Research of the analgesic effects and central nervous system impact of electroacupuncture therapy in rats with knee osteoarthritis

Xiahai Zheng [*], Jing Lin, Zhenzhen Wang, Zhenming Zeng, Haoxiong Chen

Department of Rehabilitation, The Third Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, 510375, Guangdong Province, China

A R T I C L E   I N F O

A B S T R A C T

It aimed to observe the effects of TongDu TiaoShen (TDTS) electroacupuncture (EA) on the analgesia and central system of knee osteoarthritis (KOA) rats and explore its mechanism. SD rats were rolled into the blank group, model group (KOA), control group (duloxetine 500 mg/kg/d, Ctrl), conventional EA group, and TDTS-EA group. Radiometric pain measurements and the Lequesne MG scale were used to evaluate the behavioral performance of the rats. Dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT), β-endorphin (β-EP), and leucine-enkephalin (L-ENK) were detected in the midbrain and spinal cord of lumbar enlargement. Interleukin (IL)-1β protein expression was detected by Western blot. The incubation period of thermal pain and foot contraction was decreased in the KOA group versus blank group, the Lequesne MG score was increased, DA, NE, 5-HT, β-EP, and L-ENK in the midbrain and spinal cord were increased, and synovial tissue IL-1β protein expression was increased ($P < 0.05$). EA group and TDTS-EA group had an increased incubation period of thermal pain contraction, decreased Lequesne MG score, decreased DA, NE, etc. In the midbrain, increased 5-HT and NE in the spinal cord, and decreased IL-1β in the synovial tissue versus KOA group ($P < 0.05$). The Lequesne MG score and midbrain DA, NE, 5-HT, β-EP, and synovial tissue IL-1β expression were decreased in TDTS-EA group versus EA group ($P < 0.05$). EA can effectively improve the behavioral score of KOA and participate in central analgesia by regulating central DA, NE, 5-HT, β-EP, and L-ENK.

1. Introduction

Knee osteoarthritis (KOA) is common in elderly individuals, and patients often show pain around the knee. However, acute pain has a high risk of being converted into chronic pain. If this conversion is not treated with timely intervention, it will lead to damage to the nervous system of patients, which will progress to chronic refractory pain [1]. Knee osteoarthritis (OA) is a degenerative osteoarthritis disease characterized by knee soft tissue inflammation, with a worldwide incidence rate of 3.6 % [2], while the incidence rate of people over 40 years old is as high as 15.6 % [3], which is increasing year by year [4]. The pathology of KOA shows pathological changes in articular cartilage, as well as changes in tissue structures and pathological cells, such as the synovial membrane, subchondral bone, and

* Corresponding author. Department of Rehabilitation, The Third Affiliated Hospital of Guangzhou University of Chinese Medicine, No. 261 Longxi Avenue, Liwan District, Guangzhou City, Guangdong Province, China.
E-mail address: zhengxiahai_2013@163.com (X. Zheng).

meniscus. Therefore, studies have confirmed that severe KOA may lead to disability in patients [5,6]. At present, the pathogenesis of KOA is not completely clear. The treatment of KOA in Western medicine is classified into nondrug treatment and drug treatment. Nondrug treatment of KOA includes hyperthermia, electrotherapy, percutaneous nerve electrical stimulation, and intra-articular injection of sodium hyaluronate [7–9]. Drugs for the treatment of KOA include oral diacerein and diclofenac sodium. The short-term effect of drugs for the treatment of KOA is good, but the pain can't be improved [10,11]. However, traditional Chinese medicine (TCM) believes that the root causes of knee OA are "deficiency of liver and kidney", "wind-cold-damp pathogen", and "blood stasis" [12]. External pathogens easily enter the body due to body deficiency and deficiency of qi and blood. The deficiency of qi and blood promotes the formation of blood stasis, while chronic nontreatment aggravates body deficiency and is a vicious cycle [13]. Therefore, tonifying the liver and kidney, dispelling wind-cold, dredging collaterals and activating blood circulation are the fundamental treatments for KOA.

Electroacupuncture (EA) is an innovative treatment method that combines pulse electrical stimulation with traditional acupuncture therapy. By introducing specific electrical currents and stimulating specific acupoints, it enhances the therapeutic efficacy of acupuncture [14]. Numerous research findings have demonstrated that EA, as an analgesic therapy, promotes smooth meridian flow, invigorates blood circulation, and significantly improves clinical treatment outcomes while reducing disease recurrence and the incidence of complications [15,16]. Consequently, EA holds vast potential for broad applications. TongDu TiaoShen (TDTS) mainly selects head Baihui, Shenting, Fengfu point, etc., which has the function of awakening the brain and dredging the brain. Sun et al. (2021) used TDTS EA to treat poststroke depression rats and found that it can improve depression-like behavior in rats by activating the PI3K/Akt/mTOR pathway and inhibiting autophagy in hippocampal neurons [17]. Wu et al. (2022) clinically treated chronic insomnia with TDTS EA, which could inhibit the overactivation of the hypothalamic–pituitary–adrenal axis and play a role in the treatment of chronic insomnia [18]. The results showed that TDTS EA could play a role in the treatment of diseases by regulating the state of the brain center or neurons. Many clinical practices and animal studies have already confirmed [19,20] that EA can significantly alleviate pain symptoms, improve treatment outcomes, and reduce the recurrence rate and incidence of complications. EA therapy achieves these effects by stimulating acupoints and nerve fibers, influencing the conduction and regulation of the nervous system, generating nerve impulses, and modulating the transmission and blockade of pain signals through the release of neurotransmitters [21]. However, there is no relevant research on the efficacy evaluation of TDTS EA in the treatment of KOA.

In this study, a rat model of KOA was prepared, and KOA pain was inhibited by conventional EA and TDTS EA. Changes in important neurotransmitters in the midbrain and lumbar dilatation spinal cord were detected, aiming to explore the mechanism of EA in the treatment of KOA from the perspective of molecular biology and provide a theoretical reference for the clinical treatment of KOA with EA.

2. Materials and methods

2.1. Materials

SPF-grade Sprague Dawley male rats, weighing 180–220 g, were provided by Guangdong Medical Laboratory Animal Center. Dopamine (DA) (H170-1-1), norepinephrine (NE) (H096-1-1), and serotonin (H104-1-1) enzyme-linked immunosorbent assay (ELISA) kits were all procured from Nanjing Jiancheng Bioengineering Institute. The Leucine-enkephalin (HY126) radioimmunoassay kit was provided by Beijing Huaying Biological Technology Research Institute. The β-endorphin (XP9797) radioimmunoassay kit was supplied by Shanghai Xinfan Biotechnology Co., Ltd.

2.2. Model preparation of KOA

The model of KOA was prepared by 4 % papain (Sigma Corporation, USA) injection in the joint cavity. First, 30 mg/kg of 3 % pentobarbital sodium (Beijing Solarbio Technology Co., LTD) was injected intraperitoneally to anesthetize the rats. The hair on the right knee was removed, and the skin was disinfected. Then, 0.2 mL of 4 % papain solution was injected into the joint cavity and pressed into the needle position for 1 min after injection. After flexion and extension of the knee joint, 10 injections of drugs were made to fill the joint cavity, and disinfection was performed. The degree of knee joint dysfunction was evaluated by the Lequesne MG score on the 1st, 3rd, and 7th days after modeling. A rat model of KOA was successfully established when the right knee joint showed swelling, local tingling symptoms, and an increased Lequesne MG score. All animal procedures of this experiment were approved by the Experimental Animal Management Committee (approval number: 20210826), and the experimental methods were carried out in accordance with the approval guidelines.

