EXPERIMENTAL STUDY

Investigation of the effects of umbilical cord-derived mesenchymal stem cells and curcumin on Achilles tendon healing – can they act synergistically?

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ABSTRACT

BACKGROUND: It is known that curcumin and umbilical cord-derived mesenchymal stem cells (UC-MSCs) positively affect experimental tendon injury healing. This study investigated individual effects and potential synergistic effects of using curcumin and UC-MSCs alone and together.

METHODS: Eighty female Wistar albino rats were randomly divided into five groups: Control, curcumin, sesame oil, MSCs, and Curcumin+MSCs groups. In all rats, punch tendon defect was created in both right and left Achilles tendons. While no additional treatment was applied to the control group, curcumin, sesame oil used as a solvent for curcumin, MSCs, and MSCs and curcumin combination were applied locally to the injury site, respectively, in the other groups. Curcumin was solved in sesame oil before application. In each group, half of the animals were euthanized in the post-operative 2nd week while the other half were euthanized in the post-operative 4th week. The right Achilles was used for biomechanical testing, while the left Achilles was used for histological evaluation and immunohistochemical analysis of type I, Type III collagen, and tenomodulin.

RESULTS: Histologically, significant improvement was observed in the curcumin, MSCs, and Curcumin+ MSCs groups compared to the control Group in the 2nd week. In the 2nd and 4th weeks, Type III collagen was significantly increased in the curcumin group compared to the control group. In week 4, tenomodulin increased significantly in the curcumin and MSCs groups compared to the control group. Tendon tensile strength increased significantly in MSCs and Curcumin+MSCs groups compared to the control group in the 4th week. No superiority was observed between the treatment groups regarding their positive effects on recovery.

CONCLUSION: Locally used curcumin and UC-MSCs showed positive effects that were not superior to each other in the healing of injury caused by a punch in the Achilles tendons of rats. However, synergistic effects on healing were not observed when they were applied together.

Keywords: Achilles tendon rupture; curcumin; mesenchymal stem cell; umbilical cord.

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INTRODUCTION

Tendons are commonly injured during personal or professional sportive activities.[1] Challenge is experienced during the treatment of tendon injuries due to their poor healing nature. Moreover, recent approaches to managing tendon injuries are insufficient for complete recovery. For this reason, interventions improving tendon healing have been investigated. For instance, various mechanisms have been tried to create a suitable environment for tendon regeneration by modulating inflammation in the early stages of tendon healing.[2]

In recent years, experimental studies have widely used herbal extracts to enhance healing after tissue injury. Naturally occurring substances found in the spice turmeric and curcumin have also gained widespread use to improve injured tissue repair.[3] The growing popularity of curcumin is bound up with its several properties, including anti-inflammatory and antioxidant behavior.[3,4] Based on the positive effects of anti-inflammatory and antioxidant therapies on tendon healing, curcumin has also been used in several tendon regeneration studies, and its beneficial effects have been demonstrated.[5-8] These beneficial effects were attributed to curcumin's anti-inflammatory, ROS-reducing, chondrogenetic properties, a pleiotropic agent, and its ability to reduce matrix degradation and improve collagen organization.[5]

As self-regenerative and multipotent cells, MSCs differentiate into mesodermal tissues, including tendons. These cells exert their effect by homing to the target site and revealing the ability to reconstitute the injured tissue and also provide modulation of the immune system that regulates inflammatory response,[9] which are mechanisms pivotal for a shift from pro-fibrotic tendon repair to tissue regeneration. Besides differentiation, MCSs also exert their regenerative effects by secreting paracrine factors such as growth factors and cytokines.[10] Therefore, MSCs have been studied for musculoskeletal tissue engineering and regeneration, including regenerative approaches for tendon healing.

There are some studies on the concomitant use of substances whose synergistic effects are expected in tendon regeneration studies.[5] These available and affordable substances combined include MSCs and traditional herbal extracts such as curcumin. A study has shown that the coadministration of curcumin and stem cell therapy synergistically positively affects severe experimental spinal cord injury.[11] Another study demonstrated the synergistic effects of curcumin and MSCs on tissue damage healing.[12,13] This synergistic effect can be attributed to the protective effects of curcumin on stem cell proliferation, differentiation, and senescence.[14]

Considering the above information, this study was conducted with the hypothesis that curcumin and MSCs would have positive and synergistic effects on tendon healing.

MATERIALS AND METHODS

This work was supported by the Scientific Research Project Coordination Unit of Erciyes University (Project number: TTU-2020-9689).

Umbilical cord-derived mesenchymal stem cells (UC-MSCs)

In the study, UC-MSCs, which were isolated from the umbilical cord of volunteers and characterized by immunofluorescence staining and flow cytometry, as specified in the study of Ülger et al.,[15] were used with the approval of the Erciyes University Clinical Research Ethics Committee dated April 4, 2018, and numbered 2018/182. In the second passage, UC-MSCs that highly express positive markers that should be found in MSCs in flow cytometry but do not express negative markers show adipogenic and osteogenic differentiation by immunofluorescence staining were kept in a nitrogen tank at −196°C to be used in the study. Afterward, these cells were prepared for culture and incubated. In the sixth passage, enough were reached and used.

