Effect of acupotomy versus electroacupuncture on ethology and morphology in a rabbit model of knee osteoarthritis

Zhang Wei, Gao Yang, Guo Changqing, Ibrahim Zeyad Ali Khattab, Farid Mokhtari

Zhang Wei, Gao Yang, Guo Changqing, Ibrahim Zeyad Ali Khattab, Farid Mokhtari, School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China

Supported by Grants from the National Natural Science Foundation of China: Research for the Relation Between Acupotomy’s Effective Mechanism of Treating Knee Osteoarthritis and Fak-pi3k-akt Pathway by the Theory that Modulating Sinew (Jin) and Treating Bone (Gu) (81574067)

Correspondence to: Prof. Guo Changqing, School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing 100029, China. guochangqing2017@yeah.net
Telephone: +86-13661022668
Accepted: November 17, 2018

Abstract

OBJECTIVE: To evaluate the treatment effect of acupotomy (Apo) in a rabbit model of knee osteoarthritis (KOA), compare the results of Apo versus electroacupuncture (E-Apu) on ethology, morphology, and structure of the articular cartilage surface in a rabbit model of KOA, and analyze the differences in the treatment effects of Apo versus E-Apu.

METHODS: Twenty-eight male New Zealand white rabbits were randomized into four groups: normal control, blank model, Apo, and E-Apu (n = 7 in each group). Except for the normal control group, the left hindlegs of all rabbits were fixed in an extended position for 5 weeks to establish the KOA model. The passive range of motion (PROM) and Lequesne index were measured before and after the establishment of the KOA model to assess the ethology in all groups. Safranin O-fast green staining and the Mankin score were used to assess the morphological cartilaginous changes to compare the effect of Apo versus E-Apu on the degeneration of articular cartilage, and to identify which therapy was superior in treating KOA.

RESULTS: Compared with before the establishment of the KOA model, the Lequesne index of the KOA model rabbits was significantly increased (P < 0.01), while the PROM was significantly decreased (P < 0.01). The articular cartilaginous tissue in the three model groups exhibited pathological variations in the form of laminar derangement of cartilage cells, and so the Mankin score was significantly increased compared with the control group (P < 0.01). At 1 week after the final treatment session, compared with the blank model group, both the Apo and E-Apu groups showed a significant decrease in the Lequesne index (P < 0.01), and attenuation in the degree of morphologic pathological changes (P < 0.05); The Apo improved the PROM significantly compared with the blank model group (P < 0.05), while the E-Apu had no effect (P > 0.05). Furthermore, compared with the E-Apu group, the Apo group had a significantly lower Lequesne index (P < 0.05), and a significantly greater PROM (P < 0.05).

CONCLUSION: In a rabbit model of KOA, both Apo and E-Apu reduce disorders of ethology and morphology, and improve the condition of the articular cartilage. The results suggest that Apo is more effective than E-Apu in improving the PROM and alleviating symptoms resulting from cartilage damage in a rabbit model of KOA.

© 2019 JTCM. All rights reserved.

Keywords: Osteoarthritis, knee; Acupuncture therapy
INTRODUCTION

Knee osteoarthritis (KOA) is the most common joint/bone disease, and is the fourth leading cause of disability. KOA is characterized by chronic degenerative changes in joint structure, osteoarthritis (OA) are strongly associated with aging. Advanced age and obesity are considered the primary causes of KOA. By age 65, around 30% of the population has osteoarthritis. KOA is an expensive disease with significant socioeconomic burden due to its high prevalence, worker absenteeism and costly health care utilization. As the knee is the major weight-bearing peripheral joint, the articulation of the knee is the site most frequently affected by OA. Pathological changes in KOA include damage of the articular cartilage surfaces, cartilage degradation, subchondral bone sclerosis, structural or metabolic changes, and synovial membrane inflammation. These changes result in pain, stiffness, swelling, and tenderness, which then reduced physical function and impaired quality of life.

Various therapies have been developed to address the etiological factors of KOA, including relief of the inflammatory reaction, protection or lubrication of the cartilage, and strengthening of proteoglycan in articular cartilage to limit the activity of cartilage-damaging enzymes and supply protection to free radicals. Conventional treatments for KOA include medication and surgery. In some cases, routine treatment approaches produce only mild and brief analgesia, and cause considerable adverse effects. For example, intra-articular corticosteroid injections are commonly used to provide pain relief in patients with OA, and may reduce cartilage degradation in KOA in the short-term. However, surgical procedures and intra-articular injections can also induce OA.