2.3. Grouping and interventions

Fifty rats were divided into a blank group, model group (KOA), control group (Ctrl), EA group (EA), and TDTS-EA group, with 10 rats in each group. Rats in different groups were housed in cages in an independent ventilated cage system in the animal laboratory center under constant temperature and humidity conditions and given 12 h/12 h alternating light and free diet. Blank group rats were not treated. The model group, control group, EA group, and TDTS EA group were established to model KOA according to section 2.2. Control group rats were given duloxetine 500 mg/kg/d (Eli Lilly and Company, USA) by intragastric administration based on a KOA model, once a day, 6 days a week, for 4 weeks. SDZ-IIS electronic needle therapy instrument (Suzhou Medical Supplies Factory Co., LTD.) was used for EA therapy. The rats in the EA group were given conventional EA treatment based on a KOA model, and the

acupoints of "Neixiyan" and "Dubi" were located according to Experimental Acupuncture and Moxibustion Science. The rats were fixed in the supine position, connected to the EA apparatus, and given continuous waves with a frequency of 2 Hz and a current intensity of 1 mA for 15 min each time, once a day, and 6 d once a week. The treatment continued for four weeks. Based on the conventional EA group, Baihui, Dazhui, and Shenting were added to the acupoint prescription for EA treatment of KOA model rats. The rats were fixed in the supine position, and a continuous wave with a frequency of 2 Hz was given after the EA apparatus was connected. The current intensity was 1 mA, each EA treatment lasted for 15 min, once a day, a week for 6 days, and the continuous administration lasted for 4 weeks.

2.4. Evaluation of behavior

The rats were placed in the pain threshold test environment 30 min in advance 3 d before modeling (T0) and 1 d (T1), 3 d (T2), and 7 d (T3) after modeling. After the rats adapted to the environment, the plantar pain threshold was measured by the radiometric pain measurement method. The Lequesne MG scale [22] was adopted to evaluate the behavioral performance of the rats. I. Radiant thermal pain measurement method. The plantar pain measurement instrument was employed to measure the thermal pain threshold. The radiant thermal light source was gathered at the central position of the rat plantar to be detected, and the time from the beginning of irradiation until the thermal stimulation caused pain and foot contraction reflex was recorded. The average value was measured three times, and the interval of light irradiation was 10 min. II. Lequesne MG scale. The evaluation included gait, pain stimulation, joint swelling, and range of motion. Grade I (0): no pain response, no lameness of the affected limb, normal and vigorous movement, knee motion greater than 90°, and no joint swelling. Grade II (1 point): the affected limb is contractile, the affected limb is mildly lame and vigorous, the knee joint has a range of motion of 45–90°, and the joint is mildly swollen. Grade III (2 points): spasm and contraction of the affected limb with mild systemic reactions, significant lameness of the affected limb, range of motion of the knee joint of 15–45°, and moderate swelling of the joint. Grade IV (3 points): the affected limb spasms, tremors, scurriness, and abnormal noise, the affected limb can't touch the ground and can't walk, the knee joint has a range of motion less than 15°, and the joint is severely swollen.

2.5. Spinal cord index detection

After the threshold of foot pain was determined, the rats were anesthetized by an intrabitoneal injection of 30 mg/kg 3 % sodium pentobarbital. The rats were killed by neck breaking, and the midbrain and spinal cord were separated and then stored in a −80 °C refrigerator. The levels of DA, NE, and 5-hydroxytryptamine (5-HT) in the spinal cord were detected according to the ELISA kit instructions. The protein in the sample was extracted and tested, and the sample was added successively and mixed with the reagent for 10 min. The standard products and samples were tested after the zero value was set using the blank tube. The samples were added successively and mixed with the reagent, and the reaction was carried out at 37 °C for 1 h. Then, the horseradish peroxidase (HRP) was washed and incubated at 37 °C for 30 min with the affinity streptomycin. After washing, the substrate was added and incubated for 10 min at 37 °C. The termination solution was added, and the absorbance of each hole was measured at 450 nm by a multifunctional enzyme marker. Standard curves were plotted to calculate the concentrations of DA, NE, and 5-HT.

The levels of β-endorphin (β-EP) and leucine-enkephalin (L-ENK) in the spinal cord were measured by radioimmunoassay following the instructions of the release kit. The samples were added in turn, mixed with the reagent, and incubated at 4 °C for 1 day. Then, the separation reagent was added and mixed, left for 30 min at 4 °C, and centrifuged at a low temperature of 4000 rpm for 15 min. The supernatant was discarded, and the cpm value in the precipitate was detected. After the standard curve was plotted, the concentrations of β-EP and L-ENK were calculated.

2.6. Morphological analysis of synovial tissue

Hematoxylin-eosin (HE) staining was performed to observe the morphological changes of the synovial tissue of the knee joint. The rat joint capsule was opened and the synovial tissue was removed. Four synovial tissues were randomly selected from each group and stored in the refrigerator at −80 °C for subsequent test, and the remaining six synovial tissues were cut along the transverse section. The upper part was fixed in 4 % paraformaldehyde solution for 24 h, rinsed with PBS buffer solution for 10 min × 3 times, dehydrated with gradient ethanol, embedded in paraffin, and sliced in transverse section with a thickness of 4 μm. Some slices were taken, dewaxed with xylene, then subjected to gradient ethanol dehydration, hematoxylin staining, distilled water washing, 1 % hydrochloric ethanol color separation, blue-turning with distilled water, eosin staining, gradient ethanol dehydration, xylene transparent, and sealing. The morphology of synovium was observed under optical microscope.

2.7. Western blot

The level of interleukin (IL)-1β in rat synovial tissue was detected by Western blot, and the protein in synovial tissue was extracted by RIPA lysis buffer. The concentration level of the extracted protein in the cartilage tissue was detected according to the instructions of the bicinchoninic acid (BCA) concentration detection kit. The separation glue and concentrate glue were configured with sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE). After sample loading, the protein was separated by electrophoresis with a voltage of 80 V and a current of 200 mA. The target protein was separated and transferred to PVDF membranes with a voltage of 200 V and a current of 200 mA. The protein was placed in a sealing solution containing 5 % skim milk powder and incubated at room temperature for 1 h. After Tris buffered saline Tween (TBST) film washing, mouse anti-interleukin (IL)-1β (1:2000) and mouse anti-

β-Actin (1:5000) primary antibodies were added and incubated overnight in a 4 °C refrigerator. After TBST film washing, horseradish peroxidase-labeled IgG secondary antibody (1:10,000) was added and incubated at room temperature for 1 h in the dark. After TBST film washing, the target protein bands were developed according to the ECL chemiluminescence detection kit. The bands were developed in the gel imaging system, and the relative gray values of the target protein bands were detected by ImageJ gray analysis software. β-actin was used as the internal reference gene, and the relative expression level of IL-1β protein was calculated.

2.8. Statistical processing

SPSS 19.0 was employed for data statistics and analysis. The mean plus or minus standard deviation ($\overline{x} \pm s$) was used to express the measurement data. Quantitative data comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA), and pairwise comparisons of quantitative data between two groups were performed using the *t*-test. A significance level of $P < 0.05$ was considered statistically significant for all analyses.

