Basic Investigation

Kazakh Therapy on Differential Protein Expression of Achilles Tendon Healing in a 7-Day Postoperative Rabbit Model

Objective: To compare the effect of cast immobilization with that of early Kiymil arkili emdew (Kazakh exercise therapy) on the post-operative healing of Achilles tendon rupture in rabbits, and to observe the influence of early Kiymil arkili emdew on the differentially expressed proteins in the healing tendon.

Methods: Forty-five New Zealand white rabbits were randomly divided into three groups (Arm A: control group; Arm B: postoperative immobilization group; and Arm C: postoperative early Kiymil arkili emdew group). After tenotomy, the rabbits of the two experimental groups received microsurgery to repair the ruptured tendons, and then received either cast immobilization or early Kiymil arkili emdew treatment. Achilles tendon tissue samples were collected 7 days after the surgery, and two-dimensional gel electrophoresis and MALDI-TOF-MS technique were used to analyze differentially expressed proteins in the tendon tissue of the three Arms.

Results: A total of 462.67±11.59, 532.33±27.79, and 515.33±6.56 protein spots were detected by the two-dimensional polyacrylamide gels in the Achilles tendon samples of the rabbits in Arms A, B, and C, respectively. Nineteen differentially expressed protein spots were randomly selected from Arm C. Among them, 7 were unique, and 15 had five times higher abundance than those in Arm B. These included annexin A2, gelsolin isoforms and α-1 Type III collagen. It was confirmed by western blot that gelsolin isoform b, annexin A2, etc. had specific and incremental expression in Arm C.

Conclusions: The self-protective instincts of humans were overlooked in the classical postoperative treatment for Achilles tendon rupture with cast immobilization. Kiymil arkili emdew induced the specific and incremental expression of proteins in the repaired Achilles tendon in the early healing stage in a rabbit model, compared with those treated with postoperative cast immobilization. These differentially expressed proteins may contribute to the healing of the Achilles tendon via a mechanobiological mechanism caused by the application of Kiymil arkili emdew.

Keywords: Kazakh therapy; Achilles tendon rupture; ‘Pa’-bone suture; “six-element” theory; Kiymil arkili emdew; Centrifugal practice; Mechanobiology; Proteomics; Systems biology

The theory of Kazakh medicine uses the six elements: “Space, Earth, Light, Dark, Hot and Cold,” and the natural intergrowth, intertransformation, and interdependence between them to explain the origins of life and the physiological functions of the human body. These function in response to warm-ardent, cambrian-cold, sicca-dryness, thin-wetness, tight-hardness and loose-softness properties, as well as energy absorption capacity of the AT also changes: tight-hardness and loose-softness properties, as well as...
wake-active and quiet-sleep, become unbalanced when the foot systems move in space. When the musculotendinous unit experiences excessive loading, the foot system undergoes an instantaneous loss of kick-climb archaea. Consequently, muscle systems energize the archaea, therefore the AT rupture (ATR) occurs.

It has long been recognized that as a condition, ATR can result in major disruptive injury, and ATR patients can experience great pain. A neglected ATR occurs if patients remain untreated for a period of four weeks following the injury, and this an result in significant dysfunction. The typical techniques available for the treatment of AT rupture can be classified into surgical (open surgery, percutaneous surgery, and mini incision surgery) and non-surgical (cast, splint, and brace immobilization). Although there is still no consensus regarding the best method for AT rupture, a few randomized prospective trials have been performed that suggested the superiority of open surgical treatment, especially in athletes and young patients with a high level of physical activity.

Postoperative functional treatment involving early motion and weight-bearing exercises has been recently reported to shorten the rehabilitation period and result in an improved clinical outcome. Early Kiymil arkili emdew of the ankle joint was indicated as an important factor in optimizing the treatment of ATR, and other treatments such as cast immobilization, orthosis or the synthetic anterior below-the-knee slab were not needed. The rationale behind the success of early postoperative motion was potentially due to the promotion of proliferation, migration and alignment of tendon cells, which resulted in an increase in the circumference of collagen fibers and overall reconstruction of the AT. It has been indicated that tendons are able to respond to mechanical forces by altering their structure, composition and mechanical properties, but a detailed understanding of the mechanobiology involved in the tendon healing process following microsurgery has not yet been achieved. Recent research has suggested that differential protein expression in the ruptured AT treated with different methods contributes to the development of the different clinical outcomes. Proteins, which are biological macromolecules present in the human body, are essential components of an organism and participate in virtually every cellular process. The protein expression profile is considered to provide important functional genomics information and can be used to reflect dynamic changes of the biological system. However, a literature survey revealed a paucity of research utilizing proteomic analysis of the AT. Therefore, the present study aimed to investigate the significance of early postoperative Kiymil arkili emdew (a form of kinesitherapy) in the AT healing process by using proteomic analysis to assess differential protein expression in the AT in a 7-day postoperative rabbit model.

