

Electroacupuncture potentiates peripheral CB2 receptor-inhibited chronic pain in a mouse model of knee osteoarthritis

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Purpose: Knee osteoarthritis (KOA) is a highly prevalent, chronic joint disorder, with chronic pain as its typical symptom. Although studies have shown that an activated peripheral CB2 receptor can reduce acute pain, whether the CB2 receptor is involved in electroacupuncture (EA) inhibiting chronic pain and the involved mechanism remains unclear. The aim of this study was to investigate whether EA may strengthen peripheral CB2 receptor-inhibited chronic pain in a mouse model of KOA.

Materials and methods: KOA was induced by intra-articular injection of monosodium iodoacetate (MIA) into the left knee joint of mice. Thermal hyperalgesia was tested with the hot plate test, and mechanical allodynia was quantified using von Frey filaments. The expression of CB2 receptor and IL-1 β were quantified by using immunofluorescence labeling.

Results: EA treatment at 2 Hz+1 mA significantly increased the expression of CB2 receptor in fibroblasts and decreased the expression of IL-1 β in the menisci compared with that in the KOA group. However, EA had no effect on the expression of IL-1 β in CB2^{-/-} mice. At 2 Hz+1 mA, EA significantly increased mechanical threshold, thermal latency, and weight borne after KOA modeling. However, knockout of the CB2 receptor blocked these effects of EA. After 2 Hz+1 mA treatment, EA significantly reduced the Osteoarthritis Research Society International (OARSI) score after KOA modeling. However, EA had no significant effect on the OARSI score in CB2^{-/-} mice.

Conclusion: EA reduced the expression of IL-1 β by activating the CB2 receptor, thus inhibiting the chronic pain in the mouse model of KOA.

Keywords: cannabinoid, acupuncture, inflammatory pain, IL-1 β

Introduction

Chronic pain is often persistent and poorly treated by existing therapies in clinic, which is an important focus of research on the mechanism of pain and acupuncture analgesia.^{1,2} It has been proved that electroacupuncture (EA) is effective in relieving chronic pain in patients with knee osteoarthritis (KOA).³ However, the involved mechanisms remain unclear.

The endocannabinoid (EC) system consists of the following two main receptors: CB1 receptor, which is localized primarily on the central nervous system and peripheral neurons,^{4,5} and CB2 receptor, which is found principally in the immune system and to a lesser extent in the central nervous system.⁶⁻⁹ In our previous studies, we found that endogenous cannabinoids and peripheral CB2 receptor are involved in the antinociceptive effect of EA on acute inflammatory pain.¹⁰⁻¹⁴ Moreover, other studies

also found that activated peripheral CB2 receptor reduces inflammatory pain and neuropathic pain.^{15,16} KOA is a highly prevalent, chronic joint disorder, with chronic pain as its typical symptom.^{17,18} Furthermore, the overexpression of CB2 receptor is associated with a reduced pain phenotype in monosodium iodoacetate (MIA)-treated mice.¹⁹ Thus, we wondered whether EA may inhibit chronic pain via the CB2 receptor in a mouse model of KOA.

Oversecretion of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β , contributes to the severity and the progression of osteoarthritis.²⁰ High levels of pro-inflammatory cytokines in bones and joints induce pain, cartilage loss, and even joint dysfunction.^{20,21} Furthermore, we have shown that EA inhibited the release of inflammatory cytokines IL-1 β , IL-6, and TNF- α through the CB2 receptor in inflammatory skin tissues, thus attenuating acute inflammatory pain.¹¹ Therefore, reducing the release of pro-inflammatory cytokines is an effective therapy for KOA.

Therefore, in this study, we first determined the level of CB2 receptor and inflammatory cytokine IL-1 β . There was no significant difference in pain behavior, Osteoarthritis Research Society International (OARSI) scores, and body weight between the CB2 receptor knockout mice (CB2^{-/-} mice) and wild-type mice in different KOA models or was there any obvious difference between genotypes in general health status, behavior, or locomotor activity.^{19,22} So, we used the CB2^{-/-} mice to determine whether the CB2 receptor was involved in the effects of EA inhibiting the chronic pain in the model of KOA.

Materials and methods

Animals

All animal experiments were approved by the Animal Care and Use Committee at the Huazhong University of Science and Technology and conformed to the ethical guidelines of the International Association for the Study of Pain.²³ Eight-week-old female C57BL/6 mice were obtained from the Experimental Animal Center of Tongji Medical College of Huazhong University of Science and Technology. CB2 receptor knockout mice (CB2^{-/-} mice) maintained on a C57BL/6 congenic background were kindly provided by Dr Nancy E Buckley (Basic Neuroscience Program, NINDS, National Institutes of Health, Bethesda, MD, USA). Female CB2^{-/-} mice and their wild-type littermate (CB2^{+/+}) mice were generated as described previously.²⁴ The mice were individually housed in cages with a 12-hour light/dark cycle and had free access to food and water.