3. Results

3.1. Analysis of HE staining results of synovial tissue of knee joint in different groups

The synovial tissue structure of the knee joint of rats in different groups was analyzed (Fig. 1). The synovial structure of the Blank group (Fig. 1-A), was normal and the cells were evenly distributed. The synovial structure of KOA group (Fig. 1-B) was abnormal, the lining cells were proliferated, the collagen fibrous tissue was proliferated in a large area, a large number of macrophages, lymphocytes, neutrophils, and other inflammatory cells were infiltrated, and multiple angiogenesis was observed. The synovial structure of Ctrl group (Fig. 1-C), EA group (Fig. 1-D), and TDTS-EA group (Fig. 1-E) was better, the number of lining cell layers increased less, the hyperplasia area of collagen fibrous tissue was smaller, there was a small amount of inflammatory cell infiltration, and angiogenesis was occasionally observed.

3.2. Measurement of the incubation period of radiant heat pain retraction

With the increase in modeling time, the latency of radiant heat foot contraction of the blank group had no significant change, while that of the KOA group decreased gradually and that of the Ctrl group and the EA group decreased first and then increased. Compared with the five groups at T3, the latent period of foot contraction induced by radiation heat in the KOA group, Ctrl group, and EA group was significantly shorter than that in the blank group ($P < 0.05$). The incubation period of radiant heat in the Ctrl group, EA group, and TDTS-EA group was significantly longer than that in the KOA group ($P < 0.01$). The latency of radiant heat foot retraction in the TDTS-EA group was significantly longer than that in the Ctrl group ($P < 0.05$) (Fig. 2).

3.3. Behavioral lequesne MG scale score

The Lequesne MG scale was used to evaluate the behavioral changes of the five groups of rats. The Lequesne MG scale score in the KOA group was significantly higher than that in the Blank group ($P < 0.01$). The Lequesne MG scale score of the Ctrl group, EA group, and TDTS-EA group was significantly lower than that of the KOA group ($P < 0.01$). The Lequesne MG scale score of the EA and TDTS-EA groups was significantly lower than that of the Ctrl group ($P < 0.05$). The Lequesne MG scale score of the TDTS-EA group was significantly lower than that of the EA group ($P < 0.05$). There was no significant difference in Lequesne MG scale scores between the Blank group and the TDTS-EA group ($P < 0.05$) (Fig. 3).

3.4. Measurement of the levels of 5-HT, NE, and DA in the midbrain and spinal cord

The levels of 5-HT in the brain and spinal cord of the 5 groups of rats were detected and compared. The level of 5-HT in the brain and spinal cord in the KOA group was significantly higher than that in the Blank group ($P < 0.05$). The level of 5-HT in the midbrain of the EA and TDTS-EA groups was significantly lower than that of the KOA group, but the level of 5-HT in the spinal cord was significantly higher than that of the KOA group ($P < 0.01$). Similarly, the level of 5-HT in the midbrain of the TDTS-EA group was significantly lower than that of the EA group, but the level of 5-HT in the spinal cord of the TDTS-EA group was significantly higher
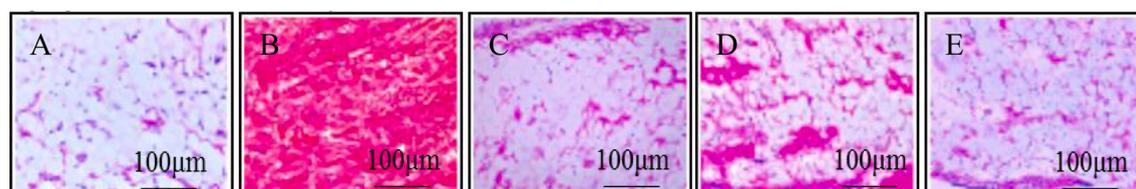

Fig. 1. HE staining of synovial tissue of knee joint of rats in the same group (× 200; A: Blank group; B: KOA group; C: Ctrl group; D: EA group; E: TDTS-EA group).

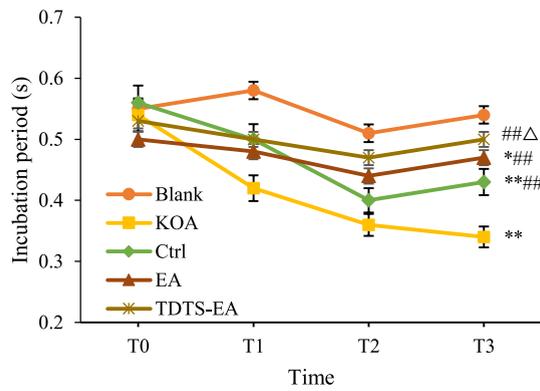**Fig. 2.** Comparison of incubation period radiant heat shrink foot pain at each time point of each group of rats. ($\bar{x} \pm$ s, n = 10).
Note: *$P < 0.05$, **$P < 0.01$ vs. blank group; ##$P < 0.01$ vs. KOA group; $^{\Delta}P<0.05$ vs. Ctrl group.

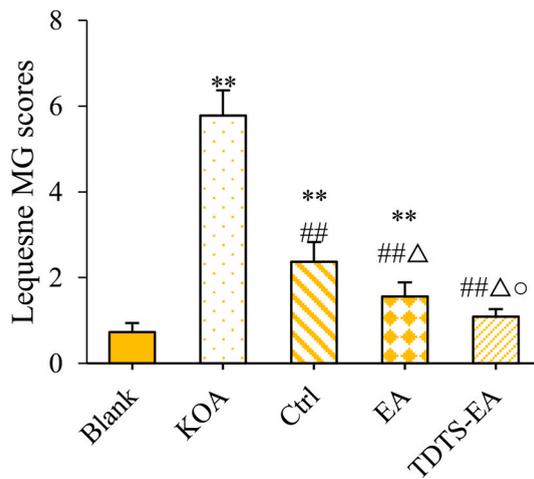**Fig. 3.** Lequesne comparison of MG rating scale for each group of rats. ($\bar{x} \pm$s, n = 10).
Note: **$P < 0.01$ vs. blank group; ##$P < 0.01$ vs. KOA group; $^{\Delta}P<0.05$ vs. Ctrl group; $^{\circ}P < 0.05$ vs. EA group.
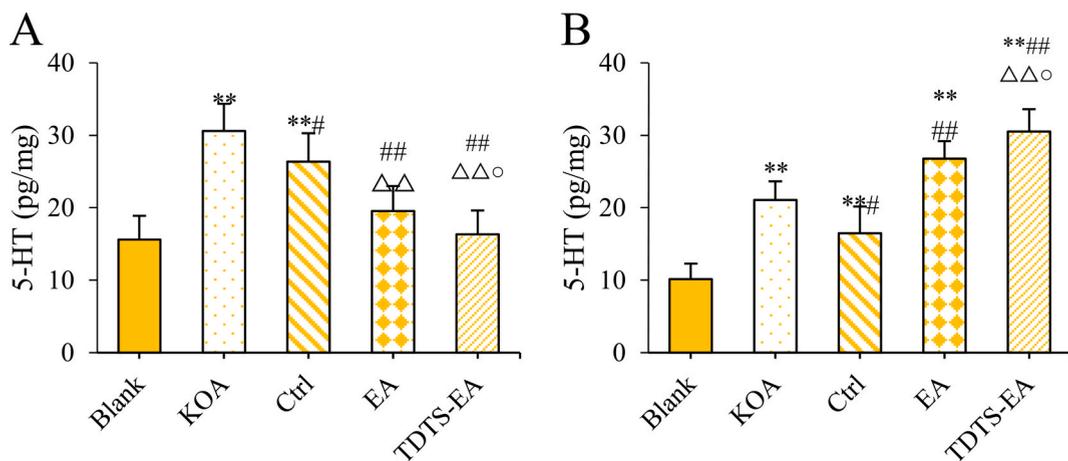

Fig. 4. Comparison of 5-HT levels in the midbrain and spinal cord of rats in each group. ($\bar{x} \pm$s, n = 10)
(A is midbrain level; B is the level of spinal cord) Note: **$P < 0.01$ vs. blank group; #$P < 0.05$, ##$P < 0.01$ vs. KOA group; $^{\Delta\Delta}P<0.01$ vs. Ctrl group; $^{\circ}P < 0.05$ vs. EA group.

than that of the EA group ($P < 0.05$) (Fig. 4-A, B).

