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Electroacupuncture Alleviates Pain in Rats with Knee Osteoarthritis by Modulating Autophagy via the PI3K/Akt/mTOR Signaling Pathway

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Abstract

Background: This study aimed to investigate the effects of electroacupuncture (EA) on autophagy in the brain of rats with knee osteoarthritis (KOA) and to elucidate the potential mechanisms underlying its analgesic effects.

Methods: Sixty Sprague–Dawley rats were randomly assigned to six groups (n = 10 per group): normal control (Normal), KOA model (KOA), sham electroacupuncture (SA), electroacupuncture (EA), 740Y-P agonist (740Y-P), and electroacupuncture plus 740Y-P (EA + 740Y-P) groups. Except for the Normal group, all rats underwent

induction of KOA. After successful model establishment, rats in the EA group received electroacupuncture at Futu (ST32)–Zusanli (ST36) and Yinlingquan (SP9)–Yanglingquan (GB34). Rats in the SA group were needled at non-acupoint sites located 3 mm lateral to ST32, ST36, SP9, and GB34, with all other procedures identical to those used in the EA group. Rats in the 740Y-P group received intraperitoneal injections of 740Y-P, whereas rats in the EA + 740Y-P group received both EA and 740Y-P treatment. Interventions were administered every other day, three times per week, for 8 weeks (24 sessions in total). Behavioral assessments were performed before modeling and on days 7, 21, 35, 49, and 63 after modeling. Following treatment, knee joint histopathology was evaluated by hematoxylin and eosin (H&E) staining. Serum levels of interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF)- α were measured by ELISA. Autophagosomes in the hippocampus were examined by transmission electron microscopy, and ATP levels in brain tissue were determined using biochemical assay kits. The protein expression levels of PI3K, phosphorylated PI3K (p-PI3K), Akt, phosphorylated Akt (p-Akt), mTOR, p62, Beclin-1, Bcl-2, and Bax in brain tissue were assessed by Western blotting, while the mRNA expression levels of p62 and Beclin-1 were determined by RT-qPCR.

Results: Compared with the Normal group, rats in the KOA group exhibited significantly reduced thermal and mechanical pain thresholds, whereas these thresholds were increased following EA and/or 740Y-P treatment. KOA rats also showed marked knee joint structural damage, elevated serum levels of IL-1 β , IL-6, and TNF- α , increased numbers of autophagosomes, and significantly reduced ATP levels. In addition, the protein expression levels of p-PI3K, p-Akt, mTOR, p62, and Bcl-2 were significantly decreased, whereas those of Beclin-1 and Bax were significantly increased. Beclin-1 mRNA expression was increased, while p62 mRNA expression was decreased ($P < 0.05$). Compared with the KOA group, the EA, 740Y-P, and EA + 740Y-P groups exhibited ameliorated cartilage surface damage, decreased serum levels of IL-1 β , IL-6, and TNF- α , reduced numbers of autophagosomes in brain tissue, and significantly increased ATP levels. Moreover, Beclin-1 and Bax protein expression was significantly reduced, whereas p-PI3K, p-Akt, mTOR, p62, and Bcl-2 protein expression was

significantly increased. Beclin-1 mRNA expression was decreased, whereas p62 mRNA expression was increased ($P < 0.05$).

Conclusion: Electroacupuncture may alleviate pain in rats with KOA by activating the PI3K/Akt/mTOR signaling pathway and suppressing excessive autophagy in the brain, thereby contributing to the attenuation of KOA-related pathological changes.

Keywords: knee osteoarthritis; electroacupuncture; autophagy; PI3K/Akt/mTOR signaling pathway; pain; central nervous system

Introduction

Knee osteoarthritis (KOA) is a common degenerative joint disorder characterized by progressive articular cartilage degeneration, fibrosis, fissuring, and ulceration, with pain being its predominant clinical manifestation [1]. With increasing global life expectancy and population aging, KOA has become a major cause of disability, substantially impairing quality of life in older adults and imposing a considerable burden on healthcare systems [2]. As no curative treatment is currently available for KOA, pain relief and functional improvement remain the primary goals of clinical management [3]. Current pain management strategies include both pharmacological and non-pharmacological approaches. Oral medications are commonly used as first-line treatments; however, their long-term use may be limited by adverse effects and diminishing therapeutic efficacy [4]. For patients with advanced KOA, total knee arthroplasty can provide substantial pain relief, but postoperative complications and incomplete functional recovery remain important clinical concerns [5]. Therefore, safer and more effective therapeutic strategies for KOA-associated pain are urgently needed.

Increasing evidence suggests that KOA pain is not solely attributable to peripheral joint pathology. Persistent nociceptive input arising from the affected joint can induce sustained hyperexcitability of pain-processing neurons at multiple levels of the central nervous system, a phenomenon known as central sensitization. Central sensitization is considered an important pathophysiological mechanism underlying the persistence and amplification of chronic pain and may also contribute to the frequent coexistence of affective disturbances, including anxiety and depression [6]. Accordingly, therapeutic

strategies capable of preventing or reversing central sensitization may provide important benefits for the management of chronic KOA pain. Acupuncture has long been used as a non-pharmacological intervention for KOA and has shown potential benefits in reducing pain, alleviating inflammation, and improving joint function [7]. Electroacupuncture (EA), which combines traditional acupuncture with controlled electrical stimulation, applies electrical currents of defined frequencies and waveforms through acupuncture needles inserted at specific acupoints. Accumulating evidence suggests that EA can effectively relieve KOA-related pain and may provide advantages over manual acupuncture in certain settings. Its analgesic effects have been attributed to multiple mechanisms, including improvement of local microcirculation, attenuation of joint tissue damage, promotion of endogenous analgesic neurotransmitter release, enhancement of endogenous pain-inhibitory pathways, and modulation of relevant signaling pathways, gene expression, peripheral and central nociceptive processing, and biomechanical function [8,9]. Previous studies have primarily focused on the peripheral effects of EA, such as suppression of pro-inflammatory mediators, including IL-1 and TNF- α , and preservation of mitochondrial function in chondrocytes. In contrast, the mechanisms through which EA regulates pain processing within the central nervous system remain incompletely understood. In particular, the role of the hippocampus, a brain region involved in the affective and cognitive dimensions of chronic pain, warrants further investigation.