In our previous study, we have demonstrated that the optimized parameters of EA inhibiting chronic pain were the low frequency and high intensity (2 Hz+1 mA) and that the sham EA group had no significant effect on pain behavior of KOA mice.²⁵ Therefore, in this study, the following three groups of mice were used: control, KOA, and 2 Hz+1 mA EA.

Induction of KOA

KOA was induced by intra-articular injection of MIA (Sigma-Aldrich Co., St Louis, MO, USA) into the left knee joint after mice were briefly anesthetized with 10% chloral hydrate. The knee joint was shaved and flexed at a 90° angle. 5 μ L of 5 mg/mL MIA in sterile saline (0.9%) was injected through the infrapatellar ligament into the joint space of the left knee with a 30 G needle.¹⁹ This concentration of MIA causes histological changes in the cartilage²⁶ and induces joint pain²⁷ in mice. The control mice received an intra-articular injection of vehicle (5 μ L of sterile saline, 0.9%).

EA treatment

The animals were habituated to the restricting bag for 3 days before KOA induction, 30 minutes each day. In the EA treatment group, mice received EA administration on the left “Neixiyan” (Ex-LE4) and “Dubi” (ST35) once every other day for 4 weeks, starting from 2 days after MIA injection. EA (1 mA and 0.1 ms) was administered at the 2 Hz frequency for 30 minutes. The EA treatment and behavioral analysis are not carried out on the same day. The mice received EA administration in the even-numbered days after MIA injection, and behavioral analysis was done in the odd-numbered days after MIA injection. Current was delivered with a Han’s Acupoint Nerve Stimulator (LH202; Huawei Co., Ltd., Beijing, China).

Two acupuncture needles were inserted into two acupoints corresponding to Ex-LE4 and ST35 in humans. Ex-LE4 is located at the medial cavity of the patella and the patellar ligament, and ST35 lies on the lateral cavity of the patella and patellar ligament. Ex-LE4 and ST35 were chosen, because they are frequently used in KOA and are specific acupoints for treating knee problems.^{28,29}

Nociceptive behavioral tests

Mechanical allodynia and heat hyperalgesia were also demonstrated in the hind paw of animals with KOA, using von Frey filaments and the hot plate test, respectively.^{23,30} Animals were first allowed to acclimate to the testing apparatus for 30 minutes a day, 3 days before MIA injection. The baseline nociceptive thresholds were tested for 3 days before MIA

injection, and the mean value was calculated as baseline. The nociceptive thresholds were tested once every other day, starting from the first day after KOA induction for 4 weeks.

The hot plate test was used to measure the response latencies according to a previously described method.³¹ A glass cylinder (40 cm high, 20 cm diameter) was used to keep mice on the hot surface of the plate, which was maintained at a temperature of $55^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$. The time between placement of the mouse and the shaking or licking of paws or jumping was recorded as the index of response latency. A latency period (cutoff) of 30 seconds was defined as complete analgesia.³² The test was repeated three times in mice, and the mean value was calculated.

Mechanical allodynia was assessed by placing mice on an elevated mesh floor, and the tactile threshold was measured by using the “up-down” method.³³ After an acclimation period of 30 minutes, a series of calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA) were applied perpendicularly to the plantar surface of the left hind paw with sufficient force to bend the filament for 6 seconds. Brief withdrawal or paw flinching was considered as a positive response. The test was repeated two times in mice, and the mean value was calculated.

Weight-bearing assessment

The spontaneous joint pain behavior was assessed using a hind limb weight-bearing apparatus (Model-600M/R; IITC Life Science, Woodland Hills, CA, USA). Mice were allowed to acclimate to the testing apparatus for 30 minutes a day, 3 days before MIA injection. The amount of weight supported by each hind leg was measured automatically. The test was repeated five times in mice, and the mean value was calculated. Weight-bearing difference between the two legs was presented as the percentage of weight borne by the left leg and was determined using the following formula: % weight on the left leg = $\frac{\text{weight on the left leg}}{\text{weight on the left leg} + \text{weight on the right leg}} \times 100\%$.³⁴

Safranin O-Fast Green staining

Four weeks after the experimental induction of KOA, mice were deeply anesthetized with 10% chloral hydrate and intracardially perfused with 37°C normal saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4; 4°C). The left knee joints were subsequently removed, post-fixed in 4% paraformaldehyde for 24 hours, and then the knee joints were decalcified in 10% EDTA for 2 weeks on a plate shaker at room temperature (RT).³⁵ The decalcified knee joints were washed in 0.1 M phosphate buffer (pH 7.4) overnight. After dehydrating the tissues with increasing

concentrations of ethanol and xylene transparent, the joints were subsequently embedded in wax. Coronal $4\ \mu\text{m}$ sections were cut in a pathological slicer from the frontal plane toward the back of each joint and mounted on gelatinized slides.