The differences in NE levels in the midbrain and spinal cord of the five groups were detected and compared. The NE levels in the midbrain and spinal cord of rats in the KOA group were significantly higher than those in the Blank group ($P < 0.05$). The NE levels in the midbrain and spinal cord of rats in the EA group and TDTS-EA group were significantly lower than those in the KOA group ($P < 0.01$). Similarly, NE levels in the midbrain and spinal cord of rats in the TDTS-EA group were significantly lower than those in the EA group, but NE levels in the spinal cord were significantly higher than those in the Blank group ($P < 0.05$) (Fig. 5-A, B).

The differences in DA levels in the midbrain and spinal cord of the five groups were detected and compared. The DA levels in the midbrain and spinal cord of rats in the KOA group were significantly higher than those in the Blank group ($P < 0.05$). The DA levels in the midbrains of the EA group and TDTS-EA group were significantly lower than those of the KOA group ($P < 0.01$). Similarly, the DA level in the midbrain of rats in the TDTS-EA group was significantly lower than that in the EA group, but the EA level in the spinal cord was significantly higher than that in the EA group ($P < 0.05$). The DA level of the spinal cord in the TDTS-EA group was significantly higher than that in the Blank group ($P < 0.05$) (Fig. 6-A, B).

3.5. Measurement of β-EP and L-ENK levels in the midbrain and spinal cord

The levels of β-EP in the midbrain and spinal cord were detected and compared among the five groups. The β-EP levels in the midbrain and spinal cord of rats in the KOA group were significantly higher than those in the Blank group ($P < 0.05$). The levels of β-EP in the midbrain of the EA group and TDTS-EA group were significantly lower than those of the KOA group ($P < 0.01$). The β-EP levels in the midbrain and spinal cord of rats in the TDTS-EA group were significantly lower than those in the EA group ($P < 0.05$). However, the level of β-EP in the spinal cord of rats in the TDTS-EA group was significantly higher than that in the Blank group ($P < 0.05$) (Fig. 7-A, B).

The differences in L-ENK levels in the midbrain and spinal cord of the five groups were detected and compared. The level of L-ENK in the midbrain and spinal cord of the KOA group was significantly higher than that of the Blank group ($P < 0.05$). The L-ENK levels in the midbrains of rats in the EA group and TDTS-EA group were significantly lower than those in the KOA group ($P < 0.01$), while the L-ENK level in the spinal cord was not significantly different from that in the KOA group ($P > 0.05$). The L-ENK levels in the midbrain and spinal cord of rats in the TDTS-EA group were significantly lower than those in the Ctrl group ($P < 0.05$) but not significantly different from those in the EA group ($P > 0.05$). The level of L-ENK in the spinal cord of rats in the TDTS-EA group was significantly higher than that in the Blank group ($P < 0.05$) (Fig. 8-A, B).

3.6. Analysis of IL-1β protein level in synovial tissue

The relative expression levels of IL-1β protein in the synovial tissue of the five groups were detected by Western blot. The results showed that the relative expression levels of IL-1β in the synovial tissue of the KOA group, Ctrl group, and EA group were significantly higher than those of the Blank group ($P < 0.01$). The relative expression levels of IL-1β in the synovial tissue of the Ctrl group, EA group, and TATS-EA group were significantly lower than those of the KOA group ($P < 0.01$). The relative expression levels of IL-1β in synovial tissue of the EA group and TDTS-EA group were significantly lower than those of the Ctrl group ($P < 0.05$). The relative expression level of IL-1β in the synovial tissue of the TDTS-EA group was significantly lower than that of the EA group ($P < 0.05$) (Fig. 9-A, B).
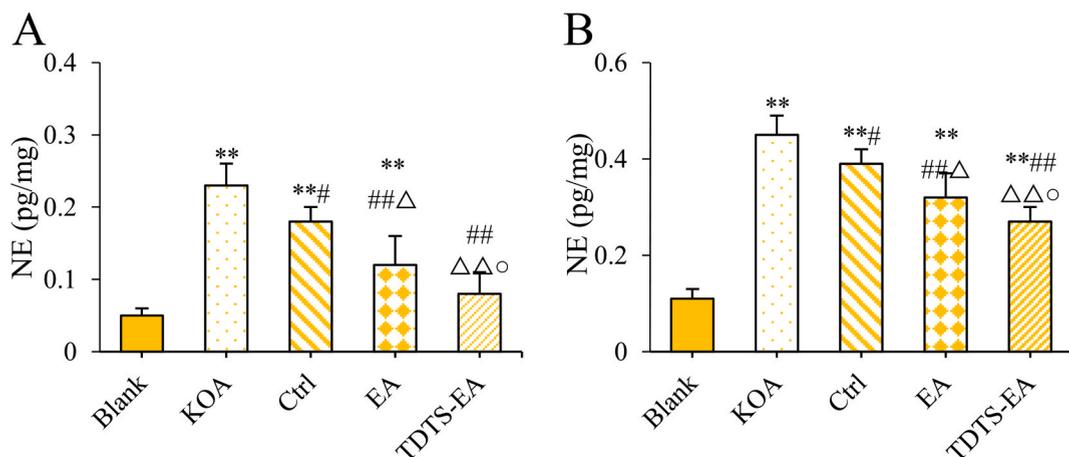

Fig. 5. Comparison of NE levels in the midbrain and spinal cord of rats in each group. ($\bar{x} \pm s$, n = 10) (A is midbrain level; B is the level of spinal cord) Note: compared with the blank group, **P < 0.01; compared with the KOA group, #*P* < 0.05; compared with the Ctrl group, △*P*<0.05; compared with the EA group, °*P* < 0.05.
Note: **P < 0.01 vs. blank group; #*P* < 0.05, ##*P* < 0.01 vs. KOA group; △*P*<0.05, △△*P*<0.01 vs. Ctrl group; °*P* < 0.05 vs. EA group.

