

—Original—

A rat model of knee osteoarthritis suitable for electroacupuncture study

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Abstract: Acupuncture is widely used for knee osteoarthritis (KOA) treatment in clinical practice. In the present study, we aimed to set a standard KOA animal model for electroacupuncture (EA) study and provide an acupuncture recipe for further KOA studies. Rats intra-articularly administered monosodium iodoacetate (MIA, 0.3, 1 or 3 mg respectively, n=12 each) were evaluated for pain-like behavior: paw withdrawal mechanical threshold, weight bearing deficit, and joint pathological changes (OARSI score) until 28 days after injury. Then by using the suitable dose (1 mg MIA), therapeutic effects of EA treatment (bilateral ST36 and ST35 acupoints, 2/10 Hz, 30 min/d, 6d/w, 2w) were evaluated in 3 groups (n=16 each): Early-on EA, Mid-term EA and Delayed EA, in which EA was started on day 1, day 7 or day 14 after MIA injection. Both 1 mg and 3 mg MIA induced significant joint damage and persistent pain behavior. But animals accepted 3 mg MIA rapidly developed cartilage and bone damage within 14 days. Early-on EA treatment provided significant pain relief and joint structure preservation in KOA rats. Mid-term EA treatment only reduced pain, while delayed EA treatment resulted in no effects in both aspects. 1 mg of MIA produces steady pain behavior and progressive joint damage, which was suitable for EA treatment evaluation. Early-on EA treatment provided both joint protection and pain reduction, while Mid-term EA could only be used for studying EA-induced analgesia in KOA.

Key words: animal model, electroacupuncture, knee osteoarthritis

Introduction

Knee osteoarthritis (KOA) is one of the leading causes of chronic disability [5, 29]. Major clinical symptoms of KOA include joint pain, limitation of activity and stiffness. Current managements such as articular lubrication, chondroitin sulfate and non-steroidal anti-inflammatory drugs (NSAIDs) analgesics mainly focus on

relieving symptoms, especially pain [33]. Acupuncture or electroacupuncture (EA) has been widely used for osteoarthritis in patients [1, 12, 17, 24, 26]. With many unsolved questions such as the best acupuncture recipe, underlying mechanisms need to be answered. To establish a standard KOA animal model suitable for acupuncture study will help to have more scientific evidences for acupuncture treatment on KOA.

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Among several distinct KOA models [4, 7, 13, 27], the monosodium iodoacetate (MIA) intra-articular injection model is widely used for pain research and efficacy evaluation of therapeutic interventions [13]. The dose of MIA used to induce KOA varies from 0.1 mg to 4.8 mg [20]. But it is unclear which dose is appropriate for EA treatment study. Also, the efficacy of EA treatment initiated at different stage after MIA injection has not been tested. Therefore, this study aims to provide evidences to suggest a suitable KOA model for acupuncture study and to provide choices of acupuncture recipe for further research regarding EA-induced analgesia and joint protection.

Materials and Methods

Animals and KOA induction

Male Sprague-Dawley (National Institutes for Food and Drug Control, Beijing, CHINA) rats weighing 200–225 g were used. Rats were maintained on a 12-h light/dark cycle with food and water available *ad libitum*. Experimental protocols were approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University (Xi'an, China) and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Under 1.5% isoflurane anesthesia, 0.3 mg, 1 mg or 3 mg of MIA (Sigma, USA) dissolved in 50 μ l sterile 0.9% saline or saline only (for sham group) was administered into the left knee articular cavity of the rats by inserting a 31-gauge needle through the patellar tendon. After stretching and flexing the injected hind limb for 5 times, rats were returned to home cage for recovery.

Experimental design

Experiment 1: Forty-eight rats were randomly divided into four groups (n=12 each): Sham group, 0.3 mg, 1 mg and 3 mg MIA groups (intra-articular injection of 0.9% saline, 0.3 mg, 1 mg or 3 mg MIA, respectively). Paw withdrawal mechanical threshold (PWMT) tests and weight bearing tests were performed on the day before (baseline), day 3, 7, 14 and 28 after MIA injection. Rats were sacrificed and left knees were saved for pathological evaluation.

Experiment 2: Based on the result of Experiment 1, 1mg of MIA was used to induce KOA in this experiment. Forty-eight rats were randomly divided into three groups (n=16 each): Early-on EA group, Mid-term EA group

and Delayed EA group (EA treatment from day 1 to day 13, from day 7 to 19 or from day 14 to day 26 after MIA injection, respectively). Each group was randomly divided into two subgroups, MIA group (30 min daily mild restraint in the awake acupuncture apparatus without EA treatment, n=8) and MIA+EA group (n=8). Behavioral tests were performed on the day before MIA injection (baseline), and at 6 h and 24 h after each course of EA treatment. After two courses of EA treatment and behavioral tests, rats were sacrificed for articular histological assessment. The experimental designs are shown in Fig. 1.

Electroacupuncture treatment

Rats were kept in a gentle immobilization apparatus designed by our laboratory for awake rodent manipulation (patent application number: p201721299630.9). Four acupuncture needles were inserted into the bilateral acupoints of ST35 (Dubi, 2 mm deep) and ST36 (Zusanli, 5 mm deep). In humans, ST35 is located in the depression lateral to the patellar ligament. ST 36 is located on the anterior aspect of the lower leg, 3 U (based on the standard acupuncture measurement of 16 U between the knee and the ankle joint) below the knee joint and one finger-breadth (middle finger) lateral to the anterior crest of the tibia. ST35 and ST36 are located on the rat's hind limbs using the comparable anatomical landmarks [15]. These two acupoints were stimulated with 2/10 Hz frequency at 1 mA for 30 min per day (SDZ-V Huatuo Electroacupuncture Instrument, Suzhou Medical Appliances Co., Ltd., Suzhou, China), 6 days weekly for 2 weeks.

