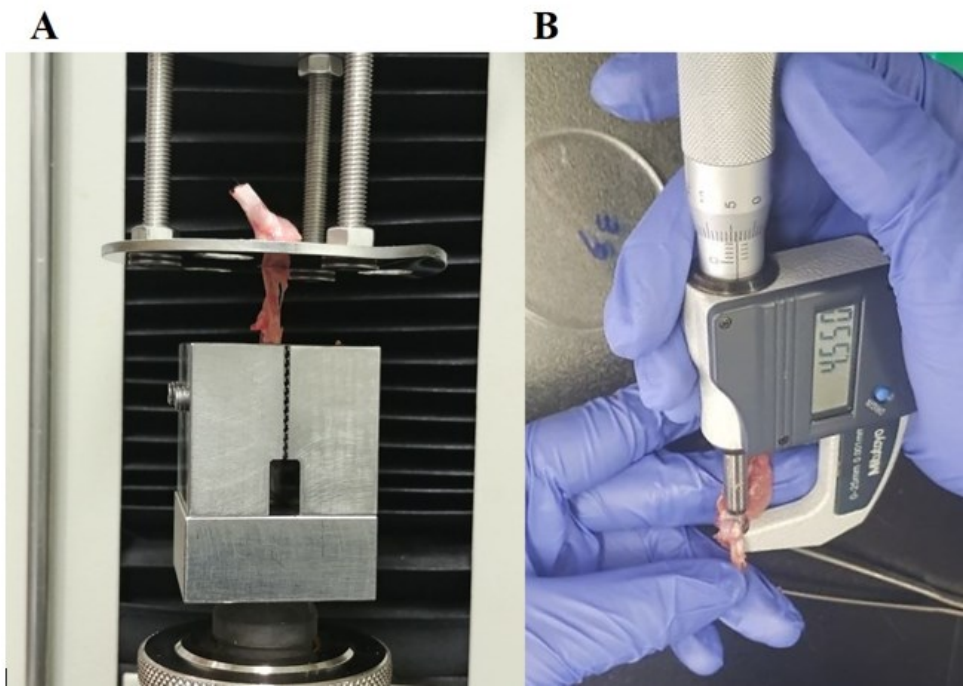
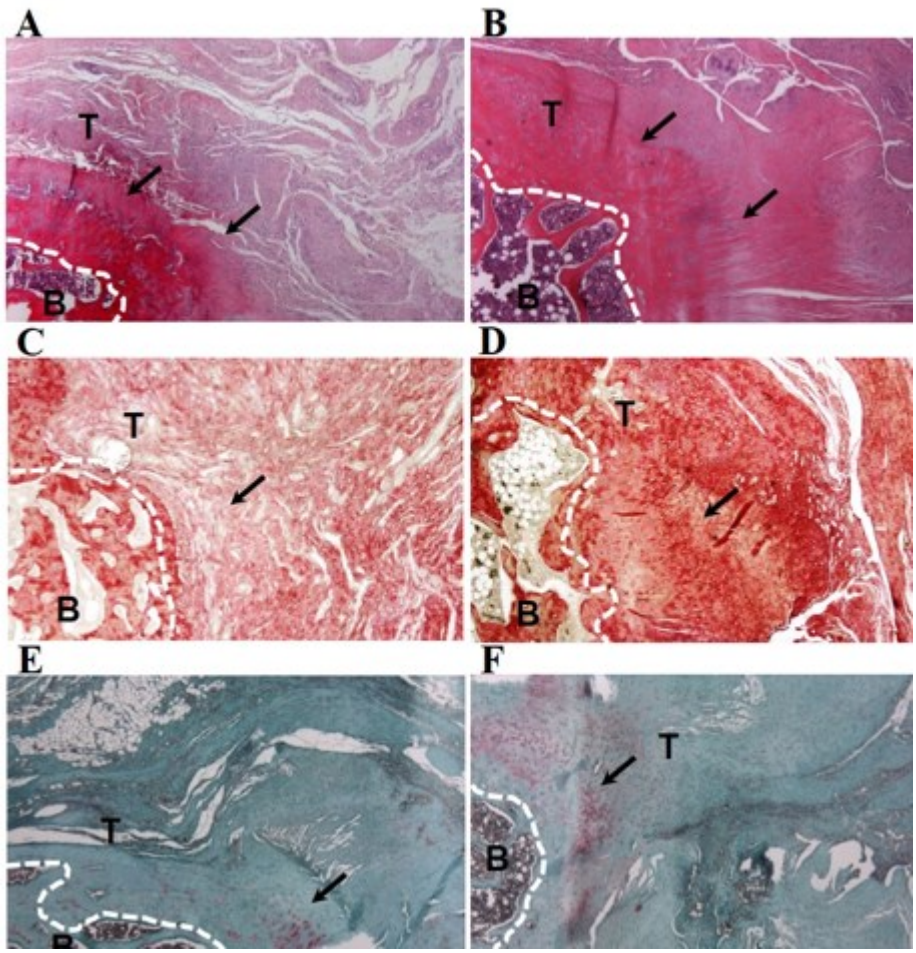