Fig. 6. Comparison of DA levels in the midbrain and spinal cord of rats in each group. ($\bar{x}$ ±s, n = 10)(A is midbrain level; B is the level of spinal cord)

Note: $*P < 0.05$, $**P < 0.01$ vs. blank group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. KOA group; $^{\Delta}P<0.05$ vs. Ctrl group; $^{\circ}P < 0.05$ vs. EA group.
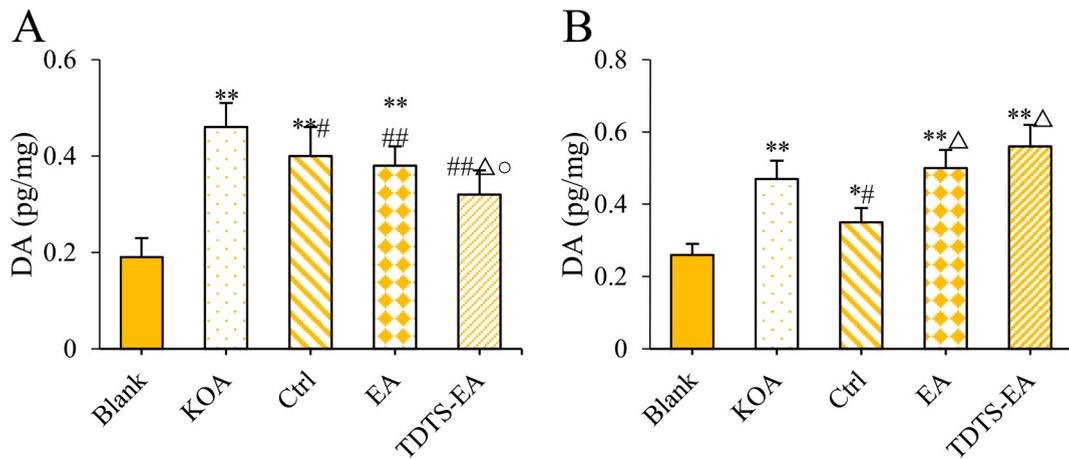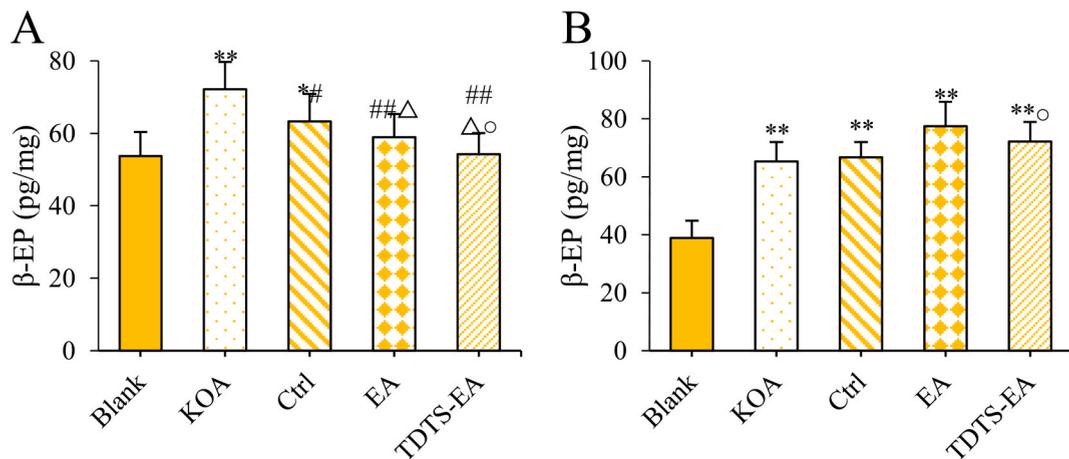

Fig. 7. Comparison of β-EP levels in the midbrain and spinal cord of rats in each group. ($\bar{x}$ ±s, n = 10)(A is midbrain level; B is the level of spinal cord)

Note: $*P < 0.05$, $**P < 0.01$ vs. blank group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. KOA group; $^{\Delta}P<0.05$ vs. Ctrl group; $^{\circ}P < 0.05$ vs. EA group.

4. Discussion

Traditional Chinese medicine believes that KOA belongs to the "bone numbness" and "knee problem" category, the disease is in the muscles and bones, the disease course is relatively long, and the disease position is deep. "Deficiency of the original and solid" is the pathogenesis of bone numbness, and the incidence of KOA is led by liver and kidney deficiency. Acupuncture has the advantages of dispelling wind cold, promoting collaterals and blood circulation, and relieving pain, so it has been applied in the clinical treatment of KOA [23]. EA is a therapeutic method combining pulse electrical stimulation on the basis of acupuncture, which has the effects of dredging collaterals, activating blood, and relieving pain, thus improving the therapeutic effect [24]. In this study, EA was used to treat a rat model with KOA, and it was found that the latency of radiant heat paw withdrawal was significantly prolonged after treatment, while the behavioral score of the Lequesne MG scale was decreased. Lv et al. (2019) [25] investigated the impact of EA therapy on chronic pain in patients with KOA and its correlation with conditioned pain modulation (CPM). The results revealed that sustained EA therapy improved CPM function in KOA patients, alleviated pain intensity, and suppressed chronic pain. This result indicated that EA could strengthen the acupuncture intensity and that the analgesic effect was more obvious. Duloxetine is a serotonin and NE reuptake inhibitor, which has effects of anti-depression, anti-anxiety, and inhibition of central pain [26]. Studies have shown that duloxetine can effectively relieve the pain caused by musculoskeletal system diseases and the symptoms of KOA, and no serious adverse reactions have been observed [27].

EA therapy can not only promote blood circulation of local tissue, activate blood, remove blood stasis, and dredge meridians and collaterals to relieve pain, but also effectively promote alternate release of analgesic substances in central nervous system (endorphin,
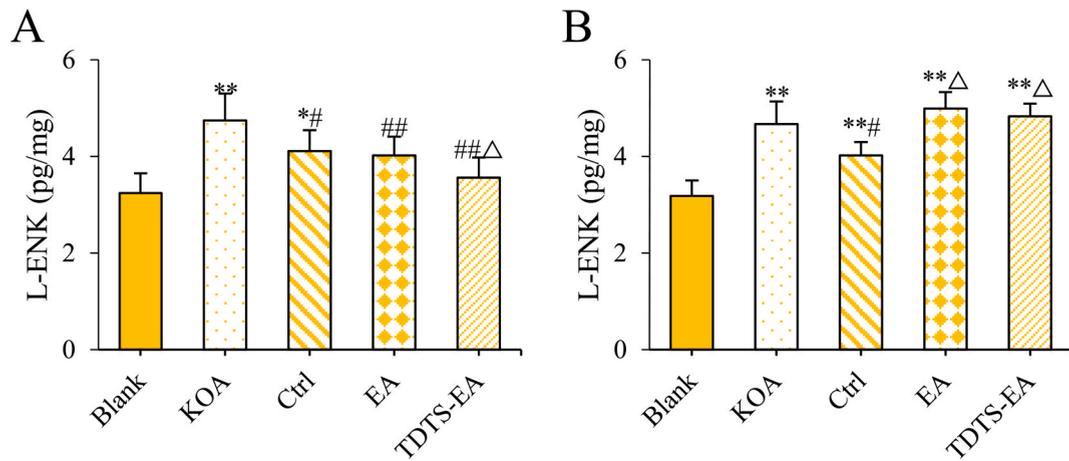
Fig. 8. Comparison of L-ENK levels in the midbrain and spinal cord of rats in each group. ($\bar{x} \pm s$, n = 10) (A is midbrain level; B is the level of spinal cord)
Note: *$P < 0.05$, **$P < 0.01$ vs. blank group; #$P < 0.05$, ##$P < 0.01$ vs. KOA group; $\triangle P < 0.05$ vs. Ctrl group.
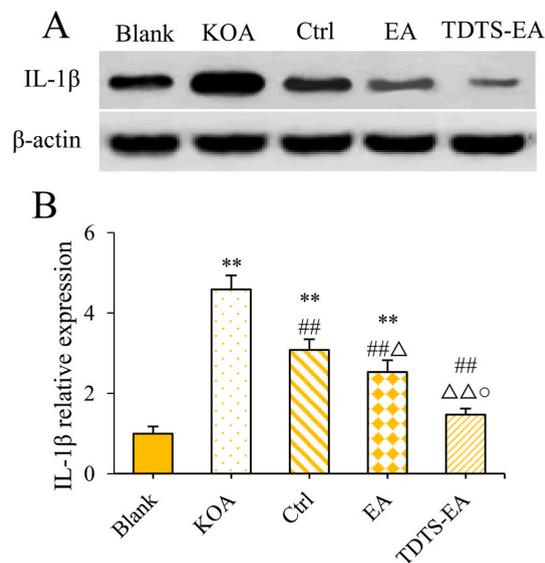