**MATERIALS AND METHODS**

**Experimental Design**

Forty-five male New Zealand white rabbits (Mean age: 6 weeks, weight: 2.5±0.2 kg) were provided by the Animal Center at the First Teaching Hospital of Xinjiang Medical University, China. The rabbits were randomized into three arms according to a pre-determined prospective animal experiment random number table. Arm A (control group, n=15), Arm B (immobilization group, n=15), and Arm C (early Kiymil arkili emdew group, n=15). The experimental protocol was approved by the local research ethics committee (No: A-20080114008).

The rabbits in Arm A were maintained intact until they were sacrificed, and protein isolation from the unilateral AT was performed. In contrast, the rabbits in Arm B and Arm C underwent a tenotomy and subsequent AT microsurgery. Prior to surgery, the rabbits all underwent antisepsis measures, hypnotic induction, and local anesthesia using a lidocaine hydrochloride injection. Tenotomy was performed 1.6 cm above the site of the unilateral tendon insertion into the calcaneus. AT microsurgery was carried out based on a novel surgical technique involving a parachute-like suture method (‘Pa’-bone suture) to repair the AT rupture. This novel technique was proposed by the present authors, and the detailed procedure is provided elsewhere. The rabbits in Arm B were treated with postoperative cast immobilization, with flexion of the knee joint at 75° and plantar flexion of the ankle joint at 90°. The rabbits in Arm C received a simulated early postoperative motion treatment by inducing for food and water, and centrifugal motion of was considered to occur during the alternation between standing up and squatting down. The movement was performed approximately 150±15 times per day.

The rabbits in each Arm were excluded from the study if any of the following occurred: 1) death; 2) loosening of the plaster cast; 3) infection of the AT site; 4) gap at the ruptured AT site larger than 1.0 mm. Not counting those excluded, 40 rabbits were accepted for further experiments, including 13, 12, and 15 rabbits in Arm A (control group), Arm B (immobilization group), and Arm C (early Kiymil arkili emdew group), respectively. Healing time errors were controlled within 1 h for each group at each time, and the rabbits were euthanized via carotid artery bloodletting. The Achilles tendon tissue was harvested in a laminar flow cabinet.

**Sample Preparation**

On day 7 (168 h) after AT microsurgery, all rabbits were sacrificed, and tendon tissues with a size of 0.5 cm³ were harvested from the healing AT area. The attached epitenon tissues were carefully removed, and the samples...
were thoroughly rinsed three times with 0.9% saline solution (at 4°C) and then snap frozen in liquid nitrogen. All of the procedures were performed rapidly to ensure that differences in the AT rehabilitation period were controlled within one hour.

The AT tissues of the rabbits were thawed, cut into small pieces with scissors, and further rinsed with phosphate-buffered saline to remove any impurities. Next, the AT tissues (weight: 50.0 mg) were crushed with a mortar and pestle, and the protein lysates were extracted using a cocktail solution (2% 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate (CHAPS), 50 mmol/L dithiothreitol, 0.1% phenylmethanesulfonyl fluoride (PMSF), 7 mol/L urea, 2 mol/L thiourea) and centrifugation at 16,000 xg for 45 min (4°C). The protein concentration was quantified according to Bradford using the ReadyPre 2-D Cleanup Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Two-dimensional Polyacrylamide Gel Electrophoresis

The proteins were first separated by isoelectric focusing (IEF). Four hundred micromolars of protein sample was loaded per 18 cm immobilized pH gradient (IPG) strip with a non-linear pH range of 3–10 by in-gel rehydration. Each strip was overlaid with 2–3 mL of mineral oil to prevent evaporation during the rehydration process. The IPG strip was then placed on the tray, and IEF was performed at 20°C using the IPGphor Isoelectric System (Amersham Pharmacia Biotech Inc., Sweden). The initial voltage was set at 250 V and raised step-wise up to 4000 V to remove salt. The proteins were focused for 8 h at 8000 V.