Behavioral tests

Assessment of mechanical hyperalgesia: Hyperalgesia was evaluated using paw withdrawal mechanical threshold test. Von Frey Hairs (Stoelting, USA, 0.4, 0.6, 1.0, 1.4, 2.0, 4.0, 6.0, 8.0 or 15 g fibers) was used to touch the plantar surface of the hind paw as previously described [3]. A cutoff of 15 g was set. Each rat was tested twice and the mean PWMT over the two trials was obtained for each rat.

Measurement of Weight Bearing Deficit: Changes in weight bearing were measured using a weight in capacitance tester (IITC Incapacitance Meter, USA) as previously described [2]. We documented five measurements of the weight borne on each hind paw and calculated the difference in weight borne by ipsilateral and

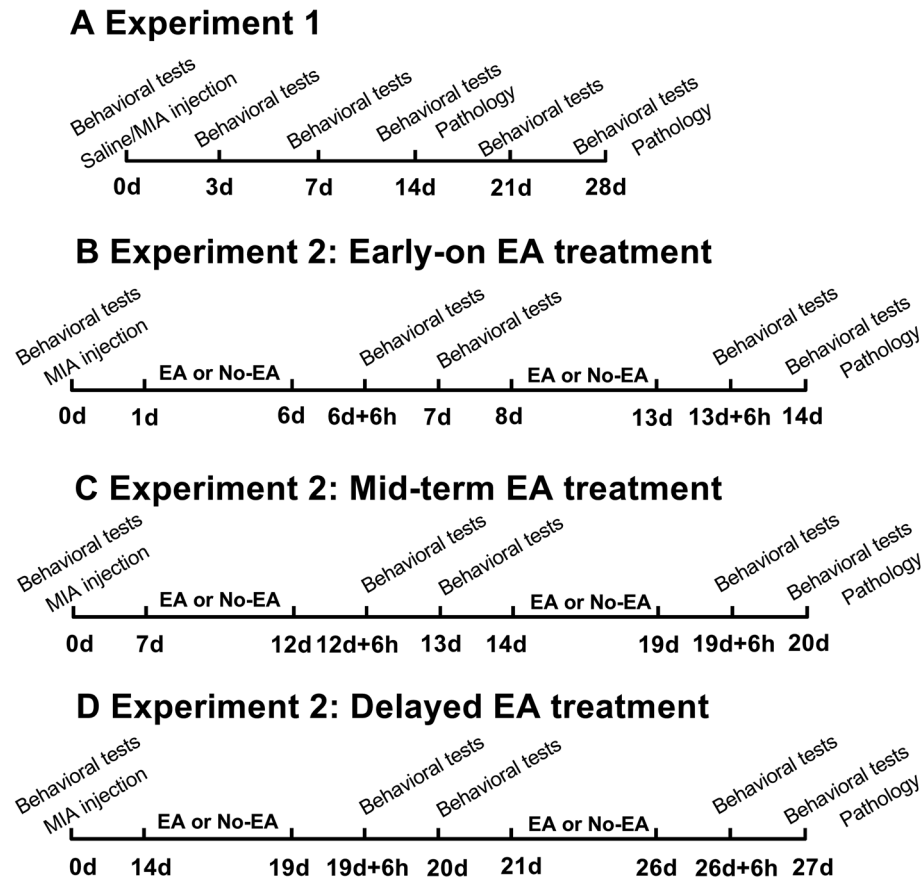


Fig. 1. Timelines of experiments. timelines of Experiment 1 (A) and Experiment 2 (B for early-on EA treatment, C for mid-term EA treatment and D for delayed EA treatment).

contralateral paws. Percentage of ipsilateral weight bearing was calculated as: [(weight borne on ipsilateral paw / sum of the weight borne on the ipsilateral and contralateral paws) \times 100]. Mean value of the 5 tests was obtained as the weight born of this rat.

Joint pathology

Rats were sacrificed with overdose pentobarbital and ipsilateral knee joints were dissected and fixed with 4% paraformaldehyde for 24 h. Decalcification was achieved with 20% EDTA immersion for 3 weeks, and then the tissues were embedded in paraffin. The joints were sagittally sectioned at 5 μ m thick and stained with hematoxylin and eosin (H&E). Degeneration of the articular cartilage was evaluated by two independent observers in a blinded manner using Osteoarthritis Research Society International (OARSI) score on a scale of 0–24 points [22].

Statistical analysis

Data were presented as mean \pm SEM for behavioral results and mean \pm SD for OARSI scores. Using Graph-Pad Prism 7.0. software, pain behaviors were analyzed with Two-way repeated measurements ANOVA, followed by Bonferroni's post hoc test. Kruskal-Wallis test followed by post hoc Dunn's tests was used for OARSI score analysis in four group comparisons. And for two groups comparison, Mann Whitney test was used. A *P* values less than 0.05 were considered statistically significant.