Fig. 9. Comparison of the relative expression level of IL-1β protein in the synovial tissue of rats in each group. ($\bar{x} \pm s$, n = 10) (A is the Western blot test chart; B is the relative expression level of b IL-1β protein)
Note: **$P < 0.01$ vs. blank group; ##$P < 0.01$ vs. KOA group; $\triangle P < 0.05$, $\triangle\triangle P < 0.01$ vs. Ctrl group; °$P < 0.05$ vs. EA group.

enkephalin, and dynorphin) to exert analgesic effect [28]. 5-HT is an important neurotransmitter in the endogenous analgesic system of the body. It can't penetrate the blood–brain barrier, but it has the effects of central system analgesia and peripheral pain [29]. The 5-HT7 receptor is mainly distributed in the dorsal root ganglion, hippocampus, thalamus, and other tissues in the lumbar spinal cord [30]. Some studies have indicated that the levels of serotonin may increase in patients with KOA, which could be associated with inflammatory responses and pain perception [31]. This study found that compared with rats with KOA, the midbrain 5-HT level was decreased, but the spinal cord 5-HT level was increased after conventional EA and TDTS EA treatments. The 5-HT levels in the brain and spinal cord of EA and TDTS were significantly lower than those of Ctrl group. This suggests that EA therapy may have a certain impact on the midbrain 5-HT (serotonin) system, and the reduction in midbrain 5-HT levels reflects the regulatory effect of EA on the midbrain 5-HT system. Spinal cord 5-HT is a neurotransmitter closely associated with pain transmission and modulation. After EA and TDTS EA treatment, spinal cord 5-HT levels increase, indicating that EA therapy may alleviate pain perception by modulating the transmission of pain signals through the regulation of spinal cord 5-HT levels. These findings demonstrate that EA and TDTS EA treatments can exert regulatory effects on pain transmission and modulation by influencing midbrain and spinal cord 5-HT levels. As the spinal cord in the lumbar enlargement is the main distribution area for 5-HT inhibiting pain receptor 5-HT7, when the concentration of 5-HT in the body increases, it inhibits 5-HT7 and thus plays a role in inhibiting pain [32]. The raphe nucleus group in the

midline of the brain stem in the central nervous system is the place where 5-HT neurons gather, from which they send out ascending and descending 5-HT energy fibers. EA can inhibit the perception of traumatic stimuli by activating 5-HT ascending fibers on the one hand, and release 5-HT in brain structures related to pain modulation on the other hand, which helps to enhance the analgesic effect of acupuncture. The descending 5-HT energy fibers can reach the posterior, lateral, and anterior horns of the spinal cord along the dorsal lateral cord of the spinal cord, and release 5-HT after activation to inhibit the transmission of the spinal cord pain impulse through presynaptic inhibition [33]. Studies have shown that 5-HT plays an inhibitory role by binding to its receptors and acting on neurons that transmit injury-related information in the superficial layers of the spinal dorsal horn (layers I and II) through different pathways [34]. DA neurons are mainly distributed in the midbrain and diencephalon.

DA is a catecholamine neurotransmitter that promotes the clinical analgesic effects of enkephalin analgesic drugs. DA in the spinal cord is involved in pain inhibition through the cAMP-PKA pathway [35]. This study also found that compared with rats with KOA, DA levels in the midbrain of rats after conventional EA and TDTS EA treatment were decreased, but DA levels in the spinal cord were increased. Moreover, the 5-HT levels in the brain and spinal cord of EA group were significantly different from those of Ctrl group. This is because the decrease in DA in the brain can enhance the endogenous analgesic effect, while the increase in DA in the spinal cord can activate the DA D2 receptor and produce analgesic effects [36]. EA therapy can promote the release or synthesis of spinal cord DA through different pathways, leading to an elevation in spinal cord DA levels [37]. The spinal cord DA system plays a crucial role in pain transmission and modulation, and EA therapy can achieve pain relief by regulating DA levels [38]. Some studies have shown that DA can counteract analgesia in brain and enhance analgesia in spinal cord. At the same time, it has been pointed out that EA can reduce joint pain in the formation of KOA by regulating the imbalance of DA synthesis and metabolism in the middle brain [39]. In addition, compared with rats with KOA, NE levels in the midbrain of rats after conventional EA and TDTS EA treatment were decreased, but NE levels in the spinal cord were increased. This suggests that EA therapy exerts a certain regulatory effect on the midbrain NE (NE) system. NE is a catecholamine neurotransmitter that acts on α-receptors in the mammalian spinal cord, thereby activating adrenergic receptors and ultimately participating in the regulation of processes such as attention, emotion, and pain transmission [40]. The reduction in midbrain NE levels reflects the inhibitory effect of EA therapy on the midbrain NE system. Spinal cord NE is a neurotransmitter closely related to pain modulation. The increase in spinal cord NE levels reflects the regulatory effect of EA therapy on the spinal cord NE system. Therefore, EA and TDTS EA treatments may alleviate pain by modulating NE levels in the midbrain and spinal cord to inhibit pain signal transmission. NE is concentrated in the medulla oblongata and pons, from which it emits ascending and descending fibers. During the formation of OA in rats, NE synthesis in the spinal cord decreases, while EA can reduce joint pain in the occurrence and development of KOA by regulating the imbalance of NE synthesis and metabolism in the spinal cord and hypothalamus [41]. The results showed that compared with conventional EA treatment, the levels of 5-HT, DA and NE in the midbrain were decreased and those in the spinal cord were increased more significantly after TDTS EA in rats. This indicates that TDTS EA therapy exerts a significant inhibitory effect on midbrain levels of 5-HT, DA, and NE, while it elicits a certain activation effect on spinal cord levels of these neurotransmitters. TDTS EA stimulation may modulate the release, metabolism, or reuptake processes of these neurotransmitters, resulting in decreased levels in the midbrain. This effect may be attributed to the interaction between the electrophysiological response generated by TDTS EA in the midbrain and the neurotransmitter systems. In contrast to its inhibitory effect on the midbrain, TDTS EA may activate the 5-HT, DA, and NE systems in the spinal cord through the regulation of their activities. The adjustment of the position and parameters of the stimulating electrode may lead to increased release of these neurotransmitters or alterations in their metabolic processes, consequently elevating their levels. DA D2 receptors are among the major receptors for DA in the central nervous system. DA can influence the expression levels of 5-HT, DA, and NE by regulating their synthesis, release, and reuptake. Additionally, DA and these neurotransmitters may also share common receptors such as 5-HT1A receptors, DA1 receptors, and α2 receptors. Therefore, changes in the expression of DA D2 receptors in the central nervous system may affect the expression of 5-HT, DA, and NE. α2 adrenergic receptors are one of the main receptors for NE and also interact with 5-HT and DA. These receptors play a role in regulating the release and effects of NE, 5-HT, and DA in the central nervous system [42]. These results indicated that EA treatment with TDTS could promote the expression of the central neurotransmitters 5-HT, DA, and NE in the spinal cord and then activate the DA D2 receptor and α2 NE receptor, thereby inhibiting pain.