Fig. 3 Hematoxylin and eosin (H&E), Picro Sirius red, and Safranin-O staining of the supraspinatus (SSP) tendon repair site. **A, B** H&E staining after 8 weeks of selective serotonin reuptake inhibitor (SSRI) treatment. **C, D** Picro Sirius red staining after 8 weeks of SSRI treatment. E, F Safranin-O staining after 8 weeks of SSRI treatment. Arrows indicate the collagen feature in each group; B, bone; T, tendon (magnification 40×); group-SSRI, SSP repair with systemic SSRI injection; group-C, conventional SSP repair without systemic SSRI injection



국문초록

동물모델에서 선택적 세로토닌 재흡수 억제제인 Sertaline이 회전근 개 건골 봉합부위의 조직 재생에 미치는 영향분석

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서론: 선택적 세로토닌 재흡수 억제제(Selective serotonin reuptake inhibitor, SSRI)는 상처 치유를 촉진하는 것으로 알려져 있어 회전근 개 파열의 회복에 긍정적인 영향을 미칠 것으로 기대된다. 본 연구에서는 SSRI가 BTI(Bone-Tendon Interface)의 치유에 긍정적인 영향을 미치고 이로 인해 회전근 개 파열의 치유과정에서 생역학 및 조직학적으로 더욱 우수한 결과를 보일 것이라는 가설을 세웠다.

재료 및 방법: 본 연구는 40마리의 성체 수컷 Sprague-Dawley 쥐를 사용하여, 두 그룹의 실험을 시행하였다. SSRI군 (극상건 (SSP) 봉합 후 SSRI 주사를 사용한 그룹) 및 대조군 (SSRI 주사없이 통상적인 극상건 봉합만 시행한 그룹)에 각각 20마리씩 배정한 뒤, 각 개체에 대해서 수술 후 8주에 생역학 및 조직학적 분석을 시행하였다.

결과: 생역학적 분석에서 극한하중(N)은 대조군보다 SSRI군에서 유의하게 높았다(54.8 ± 56.9 Vs 25.1 ± 11.1, P = .031). 조직학적

분석에서 Bonar score는 콜라겐 섬유 밀도(대조군: 0.1 ± 0.6, SSRI군: 0.6 ± 0.5, P = .024), 혈관형성(대조군: 0.1 ± 0.2, SSRI군: 0.3 ± 0.4, P = .024) 및 세포생성(대조군: 1.7 ± 0.4, SSRI군: 2.0 ± 0.0, P = .023) 모두 유의하게 SSRI군에서 높은 결과를 보였다. 총점 기준으로 SSRI군이 대조군에 비해서 유의하게 우수하였다(6.3±2.7 Vs 4.3±1.9, P=.019).

결론: 본 연구는 SSRI 치료군이 회전근 개 봉합술 후 8주째 시행한 생역학 및 조직학적 분석 결과에서 더욱 우수한 결과를 보여주었으며, 따라서 SSRI는 봉합 부위에서 일어나는 치유 과정을 개선하고 회전근 개의 복원을 촉진할 수 있을 것으로 기대된다.

색인 단어: 어깨 통증, 회전근 개, 건-골 접합부, 항염증, 조직학, 생역학

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