Results

MIA-induced KOA related pain behaviors are dose and time dependent

Figure 2A showed a time course of paw withdrawal mechanical threshold in the ipsilateral following 0.3–3 mg MIA or saline intra-articular administration. All three doses induced hypersensitivity in the ipsilateral hind

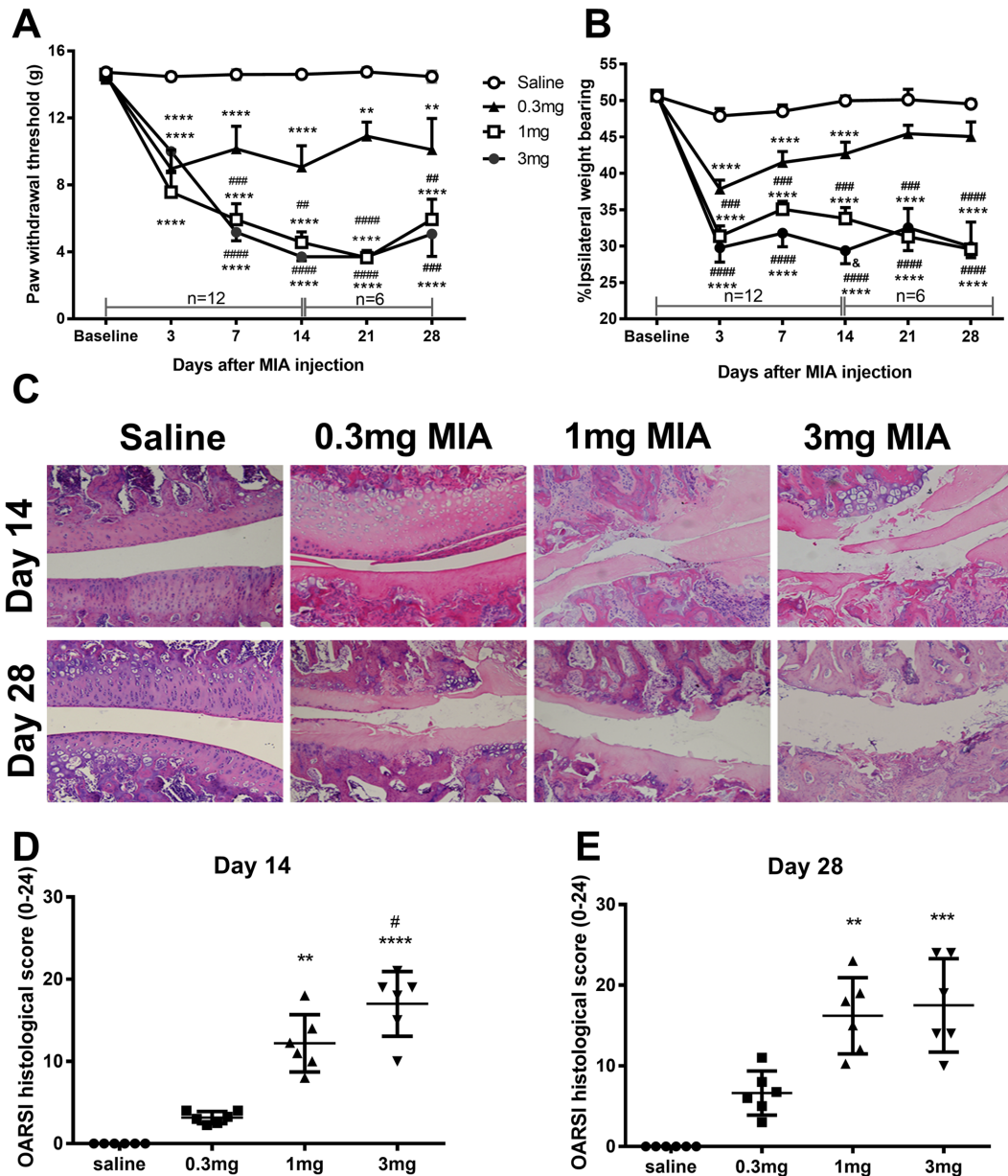


Fig. 2. MIA induces KOA-related pain behavior and cartilage damage at different dose. Paw withdrawal thresholds of the ipsilateral hind paws (A) and weight bearing deficits (B) were assessed before and after MIA (0.3, 1, and 3 mg/ rat, n=12/group) or saline (0.9% NaCl, n=12) injection. At 14 and 28 days after MIA injection, 6 rats in each group were randomly selected for articular histological assessment. Data are presented as mean \pm SEM. ** P <0.01, **** P <0.0001, vs. saline-treated group; ### P <0.01, #### P <0.001, ##### P <0.0001, vs. 0.3 mg MIA group; & P <0.05, vs. 1 mg MIA group at the same tested time point. Two-way repeated measurements ANOVA followed by Bonferroni's post hoc test was used. C. Representative articular pathology image at 14 and 28 days after Saline or MIA injection. D and E: OARSI score on day 14 (D) and day 28 (E) after MIA injection. ** P <0.01, *** P <0.001, **** P <0.0001, vs. saline-treated group; # P <0.05 vs. 0.3mg MIA group. Data were presented as mean \pm SD, n=6 per group. Kruskal-Wallis test followed by post hoc Dunn's tests was used.

paw. While animals in 1 mg and 3 mg MIA group exhibited lower PWMT than animals of 0.3 mg MIA group from day 7–28. The lowest dose of MIA (0.3 mg) reduced PWMT from 14.46 ± 0.04 to 8.96 ± 1.14 on day 3, and

remained at similar level until day 28. Both 1 mg and 3 mg MIA also induced PWMT reduction on day 3 (7.57 ± 1.17 and 10.00 ± 1.17) and a further decrease on day 7 (5.93 ± 0.94 and 5.18 ± 0.51) and day 14 (4.57 ± 0.62