Contemporary Traditional Chinese Medicine (TCM) is a model of biological systems based on a logical, theoretical background. TCM integrates several therapeutic approaches, including acupuncture, which has proven to be effective in the treatment of KOA, minimizing pain, improving functionality, and consequently improving quality of life. Worldwide, acupuncture has been widely accepted as an important mainstream complementary and alternative therapy that can relieve pain, improve knee joint function, and slow the degeneration of articulation, thus preventing the progression of KOA.

Acupotomy (Apo) and Electroacupuncture (E-Apu) are two main types of acupuncture. Apo is also referred to as Needle-Knife acupuncture, and is a closed-type surgery with an approach between surgical and nonsurgical therapy that combines the "needle" concept of TCM with the "knife" idea in modern western medicine. Apo can dissolve adhesions or contracture of the soft tissue around the knee joint where the high-stress points are located. Apo reduces abnormal joint stress, and rebalances the biodynamic mechanics of articulation. Thus, Apo has unique advantages over other non-pharmacotherapy treatments in treating KOA.

E-Apu therapy, a needle is inserted into an acupoint, and an electrical pulse is administered to the tail of the needle after the attainment of acuesthesia. The strength of the electric current applied is close to the normal bioelectricity in humans. The quantity of the stimulus can be objectively controlled using a combination of needle and electrical stimulation. Both Apo and E-Apu therapies have obtained excellent clinical curative effects in KOA; Apo loosens tissue adhesions, while E-Apu is used to effectively treat a diverse range of painful conditions.

The present study aimed to compare the therapeutic effects of Apo versus E-Apu in KOA by observing the ethology and morphological changes in a rabbit model of KOA after Apo versus E-Apu.

MATERIALS AND METHODS

Animals and surgical protocol

The present study included 49 healthy, mature, male, adult, specific pathogen-free, New Zealand white rabbits. The rabbits were 6 months old, with a weight range of (2.5 ± 0.1)-(3.0 ± 0.1) kg. All rabbits were purchased from Golden Shepherd Limited Liability Company, [SCXK (Beijing, China) 2015-0005]. The animals were raised in the Orthopedics Experimental Center of the Military District General Hospital in Beijing. They were kept in separate cages at a fixed temperature (22±2 °C) and humidity (40%-60%), with food and water provided in accordance with the requirements of the provisions and general recommendations in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the Animal Experiment Management Committee of the Beijing University of Chinese Medicine, and the Animal Research Ethics Board of Beijing University of Chinese Medicine. This study was approved by the ethical review committee of the Chinese Center for Disease Control and Prevention (Beijing, China), and the Oxford Tropical Research Ethics Committee, University of Oxford (UK).

Based on previous experience and the calculated sample size, we divided the 28 rabbits into four groups (n = 7 in each group) using a random contrast method and random number tables using the CHISS program (Beijing Yuanyida Co., Ltd., Beijing, China). The groups were: normal control, blank model, Apo, and E-Apu. All rabbits except those in the control group underwent the KOA model establishment procedure. Within each group, six rabbits were used for the final index assessments, while one underwent morphological...
examination to determine whether the model had been successfully established. In the course of the experiment, the animals were euthanized in strict accordance with the requirements of the Animal Experiment Management Committee.

The rabbit KOA model was established using the modified Videman’s method in all rabbits in the blank model, Apo, and E-Apu groups. The procedure of model establishment was introduced as below: (a) The knee joint was fixed in an extended position. (b) Resin bandage was fixed using medical gauze. (c) Two layers of macromolecule resin bandage were applied. (d) Two layers of anti-nibbling bandages were applied. (e) The toes were exposed to enable the evaluation of blood supply and swelling. Before anesthesia, the rabbits were kept in a room with no access to water or food for 10-16 h. The rabbits received an intravenous injection of 30 mg/kg of 3% pentobarbital sodium (Tianjin Pharmaceutical Jiaozuo Co., Ltd., Jiaozuo, China) in an ear vein.