Study Design

Eighty adult female Wistar albino rats (weight 300–350 g) obtained from the Experimental Research and Application Center of Erciyes University (Kayseri, Türkiye) were used in this study. All animal experiments were approved by the Erciyes University Local Ethics Committee for Animal Experiments (May 15, 2019-19/114). The rats were anesthetized by intraperitoneal injection of xylazine (5 mg/kg) and ketamine (50 mg/kg). The hind limbs of rats were shaved and cleaned with betadine. Surgery was performed under aseptic conditions. A longitudinal midline incision of approximately 2 cm was made on the lower limb to expose the Achilles tendon in each rat's lower limbs. A 1-mm diameter, full-thickness window defect on the Achilles tendon was created using a 1-mm diameter punch designed for this study. After the punch injury was made, local treatment was applied around the wound site following the treatment group, and the wound was immediately closed by skin sutures. According to treatment methods, rats were divided into five groups, with 16 rats for each group as follows:

- Control Group: Rats received no treatment after the punch injury.
- Sesame Oil Group: Rats received 0.05 mL sesame oil for each Achilles tendon after the punch injury.
- Curcumin Group: Curcumin (Curcumin for synthesis, Merck-Millipore) dissolved in 0.05 mL sesame oil (Karden-Susam yağı) at a dose of 0.22 mg/kg was injected into each rat's Achilles tendon before skin suturation. The dose of curcumin was determined based on the study of Zhang et al.[8]
- MSC Group: In this group, 6.8 × 105/0.1 mL MSCs[16] were delivered to the injured area for each Achilles tendon after the punch injury.
- Curcumin+MSC Group: Curcumin and MSCs were applied to each rat's Achilles tendon as described above.

After surgery, no dressing was applied, and the operated limbs were not immobilized. Rats were allowed to access food and water ad lib. In the Curcumin+MSC Group, one animal died for unknown reasons.

Tendon Harvesting

In each group, half of the animals were euthanized at post-operative 2 weeks, and the other half were euthanized at post-operative 4 weeks. At both time points, after sacrification, ensured isolation of the plantaris tendon and punch injured Achilles tendon area and surrounding regions were dissected en bloc, including myotendinous and osteotendinous junction. While the right Achilles tendons are used for biomechanical analysis, the left ones are used for histological evaluation for each subject.

Histological Analysis

For histological and immunohistochemical evaluation, left Achilles tendons were dissected from the musculotendinous junction to the osteotendinous junction, fixed in 10% formalin solution, and embedded in paraffin blocks after dehydration through phases of alcohol, acetone, xylene, and paraffin. Then, paraffin blocking was conducted, and cuts were taken of 4–5 µm from the main area of the tendon defect. Each section was stained with Masson trichrome and Alcian blue-PAS stainings for the Bonar scale, which evaluates tendon healing histopathologically through criteria including tenocyte morphology, cellularity, collagen fiber arrangement, vascularity, and ground substance; the numbers ranged from 0 (best) to 3 (worst) (Table 1).[17]

Immunohistochemistry Staining

Immunohistochemical staining of tenomodulin Type I collagen and Type III collagen was performed on 4–5 µm thick sections of each subject using the avidin-biotin complex method to objectively examine the tendon healing process. All sections were obtained from the main area of the tendon defect, and 3% hydrogen peroxide was applied for 10 min to block endogenous peroxidase activity. For overnight incubation at 4°C, slides were incubated with primary antibodies (Tenomodulin Polyclonal Antibody, bs-7525R, Bioss; Anti-Collagen I/COL1A1 Antibody, PA2140-2, BosterBio; Anti-Collagen III antibody, ab7778, Abcam). After overnight, slides were incubated with a seconder antibody (Lab Vision™ UltraVision™ Large Volume Detection System: Anti-Polyvalent, HRP (Ready-To-Use), TL-125-HL, Thermo). As a chromogen, 3,3' p-diaminobenzidine tetrahydrochloride (Thermo

Table 1. Modified Bonar score

1	2	3	4	5
Cell morphology (4 fields of view, 200x)	Inconspicuous elongated spindle shaped nuclei with no obvious cytoplasm at light microscopy	Increased roundness: Nucleus becomes more ovoid to round in shape without conspicuous cytoplasm	Increased roundness and size; the nucleus is round, slightly enlarged and a small amount of cytoplasm is visible	Nucleus is round, large with abundant cytoplasm and lacuna formation (chondroid change)
Collagen morphology (one field of view, 100x)	Collagen arranged in tightly cohesive well demarcated bundles	Separated of individual fiber bundles but with maintenance of overall bundle architecture	Bundle changes; separation and loss of demarcation of fiber bundles, the tissue	Marked separation of fiber bundles with complete loss of architecture
Cellularity (one field of view, 100x)	Mainly discrete cells	Hypercellular[a], in runs and/or increased cell numbers	Areas of hypo[a] as well as hypercellularity[a]	Area of assessment is mostly acellular
Vascularity (≤10 fields of view, 400×)	Inconspicuous blood vessels coursing between bundles	Occasional cluster of vessel, <2/10 field	2–3 cluster of capillaries per 10 field	Areas with greater than 3 clusters per 10 field And/or areas of pathological avascularity
Ground substance (one field of view, 100x)	Not stainable ground substance	Stainable mucin between bundles but bundles still discrete	Stainable mucin within bundles with loss of clear demarcation of bundles	Abundant mucin throughout the section with inconspicuous collagen staining

[a]Hypocellular: <20 nuclei per field; hypercellular: >30 nuclei per field. Plus 2.5 points for each of: calcification; adipocytes (intratendinous). Total score: a tendon with the most pathology will score 20. A tendon with no observable pathology will score 0.

