Original Article
Electroacupuncture with different current intensities to treat knee osteoarthritis: a single-blinded controlled study

Ziyong Ju[1,2*], Xianhui Guo[3*], Xu Jiang[2], Xin Wang[4,5], Shimin Liu[1], Jinsen He[1], Huashun Cui[2], Ke Wang[4,5]

[1]College of Acumox and Tuina, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China; [2]Department of Acupuncture, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China; [3]Department of Massage, Third Affiliated hospital of Henan University of Traditional Chinese Medicine, Henan, China; [4]Laboratory of Integrative Medicine Surgery, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China; [5]Shanghai-MOST Key Laboratory of Health and Disease Genomics, Chinese National Human Genome Center at Shanghai, Shanghai 201203, China. *Equal contributors.

Abstract: Background: To assess the efficacy of Electroacupuncture (EA) stimulation with high-intensity compared with low-intensity on knee osteoarthritis (KOA). Methods: Participants with KOA were randomized to either high-intensity EA group or low-intensity EA group. EA was applied unilaterally on the affected leg with the local points GB34, ST34, EX-LE4, EX-LE5, ST36, and SP9. The visual analogue scale (VAS) and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) were measured before and after participation. Plasma TNFα, IL-1β, IL-6, and apelin levels were also assessed by enzyme immunoassay (ELA) before and after treatment. Results: Of 80 participants who consented to study participation, 77 completed the program. The patients showed a significant improvement in their pain, stiffness, and physical function on the VAS and WOMAC, accompanying with a significantly reduction in plasma levels of apelin and TNFα. Furthermore, high-intensity group exhibited statistically significant improvements in stiffness and physical function symptoms compared with low-intensity group. Plasma level of IL-6 was significantly decreased only after high-intensity EA treatment. Furthermore, apelin level was significantly inhibited in high-intensity EA group than in low-intensity EA group. Conclusions: Both high- and low-intensity EA treatments alleviate the clinical symptoms of KOA patients. High-intensity EA is more effective than low-intensity EA. Changes in plasma levels of TNFα, apelin and IL-6 may be involved in the therapeutic effect of EA on KOA.

Keywords: Electroacupuncture, knee osteoarthritis, pain, current intensity

Introduction

Knee osteoarthritis (KOA) is a common painful chronic disease that causes pain, affects joint cartilage and loss of functional independence, and reduces the overall quality of life of affected individuals. The World Health Organization (WHO) estimates that 10% of the world's population older than 60 years has clinically relevant osteoarthritis symptoms, and the lifetime risk of developing symptomatic KOA is estimated to be ~45% (40% in men and 47% in women) based upon Johnston County Osteoarthritis Project data [1, 2]. The direct and indirect medical costs of KOA are a substantial economic burden [3, 4]. KOA is a degenerative disorder caused by the interplay of genetic, metabolic, biochemical and biomechanical factors and results in pain, swelling and malformation of the knee joint. Because there is no cure for KOA, treatments currently focus on the management of symptoms. Pain relief, improved joint function and joint stability are the primary goals of therapy. During rehabilitation, patients with KOA require effective pain control and hope no adverse events (AEs) during rehabilitation [5]. Therefore, clinical guidelines advocate conservative non-drug strategies for the treatment of KOA, which could control the pain as well as reduce physical disability and impair-

ment, while minimize the potentially harmful side effects of medication [6, 7].

Electroacupuncture (EA), as an effective and simple approach, has been widely practiced for treating KOA, not only to alleviate pain and disability, but also to decrease inflammation and progression of pathogenesis [8-11]. The intensity of the EA stimulation has recently been shown to play a critical role in EA effective in other diseases. In general, lower intensities of stimulation producing minimal effects and higher intensities producing greater effects in Clinical and animal experiments [12-15]. However, some studies have reached the opposite conclusion [16, 17]. This inconsistency therefore raises an important question that needs to be explored further regarding the relationship between the EA stimulation intensity and the therapeutic effect in clinical KOA treatment.

Inflammation has been thought to involve the development and progression of osteoarthritis even in the early stages of the disease [18]. Tumor necrosis factor α (TNFα), interleukin 1β (IL-1β) and interleukin 6 (IL-6), are the major proinflammatory cytokines, which involved in the pathophysiology of osteoarthritis [19]. Furthermore, TNFα and IL-6 were associated with radiographic osteoarthritis and knee cartilage loss in middle-aged to older people and were independent predictors of worsening knee pain [20, 21]. KOA patients also present with higher concentration IL-1β in serum which correlated with worsened joint morphology [22]. Furthermore, the EA could significantly reduce the TNFα, IL-1β and IL-6 level in patients with inflammatory, pain and KOA [23, 24]. Apelin is an adipokine which is widely distributed in periphery tissues and central nervous system and involved in a variety of physiological responses and pathological states (inflammation, pain and osteoarthritis) [25-29]. Our previously study suggested that apelin may be a potential target of EA [30].