All the serial sections were stained with the Safranin O-Fast Green staining protocol. Briefly, after hydrating sections with decreasing concentrations of ethanol, the sections were stained with 1% Safranin O and subsequently with 0.5% Fast Green solutions. Finally, the sections were dehydrated and cleared with increasing ethanol concentrations and xylene, and then mounted with rhamsan gum and a covering glass. All the stained sections were viewed at $10\times$ objective with an Olympus BX51 fluorescence microscope (Olympus Corporation, Tokyo, Japan). Images were captured using a Qimaging Micropublisher RTV 5.0 microscope camera and QCapture Pro 6.0 software (Qimaging, Surrey, BC, Canada). A total of five to six slides were randomly selected in each knee joint. All images of the obtained sections spanning the central load-bearing region of the knee were taken for both medial and lateral sides of each joint and used for histological scoring.

Histological evaluation of the severity of KOA was performed by an observer (XCY) blinded to genotype and pharmacological treatment according to the OARSI scoring system.³⁵ All the following four quadrants of the knee joint were evaluated: medial tibial plateau (MTP), medial femoral condyle (MFC), lateral tibial plateau (LTP), and lateral femoral condyle (LFC). The scores were expressed as the summed histological score. The summed score represents the additive scores for each quadrant of the joint on each section through the joint of each animal. Then, the average summed score for each experimental group was calculated.

A randomly selected sample of 12 joints was assessed by a second observer (FG), who was also blinded to genotype and treatment allocation to test reproducibility of the scoring system.

Immunofluorescence labeling

The knee joints were decalcified as described previously.³⁵ The decalcified knee joints were cryoprotected in 30% sucrose in 0.1 M phosphate buffer for 24 hours at 4°C . The sections were cut at $20\ \mu\text{m}$ on a cryostat, which were mounted onto gelatin-coated slides and dried overnight.

The sections were rinsed in 0.01 M PBS, blocked for 1 hour with 5% donkey serum and 0.2% Tween-20 in PBS and then incubated with the following primary antibodies at 37°C for 1 hour and at 4°C overnight: rabbit anti-CB2 (1:100; Abcam, Hong Kong, China); mouse anti-S100A4 (1:100; Abcam); and rabbit anti-IL-1 β (1:50; Santa Cruz

Biotechnology Inc., Dallas, TX, USA). Subsequently, the sections were washed four times in PBS for 5 minutes and incubated with the corresponding secondary antibodies from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA): donkey anti-rabbit IgG conjugated with Dylight 488 (1:400) and donkey anti-mouse IgG conjugated with Dylight 594 (1:400). The sections were washed four times in PBS for 5 minutes and then incubated with DAPI (1:1,000; Abcam). The sections were washed four times in PBS for 5 minutes and then coverslipped. Negative controls were included by omitting the primary antibodies and with primary antibodies preabsorbed with their specific blocking peptides in the abovementioned procedures, which resulted in no positive labeling in the knee joint tissues. For quantification, a total of 5–6 sections from the knee joint were randomly selected for each mouse. Digital images were acquired using an Olympus BX51 fluorescence microscope (Olympus Corporation). Images were captured using a Qimaging Micropublisher RTV 5.0 microscope camera and QCapture Pro 6.0 software (Qimaging). All images for each experiment were taken at the same time with the same camera settings, and the authors performing the image analysis were blinded to the group. The number of CB2 with DAPI or S100A4-positive cells and the area of IL-1 β in the menisci were measured by using Image J software.

Statistical analyses

Using pilot data, we estimated the sample size based on at least 80% power and $\alpha=0.05$. Data are presented as mean \pm SD. To determine the statistical difference in the withdrawal thresholds between different groups and time points, we used two-way repeated ANOVA followed by Bonferroni's post hoc test. One-way ANOVA and Newman–Keuls post hoc test were used in biochemical data. A *P*-value of less than 0.05 was considered statistically significant.

Results

EA reverses the reduction of CB2 receptor and the increase in IL-1 β expression in the menisci of KOA mice

The CB2 receptor-positive cells were present in the menisci (Figure 1A). The number of CB2 receptor-positive cells in the menisci was significantly lower in the KOA group than the control group (Figure 1B). EA treatment at 2 Hz+1 mA significantly increased the number of CB2-positive cells in the menisci compared with that in the KOA group (Figure 1B).

The area of IL-1 β -positive cells in the menisci was significantly increased in the KOA group than the control group (Figure 1C). EA treatment at 2 Hz+1 mA significantly decreased the area of IL-1 β -positive cells in the menisci compared with that in the KOA group (Figure 1C).

EA reverses the reduction of CB2 receptor expression in fibroblasts of KOA mice

Previous studies have proved that the expression of CB2 was demonstrated in synovial fibroblasts (SF).^{36,37} To dissect the role of CB2 receptor in fibroblasts, we investigated the co-localization between CB2 and the fibroblast marker S100A4 immunoreactivities.