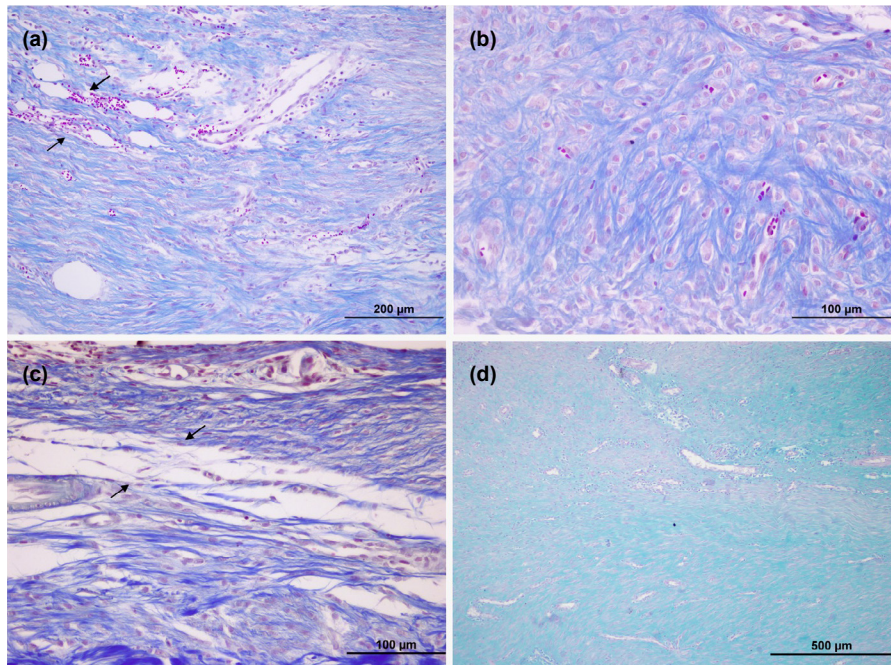

Figure 1. Representative histopathological images of tendon sections: **(a)** Arrows show capillary clusters support hypervascularity in healing tendon site (Sesame Oil Group, 2nd week); **(b)** The tenocytes' nucleus is seen as round shaped in contrast to natural spindle tenocyte morphology due to tendon healing (Sesame Oil Group, 2nd week); **(c)** Arrows show disruption of collagen bundles (Control Group, 2nd week); **(d)** The blue-green color represents abundant musin in the tendon healing site (Curcumin+MSC Group, 2nd week). Staining: (a-c) Masson trichrome; (d): Alcian blue-PAS. Magnification: a :20×, b and c: 40×, d: 4×.

Scientific, Waltham, MA) was used, and nuclear staining was performed with Gill's hematoxylin.

For each antibody, images were taken from an average of randomly selected six fields with an Olympus BX51 microscope and Olympus DP70 camera for each subject. The mean intensity of immunoreactivity was calculated using Image J software (National Institutes of Health, Bethesda, MD).

Biomechanical Evaluation

After harvesting, the right Achilles tendons were kept moist in saline and subjected to biomechanical evaluation immediately. Biomechanical experiments were conducted on the Instron® tensometer. For each tendon, the diameter was measured from the tendon injury area with a digital compass (Mitutoyo®, Japan), and the cross-sectional area was calculated in mm2 by assuming a circular shape. The tendon was fixed in a claw with sandpaper from its proximal end by muscles. At the distal end, after being held with a lung clamp, the calcaneus bone was fixed in the other claw of the tensometer. The tendons were subjected to a gradual increase of load at a displacement velocity of 10 mm/s using a load of 1 N/kg until the tendon ruptured. Maximum tensile load (N) was recorded using Instron Series IX Automated Material Testing System Version 5.33 software.

Statistical Analysis

Statistical analysis was performed with TURCOSA® software. After confirming the normal distribution of data, statistical analysis among multiple groups was conducted using one-way analysis of variance. According to the results, the Tukey test was used to determine which groups were different. For comparison of all second and fourth results of each treatment, a Student's t-test was conducted. Furthermore, the Pearson correlation test was performed to determine correlations between the tested variables. $P<0.05$ was considered statistically significant.

RESULTS

Histopathological Findings

Bonar scoring was performed using Masson trichrome and Alcian blue-PAS stained slides; histopathological changes shown in Figure 1 were evaluated. When the tendons were evaluated histopathologically by Bonar scoring, there was a statistically significant difference in curcumin, MSC, and Curcumin+MSC groups in comparison with the control group, in the 2nd week ($P<0.05$) (Fig. 2). There was no statistically significant difference among experimental groups in Bonar score in the 4th week. When the groups' individual Bonar scores in the 2nd and 4th weeks were compared, control, sesame oil, and Curcumin+MSC groups showed better results which were statistically significant ($P<0.05$) in the 4th week (Fig. 2).