After focusing, the IPG strip was removed, and the mineral oil was drained using wet filter paper. Proteins immobilized to the IPG strip were then placed on a 12% SDS-PAGE gel (thickness: 1 mm) and separated based on their molecular weights using the Protean II electrophoresis system (Bio-Rad) at 15 mA/gel. The two-dimensional gel was then silver stained using a modified method that enables direct mass spectrometry characterization.25

The gel was then scanned using a GS-800 molecular imaging system (Bio-Rad), after which spot detection, spot matching, and quantitative intensity analysis were performed using PDQuest software version 7.0. The gel images were normalized according to the total abundance in the analysis set, and differentially expressed proteins were determined by measuring the density values of the protein spots. A five-fold difference in the abundance of protein spots was established as the threshold. The results were analyzed using the Student’s t-test, based on six density values obtained for one protein spot, and a probability value <0.05 was considered statistically significant.

Mass Spectrometry Analysis

Differentially expressed spots were excised from the gel using a spot cutter (Amersham Pharmacia Biotech Inc., Sweden), and the proteins were in-gel digested with trypsin and extracted as peptides based on the method described by Gharahdaghi et al.26 Each sample was suspended in 0.8 μl of 0.5 g/L matrix solution (a-cyano-4-hydroxycinnamic acid (CHCA) in acetonitrile/water (1:1, v/v) acidified with 0.1% (v/v) trifluoroacetic acid (TFA)). The mixture was immediately spotted onto a stainless steel MALDI target plate and allowed to dry and crystallize at room temperature. Mass spectrometry was performed using a 4700 Proteomics Analyzer (TOF/TOF™, Applied Biosystems, USA) equipped with a 337 nm Nd:YAG laser.

The proteins were identified by peptide mass fingerprinting (PMF) and tandem mass spectrometry using the MASCOT version 1.9 program (Matrix Science, London, UK) against the SWISS-PROT database with GPS explorer software (Applied Biosystems).

Validation of Differentially Expressed Protein Spots by Western Blotting

Western blotting was performed three times to verify whether the differentially expressed proteins were specific on the seventh day after surgery. Alpha-1 type III collagen protein, Gelsolin isofom b protein and Annexin A2 proteins were separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane. The blots were probed with the anti-Alpha-1 type III collagen protein antibody, anti-Gelsolin isofom b protein antibody and Annexin A2 protein antibody (Santa Cruz Biotechnology, CA, USA). The Pierce ECL detection system was used to visualize the results. The digital image was obtained by scanning the film and performing a gray value analysis.27

RESULTS

Two-dimensional Electrophoresis Protein Comparison

The two-dimensional electrophoresis maps of the proteins are the same. Most of the protein spots were concentrated in the region comprising pH 3.5-9. A total mean of 462.67, 532.33, and 515.00 protein spots were detected in the gels for the AT samples obtained from rabbits in Arm A (control group), Arm B (immobilization group), and Arm C (early Kiymil arkili emdew group), respectively.

Based on the nature comparison, there were 19, 47, and 21 unique protein spots in Arms A, B and C, respectively. According to the quantity comparison (a greater than five-fold difference of expression abundance), the following information was obtained: 1) There were 29 protein spots with expression levels in Arm B (immobilization group) that were five times higher than those in Arm C (early Kiymil arkili emdew group); 2) There were 15 protein spots with expression levels in...
Arm C that were five times higher than those in Arm B; 3) There were 9 protein spots with expression levels in Arm B that were five times higher than those in Arm A (control group); 4) There were 8 protein spots with expression levels in Arm C (that were five times higher than those in Arm A; 5) There were 30 protein spots with expression levels in Arm A that were five times higher than those in Arm B; 6) There were 61 protein spots with expression levels in Arm A that were five times higher than those in Arm C.