The CB2 and S100A4-positive cells were present in the menisci (Figure 2A). Compared with the control group, MIA significantly decreased the percentage of double-stained CB2 and S100A4 in the menisci (Figure 2B). EA treatment significantly increased the percentage of double-stained CB2 and S100A4 in the menisci (Figure 2B). In addition, the number of S100A4-positive cells in the menisci did not differ significantly among different groups (Figure 2C).

CB2 receptor is involved in the EA effects on pain hypersensitivity in KOA mice

In the wild-type mice, KOA induction significantly reduced mechanical withdrawal threshold and thermal withdrawal latency (Figure 3A and C). At 2 Hz+1 mA, EA significantly increased tactile threshold and thermal latency after KOA modeling (Figure 3A and C). In CB2^{-/-} mice, KOA modeling still decreased tactile threshold and thermal latency. However, EA had no significant effects on mechanical threshold and thermal latency after KOA (Figure 3B and D).

Before MIA was injected into the knee joint cavity, there was no significant difference between right and left hind limb weight bearing in the groups (Figure 3E and F). In the wild-type mice, KOA induction significantly reduced the percentage of weight borne (Figure 3E). At 2 Hz+1 mA, EA significantly increased the percentage of weight borne in KOA mice (Figure 3E). In CB2^{-/-} mice, KOA modeling also decreased the percentage of weight borne. However, EA had no significant effect on the percentage of weight borne after KOA (Figure 3F). It suggested that the CB2 receptor participates in EA-inhibited chronic pain in KOA mice.

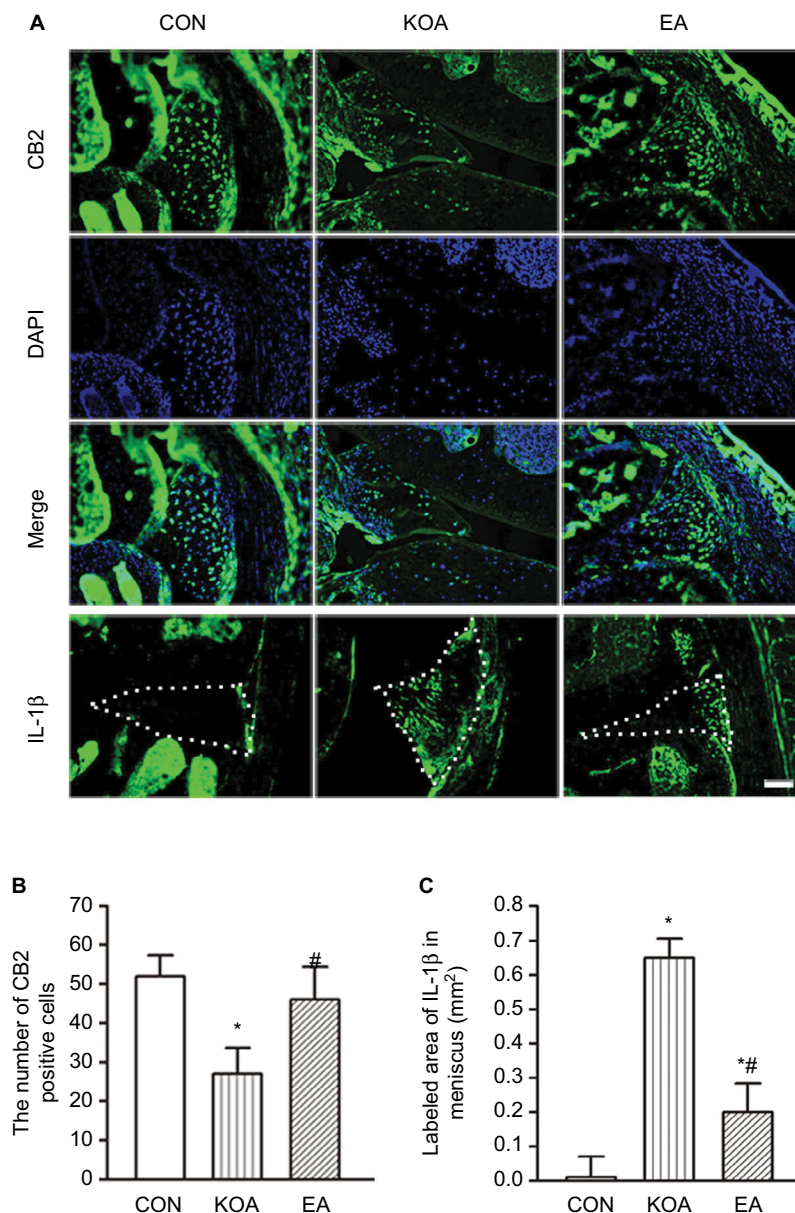


Figure 1 Quantitative analysis of the expression of CB2 receptor and IL-1 β in the menisci.