Regarding Type I collagen staining intensity in immunohistochemical analysis, there was no statistically significant difference among groups in the 2nd week ($P=0.057$).

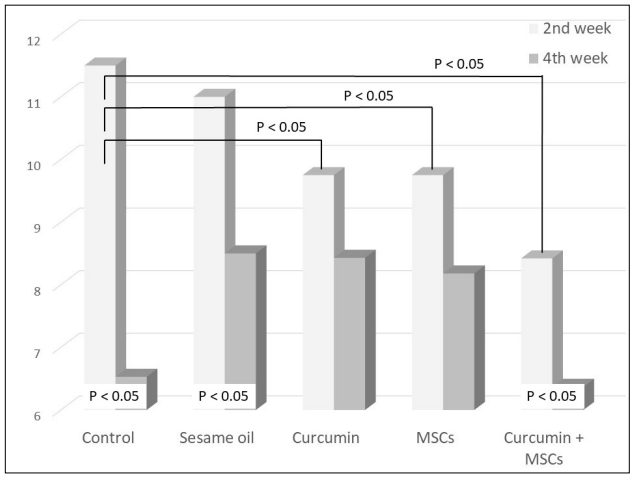

Figure 2. Bonar scores of groups in the 2nd and 4th week and their comparisons.

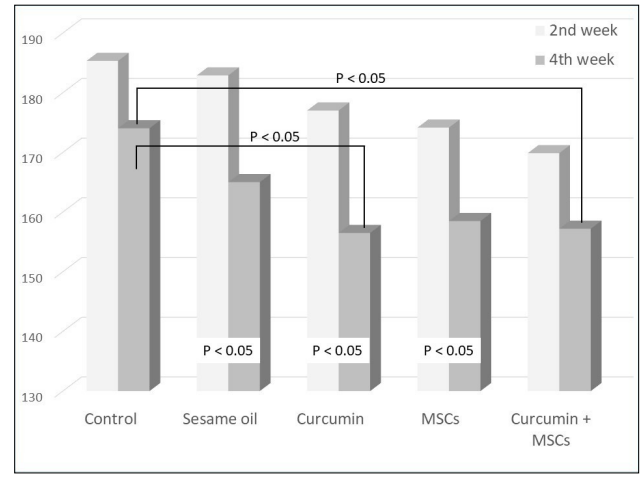

Figure 3. Type I collagen staining results of groups in the 2nd and 4th week and their comparisons.

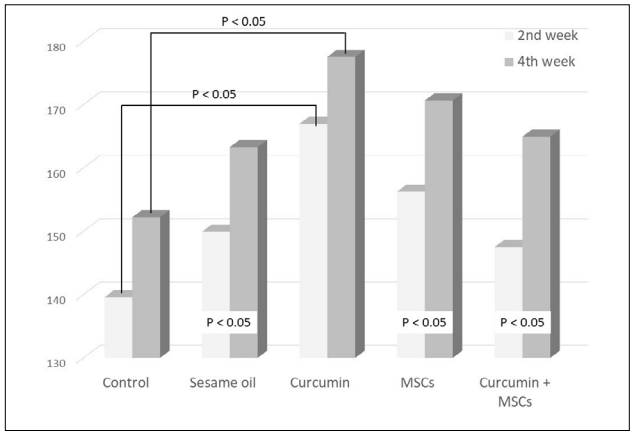

Figure 4. Type III collagen staining results of groups at the 2nd and 4th week and their comparisons

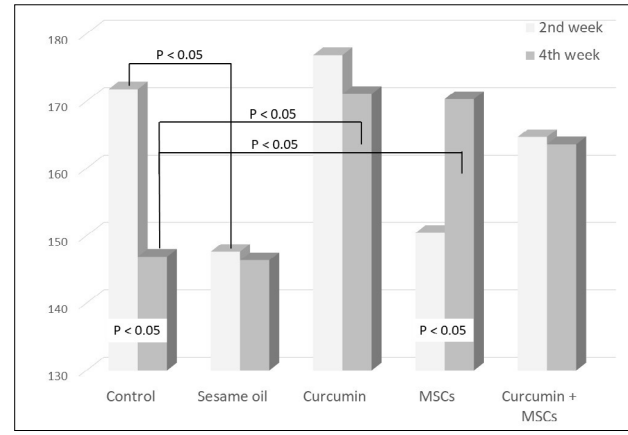

Figure 5. Tenomodulin staining results of groups in the 2nd and 4th week and their comparisons

In the 4th week, there were lower scores in curcumin and Curcumin+MSC groups according to the control group, which were statistically significant (P<0.05). Comparing Type I collagen staining intensity for the 2nd and 4th weeks, there were lower scores in the 4th week in sesame oil, curcumin, and MSC groups, which were statistically significant (P<0.05) (Fig. 3).

When Type III collagen staining intensity results were compared, according to the control group, there were statistically significant and higher scores in only the curcumin group in the 2nd week (P<0.05). There were also statistically significant and higher scores in the curcumin group compared to the control group in the 4th week (P<0.05). When it comes to the comparison of weeks 2nd and 4th regarding Type III staining intensity, there were higher scores in the Sesame Oil, MSC, and Curcumin+MSC groups, which were statistically significant, in the 4th week (P<0.05) (Fig. 4).