Identification of Differentially Expressed Proteins and Bioinformatics
AT proteins that demonstrated up to five-fold differences in expression between two Arms were considered differentially expressed proteins. According to these criteria, many differentially expressed proteins were identified in Arm A (control group), Arm B (immobilization group), and Arm C (early Kiymil arkili emdew group).

Some of these proteins are involved in various metabolic pathways and may play an important role in AT healing. For example, 6 of 19 unique proteins were identified in Arm B; 10 of 29 proteins showed expression levels in Arm B that were five times higher than those in Arm C; 1 of 9 proteins showed expression levels in Arm B that were five times higher than those in Arm A; 1 of 15 proteins showed expression levels in Arm B that were five times higher than those in Arm A and Arm C. Seven of 47 unique proteins were identified in the AT samples from Arm C; 13 of 15 proteins showed expression levels in Arm C that were five times higher than those in Arm B; 2 of 8 proteins showed expression levels in Arm C that were five times higher than those in Arm A; 2 of 61 proteins showed expression levels in Arm A that were five times higher than those in Arm C; 1 of 7 protein showed an expression level in Arm B and Arm C that was five times higher than that in Arm A; 4 of 11 proteins showed expression levels in Arm A that were five times higher than those in Arm B and Arm C.

Some of the uniquely expressed proteins in Arm C (early Kiymil arkili emdew group) are involved in various metabolic pathways and may play an important role in AT healing, e.g., glyceraldehyde-3-phosphate dehydrogenase, AMBP protein, procollagen C-proteinase enhancer protein precursor, tropomyosin, skeletal muscle alpha-tropomyosin, prepro-alpha-1 collagen type I, NCB Inr index code to gi|47077820, peptidoglycan glycosyltransferase (PGTs), annexin A2, and chaperonin containing TCP1 subunit 7 isoform b.

The proteins in Arm C that were expressed at levels five times greater than those in Arm B (immobilization group) were heat shock 70 kDa protein 12A, toll-like receptor 4, Radical SAM, 1-acyl-sn-glycerol-3-phosphate acyltransferase (AGPAT), envelope glycoprotein, fibrinogen A alpha-chain, phosphoglycerate mutase 1 (brain) isoform 1, serum albumin precursor, hypothetical protein, and gelsolin isoform b. Figure 1 shows the results obtained for peptide 5223, which was detected in the early Kiymil arkili emdew Arm. This peptide was identified as annexin A2. The unidentified spots were considered protein mixtures needing further separation and identification.

Western Blotting
Some of the differentially expressed proteins, such as annexin A2, gelsolin isoform b and alpha-1 type III collagen, were selected for validation by western blotting. Seven samples were randomly selected from each Arm, and the experiment was repeated three times. Figure 2 (a-c) shows the western blotting results, demonstrating the comparison of annexin A2, gelsolin isoform b and alpha-1 type III collagen (Santa Cruz Biotechnology) for Arm A (control group), Arm B (immobilization group), and Arm C (early Kiymil arkili emdew group) and their relative protein expression.

The variance analysis of the OD values ratio between the two protein bands in each Arm, (Figure 2 (a-b)), shows the validation results. For annexin A2 and alpha-1 type III collagen protein, the overall variance between the two Arms did not differ significantly (P=0.000) in pairwise comparisons using the least significant difference (LSD) method. Gelsolin isoform b showed protein variance nonhomogeneity, according to variance analysis using the Welch method (P=0.000), using the Dunnett T3 pairwise comparison. Table 1 shows the verification test results. For annexin A2 protein, there were statistical differences between the three Arms by pairwise comparisons. In arm A, annexin A2 was expressed 4.55 times more than in Arm B and 0.25 times more than in Arm C. In Arm C, annexin A2 protein expression abundance was 4.00 times higher than in Arm A and 18.18 times higher than in Arm B. For gelsolin isoform b protein, there was no difference between Arms A and C, but there were statistically significant differences between Arms A, C and B. The average gelsolin isoform b protein abundance ratios in Arm A were 2.65 times higher than in Arm B, and 1.04 times higher than in Arm C. In Arm C, the average protein abundance ratios were 0.96 times higher than in Arm A, so there was no difference between Arms C and A. Average gelsolin isoform b protein values of Arm C were 2.55 times higher than that of Arm B. For alpha-1 type III collagen protein, there were statistically significant differences between the three arms by pairwise comparisons. In Arm A, the average protein expression abundance was 1.70 times higher than in Arm B, and 0.24 times higher than in Arm C. In Arm C, the protein expression abundance was 4.24 times higher than in Arm B in 7.22 times higher than in Arm B. The results showed that the value of these proteins in Arm C were higher than in both Arms A and B.