β-EP is an endogenous opioid peptide that regulates endocrine, stress response, and analgesia and is mainly involved in the regulation of pain information at the spinal cord level and other levels [43]. ENK is mainly distributed in the hypothalamus, midbrain, and spinal cord, and it is also an endogenous analgesic substance [44]. In the present study, it was found that β-EP and L-ENK levels in the midbrain and spinal cord of rats with KOA were significantly increased, while those levels were significantly decreased and those in the spinal cord were significantly increased after conventional EA and TDTS EA treatments. The increased level of β-EP initiates the expression of endogenous analgesic activities, and the release of β-EP can inhibit the occurrence of pain [45]. EA stimulation can enhance the release of β-EP in the spinal cord and enhance the effect of the spinal cord on the primary integration center of the pain signal, thereby inhibiting the upward conduction of the pain signal.

IL-1β is a hormone-like polypeptide, and IL-1β is a major pathogenic factor for the development of KOA [46]. Oliviero et al. (2020) found an increase in serum IL-1β levels in patients with KOA [47]. Xu et al. (2021) established an osteoarticular cell model by inducing chondrocytes with IL-1β in vitro and inducing apoptosis and inflammatory responses [48], which was consistent with the finding in the present study that the relative expression level of IL-1β protein in KOA rats was significantly higher than that in normal rats. Second, in this study, the relative expression level of IL-1β protein in rats treated with conventional EA and TDTS EA was significantly reduced, while the relative expression level of IL-1β protein in rats treated with TDTS EA was decreased more significantly. These results indicated that TDTS EA could inhibit the expression of inflammatory cytokines and thus play a role in the treatment of KOA.

5. Conclusion

TDTS EA treatment in KOA can significantly improve pain symptoms. It inhibits the expression of pain receptor distribution by promoting the central neurotransmitters 5-HT, DA, NE, β-EP, and L-ENK and plays a central analgesic role. TDTS EA can also inhibit the expression of the inflammatory factor IL-1β and thus play a role in the treatment of KOA. However, this study only analyzed the expression of neurotransmitters in the midbrain and spinal cord, and did not investigate the expression of neurotransmitters in other brain regions such as the cortex, hypothalamic nuclei, thalamus, and periaqueductal gray. Additionally, the study did not explore the effects of different electrical frequencies or intensities on central analgesia. In future research, we plan to further investigate the expression of neurotransmitters in various brain regions and explore the mechanisms underlying the effects of EA therapy with different electrical frequencies or intensities on KOA. In conclusion, this study can provide a reference for understanding the pain mechanism of KOA and the promotion and application of TDTS EA therapy in clinical treatment.

Author contribution statement

All authors of this study participated in the design of this study. Xiahai Zheng, Jing Lin, Zhenzhen Wang, and Zhenming Zeng all participated in the preparation of the KOA model, behavioral evaluation, index detection, and other experimental processes. Haoxiong Chen provided corresponding guidance for the experimental process of this study. Xiahai Zheng, Jing Lin, and Zhenzhen Wang participated in the data acquisition and analysis of this study. Zhenming Zeng and Hao Xiong participated in the analysis and interpretation of the data in this study. Xiahai Zheng, Jing Lin, Zhenzhen Wang, and Zhenming Zeng all participated in the writing and revision of the research paper, and Hao Xiong provided technical guidance for the compilation of this research paper.

Financial details

Acknowledge all financial support (funds, funding, sponsorships) received by the study.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, [X], upon reasonable request.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgement

References

[1] D. Primorac, V. Molnar, E. Rod, et al., Knee osteoarthritis: a review of pathogenesis and state-of-the-art non-operative therapeutic considerations, Genes 11 (8) (2020) 854.
[2] H. Chen, X. Zheng, H. Huang, et al., The effects of a home-based exercise intervention on elderly patients with knee osteoarthritis: a quasi-experimental study, BMC Muscoskel. Disord. 20 (1) (2019) 160.
[3] M.L.A. Landsmeer, J. Runhaar, M. van Middelkoop, et al., Predicting knee pain and knee osteoarthritis among overweight women, J. Am. Board Fam. Med. : JABFM 32 (4) (2019) 575–584.
[4] A. Mahmoudian, L.S. Lohmander, A. Mobasheri, et al., Early-stage symptomatic osteoarthritis of the knee - time for action, Nat. Rev. Rheumatol. 17 (10) (2021) 621–632.
[5] Y. Xiaoyu, X. Hui, L. Xiaolong, et al., The mechanism of Danzikang Knee Granule in regulating the chondrogenic differentiation of mesenchymal stem cells based on TGF-β signaling pathway in cartilage repair in knee osteoarthritis, Cell. Mol. Biol. 67 (5) (2022) 164–173.
[6] M. Hall, M. van der Esch, R.S. Hinman, et al., How does hip osteoarthritis differ from knee osteoarthritis? Osteoarthritis Cartilage 30 (1) (2022) 32–41.
[7] H. Iijima, R. Eguchi, K. Shimoura, et al., Transcutaneous electrical nerve stimulation improves stair climbing capacity in people with knee osteoarthritis, Sci. Rep. 10 (1) (2020) 7294.
[8] K.L. Bennell, K.L. Paterson, B.R. Metcalf, et al., Effect of intra-articular platelet-rich plasma vs placebo injection on pain and medial tibial cartilage volume in patients with knee osteoarthritis: the RESTORE randomized clinical trial, JAMA 326 (20) (2021) 2021–2030.
[9] E. Kaya Mutlu, E. Ercin, A. Razak Ozdıncler, et al., A comparison of two manual physical therapy approaches and electrotherapy modalities for patients with knee osteoarthritis: a randomized three arm clinical trial, Physiother. Theory Pract. 34 (8) (2018) 600–612.
[10] J.P. Pelletier, J.P. Raynauld, M. Dorais, et al., DISSCO Trial Investigator Group. An international, multicentre, double-blind, randomized study (DISSCO): effect of diacerein vs celecoxib on symptoms in knee osteoarthritis, Rheumatology 59 (12) (2020) 3858–3868.
[11] H. Huang, M. Luo, H. Liang, et al., Meta-analysis comparing celecoxib with diclofenac sodium in patients with knee osteoarthritis, Pain Med. 22 (2) (2021) 352–362.
[12] M. Yang, L. Jiang, Q. Wang, et al., Traditional Chinese medicine for knee osteoarthritis: an overview of systematic review, PLoS One 12 (12) (2017), e0189884.
[13] F. Yang, Y. Chen, Z. Lu, et al., Treatment of knee osteoarthritis with acupuncture combined with Chinese herbal medicine: a systematic review and meta-analysis, Ann. Palliat. Med. 10 (11) (2021) 11430–11444.
[14] L. Ulloa, Electroacupuncture activates neurons to switch off inflammation, Nature 598 (7882) (2021) 573–574.
[15] J.J. Mao, K.T. Liou, R.E. Baser, et al., Effectiveness of electroacupuncture or auricular acupuncture vs usual care for chronic musculoskeletal pain among cancer survivors: the PEACE randomized clinical trial, JAMA Oncol. 7 (5) (2021) 720–727.

[16] S. Park, Y.R. Lyu, S.J. Park, et al., Electroacupuncture for post-thoracotomy pain: a systematic review and meta-analysis, PLoS One 16 (7) (2021), e0254093.

[17] P.Y. Sun, P.F. Li, T. Wang, et al., Effect of Tongdu Tiaoshen acupuncture on PI3K/Akt/mTOR signaling pathway and autophagy-related proteins of hippocampus in rats with post-stroke depression, Zhongguo Zhen Jiu 40 (11) (2020) 1205–1210.