The purpose of the present study was to investigate whether different stimulation intensities of EA result in diverse therapeutic effects for managing KOA, and to evaluate the relationship between circulating proinflammatory cytokine levels and the effects of EA.

Methods and materials

Participants

The prospective controlled trial was conducted from September of 2012 to October of 2013 at Shu Guang Hospital affiliated with the Shanghai Traditional Medicine University. This trail was conducted in accordance with the principles of the Declaration of Helsinki (Version 2010) [31] and the CONSORT statement (Version 2010) [32]. The study protocol was approved by the Ethics Committee of Shu Guang Hospital.

The inclusion criteria included the following: (1) individuals diagnosed with KOA according to the criteria in the American College of Rheumatology and the presence of a severity grade of II or III according to the radiological Kellgren classification [33, 34]; (2) male or female between 38 and 80 years of age; (3) participants will be informed of the research and a signed the informed consent form is required for each participant.

The exclusion criteria included the following: (1) patients who received medical treatment with steroids, physical therapy, or acupuncture within the past four weeks; (2) participants who had experienced a malignancy of any kind, psychiatric disease or suffered from serious life-threatening disease, such as the heart disease or disease of brain and blood vessels, liver, kidney, and hematopoietic system; (3) participants who complicated with serious genu varus/valgus and flexion contraction or had vascular or nerve injury history in ipsilateral limb; (4) systemic inflammatory disease such as rheumatoid arthritis; (5) patients during pregnancy and lactation period.

Patients have a choice whether to participate in the study, and may withdraw their consent at any time. Their consent or lack thereof will not affect their other deserved treatments.

Randomization and blinding

The participants were randomly assigned to two groups through complete randomization at a 1:1 ratio: high current intensity group, or low current intensity group. The randomization sequence was generated using the Statistical Package for the Social Sciences, version 19.00 (SPSS Inc., Chicago IL). All the participating
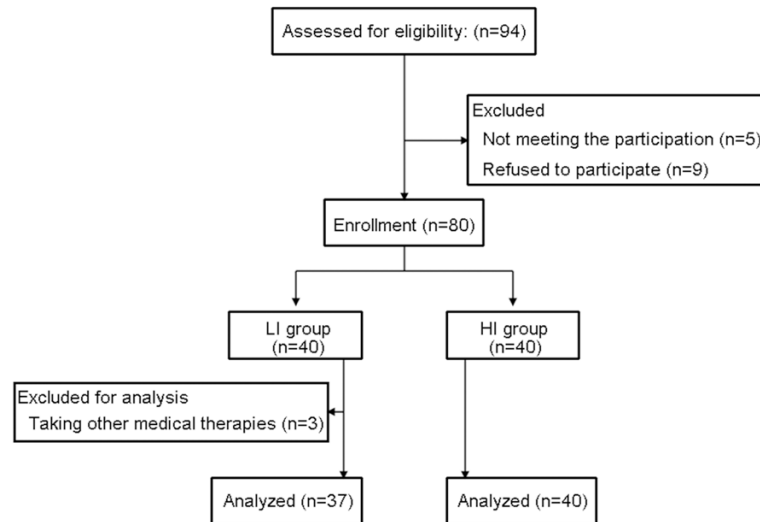
Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram.

patients were evaluated before and after intervention by the same experienced physician (Dr. Liu Shimin), who was blinded for the treatment assignment.

EA treatment

The protocol for EA was to insert single use, sterile, 30-mm-long and 30-gauge acupuncture needles into the local points GB34, ST34, EX-LE4, EX-LE5, ST36, and SP9 at the affected lower limb of the KOA patients. De qi sensation will be achieved at each point through lifting and thrusting movements combined with twirling and rotating the needles. Then, the HANS-200E stimulators (Nanjing Jisheng Co., China) (2Hz) was used to stimulate the needles in pairs GB34-ST34, EX-LE4-EX-LE5, and ST36-SP9. The three pairs of six acupoints were simultaneous stimulated. The stimulation was 30 min per session. In the high intensity group (HI group), the intensity of the stimulation was strong enough to reach the patients' tolerance threshold value (5-6 mA). The intensity of the stimulation applied to the low intensity group (LI group) was relatively weak, which the participants could begin to feel the EA stimulus and plus 1 mA (2-2.5 mA). Participants will receive 16 EA treatments: five times per week (once a day for 5 days continuously, followed by a 2-day interval) during the first 2 weeks and three times per week (once every 2-3 days) during the following 2 weeks. For patients with pain in both knees, treatments were provided bilater-