Notes: (A) Representative images showing CB2-immunoreactive cells, DAPI, and IL-1 β in the menisci of CON, KOA, and EA groups. Scale bar, 100 μ m. Dotted line marks the menisci. (B) Summary data show the number of CB2-immunoreactive cells in different groups. (C) Summary data show the area of IL-1 β in different groups. Data are expressed as mean \pm SD (n=6 mice in each group). * P <0.05, compared with the control group; # P <0.05, compared with the KOA group.

Abbreviations: CON, control; EA, electroacupuncture; KOA, knee osteoarthritis.

CB2 receptor is involved in the improving effect of EA on histopathological alterations in KOA mice

The OARSI scoring system is used for evaluating cartilage alterations. The primary slides were stained for cartilage proteoglycan using a Safranin O–Fast Green technique, which can be utilized with the scoring paradigm.^{19,35} Reproducibility of the OARSI scoring system used to assess severity of KOA was independently assessed by two observers (XCY

and FG) in 12 out of 36 samples (33.3%). This showed an inter-observer kappa co-efficient of 0.786 (P <0.001), which is considered substantial.³⁸

Representative images from serial histological sections of the wild-type and CB2^{-/-} mice stained with Safranin O–Fast Green are presented in Figure 4A. There was no significant difference in OARSI scores for the saline injection joints between the wild-type and CB2^{-/-} mice (Figure S1). The OARSI score for cartilage damage indicated that the saline injection did not

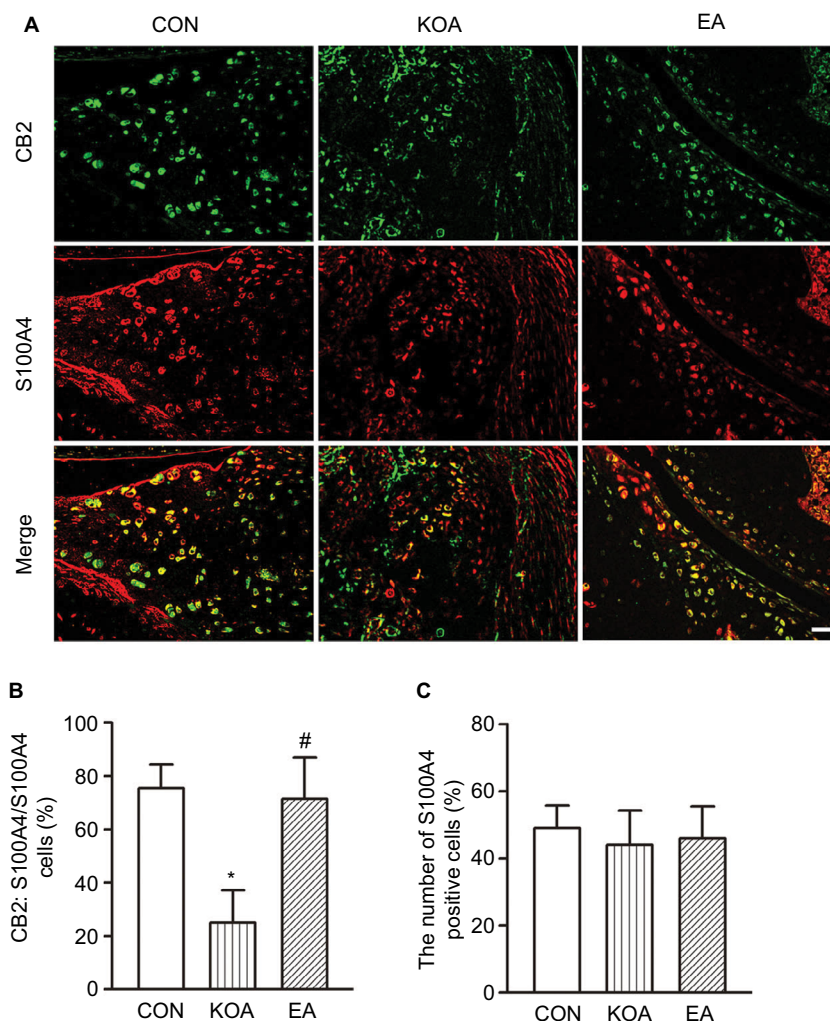


Figure 2 Double immunolabeling of CB2 and the fibroblast marker S100A4 in the menisci.

Notes: (A) CB2 labeling (green); S100A4 labeling (red); and double-labeled cells (yellow). Scale bar, 20 μ m. (B) Summary data show the percentage of double-stained cells % in the number of total S100A4-immunoreactive cells. (C) Summary data show the number of S100A4-immunoreactive cells in different groups. Data are expressed as mean \pm SD (n=6 mice in each group). *P<0.05, compared with the control group; #P<0.05, compared with the KOA group.