A significant difference was observed between Arm A and Arm B with respect to the expression levels of these
three proteins. However, significant differences were also observed when comparing the expression levels of these three proteins between Arm B and Arm C. These data were consistent with the results obtained by two-dimensional electrophoresis.

Figure 1. Characterization of peptide 5223 detected in AT samples from rabbits in the early Kiymil armb Walsh arm; a) peptide mass fingerprinting; b) mass spectrometry sequence analysis. The peptide was identified as annexin A2.

Table 1. Western blot analysis of mean protein abundance expressions

<table>
<thead>
<tr>
<th>Protein name</th>
<th>Arms</th>
<th>Statistics</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (n=7)</td>
<td>B (n=7)</td>
<td>C (n=7)</td>
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<tr>
<td>Annexin A2</td>
<td>0.50±0.02</td>
<td>0.11±0.02</td>
<td>2.00±0.02</td>
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<tr>
<td>Gelsolin isoform b</td>
<td>1.06±0.02</td>
<td>0.40±0.02</td>
<td>1.02±0.07</td>
</tr>
<tr>
<td>Alpha-1 type III collagen</td>
<td>0.46±0.03</td>
<td>0.27±0.02</td>
<td>1.95±0.04</td>
</tr>
</tbody>
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Notes: * Homogeneity of variance test F=14.792, P=0.000, variance nonhomogeneity, Welch method was used for analysis of variance. Pairwise comparison of each set of data as follows: 1. Annexin A2: Pairwise comparison among the three groups were statistically different. 2. Gelsolin isoform b: there were no differences between the A and C Arms, there were statistically significant differences between A, C and B Arms. 3. Alpha-1 type III collagen: Three pairwise comparisons between Arms were statistically different. The first and third protein pairwise comparison using the LSD method, the second protein variance nonhomogeneity, using Dunnett T3 pairwise comparison method.
DISCUSSION

In Kazakh medicine, the cause of ATR, according to Bolijawsiz, is the occurrence of a sports related accident. ATR has long been recognized as a major disruptive injury, and many complications can occur if the patient is not properly treated.2-21 In the theory of the six elements in Kazakh medicine, the “dark element” has another meaning, which indicates exploration of the unknown and knowledge of the process, and an alternative meaning of the “space element” indicates movement and transportation. Kazakh medicine also advocates the use of Kiymil arkili emdew.1

Early postoperative motion has been generally accepted as a method to treat ATR with the introduction of new surgical techniques. However, it has not yet been clear how Kiymil arkili emdew contributes to the healing of ATR according to Kazakh medicine. The newly developed proteomic analysis technique could be a potentially useful tool to elucidate the rationale behind the success of Kiymil arkili emdew. However, a literature survey revealed that research investigating proteomic analysis in AT is lacking.22

In the present study, proteomic analysis of differential protein expression was performed based on two-dimensional polyacrylamide gel electrophoresis and mass spectrometry in ruptured ATs of rabbits treated either with postoperative cast immobilization or early Kiymil arkili emdew. The differential expression levels of the detected proteins were validated by western blotting. Analysis of the proteins identified in Arm A (control group), Arm B (immobilization group), and Arm C (early Kiymil arkili emdew group) demonstrated that some of the proteins might contribute to healing of the AT rupture, especially the 17 proteins detected in the early Kiymil arkili emdew Arm, such as gelsolin isoform b, heat shock protein, annexin A1, radical SAM, hypothetical protein, gi|6807898 and gi|55728420, and others.

Three proteins, including the specific and incrementally expressed annexin A2, were verified by western blot.