ally. The EA was operated by the same researcher in each run to ensure the consistency of the procedure. All patients were encouraged to self-exercise, and paid attention to maintaining good posture. Meanwhile, all patients received 30 mg etoricoxib tablets once a day during the study.

Outcomes

The scale for the primary outcome measure was the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (range, 0-240), and its 3 subscales (pain, stiffness, and physical function) [35, 36]. Additional outcome measures were the visual analogue scale (VAS) from 0-10 with terminal descriptors of 'no pain' and 'worst pain possible', for which reliability has been demonstrated in KOA studies [37-39]. The outcome measures above were assessed before the treatment (month 0), at the end of the treatment period (month 1).

Laboratory tests

The blood sample collection took place in the morning between 8 and 9 AM. 2 mL Blood samples were obtained from the antecubital foci and collected into EDTA (1 mg/mL blood) tubes containing aprotinin (500 KIU/mL of blood) (AppliChem, Darmstadt, Germany). The samples were kept on ice for 15 min and then centrifuged at 1600 g for 15 min at 4°C. The plasma was separated into six tubes and immediately frozen at -80°C until assayed.

Plasma apelin concentrations were measured by using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Phoenix peptides, Burlingame, CA, USA), according to the manufacturer's instructions. The minimal detectable concentration was 0.09 ng/mL. The ELISA kit has a reported 100% cross-reactivity with human apelin-12, apelin-13 and apelin-36. Plasma TNFα, IL-1β, and IL-6 levels were determined by ELISA kits (Yuanye Bio-Tech, Shanghai, China) by the same individual who was also blinded for the treatment assignment. The

Table 1. Baseline characteristics

Characteristic	LI group (n = 37)	HI group (n = 40)	P value
Gender (M/F)	10 (27%)/27 (73%)	14 (35%)/26 (65%)	0.482
Age, mean (SD), y	61 (10)	62 (6)	0.362
BMI, mean (SD), (kg/m^2)	26.7 (3.8)	27.4 (3.4)	0.355
VAS, mean (95% CI)	4.62 (4.17-5.08)	4.79 (4.46-5.11)	0.322
WOMAC index mean (95% CI)	87.54 (79.16-95.92)	87.58 (81.75-93.40)	0.460
WOMAC pain mean (95% CI)	18.84 (16.63-21.05)	19.15 (17.59-20.71)	0.344
WOMAC stiffness mean (95% CI)	5.11 (4.50-5.72)	5.20 (4.76-5.64)	0.253
WOMAC physical function mean (95% CI)	63.59 (57.16-70.03)	63.22 (59.05-67.40)	0.456
Apelin (ng/mL)	1.14 (1.11-1.16)	1.15 (1.11-1.19)	0.166
IL-1β (pg/mL)	27.51 (22.40-32.62)	26.03 (23.25-28.81)	0.980
IL-6 (pg/mL)	16.99 (14.58-19.40)	17.56 (15.09-20.03)	0.683
TNFα (pg/mL)	39.62 (32.78-46.46)	39.01 (32.85-45.17)	0.866

Abbreviations: F, female; M, male; BMI, body mass index; VAS, Visual Analogue Scale for pain; WOMAC, Western Ontario and McMaster Universities Osteoarthritis.

detection limits for the ELISA kits are 1.0 pg/mL for TNFα assay, 1.0 pg/mL for IL-1β assay, and 1.0 pg/mL for the IL-6 assay. In each assay, all of the samples were run at the same time, and the r-value of standard curves was greater than 0.99 for all assays.

Statistical analyses

The initial analysis examined the demographic and baseline characteristics of participants in the two groups with an independent two-sample *t* test (body mass index, age), χ^2 analysis (gender), and Mann-Whitney *U* test (VAS, WOMAC, and laboratory tests).

Since most of the data were not normally distributed, Mann-Whitney *U* test was employed to compare the difference in pain scores and plasma TNFα, IL-1β, and IL-6 levels between groups. Meanwhile, Wilcoxon signed rank test were performed for changes in all efficacy evaluations between post-treatment data and baseline data within each treatment group. All statistics were completed with two-tailed tests and *P* < 0.05 was considered to indicate statistical significance. All analyses were performed using the SPSS 19.0 software. Results are presented as the mean ± standard deviation (SD) or 95% confidence interval (CI).