Autophagy is an evolutionarily conserved intracellular degradation and recycling process that maintains cellular homeostasis by removing damaged proteins and organelles. Under physiological conditions, autophagy generally serves a cytoprotective function; however, excessive or dysregulated autophagic activity may contribute to cellular dysfunction and death [10]. In KOA, appropriate activation of autophagy, including mitophagy, can protect chondrocytes against cellular injury and inflammatory stress [11]. In contrast, excessive or aberrant autophagy within the central nervous system may promote degradation of synaptic proteins, structural alterations in neurons, and even neuronal death, thereby contributing to chronic pain and neurological dysfunction [12]. Thus, the biological consequences of autophagy may be highly

dependent on its magnitude, duration, and tissue context. The PI3K/Akt/mTOR signaling pathway is a major intracellular pathway regulating cell survival, metabolism, and autophagy. Activation of phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) promotes activation of the mechanistic target of rapamycin (mTOR), which acts as a key negative regulator of autophagy and suppresses autophagosome formation [13]. Previous studies have demonstrated that chronic pain may be accompanied by abnormally enhanced autophagic activity in specific brain regions, together with reduced activity of the PI3K/Akt/mTOR pathway [14]. These observations raise the possibility that modulation of central autophagy through the PI3K/Akt/mTOR pathway may represent an important mechanism underlying pain regulation. However, whether EA exerts its central analgesic effects in KOA by activating the PI3K/Akt/mTOR pathway and consequently suppressing excessive autophagy remains largely unexplored. Based on these considerations, the present study established a rat model of KOA and applied EA at Futu (ST32), Zusanli (ST36), Yinlingquan (SP9), and Yanglingquan (GB34), four acupoints commonly used in the clinical management of KOA. The PI3K agonist 740Y-P was additionally employed to investigate the involvement of the PI3K/Akt/mTOR pathway. Behavioral pain responses and knee joint pathology were evaluated, while changes in hippocampal autophagosomes, brain ATP levels, PI3K/Akt/mTOR signaling, autophagy-related proteins (Beclin-1 and p62), and apoptosis-related proteins (Bax and Bcl-2) were systematically examined. Through these analyses, this study aimed to clarify whether EA alleviates chronic KOA pain by regulating central autophagy through the PI3K/Akt/mTOR signaling pathway and to provide mechanistic evidence supporting the clinical application of EA for KOA-associated pain.

1 Materials and Methods

1.1 Experimental Animals

Sixty SPF-grade female SD rats (6–8 weeks old, body weight 180–200 g) were purchased from SPF (Beijing) Biotechnology Co., Ltd. (Animal license No.: SCXK [Beijing] 2024-0001). The animals were housed under standard conditions (temperature

22 °C, humidity 55%, 12 h light/12 h dark cycle) with free access to food and water and were acclimatized for 7 days before the experiment. All experimental procedures were approved by the Ethics Committee of Henan Provincial Hospital of Traditional Chinese Medicine (Approval No.: PZ-HNSZYY-2025-013).

1.2 Reagents and Instruments

PI3K activator 740Y-P (Cell Signaling Technology); hematoxylin and eosin (HE) staining solution (Servicebio); ELISA kits for interleukin-6 (IL-6), IL-1 β , and tumor necrosis factor- α (TNF- α) (Union Biological, China); TRIzol reagent (Beyotime, Shanghai, China); antibodies against p-PI3K, PI3K (Proteintech), p-Akt, Akt, mTOR, p62, Beclin-1, Bcl-2, Bax, and GAPDH (Cell Signaling Technology); EL204 analytical balance (Mettler Toledo, Switzerland); 2H12003 microscope (Olympus, Japan).

1.3 Animal Grouping and Model Establishment

Sixty SD rats were randomly divided into six groups using a random number table: Normal group, Model group, Sham electroacupuncture group (SA), Electroacupuncture group (EA), Agonist group, and EA plus agonist group, with 10 rats in each group. A total of 60 rats were initially allocated to six experimental groups, with 10 rats in each group. Different numbers of biological samples were included in subsequent biochemical and molecular analyses. Specifically, 4 – 7 serum samples per group were analyzed by ELISA, six brain tissue samples per group were used for ATP determination, and three samples per group were included in RT-qPCR analysis. The actual sample size for each assay is presented in the corresponding tables. Except for the Normal group, KOA models were established in all other groups using the destabilization of the medial meniscus (DMM) surgery. DMM model establishment: Rats were anesthetized with sodium pentobarbital (35 mg/kg, intraperitoneal injection). After shaving and disinfecting the surgical area, the rats were placed in a supine position with the right hind limb fixed and flexed at 90°. The skin was incised to expose the patellar ligament, and the joint capsule was opened along the medial edge of the patellar ligament. After blunt removal of intra-articular fat, the medial meniscus, which is connected to the tibial plateau by the medial meniscotibial ligament (MMTL), was exposed. The MMTL was transected, the wound was irrigated, the joint cavity was sutured, and the skin was

closed. Erythromycin ointment was applied locally to prevent infection.

Intervention methods: Acupoints on the affected side included Futu (ST32), Zusanli (ST36), Yinlingquan (SP9), and Yanglingquan (GB34), located according to the 2021 Chinese Acupuncture Society standards for rat acupoints and the Experimental Acupuncture Atlas [15,16]. EA group: After needle insertion, electrodes were connected to Futu–Zusanli and Yinlingquan–Yanglingquan pairs, respectively. Stimulation was delivered for 30 min using a 2/100 Hz dense-disperse wave, with slight needle vibration and no struggling by the animals. Treatment was administered once every two days, three times per week, for 8 weeks (24 sessions in total). SA group: Needles were inserted 3 mm lateral to each corresponding acupoint at non-acupoint sites; all other procedures were identical to those in the EA group. Normal and Model groups: Rats were restrained in the same manner as the EA group but received no EA or sham intervention. Agonist group: Rats received intraperitoneal injection of 740Y-P (20 mg/kg) followed by restraint. EA + agonist group: Rats first received intraperitoneal injection of 740Y-P (as in the agonist group). After 30 min, they were restrained and treated with EA (as in the EA group).

1.4 Behavioral Assessment

Behavioral tests were conducted before modeling and on days 7, 21, 35, 49, and 63 after modeling.