After anesthesia, the rabbits were in supine position on the operating table, and the left hind leg was exposed. The skin of the left hind leg was covered with absorbent cotton to prevent damage. The left hind leg was then fixed with a resin bandage from groin to toe. The medical resin bandage was softened by hot water at 65-85 °C on the inner side of the bandage. The ankle joint was dorsiflexed to 60°, and the knee joint was fixed in an extended position. The resin bandage was fixed to medical gauze, and then wrapped with two layers of macromolecule resin bandages. After the bandage had set, a silk stocking was pulled over it to protect the bandage from being bitten. The stocking was fixed with medical adhesive tape, and was partially cut to expose the toes to enable the observation of blood supply and swelling. The motion of the left hind leg was constrained by bandages and stockings for 5 weeks to establish the KOA model. After 5 weeks, one rabbit was randomly chosen from each group, and was euthanized by air embolism under anesthesia; articular cartilage was then removed for microscopic examination and assessment of pathological indexes. All bandages and stockings were removed after the KOA model was successfully established. No rabbits contract an infection or died during the experiment.

**Treatment and techniques**

(a) The normal control group underwent no special intervention, and were fed normally. (b) The blank model group underwent no special intervention after the establishment of the KOA model. (c) The Apo group underwent Apo therapy that was commenced 1 week after the establishment of the KOA model. Rabbis were anesthetized and placed in supine position on the operating table with the left knee joint exposed. The knee joint was shaved, disinfected, and aseptically draped. Apo was performed at five Ashi acupoints that were selected during each treatment (cord-like and non-cord-like points); these acupoints were located in the tendon of the biceps femoris, the biceps femoris muscle, the anserine bursa, or the cord-like muscles around the knee joint. Apo treatment comprised longitudinal dredging and transverse stripping using a No. 4 needle-knife (40 mm long). One to two strikes were administered per acupoint. The pinprick was pressed for a few seconds, and then covered with a sticking plaster. Rabbits underwent one treatment session twice a week and last for 4 weeks. (d) The E-Apu group were anesthetized and placed on the operating table as described above. An acupuncture needle was inserted into Li-angqiu (ST 34), Xuehai (SP 10), Neixiyan (EX-LE 4), and Waixiyan (EX-LE 5), and a Hans acupoint nerve stimulator was used to apply an electrical current with a frequency of rarefaction-dense wave of 2/100 Hz, 3 mA, for 20 min each session. This procedure was carried out three times weekly for 4 weeks.

**Sampling and assessment**

Behavioral monitoring: behavioral indices assessment. The behavioral indices were each assessed twice. Each index was assessed before the beginning of treatment, and at 1 week after the final treatment session. The results were evaluated and scored separately by two independent researchers, and the average scores were used for analysis. The modified Lequesne index was used to evaluate the knee joint level and grade of pain. The gait, knee joint activity, and swelling of the knee joint were assessed.

Behavioral monitoring: evaluation of the passive range of motion of the knee joint. The passive range of motion (PROM) of the left hind leg was assessed by measuring the joint angle with a protractor before the beginning of treatment, and at 1 week after the final treatment session. The axis center of the protractor was placed on the center of the knee joint, the stationary arm of the protractor was fixed parallel to the femur, and the moving arm traced the movement of the tibia. The flexion angle (°) was recorded when the knee joint was in the extreme extended or flexed positions, and the moving arm was at maximum extension. The PROM of the knee joint was calculated as the maximum extension angle minus the maximum flexion angle.

Histologic observation of the knee joint cartilage: the cartilage specimens were rinsed in phosphate-buffered saline before being immersed in neutral EDTA-Na2 for decalcification for 1 d. The specimens were subsequently dehydrated through the use of graded ethanol, embedded in paraffin, and then dewaxed. Each specimen was cut into 6-μm-thick serial longitudinal sections and stained with hematoxylin and eosin, safranin O, and toluidine blue to enable the observation of the pathologic changes in the articular cartilage and subchondral bone. Cartilage degeneration was assessed in accordance with the Mankin scoring system. The Mankin scores of the hematoxylin and eosin-stained articular cartilage specimens were evaluated using a double-blind method.
**Data processing and statistical analysis**

All experimental data were analyzed using SPSS 21.0 software (SPSS for Windows, version 21.0, SPSS Inc., Chicago, IL, USA). All data were normally distributed, and were expressed as the mean ± standard deviation ($\bar{x} \pm s$). Single-factor analysis of variance was used to compare results among multiple groups, and least significant difference $t$-tests were used to compare results between two groups. The level of significance was $P < 0.05$, and the level of marked significance was $P < 0.01$.