[18] W.Z. Wu, S.Y. Zheng, C.Y. Liu, et al., Effect of Tongdu Tiaoshen acupuncture on serum GABA and CORT levels in patients with chronic insomnia, Zhongguo Zhen Jiu 41 (7) (2021) 721–724.

[19] G.X. Shi, J.F. Tu, T.Q. Wang, et al., Effect of electro-acupuncture (EA) and manual acupuncture (MA) on markers of inflammation in knee osteoarthritis, J. Pain Res. 13 (2020) 2171–2179.

[20] T.Q. Wang, Y.T. Li, L.Q. Wang, et al., Electroacupuncture versus manual acupuncture for knee osteoarthritis: a randomized controlled pilot trial, Acupunct. Med. 38 (5) (2020) 291–300.

[21] M. Zhou, Q. Zhang, M. Huo, et al., The mechanistic basis for the effects of electroacupuncture on neuropathic pain within the central nervous system, Biomed. Pharmacother. 161 (2023), 114516.

[22] W.F. Peter, M.J. Jansen, E.J. Hurkmans, et al., Guideline Steering Committee - hip and Knee Osteoarthritis. Physiotherapy in hip and knee osteoarthritis: development of a practice guideline concerning initial assessment, treatment and evaluation, Acta Reumatol Port 36 (3) (2011) 268–281.

[23] J.F. Tu, J.W. Yang, G.X. Shi, et al., Efficacy of intensive acupuncture versus sham acupuncture in knee osteoarthritis: a randomized controlled trial, Arthritis Rheumatol. 73 (3) (2021) 448–458.

[24] T.Q. Wang, L.R. Li, C.X. Tan, et al., Effect of electroacupuncture on gut microbiota in participants with knee osteoarthritis, Front. Cell. Infect. Microbiol. 11 (2021), 597431.

[25] Z.T. Lv, L.L. Shen, B. Zhu, et al., Effects of intensity of electroacupuncture on chronic pain in patients with knee osteoarthritis: a randomized controlled trial, Arthritis Res. Ther. 21 (1) (2019) 120.

[26] Q. Zhou, J. Chen, W. Yu, et al., The effectiveness of duloxetine for knee osteoarthritis: an overview of systematic reviews, Front. Physiol. 13 (2022), 906597.

[27] M.C. Osani, R.R. Bannuru, Efficacy and safety of duloxetine in osteoarthritis: a systematic review and meta-analysis, Korean J. Intern. Med. (Engl. Ed.) 34 (5) (2019) 966–973.

[28] S.Y. Wu, C.H. Lin, N.J. Chang, et al., Combined effect of laser acupuncture and electroacupuncture in knee osteoarthritis patients: a protocol for a randomized controlled trial, Medicine (Baltim.) 99 (12) (2020), e19541.

[29] Q.Q. Liu, X.X. Yao, S.H. Gao, et al., Role of 5-HT receptors in neuropathic pain: potential therapeutic implications, Pharmacol. Res. 159 (2020), 104949.

[30] J.L. Cortes-Altamirano, A. Olmos-Hernandez, H.B. Jaime, et al., Review: 5-HT1, 5-HT2, 5-HT3 and 5-HT7 receptors and their role in the modulation of pain response in the central nervous system, Curr. Neuropharmacol. 16 (2) (2018) 210–221.

[31] R.X. Zhang, K. Ren, R. Dubner, Osteoarthritis pain mechanisms: basic studies in animal models, Osteoarthritis Cartilage 21 (9) (2013) 1308–1315.

[32] X.C. Yuan, X.J. Yan, L.X. Tian, et al., 5-HT7 receptor is involved in electroacupuncture inhibition of chronic pain in the spinal cord, Front. Neurosci. 15 (2021), 733779.

[33] B.K. Seo, W.S. Sung, Y.C. Park, et al., The electroacupuncture-induced analgesic effect mediated by 5-HT1, 5-HT3 receptor and muscarinic cholinergic receptors in rat model of collagenase-induced osteoarthritis, BMC Compl. Alternative Med. 16 (2016) 212.

[34] X.C. Yuan, Y.Y. Wang, L.X. Tian, et al., Spinal 5-HT2A receptor is involved in electroacupuncture inhibition of chronic pain, Mol. Pain 18 (2022), 17448069221087583.

[35] R.A. Serafini, K.D. Pryce, V. Zachariou, The mesolimbic dopamine system in chronic pain and associated affective comorbidities, Biol. Psychiatr. 87 (1) (2020) 64–73.

[36] H. Yang, J.W. de Jong, I. Cerniauskas, et al., Pain modulates dopamine neurons via a spinal-parabrachial-mesencephalic circuit, Nat. Neurosci. 24 (10) (2021) 1402–1413.

[37] M. Wu, Y. Chen, Z. Shen, et al., Electroacupuncture alleviates anxiety-like behaviors induced by chronic neuropathic pain via regulating different dopamine receptors of the basolateral amygdala, Mol. Neurobiol. 59 (9) (2022) 5299–5311.

[38] Y. Li, G. Xu, S. Hu, et al., Electroacupuncture alleviates intestinal inflammation and barrier dysfunction by activating dopamine in a rat model of intestinal ischaemia, Acupunct. Med. 39 (3) (2021) 208–216.

[39] J.H. Ye, W. Zuo, J. Li, R. Fu, D.J. Eloy, A. Bekker, et al., Electroacupuncture relieves pain during alcohol withdrawal, Med. Acupunct. 32 (6) (2020) 357–361.

[40] H. Obata, Analgesic mechanisms of antidepressants for neuropathic pain, Int. J. Mol. Sci. 18 (11) (2017) 2483.

[41] G. Rösch, D. Muschter, S. Taheri, et al., β2-Adrenoceptor deficiency results in increased calcified cartilage thickness and subchondral bone remodeling in murine experimental osteoarthritis, Front. Immunol. 12 (2022), 801505.

[42] Q. Tang, Q. Jiang, S.R. Sooranna, et al., Effects of electroacupuncture on pain threshold of laboring rats and the expression of norepinephrine transporter and α2 adrenergic receptor in the central nervous system, Evid Based Complement Alternat Med 2016 (2016), 9068257.

[43] N. Mohammed, H. Allam, E. Elghoroury, et al., Evaluation of serum beta-endorphin and substance P in knee osteoarthritis patients treated by laser acupuncture, J. Compl. Integr. Med. 15 (2) (2018).

[44] L. Basso, L. Garnier, A. Bessac, et al., T-lymphocyte-derived enkephalins reduce Th1/Th17 colitis and associated pain in mice, J. Gastroenterol. 53 (2) (2018) 215–226.

[45] H. Ahn, J.H. La, J.M. Chung, et al., The relationship between β-endorphin and experimental pain sensitivity in older adults with knee osteoarthritis, Biol. Res. Nurs. 21 (4) (2019) 400–406.

[46] F.L. Lu, L.H. Kong, Improvement effect of long-term vitamin d supplementation on skeletal muscle fatigue in overtraining rats, Acta Med. Mediterr. 5 (2022) 3415.

[47] F. Oliviero, R. Ramonda, A. Hoxha, et al., Effect of an oral preparation containing hyaluronic acid, chondroitin sulfate, hydrolyzed collagen type II and hydrolyzed keratin on synovial fluid features and clinical indices in knee osteoarthritis. A pilot study, Reumatismo 72 (3) (2020) 125–130.

[48] J. Xu, Y. Pei, J. Lu, et al., LncRNA SNHG7 alleviates IL-1β-induced osteoarthritis by inhibiting miR-214-5p-mediated PPARGC1B signaling pathways, Int. Immunopharm. 90 (2021), 107150.