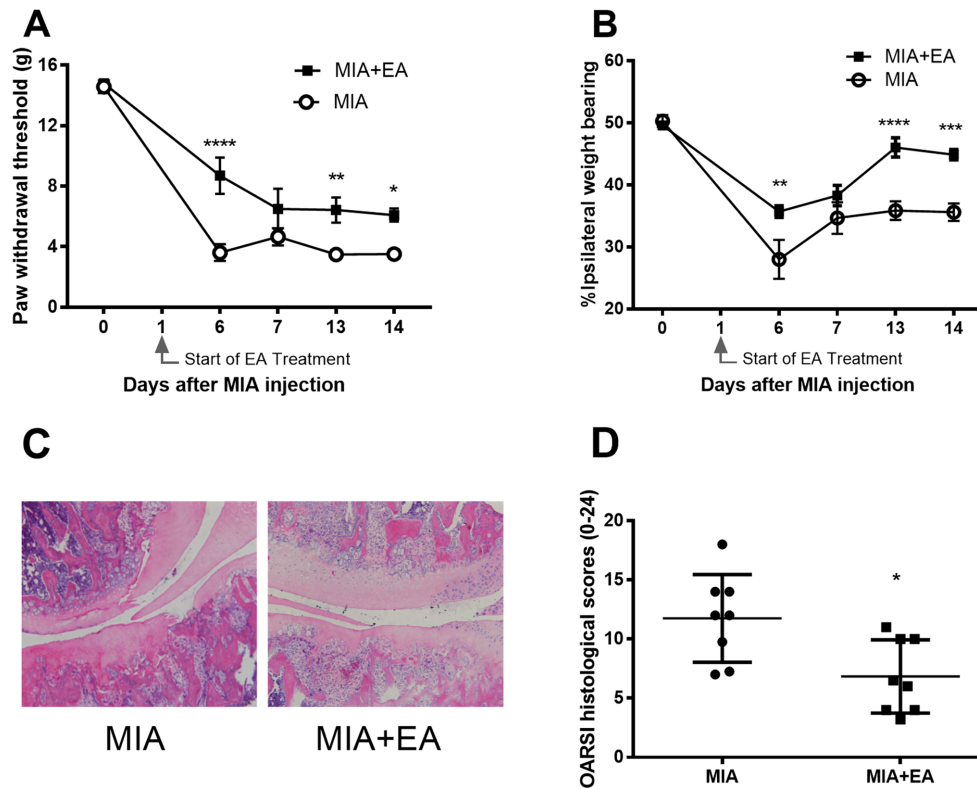


Fig. 3. Effect of early-on EA treatment. Paw withdrawal thresholds of the ipsilateral hind paws (A) and weight bearing deficits (B) were assessed before and after injection MIA. Data were presented as mean \pm SEM; $n=8$ per group; $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$ vs. MIA group. Two-way repeated measurements ANOVA followed by Bonferroni's post hoc test were used. (C) Articular cartilage pathology image in MIA and EA treated group. (D) OARSI scores were presented as mean \pm SD; $n=8$ per group; $*P<0.05$ vs. MIA group (Mann-Whitney U test).

and 3.71 ± 0.24), which sustained up to day 28 (5.95 ± 1.20 and 5.07 ± 1.33). For weight bearing deficit test (Fig. 2B), 0.3 mg MIA induced significant reduction in ipsilateral weight bearing on day 3–14, but recovered by day 21 and 28 ($P>0.05$ vs saline group). Instead, the 1 mg and 3 mg MIA induced significant reduction of weight bearing loss of the ipsilateral hind limb from day 3 until the end of the observation period. At all tested time points after MIA injection, animals in 1 mg and 3 mg MIA groups had more severe weight bearing deficit than rats in 0.3 mg MIA group. However, 1 mg and 3 mg of MIA induced similar extent of pain behavior in PWMT and percentage of weight bearing deficit test, except that on day 14 after MIA injection, rats accepted 3 mg MIA exhibited further reduction on weight bearing test compared to rats from 1 mg MIA group.

MIA-induced cartilage changes are dose and time dependent

No cartilage degeneration was observed in saline injected rats on day 14 and 28 (Fig. 2C). Chondrocyte deaths were observed in the superficial zone at day 14 (Fig. 2D) in rats with 0.3 mg MIA. By day 28, the cartilage superficial zone was mildly affected while its OARSI score (6.63 ± 2.73) didn't reach a significance compared to saline group during multiple comparison analysis (Fig. 2E). However, for 1 mg and 3 mg MIA groups, OARSI score significantly elevated at day 14 (Fig. 2D) and day 28 (Fig. 2E) compared to saline group ($P<0.01$). On day 14, chondrocyte death, matrix loss and cartilage thickness decrease were observed in animals from 1 mg MIA group, with an OARSI score of 12.21 ± 3.49 (Fig. 2D, $P<0.01$ vs. saline group). But animals from 3 mg MIA group exhibited erosion of hyaline cartilage, mineralization of cartilage and bone (Fig. 2D, OARSI score 17 ± 3.95 , $P<0.0001$ vs saline group and