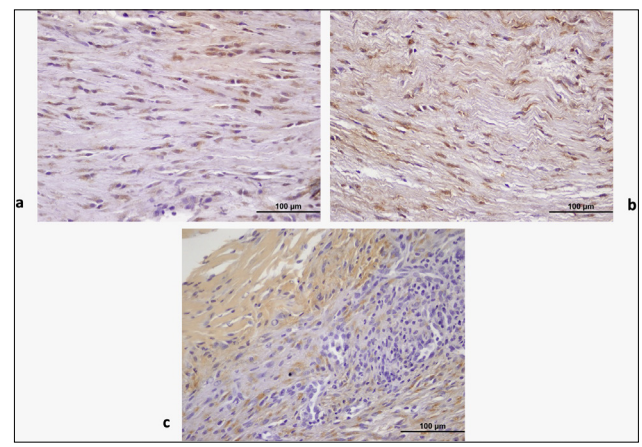

Figure 6. Immunohistochemical staining of tenomodulin (a), type III (b), and type I collagen after injury. Brown staining corresponds to the immunostained area. (a): Curcumin Group, 2nd week; (b): Sesame oil Group, 4th week; Control Group, 2nd week). Magnification: ×40.

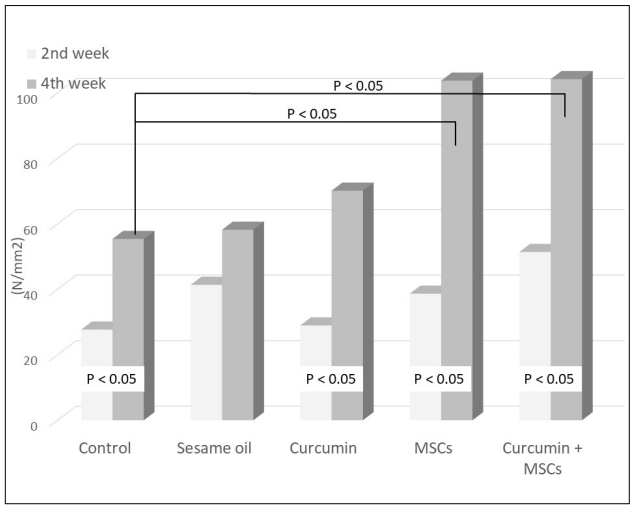

Figure 7. Tensile strength of groups in the 2nd and 4th and their comparisons.

When the staining intensity of tenomodulin is evaluated, there were statistically significantly lower scores in the sesame oil group in the 2nd week (P<0.05) and statistically significantly higher scores in curcumin and MSC groups in the 4th week, according to the control group (P<0.05). In the comparison of tenomodulin staining intensity within groups in the 2nd and 4th weeks, there were lower scores in the control group and higher scores in the MSC Group which both were statistically significant in the 4th week (P<0.05) (Fig. 5). Representative images of immunohistochemical analysis are shown in Figure 6.

Biomechanical Analysis

As a result of biomechanical analysis, although there was no difference among groups according to the control group in the 2nd week (P=0.057), there were statistically significant higher tensile strength values in MSC and Curcumin+MSC groups in comparison with the control group in the 4th week. At the same time, there was no statistically significant difference between these two groups when compared with each other (P<0.05). When the 2nd and 4th weeks, values of the groups were compared, the 4th week results were statistically significantly higher in all groups, except the sesame oil Group (P<0.05) (Fig. 7).

Mean values and statistical comparisons of all variables mentioned above are demonstrated in Table 2.

Correlation Analysis

The Pearson correlation analyses investigating the existence of a relationship between the variables in all groups are given collectively in Table 3.

According to the analysis results, no statistically significant correlation was found between the tenomodulin staining intensity and other variables.

There is a positive, weak, and statistically significant correlation between Bonar score and Type I collagen staining intensity (r=0.3204, P=0.004) and a negative, weak, and statistically significant correlation between tensile strength (r=−0.382, P<0.001).

A negative, weak, and statistically significant correlation was

Table 2. Mean values of groups at 2nd and 4th weeks (±SD)