(1) Mechanical withdrawal threshold (MWT): Using Von Frey filaments and the up–down method [17], the 50% mechanical withdrawal threshold was calculated. Rats were placed in transparent Plexiglas cages (20×20×25cm) with a mesh floor (0.5×0.5cm). After 20 min of acclimatization, filaments (6–60g) were applied vertically to the center of the right hind paw for 2 s with 15 s intervals. Each test was repeated five times. The 50%MWT was calculated according to the standard formula.

(2) Thermal withdrawal latency: Rats were placed in acrylic chambers on a glass plate and allowed to acclimatize for 20 min. After preheating the apparatus, the radiant heat source was focused on the plantar surface of the right hind paw. The time from onset of irradiation to paw withdrawal was recorded as the thermal withdrawal latency. Each rat was tested six times at 5 min intervals, and the mean value was calculated.

1.5 HE Staining for Histopathological Changes in Synovial Tissue

Rats were anesthetized with 3% sodium pentobarbital (30 mg/kg) and sacrificed by air embolism. Synovial tissues of the knee joints were carefully dissected. One portion was fixed in 4% paraformaldehyde for 24 h, dehydrated through graded ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 μ m. Sections were stained with hematoxylin and eosin and observed under a light microscope.

1.6 ELISA for Serum Inflammatory Cytokines

After anesthesia, 5 mL of blood was collected from the abdominal aorta into anticoagulant tubes. Samples were centrifuged at 1,000 r/min for 15 min, and serum was collected for determination of IL-6, IL-1 β , and TNF- α levels according to the manufacturers' instructions. The numbers of serum samples included in the ELISA analysis were 5, 5, 7, 5, 4, and 5 for the Normal, KOA, KOA + 740Y-P, KOA + SA, KOA + EA, and KOA + EA + 740Y-P groups, respectively.

1.7 Transmission Electron Microscopy for Autophagosomes in Brain Tissue

Hippocampal tissues were fixed in 2.5% glutaraldehyde and washed three times in 0.1 mol/L phosphate buffer (15 min each). Samples were post-fixed in 1% osmium tetroxide for 2 h in the dark, washed, dehydrated, embedded, sectioned, stained, and observed under a transmission electron microscope to quantify autophagosomes.

1.8 Biochemical Assay for Mitochondrial ATP in Brain Tissue

ATP concentrations were determined using a commercial biochemical kit. A standard curve was generated, and 100 μ L of ATP working solution was added to each sample. ATP levels were calculated based on the standard curve. ATP levels were measured in brain tissue samples from six rats in each experimental group ($n = 6$ per group).

1.9 Western Blot Analysis of Protein Expression in Brain Tissue

Brain tissues were homogenized in TRIzol reagent and centrifuged at 10,000 r/min for 10 min. Total protein concentration was determined using a BCA assay. Proteins were denatured, separated by SDS-PAGE, and transferred onto PVDF membranes. Membranes were blocked with 5% skim milk for 1 h, incubated overnight at 4 $^{\circ}$ C with primary antibodies (1:500), followed by HRP-conjugated secondary antibodies (1:1000)

for 2 h at room temperature. Bands were visualized using ECL reagent and analyzed with ImageJ software.

1.10 RT-qPCR for Gene Expression in Synovial Tissue

Total RNA was extracted using TRIzol according to the manufacturer's instructions. RNA was reverse-transcribed into cDNA using SweScript All-in-One SuperMix. Quantitative real-time PCR was performed using Universal Blue SYBR Green qPCR Master Mix. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method. Primer sequences used for qRT-PCR are listed in Table 1. RT-qPCR analysis was performed using three biological samples from each experimental group (n = 3 per group).

Table 1 Primer sequence

Gene	Sequence (5'-3')	长度	种属
RPL13a	F:ATGAACACCAACCCGTCTCG	138	<i>Rat</i>
	R:GCCTCTTTTGGTCTTGTGCGT		
P62	F:GGTGTCTGTGAGAGGACGAG	134	<i>Rat</i>
	R:CCTCTTACTGGGGTTCCACG		
Beclin 1	F:CATGCAGGTGAGCTTCGTGTC	108	<i>Rat</i>
	R:CCTGAATGGTCACTCGGTCC		

1.11 Statistical Analysis

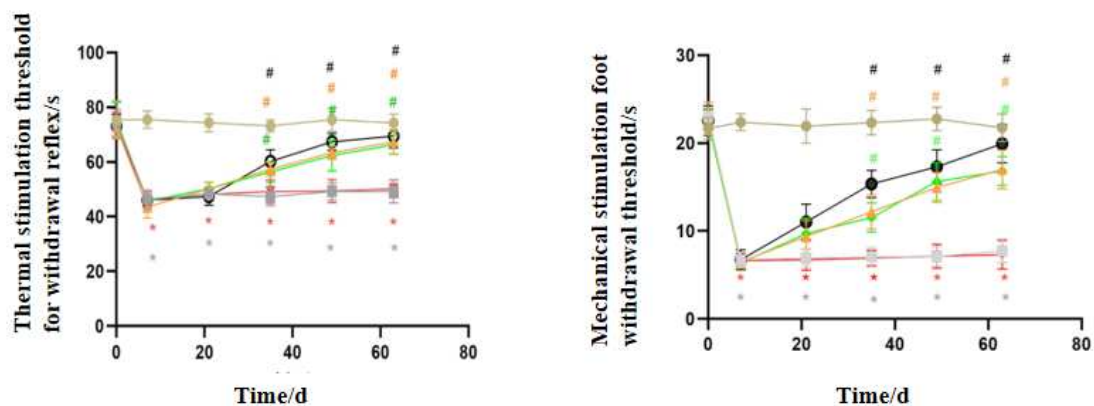
GraphPad Prism 8.0 software was used for statistical analysis of the experimental data. Quantitative data are expressed as mean $\pm$ standard deviation ($\bar{x} \pm s$). Intergroup comparisons were performed using repeated-measures analysis of variance (ANOVA) and one-way ANOVA. Multiple comparisons were conducted using the LSD-t test. A value of $P < 0.05$ was considered statistically significant. Sample sizes varied among experimental endpoints. The actual number of biological replicates (n) for each analysis is indicated in the corresponding tables and figures. The individual rat was considered the experimental unit when measurements were obtained from independent animals. Technical replicates were not considered independent biological replicates.