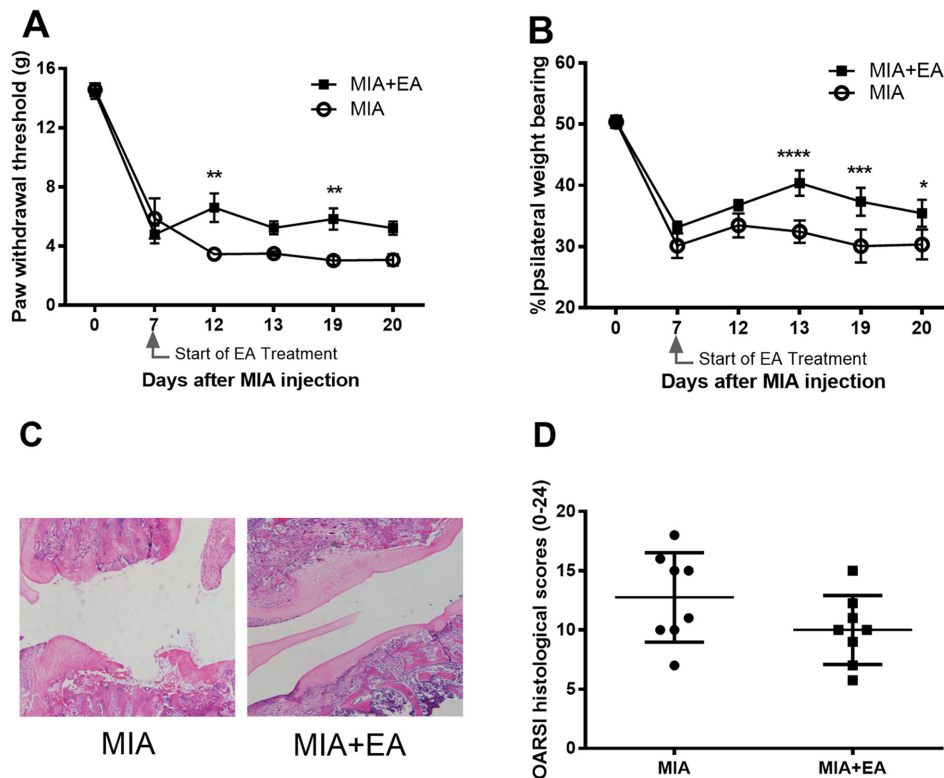


Fig. 4. Effect of mid-term EA treatment. Paw withdrawal thresholds of the ipsilateral hind paws (A) and weight bearing deficits (B) were assessed before and after injection MIA. Data were presented as mean \pm SEM; $n=8$ per group; * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ vs. MIA group. Two-way repeated measurements ANOVA followed by Bonferroni's post hoc test was used. Representative images of articular cartilage pathology of MIA and mid-term EA treated group are shown in C. OARSI score (D) were presented as mean \pm SD; $n=8$ per group; * $P<0.05$ vs. MIA group (Mann-Whitney U test).

$P<0.05$ vs 0.3 mg MIA group). By day 28, animals in 1 mg MIA group developed subchondral bone exposure (Fig. 2E, OARSI score 16.21 ± 4.73 , $P<0.01$ vs saline group). While OARSI score in 3 mg MIA group was 17.5 ± 5.79 (Fig. 2E, $P<0.001$ vs saline group).

Effect of EA treatment

Early-on EA treatment: Mechanical hyperalgesia of the ipsilateral hind paw and weight bearing deficits were observed in MIA group. Early-on EA treatment increased the PWMT (Fig. 3A), decreased the weight bearing deficits (at day 6, 13 and 14 respectively, Fig. 3B), and reduced pathological OARSI scores ($P<0.05$, Figs. 3C and D).

Mid-term EA treatment: Mid-term EA treatment increased the PWMT at 6 h after the first and second EA course ($P<0.01$ vs MIA group on day 12, and day 19 Fig. 4A), but failed to produce PWMT reduction at 24 h after

each EA course. A significant difference was observed in the weight bearing deficits (Fig. 4B) from day 13 until the end of the observation period. However, no significant difference in joint pathology was observed (Fig. 4C), indicating that EA treatment started at day 7 after MIA injury could not attenuate the progression of joint pathological changes ($P=0.12$, Fig. 4D).

Delayed EA treatment: Delayed EA treatment was administered since day 14 after MIA injection. PWMT recovery was only observed at 6 h after the first EA course ($P<0.001$ vs MIA group on day 19, Fig. 5A). And weight bearing deficit slightly recovered at 6 h after the second EA course ($29.09 \pm 2.22\%$ for MIA vs $31.27 \pm 1.63\%$ for MIA+EA group, Fig. 5B). However, this reduction could not sustain until 24 h after treatment. HE staining of the ipsilateral knee joint showed that delayed EA treatment could not reduce OARSI scores (Figs. 5C and D).

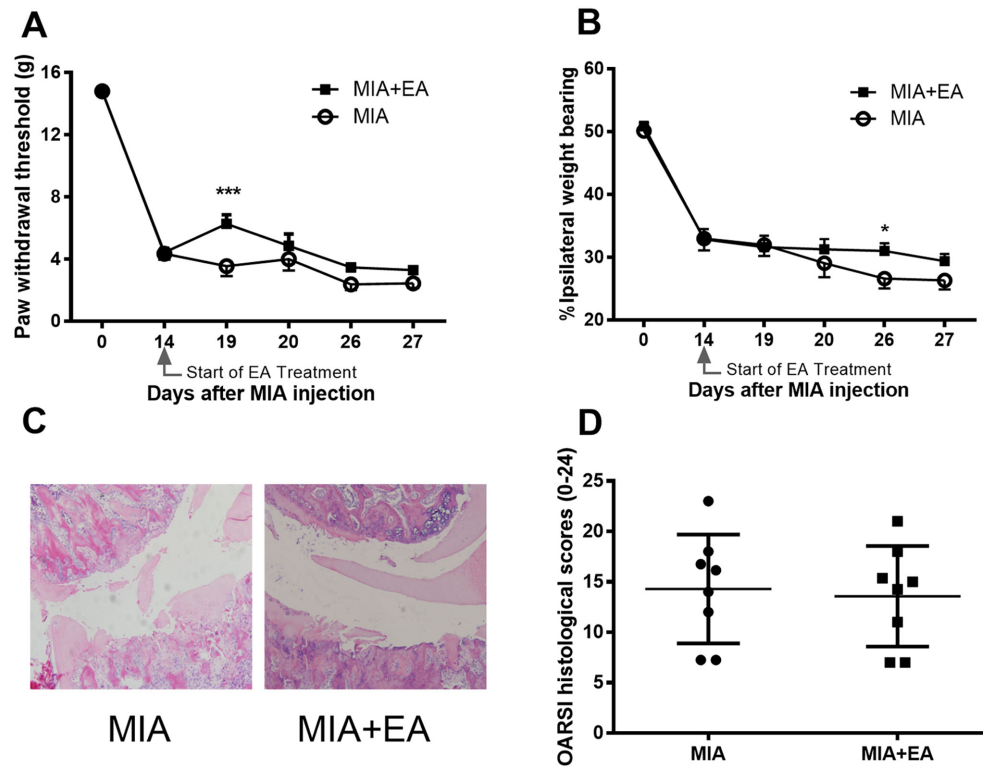


Fig. 5. Effect of delayed EA treatment. Paw withdrawal thresholds of the ipsilateral hind paws (A) and weight bearing deficits (B) were assessed before and after injection MIA. Data were presented as mean \pm SEM; $n=8$ per group; $*P<0.05$, $***P<0.001$ vs. MIA group. Two-way repeated measurements ANOVA followed by Bonferroni's post hoc test were used. (C) Representative articular cartilage pathology images of MIA and delayed EA treatment group. OARSI score (D) were presented as mean \pm SD; $n=8$ per group; $*P<0.05$ vs. MIA group (Mann-Whitney U test).