**Theory basis and practical development**

Acupotomy (Apo), a combination of acupuncture and modern surgical principles, was invented in China 1976, by professor Zhu Hanzhang. It has increasingly attracted attention for micro-traumatic concept and prominent curative effect in musculoskeletal disease including chronic neck pain, lower back pain, osteoarthritis and myofascial pain syndrome. An systematic review and meta-analysis reported that Ap used for treating KOA could receive a higher effective rate than electroacupuncture and could better relieve the pain remarkably. To explore the mechanism of Ap in treating KOA, our research team has made a series of animal researches. In vivo studies showed that immobilization increased matrix metalloproteinase 3 (MMP-3), inducing the loss of proteoglycan content and joint damage. Whereas joint movement prevented proteinase increase and proteoglycan loss. In 2008, we found improved Videman’s method of the left hind limb immobilization could induce KOA pathological changes successfully, Ap improved KOA rabbits’ knee-joint motion range, promoted the chondrocytes anabolism by decreasing MMP-3 and MMP-13 expression and increasing Col-II and Aggrecan proteins effectively. Mechanical stimulation has opposite effects on anabolism and catabolism.

**RESULTS**

**Gait and passive range of motion**

After modeling but before treatment, the Lequesne index was significantly higher in the normal control group compared with the other groups ($P < 0.01$), with no significant differences between the three KOA model groups. One week after the final treatment session, the Lequesne indexes of the three model groups were still significantly higher than that of the normal control group ($P < 0.01$). This indicates that the behavior of the normal control group still differed from that of the other three groups. The Lequesne index was improved by both Apo and E-Apu (Table 1). Compared with the blank model group, the Lequesne indexes of the Apo and E-Apu groups were significantly decreased ($P < 0.01$). Furthermore, the Lequesne index of the Apo group was significantly lower than that of the E-Apu group ($P < 0.05$).

**Histologic examination of cartilage specimens**

The articular cartilage in the normal control group was velvety and translucent, with no congestion or edema in the joint or synovial membranes (Figure 2A-D); the superficial, transitional, radiation, and calcified layers were clearly visible from the shallow to the deep layer.
The long axis of the chondrocytes was parallel to the cartilage surface. The chondrocytes in the superficial layer were small and singly distributed with a flat shape, but were spherical-shaped in the transitional layer, with similar sizes and irregular arrangement. The cells were divided and proliferated into homologous groups. Multiple cells in the radiation layer were arranged in columns perpendicular to the surface of the cartilage, with plenty of intercellular substance. The tidemark was clear and complete. No chondrocyte clusters or pannus were observed.

In the specimens collected from the three model groups after modeling but before treatment, the cartilage layers were disorderly arranged, and the four-layered structure was indistinct (Figure 3A-D). Chondrocyte detachment was observed in the superficial and transitional layers. Chondrocytes were severely clustered, and the tidemark was not visible. A small amount of chondrocytes were arranged in columns in the transitional layer.

In specimens collected from the Apo group at 1 week after the final treatment session, the superficial, transitional, radiation, and calcified layers of the cartilage structure were clearly observed (Figure 4A-D). The cartilage surface was rough, and the arrangement of chondrocytes was slightly disordered. Chondrocyte clusters were observed in some areas of the superficial and transitional layers. Cells in the radiation layer were regularly arranged in columns perpendicular to the surface of the cartilage. Chondrocytes were divided into homologous groups, and the tidemark was vaguely visible. No pannus was observed.

In specimens collected from the E-Apu group at 1 week after the final treatment session, the cartilage...
structure was reasonably clear (Figure 5A-D). The cartilage surface was irregular, and the chondrocytes were severely clustered. However, the cells in the radiation layer were still arranged in columns. The tidemark was vaguely visible, and pannus was not observed.

**Mankin scores**

Four rabbits (one from each group) were used in this assessment. Before treatment, compared with the normal control group, there were pathological changes in the knee joint cartilage specimens from the blank model, Apo, and E-Apu groups. The Mankin score in the three model groups was significantly greater than that in the normal control group (P < 0.01). Furthermore, the Mankin scores of the Apo and E-Apu groups were significantly lower than that of the blank model group (P < 0.01), and the Mankin score of the Apo group was significantly lower than that of the E-Apu group (P < 0.05) (Table 3).