	Control	Sesame Oil	Curcumin	MSC	Curcumin+ MSC	P (ANOVA)
Bonar score						
2nd week	11.50 (±1.51)	11.00 (±1.16)	9.75* (±0.46)	9.75* (±1.38)	8.42*# (±1.13)	<0.001
4th week	6.53 (±3.02)	8.50 (±0.92)	8.43 (±1.80)	8.18 (± 2.35)	6.37 (±1.59)	0.102
p (Student t test)	<0.001	<0.001	0.066	0.129	0.014	
Type I collagen staining intensity						
2nd week	185.33 (±15.32)	182.90 (±7.25)	177.02 (±8.94)	174.15 (±15.88)	169.87 (±6.65)	0.086
4th week	174.04 (± 4.79)	165.02 (±12.11)	156.50* (±10.79)	158.50 (± 8.71)	157.20* (±15.83)	0.019
p (Student t test)	0.067	0.003	0.002	0.028	0.071	
Type III collagen staining intensity						
2nd week	139.55 (±9.93)	149.92 (±12.21)	166.96* (±12.14)	156.25 (±13.08	147.50 $ (±14.19)	0.006
4th week	152.20 (±14.56)	163.28 (±8.94)	177.54* (±23.13)	170.66 (± 8.18)	164.95 (±10.32)	0.021
p (Student t test)	0.075	0.026	0.337	0.026	0.019	
Tenomodulin staining intensity						
2nd week	171.73 (±19.18)	147.63* (±12.45)	176.84 # (±8.19)	150.41 $ (±22.36)	164.67 (±8.29)	0.002
4th week	146.88 (±15.54)	146.36 (±146.36)	171.04 *# (±7.28)	170.30*# (±12.47)	163.60 (±12.77)	<0.001
p (Student t test)	0.013	0.845	0.174	0.045	0.862	
Tensile strength (N/mm2)						
2nd week	27.68 (±15.45)	41.37 (±10.24)	28.96 (±16.01)	38.66 (±11.54)	51.40 (±24.28)	0.057
4th week	55.36 (±19.02)	58.17 (±21.59)	70.10 (±19.77)	103.70*# (±43.33)	104.17*# (±17.77)	<0.001
p (Student t test)	0.011	0.083	<0.001	0.002	<0.001	

P<0.05 (Post Hoc Tukey Test). *: Relative to Control group; # :relative to Sesame oil group; $: Relative to Curcumin group

Table 3. Pearson correlation analysis of the variables

	Bonar score	Type I collagen staining intensity	Type I collagen staining intensity	Tenomodulin	Tensile strength
Bonar score	1	0.3204	−0.1921	0.0636	−0.382
Type I collagen staining intensity	0.004	1	−0.3865	0.0175	−0.4465
Type I collagen staining intensity	0.104	<0.001	1	0.1096	0.3538
Tenomodulin	0.585	0.882	0.363	1	−0.0203
Tensile strength	<0.001	<0.001	0.003	0.866	1

found between Type I and Type III collagen staining intensity (r=−0.3865, P<0.001).

There is a negative, moderate, and statistically significant correlation between tensile strength and Type I collagen staining intensity (r=−0.4465, P<0.001) and a positive, weak, and statistically significant correlation between Type III collagen staining intensity (r=0.3538, P=0.003) was determined.

DISCUSSION

In this study, based on the hypothesis that combined application of curcumin and UC-MSCs would exert synergistic positive effects, including enhancement of tendon regeneration on Achilles tendon rupture, control, curcumin, MSC, and Curcumin+MSC experimental groups were formed.

Several studies have shown that the combined use of UC-MSCs and curcumin can have a positive effect on recovery.[11-13] Ormond et al. have shown in their study[11] that the use of curcumin in combination with stem cell therapy synergistically improves recovery from severe SCI, and they claimed that results of the study indicate that the effect of curcumin extends beyond its known anti-inflammatory properties to the regulation of stem cell proliferation.

The efficacy of oral curcumin is hindered by its poor bioavailability because the hydrophobic and unconjugated curcumin molecule is not absorbed at the desired level through the gastrointestinal tract. Therefore, very low curcumin levels are detected in the blood and tissues after curcumin intake.[18] Because curcumin may not reach the injury site at the desired level due to these pharmacodynamic and pharmacokinetic properties, curcumin was administered locally, dissolved in sesame oil.[19] It is thought that the results of this study will contribute to the literature because there are only two studies in which curcumin was administered locally to the tendon injury site.[5,8]

The rationale for creating the sesame oil group in the study was to see if sesame oil had any role individually for the results to be obtained in the curcumin group, where curcumin was dissolved in sesame oil and used. Since there is no other tendon healing study in the literature in which sesame oil is used as a solvent for curcumin, this investigation is the first of its kind. In terms of the investigated variables, sesame oil-related variables did not differ from the Control Group, except for the tenomodulin values of the 2nd week. Accordingly, it can be suggested that sesame oil does not affect tendon healing, and when used as a solvent of curcumin in tendon healing studies, it will not interfere with the results.

As a type of MSCs, UC-MSCs have some superior characteristics compared to other types of MSCs, including effortless and non-invasive methods of obtaining, easy ex vivo expansion, and the absence of ethical problems.[20] Plus, studies in the literature prove the positive effects of UC-MSCs on tendon healing histologically.[21,22] Therefore, using UC-MSCs was preferred in this study.

Rapid healing can complicate analyzing factors in wound tissue in tendon healing studies using transected and sutured tendons.[23] Based on this reality, a window defect made by a punch was used in this study. In addition, the window defect model was used considering that limb immobilization is required in the models where the tendon is fully transected, and the vital activities of the rats may be impaired since we use both hind extremities of the rats. Bonar scoring is a frequently used method in the histopathological evaluation of tendon healing. In this study, a statistically significant decrease was observed in the 2nd week Bonar scores in the curcumin, MSC, and Curcumin+MSC groups (more evident in the Curcumin+MSC Group) compared to the control group, indicating that tendon healing is progressing in a positive direction. In the 4th week, there was no statistically significant difference among the groups compared to the control group. When the results of the 2nd and 4th weeks were compared, a statistically significant decrease was observed in the control, sesame oil, and Curcumin+MSC groups. With these results, the expected positive effects of curcumin and MSC on tendon healing in the experimental groups, according to the information in the literature, were confirmed.