Abbreviations: CON, control; EA, electroacupuncture; KOA, knee osteoarthritis.

induce histological alterations in the knee joint of the wild-type and CB2^{-/-} mice (Figure 4B and C). In contrast, the intra-articular injection of MIA induced significant increase in the OARSI score in the knee joint of the wild-type and CB2^{-/-} mice (Figure 4B and C). Therefore, after intra-articular injection of MIA, the CB2^{-/-} mice developed similar histological alterations to the wild-type mice in the knee joint.

After 2 Hz+1 mA treatment, EA significantly reduced the OARSI score after KOA modeling in wild-type mice (Figure 4B). However, EA had no significant effect on the OARSI score after KOA modeling in CB2^{-/-} mice (Figure 4C). It suggested that the CB2 receptor mediated the EA improvement of the histopathological alterations.

CB2 receptor is involved in the EA effect on reduction of the level of IL-1 β expression in the menisci of KOA mice

In the wild-type mice, the area of IL-1 β -positive cells in the menisci was significantly increased than that in the control group 4 weeks after KOA induction (Figure 5A and B). At 2 Hz+1 mA, EA significantly reduced the area of IL-1 β -positive cells after KOA modeling (Figure 5A and B). In CB2^{-/-} mice, KOA modeling increased the area of IL-1 β -positive cells. However, EA had no significant effect on the area of IL-1 β -positive cells after KOA modeling (Figure 5A and C).

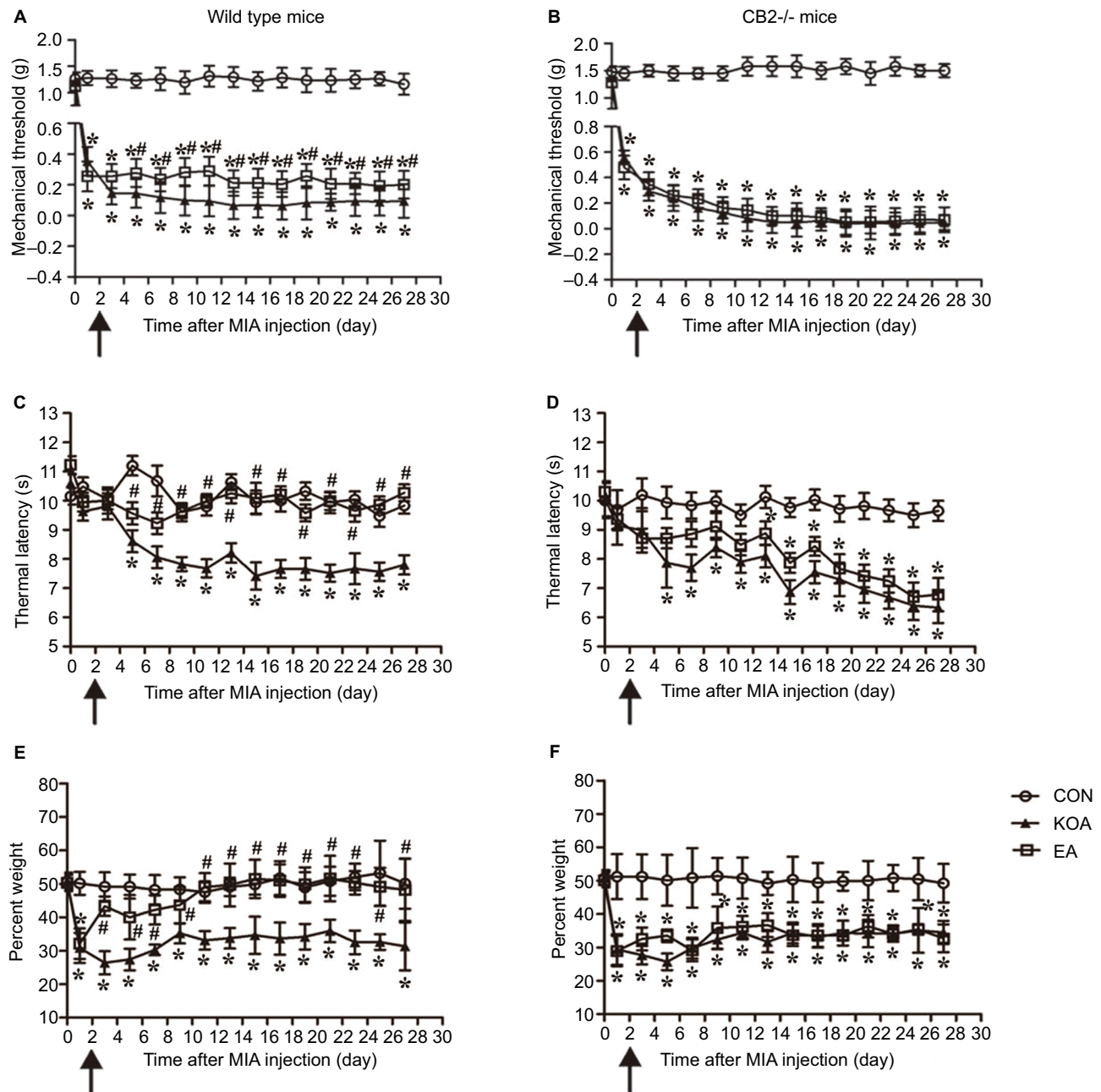


Figure 3 Comparison of the effects of EA on pain hypersensitivity in wild-type mice and $CB2^{-/-}$ mice subjected to KOA induction.