**DISCUSSION**

We compared the curative effects of Apo and E-Apu in a rabbit model of KOA using the PROM and Lequesne index to assess changes in ethology, and using Safranin O-fast green staining and the Mankin score system to assess the morphologic changes in cartilage specimens. Decades of research into the establishment of KOA models has led to no specific recommendations regarding the selection of animal species. The animal species is usually chosen in accordance with the needs of the experiment, the funding, and the modeling mechanism. KOA models have been successfully established in many animal species, including rats, mice, and rabbits. The purpose of our research was to compare the curative effects of Apo and E-Apu for KOA. As the tip of the blade used in Apo is too large to use on very small animals and may induce serious hind leg injury, we selected rabbits as the most suitable species for our research.

There are two types of animal KOA models: spontaneous and induced. The mechanism of the spontaneous KOA model is close to the process seen in human disease, but the spontaneous model takes longer to establish than the induced model. The induced model of KOA can be established via surgical therapy, articular cavity injection, or joint fixation. Surgical therapy and articular cavity injection are effective in the short-term, but cause serious knee joint damage, and pathogenic factors could occur in the long-term; this will affect the determination of the efficacy of the intervention therapy. Motor control via joint fixation changes the normal stress of the knee joint, which induces cartilage degradation to establish the KOA model; this method is more suitable for studying the curative effect of Apo therapy, which is based on releasing adhesion tendons and remitting tension. Previous studies have successfully established a rabbit KOA model via the immobilization method, inducing changes in cartilage degradation of collagen II and proteoglycan, cartilage proliferation, and osteophyte formation. Thus, we selected the immobilization method to establish the rabbit KOA model. Previous studies have confirmed that the muscle-tendon unit plays a pivotal role in human movement. In the early stage of OA, the function of the muscle-tendon unit was impaired, and the stiffness of the patellar tendon increased. The purpose of our research was to compare the curative effects of Apo and E-Apu for KOA. As the tip of the blade used in Apo is too large to use on very small animals and may induce serious hind leg injury, we selected rabbits as the most suitable species for our research. We compared the curative effects of Apo and E-Apu in a rabbit model of KOA using the PROM and Lequesne index to assess changes in ethology, and using Safranin O-fast green staining and the Mankin score system to assess the morphologic changes in cartilage specimens.
E-Apu. Therefore, combined with the Lequesne index, the advantages of Apo therapy over E-Apu in changing knee joint behavior patterns may be associated with the resultant increase in PROM. Histologic examination revealed pathological morphological changes in cartilage sections stained with safranin O-fast green in the blank model, Apo, and E-Apu groups. Compared with the normal control group, the three model groups had significantly increased Mankin scores after model establishment but before treatment; this is similar to the findings of previous studies. Apo can repair the damage caused to the knee joint cartilage to a certain extent. Both Apo and E-Apu reduced the cartilage degeneration by varying degrees. In addition, Apo had a superior protective effect on cartilage than E-Apu. As Apo and E-Apu both effectively improved the ethology and morphology of rabbits with KOA, they may achieve similar satisfactory curative effects in cervical spondylosis of the nerve root. Further research is warranted into the curative effect of Apo intervention on rabbits’ behavioral indexes to compare the differences between Apo and E-Apu therapies. In conclusion, the modified Videman’s method of fixing the left hindleg in the extension position caused the rabbits to simulate the behavior displayed by humans with KOA, such as pain and dysfunction, and caused pathological changes in cartilage degradation. Both Apo and E-Apu improved the symptoms of the rabbit KOA model, including artrosis ache, swelling, dysfunction, and deformation. Overall, Apo had a superior treatment effect to E-Apu, regarding the PROM of the knee joint and morphological features.

**REFERENCES**


One of the acupotomy lysis on cartilage matrix changes in knee osteoarthritis. Rheumatol Int 2018; 38(3): 455-459.


Yu JN. Effect of acupotomy on FAK-PI3K-AKT signaling pathways and dynamics of quadriceps femoris in KOA rabbit articular cartilages by loose the tendon to treat the bone theory. Beijing: Beijing University of Chinese Medicine, 2016: 111.