2 Result

2.1 Changes in Thermal and Mechanical Withdrawal Thresholds in Each Group

There were no significant differences in thermal pain thresholds or mechanical

withdrawal thresholds among the groups before model establishment ($P > 0.05$). After modeling, the thermal and mechanical withdrawal thresholds in all groups were significantly lower than those in the Normal group ($P < 0.05$). After intervention, the thermal and mechanical withdrawal thresholds in each group increased, and the analgesic effect became progressively more evident over time, persisting up to day 63 after modeling ($P < 0.05$). Moreover, the thermal and mechanical withdrawal thresholds in the electroacupuncture group, agonist group, and electroacupuncture plus agonist group were significantly higher than those in the Model group ($P < 0.05$), as shown in Figure 1.



Note: *VS Norma $P < 0.05$; #VS KOA $P < 0.05$;

Figure 1 Comparison of the thermal and mechanical withdrawal thresholds in each group of rats

2.2 Histopathological Changes in Knee Joint Tissues of Rats in Each Group

In the Normal group, no obvious histopathological changes were observed in the knee joint. The cartilage surface was smooth and intact, and chondrocytes were evenly distributed. In the Model group, severe cartilage damage was observed, with abnormal morphological structure, destruction of the cartilage membrane, blurred tidemark, sparse and disorganized chondrocytes. The pathological changes in the SA group were similar to those in the KOA group. Compared with the KOA group, the EA group, 740Y-P group, and EA + 740Y-P group showed marked improvement in synovial and cartilage histopathology. Cartilage damage was alleviated, the morphological structure was relatively normal, the cartilage surface was comparatively smooth, and chondrocytes

were arranged in an orderly manner (Figure 2).

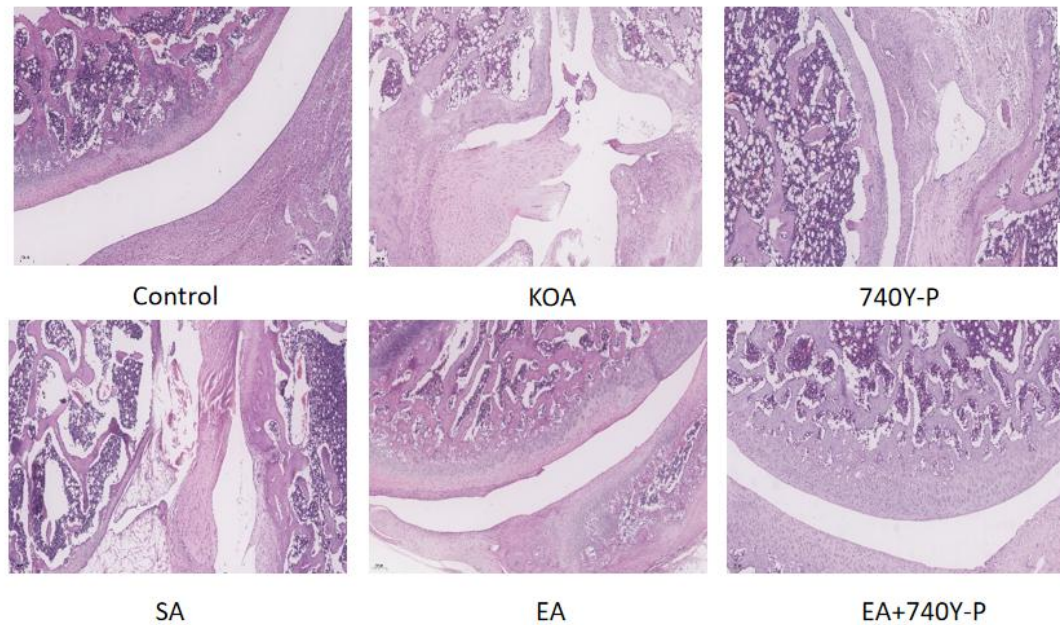


Figure 2 HE staining of knee joints in each group of rats

2.3 Changes in Autophagosomes in the Hippocampus of Rats in Each Group

In the Normal group, no autophagolysosomes or autophagosomes were observed. In the KOA group, the numbers of autophagosomes and autophagolysosomes were markedly increased. The SA group showed no obvious differences in the numbers of autophagosomes and autophagolysosomes compared with the KOA group. Compared with the KOA group, the EA group, 740Y-P group, and EA + 740Y-P group exhibited a reduction in the number of autophagosomes and autophagolysosomes (Figure 3).

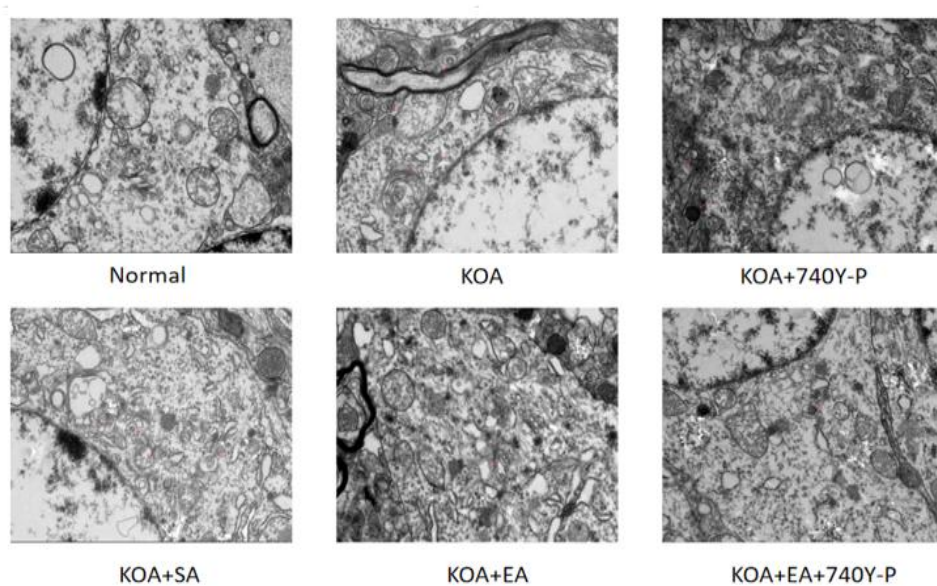


Figure 3 Transmission electron microscopy examination of autophagosome changes in the hippocampus of rats in each group (×10000)