Notes: Time course of changes in the tactile threshold in the wild-type mice (**A**) and $CB2^{-/-}$ mice (**B**) after KOA induction. Time course of changes in the thermal withdrawal threshold in the wild-type mice (**C**) and $CB2^{-/-}$ mice (**D**) after KOA induction. Effects of KOA and EA on weight bearing of wild-type mice (**E**) and $CB2^{-/-}$ mice (**F**) subjected to KOA induction. EA was administered for 30 minutes, once every other day for 4 weeks, starting from 2 days after MIA injection, as indicated by arrows. Data are expressed as mean \pm SD (n=8 mice in each group). * P <0.05, compared with the control group; # P <0.05, compared with the KOA group.

Abbreviations: CON, control; EA, electroacupuncture; KOA, knee osteoarthritis; MIA, monosodium iodoacetate.

Discussion

In this study, we demonstrated that EA treatment at 2 Hz+1 mA significantly increased the expression of CB2 receptor in fibroblast and decreased the level of IL-1 β in the knee meniscus of KOA mice. Moreover, we also found that

knockout of CB2 receptor can block the analgesic effect of EA and that EA had no effect on the expression of IL-1 β in $CB2^{-/-}$ mice. Our studies provided the evidence that EA can reduce the expression of IL-1 β through activating the CB2 receptor, thus inhibiting the chronic pain in the model of KOA.

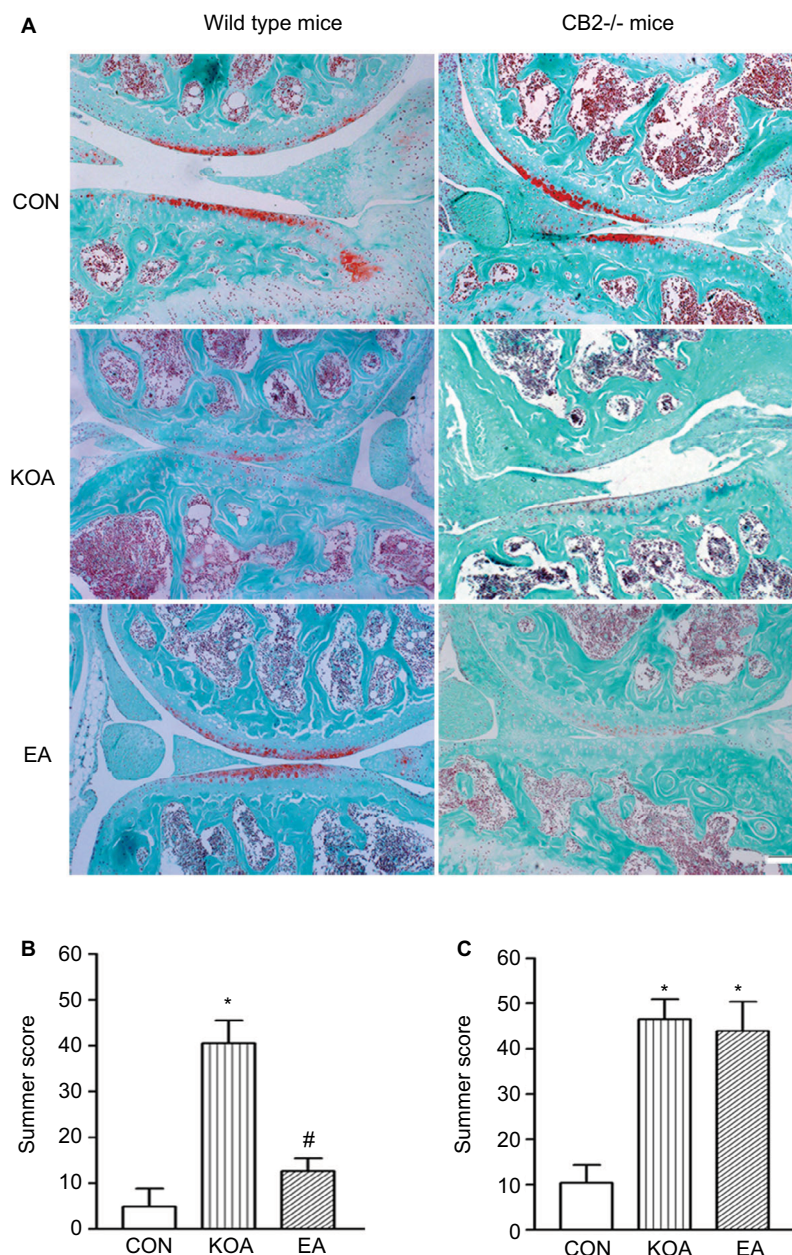


Figure 4 Comparison of the effects of EA on histological changes in wild-type mice and CB2^{-/-} mice subjected to KOA induction.