Results

The flow chart of enrollment is shown in **Figure 1**. Of the 94 participants diagnosed with KOA who were informed of the study, 9 decided not to participate. Out of the remaining 85 patients,

80 were eligible for the study and were randomly assigned either to the HI group (n = 40) or to the LI group (n = 40). Three patients in the LI group terminated prematurely due to receive other medical therapies. Seventy-seven subjects (40 in HI group, 37 in LI group) completed the four-week treatment as well as the pre- and post-treatment assessments. No statistically significant differences were found in the baseline characteristics of the randomized two groups, as showed in **Table 1**.

Compared with pre-treatment baseline level, both the high- and low intensity EA stimulations led to significantly reduced VAS, WOMAC index score and its subscales (pain, stiffness, and physical function) after EA treatment, except the WOMAC stiffness scale in LI group (*P* = 0.062) (**Table 2**), showing the effectiveness of the treatments.

For the VAS at the end of treatment, there was no statistically significant difference between LI and HI groups (**Table 2**). For the WOMAC index, there was significant difference between the LI group and HI group (*P* < 0.001) (**Table 2**). In the WOMAC subscales, the WOMAC stiffness scale and physical function scale in HI group were statistically significantly lower compared with that in LI group (*P* < 0.01, *P* < 0.05, respectively). Similar with the VAS scale, there was no statistically significant difference between LI and HI groups on the WOMAC pain scale.

There were no significant differences in the apelin, IL-1β, IL-6, and TNFα levels between

Table 2. Effects of high- and low-intensity EA stimulations on primary outcome measures

	LI group (n = 37)		HI group (n = 40)		P value (Z)‡
	mean (95% CI)	P value (Z)†	mean (95% CI)	P value (Z)†	
VAS	3.72 (3.31-4.12)	< 0.001 (-4.508)	3.29 (2.98-3.60)	< 0.001 (-5.455)	0.161 (-1.400)
WOMAC index	76.65 (68.10-85.20)	< 0.001 (-3.941)	63.15 (58.42-67.88)	< 0.001 (-5.514)	0.037 (-2.086)
WOMAC pain	16.24 (13.93-18.56)	< 0.001 (-3.303)	13.70 (12.71-16.69)	< 0.001 (-5.525)	0.362 (-0.912)
WOMAC stiffness	4.65 (3.94-5.36)	0.062 (-1.864)	3.28 (2.89-3.66)	< 0.001 (-5.484)	0.001 (-3.306)
WOMAC physical function	55.76 (49.49-62.06)	< 0.001 (-3.646)	46.18 (42.63-49.72)	< 0.001 (-5.514)	0.048 (-1.981)

Abbreviations: VAS, Visual Analogue Scale for pain; WOMAC, Western Ontario and McMaster Universities Osteoarthritis. †Compared between baseline data and post-treatment data; ‡Compared between LI and HI groups.

Table 3. Apelin and proinflammatory cytokines concentrations in plasma

	LI group		HI group		P value (Z)‡
	After treatment Mean (95% CI)	P value (Z)†	After treatment Mean (95% CI)	P value (Z)†	
Apelin (ng/mL)	1.12 (1.11-1.14)	0.055 (-1.917)	0.95 (0.92-0.97)	< 0.001 (-5.309)	< 0.001 (-7.169)
IL-1β (pg/mL)	25.21 (21.50-28.91)	0.561 (-0.581)	25.30 (22.03-28.57)	0.051 (-1.954)	0.223 (-1.219)
IL-6 (pg/mL)	16.04 (13.28-18.80)	0.492 (-0.686)	16.24 (14.02-18.45)	< 0.001 (-3.782)	0.498 (-0.678)
TNFα (pg/mL)	35.12 (29.29-40.94)	0.002 (-3.055)	34.72 (29.66-39.78)	< 0.001 (-4.837)	0.870 (-0.163)

†Compared between baseline data and post-treatment data; ‡Compared between LI and HI groups.

groups at the beginning stage (**Table 1**). Compared with baseline, the plasma levels of apelin and TNFα were decreased significantly in both the LI and HI groups after EA treatment (**Table 3**). However, the IL-1β level in plasma was not affected by the treatments. The change in the plasma level of IL-6 was significantly reduced only in HI group (**Table 3**). Furthermore, the reduction of apelin level was significantly less in the LI group than that of the HI group (**Table 3**).