2.4 Comparison of Serum Inflammatory Cytokine Levels Among Groups

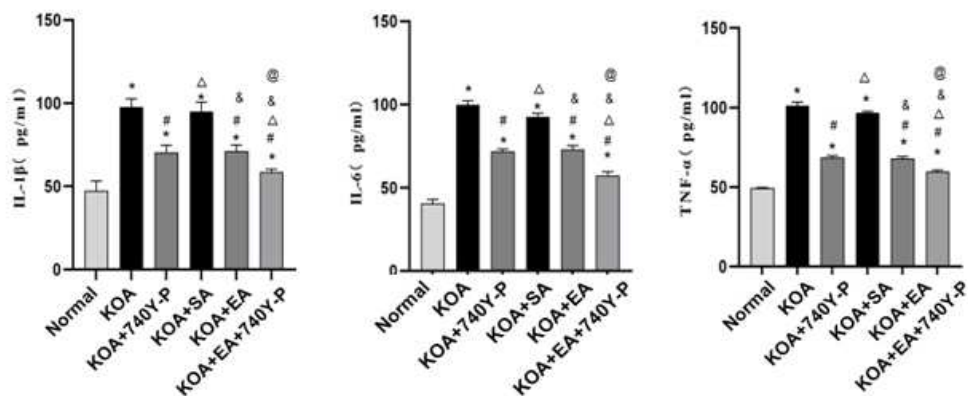
Serum levels of TNF- α , IL-6, and IL-1 β in the KOA group were significantly higher than those in the Normal group ($P < 0.05$). Compared with the KOA group, the EA group, 740Y-P group, and EA + 740Y-P group showed significantly reduced serum levels of TNF- α , IL-6, and IL-1 β ($P < 0.05$). In contrast, no significant differences in serum TNF- α , IL-6, or IL-1 β levels were observed between the SA group and the KOA group ($P > 0.05$) (Table 2 and Figure 4).

Table 2 Expression levels of IL-6, IL-1 β and TNF- α in rat serum($\bar{x} \pm s$)

Group	n	IL-6 (pg/ml)	IL-1 β (pg/ml)	TNF- α (pg/ml)
Normal	5	40.66 $\pm$ 5.27	47.41 $\pm$ 5.77	49.39 $\pm$ 0.74
KOA	5	99.78 $\pm$ 5.70*	97.65 $\pm$ 5.06*	101.35 $\pm$ 4.64*
KOA+740Y-P	7	71.90 $\pm$ 3.86*#	70.53 $\pm$ 4.24*#	68.67 $\pm$ 2.68*#
KOA+SA	5	92.65 $\pm$ 4.85* Δ	95.08 $\pm$ 5.57* Δ	96.71 $\pm$ 1.89* Δ
KOA+EA	4	73.21 $\pm$ 4.54*#&	71.13 $\pm$ 3.83*#&	68.05 $\pm$ 2.15*#&
KOA+EA+740Y-	5	57.45 $\pm$ 5.08*# Δ &@	58.72 $\pm$ 1.67*# Δ &@	59.81 $\pm$ 1.97*# Δ &@

P

Note:*VS Norma $P < 0.05$;#VS KOA $P < 0.05$; Δ VS KOA+740Y-PP < 0.05 ;&VS KOA+SAP < 0.05 ;@VS KOA+EAP < 0.05 ;



Note: *VS Norma $P < 0.05$;#VS KOA $P < 0.05$; Δ VS KOA+740Y-PP < 0.05 ;&VS KOA+SAP < 0.05 ;@VS KOA+EAP

<0.05;

Figure 4 Comparison of the expression levels of IL-6, IL-1β, and TNF-α in rat serum

2.5 Changes in Mitochondrial ATP Levels in Brain Tissue

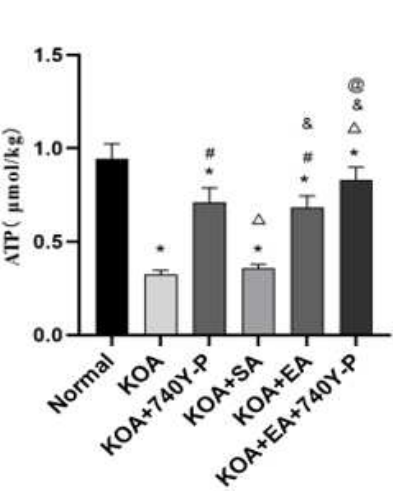
ATP synthesis in the KOA group was significantly lower than that in the Normal group ($P < 0.05$). Compared with the KOA group, ATP levels were significantly increased in the EA group, 740Y-P group, and EA + 740Y-P group ($P < 0.05$). In contrast, no significant difference in ATP levels was observed between the SA group and the KOA group ($P > 0.05$) (Table 3 and Figure 5).

Table 3 ATP Content of Mitochondria in Rat Brain Tissue ($\bar{x} \pm s$)

Group	n	ATP ($\mu\text{mol/kg}$)
Normal	6	0.94±0.08
KOA	6	0.32±0.02*
KOA+740Y-P	6	0.71±0.08**
KOA+SA	6	0.36±0.02* [△]
KOA+EA	6	0.68±0.06* ^{#&}
KOA+EA+740Y-P	6	0.83±0.07* ^{#△&@}

Note: *VS Norma $P < 0.05$; #VS KOA $P < 0.05$; [△]VS KOA+740Y-PP $P < 0.05$; &VS KOA+SAP $P < 0.05$; @VS KOA+EAP

<0.05;