Discussion

Joint degeneration and chronic pain are two major problems associated with knee osteoarthritis. Acupuncture is widely used for KOA in clinical settings for pain management [17, 23, 24]. But a standard animal model suitable for acupuncture study has not been established. In this study, we investigated the suitable MIA dose appropriate for evaluating the efficacy of acupuncture in a rat KOA model and an effective acupuncture treatment strategy.

MIA induced osteoarthritis animal model was established in 1984 [31]. More than a decade later, another group reported that only high dose (0.3–3 mg) would result in a long-term damage which correlated to human KOA [8]. A range of MIA dosage was later extended to 0.1–4.8 mg [20], MIA with 1, 2 and 3 mg were extensively used [12]. However, which dose of MIA is suitable for acupuncture study hasn't been evaluated in MIA-

induced KOA model. Literature review showed that intra-articular injection of MIA inhibits chondrocyte glycolysis and results in chondrocyte damage, subchondral bone necrosis and inflammation [21]. Nwosu LN *et al.* reported that pain behaviors were associated with OA structural severity and synovitis. Both 0.1 and 1 mg of MIA injection induced similar structural pathology while the higher dose associated better with paw withdraw behavior [19]. Another study showed that 0.2 mg MIA induced reversible synovitis [28]. We evaluated the pain behavior and joint pathological changes after 0.3, 1 and 3 mg of MIA injection in rat knees. The results indicated that 0.3 mg elicit very limited joint damage, both 1 mg and 3 mg MIA induced PWMT and weight bearing percentage reduction from day 3 after injection through the end of the study, which was in accordance with previous reports [2, 8]. However, the severity of joint damage proceeded slower in rats accepted 1 mg MIA injection which made it a better model represent the slow

onset of KOA in human.

Among acupuncture treatment strategies for KOA patients, acupoints ST35 and ST36 are most frequently used in clinical trials [26]. Stimulation at ST36 has been proven to prevent joint destruction [32] and to attenuate pain in a collagenase induced arthritis animal model [25]. Qi *et al.* treated patients with ST35 and EX-LE4 acupoints and resulted in a significant reduction in VAS and WOMAC scores, especially in patients with lower KOA stages [23]. Helianthi *et al.* used laser acupuncture at ST35, ST36, SP9, GB34, and EX-LE4 acupoints also showed a significant improvement in VAS score [9]. In addition, EA stimulation at bilateral ST36 and BL60 acupoints with 2/10 Hz frequency provided analgesic and immunomodulation effects in a bone cancer pain model [16]. Since KOA is characterized by joint inflammation and pain, we selected the bilateral ST36 and ST35 acupoints and 2/10 Hz alternative frequency EA stimulation as our EA treatment strategy.

Clinically, EA is more often used in advanced osteoarthritis to control pain. But the efficacy of acupuncture for KOA treatment is controversy [6, 10, 11, 14, 30]. The discrepancy may be related to variation in acupuncture recipes. EA treatment for different stages of joint injury may lead to different outcomes. In the present study, we tested the efficacy of two courses of EA initiated from day 1, day 7 or day 14 on pain behavior and joint pathology. Results showed that EA treatment was most effective when applied early after joint injury. The results were in consistence with a previous study that early-on EA treatment decreased the weight bearing deficits in a rat KOA model [15]. Li-A *et al.* reported that EA treatment activates serotonergic neurons in the nucleus raphe magnus and project to the spinal cord to alleviate pain. The treatment effect of EA could be blocked by 5-HT_{2A/2C} receptor blocker. They reported a short 4-day course of EA treatment and a rather strong analgesic effect that last for 3 days after EA treatment, shown as recovered weight bearing deficit. However, weight bearing test recovery was only observed 6 h after the last EA treatment on day 6 in our study. By day 7, the difference was not significant since weight-bearing recovered to similar level in untreated animals as EA treated ones. The recovery tendency at day 7 after MIA can also be observed in Li's paper. Also, we have chosen the 1 mg MIA model since it represents a progressive pathological change, but 3 mg model was used by Li *et al.* Our results did reveal alleviated joint damage after two courses of

EA treatment, but they did not observe joint pathologic changes. Their acupoint choices and EA strategy are also different from ours. Most importantly, we applied EA on awake rats with a mild restrain device while Li-A *et al.* conducted EA treatment to rats under isoflurane anesthesia. It is unknown whether anesthesia could influence the effects of EA. These differences may partly explain the behavioral difference. However, serotonergic system may be an interesting target for further investigation. Therefore, the time point of treatment initiation was crucial for treating KOA, as shown in another study researching for the importance of early medication initiation for KOA [18]. Our results also indicated that initiation time of EA treatment is very important for KOA, which will be helpful both for future EA research design and for clinical practice.