After a tendon injury, healing and scar formation occurs in three overlapping phases: Inflammation, proliferation, and remodeling. In the inflammatory phase, erythrocytes, and inflammatory cells, especially neutrophils, migrate to the wound site, and tendon cells initiate Type III collagen synthesis. A few days later, the proliferative phase begins, during which Type III collagen synthesis reaches its peak and stabilizes the repair site in its current state.[24] Furthermore, prominent high cellu-

larity is observed in this stage. After a few weeks, the remodeling phase is entered, and Type I collagen, the most abundant collagen in the tendon structure, is synthesized in this phase at the highest rate.[10] The replacement of Type III collagen with Type I collagen is critical in the natural tendon healing process and occurs mainly during the remodeling phase.[25] In this study, there was no difference between the groups in the 2nd week regarding Type I collagen staining intensity. At the same time, there was a statistically significant decrease in the curcumin and Curcumin+MSC groups (P<0.05) and close to a significant value in the MSC group in the 4th week, compared to the control group. In the literature, it has been shown that gene expression of Type I collagen gradually decreases at the beginning of tendon healing and during the proliferation phase.[24] In this context, it can be interpreted that the proliferation phase continues in the curcumin, MSC, and Curcumin+MSC groups in the 4th week. At the same time, the tendon healing has progressed to a more advanced stage in the control group, according to Bonar scoring. However, the curcumin and Curcumin+MSC groups had no superiority over each other. In the Type I collagen staining intensity of each group in the 2nd and 4th weeks, there was no difference between the two values in the control group, while the statistically significant decrease in the sesame oil, curcumin, and MSC groups confirms that the proliferation phase of the healing continued in the 4th week in those experimental groups.

The data obtained on the staining intensity of Type III collagen in tendons examined in this study confirm the known positive effects of MSCs and curcumin on tendon healing. Regarding Type III collagen staining intensity, there was an increase in all other groups compared to the control group in the 2nd and 4th weeks. The increase was statistically significant only in the curcumin group. When the results of the 2nd and 4th weeks were compared, a statistically significant increase was observed in the sesame oil, MSC, and Curcumin+MSC groups. This significant increase between weeks was consistent with the interpretation that tendons were in the proliferative healing phase.

Tenomodulin is a useful phenotypic marker for mature tenocytes and is upregulated one and 2 weeks after tendon injury; its level decreases during the next remodeling phase.[26,27] In this study, when it comes to the staining intensity of tenomodulin, a statistically significant decrease was observed in the sesame oil group compared to the control group in the 2nd week. No literature information was found to explain or support this finding. In the 4th week, although an increase was observed in all groups except the sesame oil group compared to the control group, this increase was found to be incredibly significant (P<0.02) in the curcumin and MSC groups and close to the significance level in the Curcumin+MSC Group (P=0.07). These results suggest that both curcumin and MSC cause an intense presence of tenocytes in the healing zone in the 4th week, consistent with the proliferative stage. This interpretation supports ongoing proliferative phases based on Type I and III collagen staining intensities. When the results of the 2nd and 4th weeks were compared, the statistically significant decrease in the control group was consistent with the literature. The increase in tenomodulin levels in the experimental groups is also compatible with Type I and Type III collagen values, showing that the proliferative phase continues in these groups.

According to the biomechanical evaluation, the tensile strength did not show a statistically significant difference between the groups in the 2nd week. However, an increase with a significant value of P=0.072 was determined in the Curcumin+MSC Group compared to the control group. According to the literature, an increase in tensile strength is not expected in the first 2 weeks of tendon healing.[28] Hence, the results were in line with the literature. In the 4th week, it was determined that the tensile strength was increased in the MSC and Curcumin+MSC groups compared to the control group. Considering the positive effects of curcumin and MSC on tendon healing described in the opening paragraphs of the discussion section, this increase is an expected result. When the 2nd and 4th weeks were compared, a statistically significant increase was observed in all groups except sesame oil. This increase was much more pronounced in the experimental groups than in the control group. It was in line with the literature mentioning the relationship between tensile strength and tendon healing.[28] According to these results, it is seen that both curcumin and MSC have a significant effect on the recovery of tensile strength in healing tendons.

Although it differs in the variables among groups, data supporting the continuation of the proliferative phase in the recovery region were generally obtained. In the comparison of the 2nd and 4th weeks in terms of all variables, the fact that Bonar score and tenomodulin, which gives more direct information in terms of cellular data, were significantly decreased in the control group can be interpreted as the tendon healing phases in the control group were complete or close to completion. The absence of a meaningful change in the staining intensities of Types I and III collagen in the control group was additional findings that can be interpreted as a stationary phase during the recovery period.

In this study, it was also seen that there was a statistically significant and positive correlation between the Bonar score and Type I collagen staining intensity. The improvement in better condition manifested as a decrease in the Bonar score is paralleled by a decrease in Type I collagen. A negative correlation was found between the Bonar score and tensile strength. This relationship means that better healing, expressed by a lower Bonar score, has higher tensile strength, which is expected and consistent with general literature. There is no literature available to explain this relationship directly.