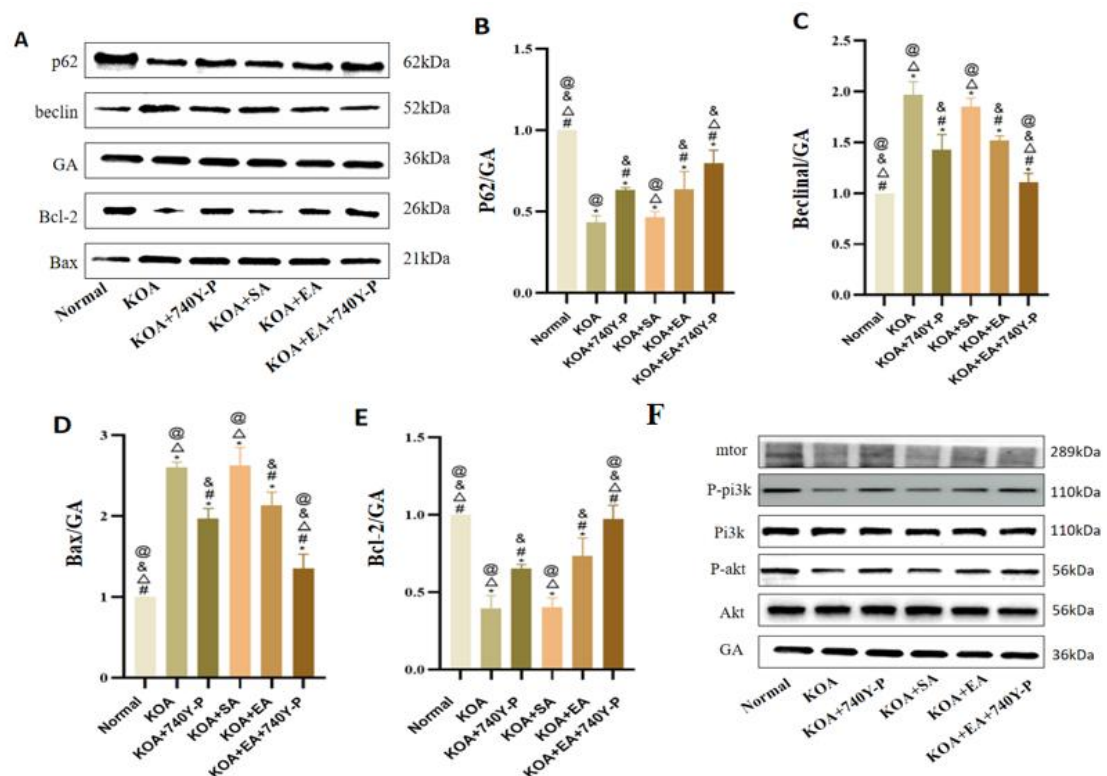
Note: *VS Norma $P < 0.05$; #VS KOA $P < 0.05$; [△]VS KOA+740Y-PP $P < 0.05$; &VS KOA+SAP $P < 0.05$; @VS KOA+EAP

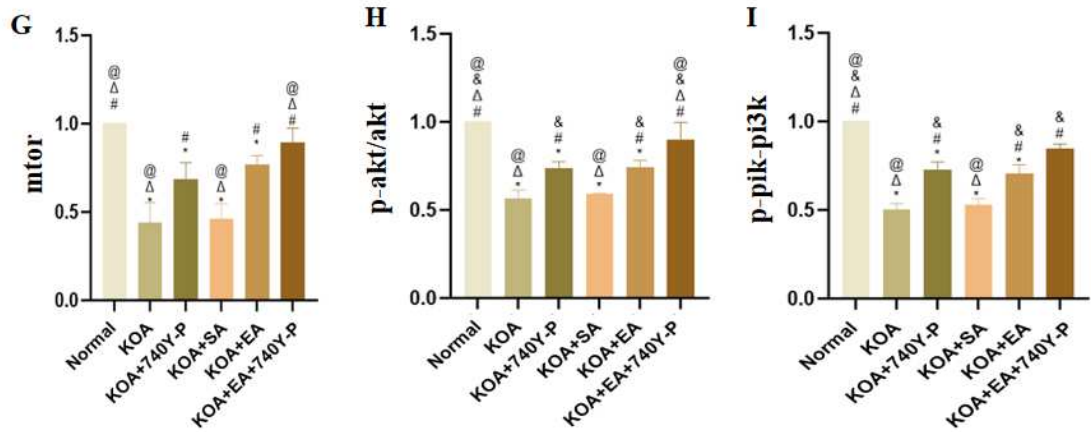
<0.05;

Figure 5 Comparison of mitochondrial ATP content in brain tissues of each group of rats

2.6 Comparison of PI3K/Akt/mTOR Pathway–Related and Autophagy-Related Protein Expression in Brain Tissue Among Groups

In the KOA group, protein expression levels of Beclin-1 and Bax in brain tissue were significantly higher than those in the Normal group, whereas the expression levels of p-PI3K, p-Akt, mTOR, p62, and Bcl-2 were significantly lower than those in the Normal group ($P < 0.05$). Compared with the KOA group, the EA group, 740Y-P group, and EA + 740Y-P group showed significantly decreased expression of Beclin-1 and Bax, and significantly increased expression of p-PI3K, p-Akt, p62, and Bcl-2 in brain tissue ($P < 0.05$). However, there were no significant differences in the expression of mTOR, Beclin-1, Bax, p-PI3K, p-Akt, p62, or Bcl-2 between the SA group and the KOA group ($P > 0.05$) (Figure 6). These findings demonstrate that electroacupuncture can inhibit autophagy by activating the PI3K/Akt/mTOR signaling pathway, thereby alleviating pain.





Note: A-E: Comparison of protein expression of P62, Beclin, Bcl-2, and Bax in the brain tissues of each group of rats;
 F-I: Expression of p-P3K, PI3K, p-AKT, AKT, and mTOR in the brain tissues of each group. *VS Normal $P < 0.05$; #VS KOA $P < 0.05$; ^VS KOA+740Y-P $P < 0.05$; &VS KOA+SA $P < 0.05$; @VS KOA+EA $P < 0.05$;

Figure 6 Comparison of protein expressions of p-P3K, PI3K, p-AKT, AKT, mtor, p62, Beclin, Bcl-2, and Bax in the brain tissues of each group of rats