The Changes in Signal Transduction Proteins
Annexin A2 is a calcium-dependent membrane phospholipid-binding protein, present in the cell membrane, cytoplasm and extracellular space. It plays an important role in signal transduction, the structural formation of cell membrane, membrane aggregation and in exocytosis and membrane fusion. Annexin A2 gene silencing siRNA is known to inhibit Jurkat cell proliferation and chemotaxis. In this study, annexin A2 in Arm C (early Kiymil arkili emdew group) was upregulated, and had 4.00 times higher abundance expression than Arm A (control group) and 18.18 times higher abundance expression than in Arm B (postoperative immobilization group) by western blotting. This indicates that annexin A2 maybe a vigorous growth bio-marker for tissue organization.

In contrast, the role of annexin A1 is involved in cell growth, proliferation, apoptosis and other important processes including inflammatory activity. It restricts cell growth by affecting the growth factor complex formation in the MAPK/ERK signaling pathway upstream and activates the ERK1/2MAPK signaling cascade and inhibition of cyclin D1 expression. Annexin A1 in Arm B (postoperative immobilization group), specifically expressed in the healing AT of rabbits, may indicate that the immobilized AT is in the “disused” state, and cell growth is in a slow or suppressed state.

The Energy Metabolism Proteins
AGPAT, PGAM1 and GAPDH, are energy metabolic proteins. PGAM1 can catalyze triglyceride and triglyceride sn-2 sites of key biosynthesis of lipid signaling molecules and can form heart muscle phospholipids and phosphatidylinositol. It plays an important role in cell proliferation, apoptosis, migration, invasion, breathing and cytokine release expression.23 Ren et al24 found it similarly upregulated in the dynamic pressure of rabbit articular cartilage cells under a metabolic enzyme. In present study, PGAM1 may have been upregulated due to the centrifugal stress on the healing AT during postoperative early Kiymil arkili emdew. Stress activates energy metabolism and higher protein expression, suggesting increased levels of glycolysis or increased reactivity of the enzyme in healing tissue. It Increased levels of PGAM1 can be considered as therapeutic targets in AT tissue growth. In contrast, postoperative immobilization inhibited energy metabolism protein expression, indicating that the cast immobilization treatment causes the AT tissue regeneration and healing to be relatively slow.18,25
The Changes in Stress Proteins
Proteins with a variety of biological functions, Heat shock 27 and 70 kDa, can act as stress reaction molecular chaperones and as intracellular multiple protein translocation assembly proteins. They are involved in the stress response, mechanical stimulation, mechanical stretching, mechanical stress-initiated signaling, mechanical stress induction and stress regulation and in oxidative stress and apoptosis and other important life-cycle activities. The expression of HSP27 and 70 may have a protective effect on tissue organization when under stress.

The study also found beta enolase protein was upregulated in Arm B (postoperative immobilization group). This protein may be involved in immobilization or non-stress conditions. Ren Yuanyuan\(^{26}\) found that beta enolase was downregulated in rat skeletal muscle in the proteome analysis after induction of atrophy by hindlimb suspension. In the present study, we considered that upregulated beta enolase protein was a biomarker of delayed healing of the AT. In contrast, alpha enolase was the key protein regulating cell biological behavior under pressure or a stressed state.\(^{23}\) Further study is needed on the specific relationship of the two proteins during both postoperative early Kiymil arkili emdew and immobilization treatment.

Changes in Cytoskeletal Proteins
Gelsolin isoform b, a cytoskeletal regulatory protein, can cut capped actin filaments, or control the actin structure by actin nucleation, etc. It was found that the protein was upregulated in Arm A (control group), at 2.65 times higher abundance expression than in Arm B (postoperative immobilization group). It was expressed 2.55 times higher in Arm C (early Kiymil arkili emdew group) than in Arm B as shown by western blotting. This indicated that Gelsolin protein expression was inhibited in immobilization treatment. Immobilization treatment inhibited part of the rabbits’ physiological functions during the healing of ATR.

Alpha-1 type III collagen is a collagen of fibrillar collagen with a C-terminal domain. The AT is a single axle, dense, collagenous connective tissue, mainly composed of type I and III collagen and elastic fibers. Type I collagen fibers are parallel to the axis of the tendon, while type III collagen is powerfully attached to type I collagen fibers, and together with the elastic fibers make a rich viscoelastic tendon.\(^{27}\) It was found that the protein expression level in Arm A (control group) was 1.70 times higher than in Arm B (postoperative immobilization group), was also 7.22 times higher in Arm C (early Kiymil arkili emdew group) than in Arm B. This indicates that the immobilization treatment inhibits the expression of the protein. Thus, immobilization treatment inhibited the rabbits’ AT tissue regeneration and growth.