Discussions

EA has been proven to be safe and efficacious for pain relief and functional improvements in KOA patients both short term and long term [8-11]. In this study, patients with KOA who underwent EA treatment had significantly improvement in pain, stiffness, and physical function after 4 weeks, compared with their baseline before treatment. In agreement with previous studies, this result also revealed that both high- and low-intensity EAs treatment is effectively reducing pain symptoms and improving function in KOA patients.

Inflammation contributes to the symptoms and progression of osteoarthritis [18, 19, 40]. The degradation of articular knee cartilage in osteoarthritis causes an inflammatory process that is responsible for the proteolytic digestion of

articular cartilage [41, 42]. Meanwhile, the inflammation may be the crucial link between local noxious stimuli and recruitment of centrally mediated pathways [43-45]. When inflammatory mediators such as cytokines are released intraarticularly from damaged tissue, they can modulate both central and peripheral nociceptors. Meanwhile, EA is also widely used for treatment of inflammatory conditions to relieve pain, such as rheumatoid arthritis (RA), inflammatory bowel disease, and inflammatory pain [46-48]. These facts suggested that the therapeutic effect of EA may be mediated by their anti-inflammatory mechanism of action. This assumption was supported by the observation in this study that following therapy with EA treatment, the plasma levels of apelin and TNFα showed significant reduction compared to baseline values (**Table 3**). Recently, it has been demonstrated that the levels of apelin in paired serum and synovial fluid are significantly higher in osteoarthritis patients than in healthy volunteers [28]. Apelin can enhance osteoblast proliferation and suppress its apoptosis [49-51]. TNFα appears to play a pivotal role in cartilage matrix degradation and bone resorption in osteoarthritis, which could suppress the synthesis of proteoglycan, link protein and type II collagen in chondrocytes [52, 53]. Taken together, our findings suggested that EA may decrease circulating apelin and TNFα and alle-

viate pain symptoms and improve function in KOA patients.

The intensity of EA is an important parameter in clinical treatment because of its influence on EA's therapeutic effects. Previous studies have shown that the EA with high-intensity stimulation is more effective than treatments with less stimulation in human subjects and experimental animals with other diseases [12, 14, 15, 54]. However, some contrary conclusions are reported in animal studies [16, 17]. Therefore, in the present study, we investigated that whether the different intensity of EA could have different therapeutic effects in treating patients with KOA. In the present study, there was no difference in reducing pain between high- and low-intensity EA treatments (**Table 2**). However, our results showed that although patients in both groups markedly improved from baseline after the EA treatment, a clear trend indicates that more improvement in WOMAC subcategories of stiffness and physical function in HI group than LI group (**Table 2**). This finding suggested that a strong but comfortable intensity might be beneficial for the functional amelioration of KOA patient populations than low-intensity.

Furthermore, the apelin level reduced more significant in HI group than in LI group ($P < 0.001$) (**Table 3**). The serum IL-6 level was only decreased in HI group after EA treatment (**Table 3**). Chondrocytes produce low levels of IL-6 under normal conditions. High baseline levels of IL-6 and CRP in patients with osteoarthritis were associated with an increased risk of cartilage loss [55]. High BMI and increased levels of circulating IL-6 were associated with the risk of developing radiographic KOA [56, 57]. Treatment of chondrocytes with IL-6 and/or soluble IL-6 receptor can decrease expression of type II collagen [58]. Given our results, the apelin and IL-6 were significantly inhibited by high-intensity EA, which may correlate with the more effective improve physical function of KOA patients. However, the relationship between plasma apelin and IL-6 levels and functional amelioration in patients with KOA may be addressed by a multicentre, double-blind RCTs with large sample sizes.

In conclusion, the current study suggests that short-term treatment (4 weeks) with high- or low-EA effectively suppresses proinflammatory cytokines and improves pain and function in patients with symptomatic KOA. Higher intensity of EA stimulation provides better improvement in the disease-specific quality of life of KOA patients and a greater modulation of cytokine secretion. Further studies investigating molecular pathways implicated in the different intensity EA response during KOA are warranted.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Huashun Cui, Department of Acupuncture, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, No. 523 Zhangheng Rd., Shanghai 201203, China. E-mail: huashunchoi@126.com; Dr. Wang Ke, Laboratory of Integrative Medicine Surgery, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, No. 523 Zhangheng Rd., Shanghai 201203, China. E-mail: wangke8430@163.com

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