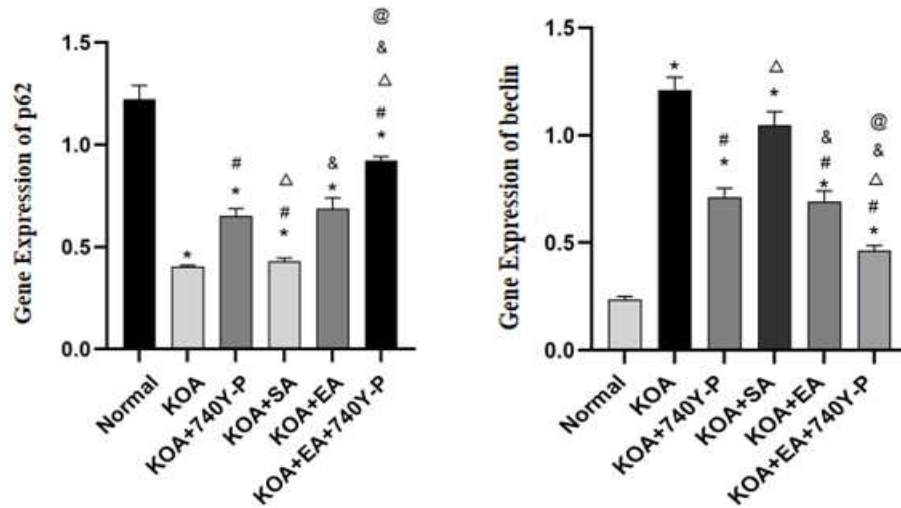
2.7 Expression of Autophagy-Related Genes in Brain Tissue of Rats in Each Group

In the KOA group, p62 gene expression in brain tissue was lower than that in the Normal group, while Beclin-1 gene expression was higher than that in the Normal group ($P < 0.05$). Compared with the KOA group, the EA group, 740Y-P group, and EA + 740Y-P group showed significantly increased p62 gene expression and significantly decreased Beclin-1 gene expression in brain tissue ($P < 0.05$). In the SA group, there were no significant changes in Beclin-1 or p62 expression compared with the KOA group ($P > 0.05$) (see Table 4 and Figure 7).

Table 4 Expression levels of p62 and beclin mRNA in rat brain tissue ($\bar{x} \pm s$)

Group	n	beclin	p62
Normal	3	0.24±0.03	1.22±0.12
KOA	3	1.21±0.08*	0.41±0.01*
KOA+740Y-P	3	0.71±0.07*#	0.65±0.06*#
KOA+SA	3	1.05±0.11*^	0.43±0.03*^
KOA+EA	3	0.69±0.08*#&	0.68±0.09*#&
KOA+EA+740Y-	3	0.46±0.04*#^&@	0.92±0.03*#^&@

Note: *VS Norma $P < 0.05$; #VS KOA $P < 0.05$; Δ VS KOA+740Y-PP $P < 0.05$; &VS KOA+SAP $P < 0.05$; @VS KOA+EAP $P < 0.05$;



Note: *VS Norma $P < 0.05$; #VS KOA $P < 0.05$; Δ VS KOA+740Y-PP $P < 0.05$; &VS KOA+SAP $P < 0.05$; @VS KOA+EAP $P < 0.05$;

Figure 7 Comparison of p62 and beclin mRNA expression levels in brain tissues of each group of rats

Discussion

Knee osteoarthritis (KOA) is not merely a peripheral joint disorder characterized by structural degeneration, but also a chronic pain condition involving complex interactions between the peripheral and central nervous systems [18]. In the present study, a rat model of KOA was established to systematically evaluate the effects of electroacupuncture (EA) on pain-related behaviors, articular cartilage pathology, and autophagy-associated changes in the brain. Our findings showed that EA significantly increased mechanical and thermal pain thresholds and ameliorated pathological damage to the knee joint in KOA rats. At the molecular level, these effects were accompanied by activation of the PI3K/Akt/mTOR signaling pathway and suppression of excessive autophagy in the hippocampus, suggesting that regulation of central autophagy may contribute to the analgesic effects of EA in KOA.

Pain is the primary reason patients with KOA seek medical attention and is a major

contributor to impaired quality of life and psychological comorbidities, including anxiety and depression. Importantly, the severity of pain in KOA varies considerably among individuals and correlates only modestly with radiographic structural abnormalities, indicating that the intensity of pain cannot be fully explained by peripheral joint damage alone [19]. Accumulating evidence suggests that persistent nociceptive input arising from the diseased joint induces neuroplastic alterations in both the peripheral and central nervous systems, resulting in enhanced neuronal excitability and amplification of nociceptive signaling. This phenomenon, generally referred to as central sensitization, is considered an important mechanism underlying persistent and disproportionate pain in KOA [20,21]. Therefore, targeting central mechanisms of pain processing may provide an important therapeutic strategy for improving chronic KOA pain.

Autophagy is a highly conserved intracellular degradation process that plays a context-dependent role in maintaining cellular homeostasis. Moderate activation of autophagy generally exerts cytoprotective effects, whereas excessive or dysregulated autophagy may contribute to cellular dysfunction and death. In cartilage, mitophagy is generally considered beneficial because it facilitates the removal of damaged mitochondria and limits oxidative stress. For example, previous studies have shown that modified Duhuo Jisheng decoction can activate PINK1/Parkin-mediated mitophagy in chondrocytes, thereby promoting the clearance of damaged mitochondria, reducing reactive oxygen species accumulation, and delaying cartilage degeneration [22]. Conversely, insufficient autophagy in chondrocytes may lead to the accumulation of damaged organelles and promote apoptosis and extracellular matrix degradation. However, the effects of autophagy may differ in the central nervous system. Under conditions of persistent pain, ischemia, hypoxia, or prolonged cellular stress, excessive or dysregulated autophagy may result in degradation of essential neuronal and synaptic components, thereby impairing neuronal structure and function and potentially contributing to chronic pain-related neuroplasticity [23].

In the present study, transmission electron microscopy revealed an increased number of autophagic structures in the hippocampus of KOA rats. Consistent with this

observation, Beclin-1 protein expression was increased, whereas p62 expression was decreased. These findings suggest enhanced autophagy-associated activity in the hippocampus following KOA induction. Autophagy and apoptosis are closely interconnected processes that jointly determine neuronal survival under pathological stress. Autophagy can promote cellular homeostasis by removing damaged proteins and organelles; however, excessive or persistent autophagic activation may interact with apoptotic signaling and contribute to neuronal injury [24]. Beclin-1 is a key regulator of autophagy initiation and participates in the formation of the Beclin-1–Vps34 complex, which is essential for autophagosome nucleation. Increased Beclin-1 expression is therefore commonly associated with enhanced autophagy initiation. In contrast, p62 is selectively incorporated into autophagosomes and subsequently degraded in lysosomes; thus, reduced p62 expression is generally consistent with enhanced autophagic degradation, although interpretation of autophagic flux ideally requires dynamic assessment [25].