The negative correlation between Type I and Type III collagen staining intensities in the experimental groups is consistent with the literature. It is an expected finding that while Type

I collagen decreases, Type III collagen increases in samples representing the first 4 weeks of tendon healing and, therefore, in the proliferation phase. The negative relationship between Type I and III collagen was also manifested in the relationship of these collagen types with tensile strength. A positive correlation was found between Type III collagen staining intensity and tensile strength; as the staining intensity of Type III collagen increased, the tensile strength also increased. A negative correlation was found between Type I collagen level and tensile strength. Although this relationship seems contradictory, it has been interpreted as follows: The samples belong to the proliferation period of tendon healing, which regeneration or increase in the level of Type I collagen is not yet expected during it, and it decreased in the environment with the disappearance of the tendon fragment during the creation of the window defect because it is the essential structural component of the tendon. For these reasons, it can be said that the tensile strength is mainly due to Type III collagen, which is newly formed and added to the defect site in this period. Since it is known that there is a negative relationship between Type III and Type I collagen in the initial period of tendon healing, it seems reasonable that Type III collagen staining intensity and tensile strength increase. In contrast, Type I collagen staining intensity decreases, as in our correlation analysis.

When all the study findings were evaluated, this study is in parallel with the studies in the literature showing the positive effects of curcumin and stem cells on tendon healing. However, we could not demonstrate in this study that the combined use of curcumin and stem cells has synergistic effects on tendon healing. Although this result seems inconsistent with studies in the literature that reported synergistic effects on curcumin and stem cells,[11-13] it has been reported that the results vary according to dose and stem cell type and that curcumin treatment may have negative effects at high doses, such as with a double-edged knife.[12] No similar synergistic effect was observed in our study due to the difference in our experimental tendon regeneration model or the fact that the ideal dose for curcumin was not used. The lack of groups in which curcumin was given at different doses seems to be a limitation of our study.

CONCLUSION

In this study, the positive effects of curcumin and UC-MSCs, which have been shown to impact tendon healing in the literature positively, were also demonstrated in recovery after punch-induced window defect in the Achilles tendon in rats. Still, synergistic effects on healing were not observed when they were applied together.

Ethics Committee Approval: This study was approved by the Erciyes University Ethics Committee (Date: 15.05.2019, Decision No: 19-114).

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DENEYSEL ÇALIŞMA - *ÖZ*

Aşil tendonu iyileşmesinde umbilikal kord-kaynaklı mezenkimal kök hücre ve kurkuminin etkilerinin incelenmesi - sinerjistik etkileri olabilir mi?

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AMAÇ: Kurkumin ve umbilikal kord-kaynaklı mezenkimal kök hücrelerin (UK-MKH) deneysel tendon hasar iyileşmesini olumlu yönde etkilediği bilinmektedir. Bu çalışma, kurkumin ve UK-MKH'lerin tek başına ve birlikte kullanılmasının bireysel etkilerini ve potansiyel sinerjistik etkilerini incelemeyi amaçlamıştır.

GEREÇ VE YÖNTEM: 80 dişi Wistar albino sıçan rastgele beş gruba ayrıldı: Kontrol, Kurkumin, Susam Yağı, MKH ve Kurkumin+MKH grupları. Tüm sıçanlarda, hem sağ hem de sol Aşil tendonlarında punch yardımıyla tendon hasarı oluşturuldu. Kontrol grubuna herhangi bir tedavi uygulanmadı. Diğer deney gruplarında yara bölgesine lokal olarak Kurkumin, kurkumin için bir çözücü olarak kullanılan susam yağı, MKH ve MKH ve kurkumin kombinasyonu uygulandı. Her bir grupta, hayvanların yarısı postoperatif 2. haftada diğer yarısı da 4. haftada sakrifiye edildi. Sağ Aşil, biyomekanik test için kullanılırken, sol Aşil histolojik değerlendirme ve tip I, tip III kollajen ve tenomodulinin immünohistokimyasal analizi için kullanıldı.

BULGULAR: 2. haftada, Kurkumin, MKH ve Kurkumin+ MKH gruplarında Kontrol grubuna kıyasla histolojik olarak iyileşmede istatistiksel anlamlı ilerleme gözlendi. 2. ve 4. haftalarda Kurkumin grubunda tip III kollajen, Kontrol grubuna kıyasla önemli ölçüde arttı. 4. haftada tenomodulin, Kurkumin ve MKH gruplarında Kontrol grubuna kıyasla önemli ölçüde arttı. Tendon gerim gücü 4. haftada, MKH ve Kurkumin+MKH gruplarında Kontrol grubuna kıyasla önemli düzeyde arttı. Tedavi grupları arasında iyileşme üzerindeki olumlu etkiler açısından birbirlerine üstünlük gözlenmedi.

SONUÇ: Lokal olarak kullanılan Kurkumin ve UK-MKH, sıçanların Aşil tendonlarında punch ile oluşturulan hasarın iyileşmesinde birbirine üstünlükleri olmayacak şekilde olumlu etkiler göstermiştir. Bununla yanısıra, birlikte uygulandıklarında iyileşme üzerine sinerjistik etkileri gözlenmemiştir.

Anahtar sözcükler: Aşil tendon rüptürü; kurkumin; mezenkimal kök hücre; umbilikal kord.

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