To exclude influences of anesthesia in EA treatment, such as central sedation and possible neuroprotection or neural injury, we designed a restriction device (Patent application No. 201721299630.9) that covered the body and head of the animal with soft cotton while allowing the hind limbs to stretch out and touch the ground. We pre-accommodated the animals in these devices 30 min/day for two days before MIA injection. Most animals accommodated well in the device. Animals that keep twisting or with tighten tails that show elevated level of stress were excluded before randomization. Six of 54 animals in experiment 2 were excluded before randomization.

In summary, we recommended a standard rat KOA mode of 1 mg MIA intra-articular injection, which is suitable for electroacupuncture treatment for further studies. This rodent KOA model mimics a similar progressive course of human KOA. Early-on EA treatment provides significant analgesic effects and reduces histological changes of the knee joint. Mid-term EA treatment provides substantial analgesia, but not joint damage alleviation. Delayed EA has no benefits on pain or joint pathology. The present study provided a stable animal model of KOA, a new therapeutic EA strategy that can be used as a standard model in future studies regarding EA treatment for KOA.

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References

- Berman, B.M., Lao, L., Langenberg, P., Lee, W.L., Gilpin, A.M.K., and Hochberg, M.C. 2004. Effectiveness of acupuncture as adjunctive therapy in osteoarthritis of the knee: a randomized, controlled trial. *Ann. Intern. Med.* 141: 901–910. [Medline] [CrossRef]
- Bove, S.E., Calcaterra, S.L., Brooker, R.M., Huber, C.M., Guzman, R.E., Juneau, P.L., Schrier, D.J., and Kilgore, K.S. 2003. Weight bearing as a measure of disease progression and efficacy of anti-inflammatory compounds in a model of monosodium iodoacetate-induced osteoarthritis. *Osteoarthritis Cartilage* 11: 821–830. [Medline] [CrossRef]
- Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., and Yaksh, T.L. 1994. Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods* 53: 55–63. [Medline] [CrossRef]
- Christiansen, B.A., Anderson, M.J., Lee, C.A., Williams, J.C., Yik, J.H., and Haudenschild, D.R. 2012. Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis. *Osteoarthritis Cartilage* 20: 773–782. [Medline] [CrossRef]
- Cross, M., Smith, E., Hoy, D., Nolte, S., Ackerman, I., Fransen, M., Bridgett, L., Williams, S., Guillemin, F., Hill, C.L., Laslett, L.L., Jones, G., Cicuttini, F., Osborne, R., Vos, T., Buchbinder, R., Woolf, A., and March, L. 2014. The global burden of hip and knee osteoarthritis: estimates from the global burden of disease 2010 study. *Ann. Rheum. Dis.* 73: 1323–1330. [Medline] [CrossRef]
- Fan, A.Y. 2015. The methodology flaws in Hinman's acupuncture clinical trial, part I: design and results interpretation. *J. Integr. Med.* 13: 65–68. [Medline] [CrossRef]
- Fang, H. and Beier, F. 2014. Mouse models of osteoarthritis: modelling risk factors and assessing outcomes. *Nat. Rev. Rheumatol.* 10: 413–421. [Medline] [CrossRef]
- Guingamp, C., Gegout-Pottie, P., Philippe, L., Terlain, B., Netter, P., and Gillet, P. 1997. Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility, morphology, and biochemistry. *Arthritis Rheum.* 40: 1670–1679. [Medline] [CrossRef]
- Helianthi, D.R., Simadibrata, C., Srilestari, A., Wahyudi, E.R., and Hidayat, R. 2016. Pain reduction after laser acupuncture treatment in geriatric patients with knee osteoarthritis: a randomized controlled trial. *Acta Med. Indones.* 48: 114–121. [Medline]
- Hinman, R.S., Forbes, A., Williamson, E., and Bennell, K.L. 2015. Acupuncture for chronic knee pain: a randomised clinical trial. Authors' reply. *Acupunct. Med.* 33: 86–88. [Medline] [CrossRef]
- Hinman, R.S., McCrory, P., Pirota, M., Relf, I., Forbes, A., Crossley, K.M., Williamson, E., Kyriakides, M., Novy, K., Metcalf, B.R., Harris, A., Reddy, P., Conaghan, P.G., and Bennell, K.L. 2014. Acupuncture for chronic knee pain: a randomized clinical trial. *JAMA* 312: 1313–1322. [Medline] [CrossRef]
- Jubb, R.W., Tukmachi, E.S., Jones, P.W., Dempsey, E., Waterhouse, L., and Brailsford, S. 2008. A blinded randomised trial of acupuncture (manual and electroacupuncture) compared with a non-penetrating sham for the symptoms of osteoarthritis of the knee. *Acupunct. Med.* 26: 69–78. [Medline] [CrossRef]
- Lampropoulou-Adamidou, K., Lelovas, P., Karadimas, E.V., Liakou, C., Triantafillopoulos, I.K., Dontas, I., and Papaioannou, N.A. 2014. Useful animal models for the research of osteoarthritis. *Eur. J. Orthop. Surg. Traumatol.* 24: 263–271. [Medline] [CrossRef]
- Lao, L. and Yeung, W.F. 2015. Treating chronic knee pain with acupuncture. *JAMA* 313: 627–628. [Medline] [CrossRef]
- Li, A., Zhang, Y., Lao, L., Xin, J., Ren, K., Berman, B.M., and Zhang, R.X. 2011. Serotonin Receptor 2A/C is involved in electroacupuncture inhibition of pain in an osteoarthritis rat model. *Evid. Based Complement. Alternat. Med.* 2011: 619650. [Medline] [CrossRef]
- Liang, Y., Du, J.Y., Fang, J.F., Fang, R.Y., Zhou, J., Shao, X.M., Jiang, Y.L., Chen, Y.T., and Fang, J.Q. 2017. Alleviating mechanical allodynia and modulating cellular immunity contribute to electroacupuncture's dual effect on bone cancer pain. *Integr. Cancer Ther.* 16: 1534735417728335. [Medline]
- Mavrommatis, C.I., Argyra, E., Vadalouka, A., and Vasilaikos, D.G. 2012. Acupuncture as an adjunctive therapy to pharmacological treatment in patients with chronic pain due to osteoarthritis of the knee: a 3-armed, randomized, placebo-controlled trial. *Pain* 153: 1720–1726. [Medline] [CrossRef]
- Mohan, G., Perilli, E., Parkinson, I.H., Humphries, J.M., Fazzalari, N.L., and Kuliwaba, J.S. 2013. Pre-emptive, early, and delayed alendronate treatment in a rat model of knee osteoarthritis: effect on subchondral trabecular bone microarchitecture and cartilage degradation of the tibia, bone/cartilage turnover, and joint discomfort. *Osteoarthritis Cartilage* 21: 1595–1604. [Medline] [CrossRef]
- Nwosu, L.N., Mapp, P.I., Chapman, V., and Walsh, D.A. 2016. Relationship between structural pathology and pain behaviour in a model of osteoarthritis (OA). *Osteoarthritis Cartilage* 24: 1910–1917. [Medline] [CrossRef]
- Okun, A., Liu, P., Davis, P., Ren, J., Remeniuk, B., Brion, T., Ossipov, M.H., Xie, J., Dussor, G.O., King, T., and Porreca, F. 2012. Afferent drive elicits ongoing pain in a model of advanced osteoarthritis. *Pain* 153: 924–933. [Medline] [CrossRef]
- Pitcher, T., Sousa-Valente, J., and Malcangio, M. 2016. The monoiodoacetate model of osteoarthritis pain in the mouse. *J. Vis. Exp.* 53746: 1–5. [Medline]
- Pritzker, K.P.H., Gay, S., Jimenez, S.A., Ostergaard, K., Pelletier, J.P., Revell, P.A., Salter, D., and van den Berg, W.B. 2006. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage* 14: 13–29. [Medline] [CrossRef]
- Qi, L., Tang, Y., You, Y., Qin, F., Zhai, L., Peng, H., and Nie,