Notably, KOA rats also exhibited increased expression of the pro-apoptotic protein Bax and decreased expression of the anti-apoptotic protein Bcl-2. Bcl-2 not only suppresses apoptosis but can also interact with Beclin-1 and inhibit autophagy initiation. Under pathological conditions, disruption of the Bcl-2/Beclin-1 interaction may facilitate autophagic activation, while increased Bax activity favors apoptotic signaling. Taken together, the simultaneous increase in Beclin-1 and Bax and decrease in p62 and Bcl-2 observed in KOA rats suggest that chronic KOA pain is associated with dysregulated autophagy and enhanced apoptotic stress in the hippocampus. Following EA treatment, the number of autophagic structures was reduced, Beclin-1 and Bax expression decreased, and p62 and Bcl-2 expression increased. These findings suggest that EA may suppress excessive autophagy-associated activity and attenuate neuronal stress, thereby contributing to reduced central pain sensitization. The PI3K/Akt/mTOR pathway is an important regulator of cell survival, metabolism, and autophagy. In the KOA group, the levels of p-PI3K, p-Akt, and mTOR were significantly lower than those in the Normal group, suggesting suppression of PI3K/Akt/mTOR signaling during chronic KOA pain. Because mTOR serves as a major negative regulator of autophagy,

reduced activity of this pathway may facilitate autophagy activation. This interpretation is consistent with the increased Beclin-1 expression, decreased p62 expression, and increased abundance of autophagic structures observed in the KOA group. In parallel, reduced Bcl-2 and increased Bax expression indicated enhanced apoptotic stress. EA markedly reversed these molecular alterations. The expression levels of p-PI3K, p-Akt, and mTOR were significantly increased following EA treatment, accompanied by reduced Beclin-1 and Bax expression and increased p62 and Bcl-2 expression. These results suggest that EA may activate the PI3K/Akt/mTOR signaling pathway and thereby suppress excessive autophagy-associated activity while enhancing neuronal survival. To further examine the involvement of this pathway, the PI3K agonist 740Y-P was used as a pharmacological intervention. Treatment with 740Y-P, either alone or in combination with EA, increased mechanical and thermal pain thresholds, improved knee joint pathology, and produced molecular changes broadly consistent with activation of the PI3K/Akt/mTOR pathway and suppression of autophagy. In contrast, sham EA produced substantially weaker effects. Collectively, these findings support the involvement of PI3K/Akt/mTOR-mediated regulation of central autophagy in the analgesic action of EA in KOA.

From the perspective of traditional Chinese medicine (TCM), KOA is generally classified within the category of “Bi syndrome,” which is traditionally attributed to invasion of external pathogenic factors, obstruction of meridians, impaired circulation of Qi and blood, blood stasis, and deficiency of the liver and kidney. Clinically, the condition is characterized by joint pain, stiffness, and restricted flexion and extension. Acupuncture treatment is traditionally used to regulate Qi and blood, unblock the meridians, relieve pain, and improve musculoskeletal function [26]. EA combines conventional acupuncture with controlled electrical stimulation and may enhance and prolong the stimulatory effects of acupuncture. Previous studies have reported that EA can effectively relieve KOA-related pain and improve knee function [27]. In the present study, Futu (ST32), Zusanli (ST36), Yinlingquan (SP9), and Yanglingquan (GB34) were selected as the principal acupoints. According to TCM theory, Zusanli (ST36), the He-Sea point of the Stomach Meridian, is commonly used to regulate Qi and blood and

strengthen the body, whereas Yanglingquan (GB34), the He-Sea point of the Gallbladder Meridian and the influential point of the tendons, is frequently selected for musculoskeletal and knee disorders [28]. Futu (ST32) is traditionally used for pain and dysfunction of the lower extremities, while Yinlingquan (SP9), the He-Sea point of the Spleen Meridian, is commonly used for disorders involving the knee and lower limbs [29]. From a modern biomedical perspective, EA may exert analgesic effects through multiple mechanisms, including modulation of peripheral inflammation, improvement of local circulation, regulation of nociceptive signaling, and alteration of central neuroplasticity [30,31]. The present findings further suggest that regulation of PI3K/Akt/mTOR signaling and autophagy within the central nervous system may represent an additional mechanism underlying the analgesic effects of EA in KOA.

Overall, our results suggest that chronic KOA pain is associated with suppression of the PI3K/Akt/mTOR pathway, enhanced autophagy-associated activity, and increased apoptotic stress in the hippocampus. EA treatment partially reversed these alterations and alleviated pain-related behaviors, indicating that activation of PI3K/Akt/mTOR signaling and regulation of central autophagy may contribute to its analgesic effects. These findings provide additional mechanistic evidence supporting the use of EA for KOA-related chronic pain and highlight central autophagy as a potential target for further investigation.

Conclusion

In summary, this study demonstrates that chronic pain in KOA is associated with inhibition of the PI3K/Akt/mTOR pathway and excessive autophagy in rat brain tissue. EA stimulation at acupoints such as Futu and Zusanli effectively activates the PI3K/Akt/mTOR signaling pathway, upregulates p-PI3K, p-Akt, and mTOR expression, inhibits Beclin-1-dependent excessive autophagy, reduces neuronal apoptosis, significantly increases pain thresholds, alleviates pain, and delays cartilage degeneration. These findings not only enrich the scientific understanding of acupuncture but also provide experimental evidence for targeting the PI3K/Akt/mTOR pathway in the treatment of KOA chronic pain, further establishing EA as a safe and effective non-pharmacological therapy for comprehensive KOA management.

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Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Henan Provincial Hospital of Traditional Chinese Medicine (Approval No.:PZ-HNSZYY-2025-013). All experiments were conducted in accordance with the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals.

Author Contributions

Chongtao Han, Jingjing Zhao, and Fuwei Pan contributed equally to this work and share first authorship. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas, took part in drafting, revising or critically reviewing the article, gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no competing interests.

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