Tropomyosin protein plays an important role in maintaining the integrity of tendon fibers, and is necessary to repair or regeneration after mechanical injury to tissues. Lack of Tropomyosin protein may increase brittleness of tendon fibers and make them more prone to damage while under conduction stress, and more likely to degenerate or degrade. In our experiment, the protein was upregulated in Arm C (early Kiymil arkili emdew group). This may be due to centrifugal stress stimulation on the healing AT, leading to Tropomyosin upregulation.

From a mechanobiology perspective, with postoperative early Kiymil arkili emdew in the 21 day healing period, the rabbit AT collagen fibril diameter reached 44.49% of that of the normal group.\(^{25}\) Maximum tensile load reached 43.41%, of that of the normal group in the same period.\(^{29}\) As Battaglia\(^{28}\) theorized, AT strength is positively correlated to the diameter of the collagen fibril. Postoperative early centrifugal practice promoted rabbit AT collagen fibrils closer to normal. Histological changes in the AT have not yet shown significant difference in the 7 day postoperative healing period.

Once the related gene transcription regulation network has been started, cells proliferate and tissues gradually grow strong. There is strong evidence that proteins such as annexin A2 and PGAM1 are upregulated in Arm C (early Kiymil arkili emdew group) and can be considered as new therapeutic targets in healing AT. It is interesting that many scholars believed that annexin A2 and PGAM1 were tumor therapeutic targets. Through the results of the present study, seeing that these proteins are upregulated in Arm C indicates they are vigorous growth markers, not tumor-specific expression markers.

In contrast, other proteins such as beta enolase and annexin A1 had incremental expression in Arm B (postoperative immobilization group), which indicated that immobilization treatment may inhibit annexin A2, PGAM1 and other key protein synthesis, thus inhibiting AT tissue regeneration and growth,\(^{3,12}\) which is counterproductive to healing the AT.

Using the “six-element” theory as a guide, the “dark element” was used in the comprehensive treatment for AT, which included an effective program of early Kiymil arkili emdew. This procedure helps the healing ATs to be tight-hard and loose-softness during wakeful movements and quiet-sleep, to achieve a balance. Therefore, early exercise improves the recovery of the AT function by early postoperative Kiymil arkili emdew. This procedure results in a shorter healing time. However, whether it can be used for repairing AT that has remained untreated for longer periods remains unknown. Further studies are needed to elucidate the “dark element” to achieve a “light element.”

The abundance of annexin A2, gelsolin isoform b,
alpha-1 type III collagen, heat shock 70 kDa protein, AMBP protein, NCBI index code to gi|47077820 protein etc., in the early Kiymil arkili emdew group was significantly higher than that in the immobilization group. Because the same AT microsurgery was used for the rabbits in the two groups, with the only difference being the postoperative treatment method following microsurgery, the significant increases were considered to be due to the application of the postoperative early Kiymil arkili emdew (centrifugal stress or mechanical strain). Previous clinical research has shown that postoperative early Kiymil arkili emdew could promote overall reconstruction of the ruptured AT through a mechanobiological mechanism.

Although the use of proteomic analysis to reveal biological changes is available, it has been rarely used to analyze the differentially expressed proteins in the treatment of AT rupture. In the present study, proteomic analysis was performed to preliminarily investigate the treatment of AT rupture in a specific 7 day postoperative rabbit model. Some differentially expressed proteins were not successfully identified. Therefore, further research is required by using alternative methods to identify the differentially expressed proteins and to investigate their functions during the early AT healing stage.

The self-protective instincts of humans were overlooked in the classical postoperative treatment for Achilles tendon rupture with cast immobilization. Kiymil arkili emdew could induce specific and incremental expression in differentially expressed proteins in the repaired Achilles tendon in the early healing stage in a rabbit model. These differentially expressed proteins may contribute to the healing of the Achilles tendon via a mechanobiological mechanism caused by the application of Kiymil arkili emdew. The present research explored the mechanobiological mechanism of early Kiymil arkili emdew on the overall reconstruction of the ruptured AT.

REFERENCES


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