- R. 2016. Comparing the effectiveness of electroacupuncture with different grades of knee osteoarthritis: a prospective study. *Cell. Physiol. Biochem.* 39: 2331–2340. [Medline] [CrossRef]
24. Sangdee, C., Teekachunhatean, S., Sananpanich, K., Sugandhavesa, N., Chiewchantanakit, S., Pojchamarnwiputh, S., and Jayasvasti, S. 2002. Electroacupuncture versus diclofenac in symptomatic treatment of osteoarthritis of the knee: a randomized controlled trial. *BMC Complement. Altern. Med.* 2: 3. [Medline] [CrossRef]
 25. Seo, B.K., Park, D.S., and Baek, Y.H. 2013. The analgesic effect of electroacupuncture on inflammatory pain in the rat model of collagenase-induced arthritis: mediation by opioidergic receptors. *Rheumatol. Int.* 33: 1177–1183. [Medline] [CrossRef]
 26. Shim, J.W., Jung, J.Y., and Kim, S.S. 2016. Effects of electroacupuncture for knee osteoarthritis: a systematic review and meta-analysis. *Evid. Based Complement. Alternat. Med.* 2016: 3485875. [Medline] [CrossRef]
 27. Suokas, A.K., Sagar, D.R., Mapp, P.I., Chapman, V., and Walsh, D.A. 2014. Design, study quality and evidence of analgesic efficacy in studies of drugs in models of OA pain: a systematic review and a meta-analysis. *Osteoarthritis Cartilage* 22: 1207–1223. [Medline] [CrossRef]
 28. Udo, M., Muneta, T., Tsuji, K., Ozeki, N., Nakagawa, Y., Ohara, T., Saito, R., Yanagisawa, K., Koga, H., and Sekiya, I. 2016. Monoiodoacetic acid induces arthritis and synovitis in rats in a dose- and time-dependent manner: proposed model-specific scoring systems. *Osteoarthritis Cartilage* 24: 1284–1291. [Medline] [CrossRef]
 29. Wallace, I.J., Worthington, S., Felson, D.T., Jurmain, R.D., Wren, K.T., Maijanen, H., Woods, R.J., and Lieberman, D.E. 2017. Knee osteoarthritis has doubled in prevalence since the mid-20th century. *Proc. Natl. Acad. Sci. USA* 114: 9332–9336. [Medline] [CrossRef]
 30. White, A. and Cummings, M. 2015. Acupuncture for knee osteoarthritis: study by Hinman et al represents missed opportunities. *Acupunct. Med.* 33: 84–86. [Medline] [CrossRef]
 31. Williams, J.M. and Brandt, K.D. 1984. Immobilization ameliorates chemically-induced articular cartilage damage. *Arthritis Rheum.* 27: 208–216. [Medline] [CrossRef]
 32. Yim, Y.K., Lee, H., Hong, K.E., Kim, Y.I., Lee, B.R., Son, C.G., and Kim, J.E. 2007. Electro-acupuncture at acupoint ST36 reduces inflammation and regulates immune activity in Collagen-Induced Arthritic Mice. *Evid. Based Complement. Alternat. Med.* 4: 51–57. [Medline] [CrossRef]
 33. Zhang, W., Nuki, G., Moskowitz, R.W., Abramson, S., Altman, R.D., Arden, N.K., Bierma-Zeinstra, S., Brandt, K.D., Croft, P., Doherty, M., Dougados, M., Hochberg, M., Hunter, D.J., Kwoh, K., Lohmander, L.S., and Tugwell, P. 2010. OARSI recommendations for the management of hip and knee osteoarthritis: part III: Changes in evidence following systematic cumulative update of research published through January 2009. *Osteoarthritis Cartilage* 18: 476–499. [Medline] [CrossRef]