Notes: (A) Representative histological knee joint sections (medial side) stained with Safranin O–Fast Green of wild-type mice and CB2^{-/-} mice. Summary data show the effect of EA on the OARSI score of wild-type mice (B) and CB2^{-/-} mice (C). Data are expressed as mean±SD (n=6 mice in each group). Scale bar: 100 μm. *P<0.05, compared with the control group; #P<0.05, compared with the KOA group.

Abbreviations: CON, control; EA, electroacupuncture; KOA, knee osteoarthritis; OARSI, Osteoarthritis Research Society International.

Previous studies have demonstrated the antinociceptive effects of CB2 agonists in different models of chronic pain and arthritis.^{39–42} We found that at 2 Hz+1 mA, EA significantly increased tactile threshold, thermal latency, and the percentage of weight borne after KOA modeling. However, EA had no significant effects on mechanical threshold, thermal latency, and the percentage of weight borne after KOA modeling in CB2^{-/-} mice. These data suggest that the CB2 receptor participates in EA-inhibited chronic pain in KOA mice.

In addition, previous studies showed that the activated peripheral CB2 receptor can reduce inflammatory pain and neuropathic pain.^{15,16} In this study, we found that EA treatment at 2 Hz+1 mA significantly increased the expression of CB2 receptor in the knee meniscus of KOA mice.

Although the CB2 receptor is mainly distributed in immune cells, evidence exists that the CB2 receptor is expressed in fibroblasts, chondrocytes, and synovial cells.^{43–45} Consistently, our data illustrate that the CB2 receptor

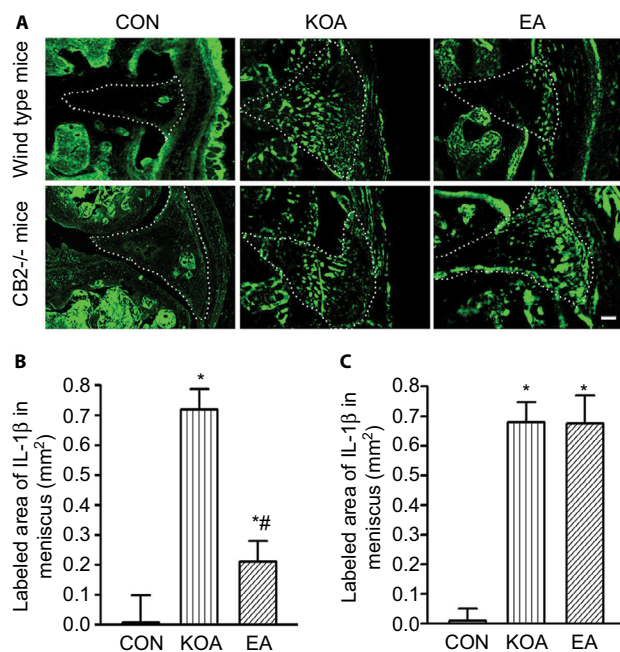


Figure 5 Quantitative analysis of the level of IL-1 β in wild-type mice and CB2^{-/-} mice subjected to KOA induction.

Notes: (A) Representative images showing IL-1 β of the wild-type mice and CB2^{-/-} mice after KOA induction. Scale bar: 100 μ m. Dotted line marks the menisci. Summary data show the area of IL-1 β in the menisci of the wild-type mice (B) and CB2^{-/-} mice (C). Data are expressed as mean \pm SD (n=6 mice in each group). *P<0.05, compared with the control group; #P<0.05, compared with the KOA group.

Abbreviations: CON, control; EA, electroacupuncture; KOA, knee osteoarthritis.

coexisted with fibroblasts in menisci and EA significantly increased the expression of CB2 receptor in fibroblasts. Oversecretion of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β , contributes to the severity and the progression of osteoarthritis.²⁰ In addition, in vitro studies have shown that cannabinoids reduce cytokine production from rheumatoid arthritis fibroblasts.⁴⁶ We found that EA significantly decreased the level of IL-1 β in the knee meniscus of KOA mice. However, EA had no effect on the level of IL-1 β in the knee meniscus after CB2 receptor knockout. These data suggest that EA activated CB2 receptor in fibroblasts and then inhibited the level of IL-1 β in the knee meniscus.

Previous studies showed that intra-articular injection of the metabolic inhibitor MIA into the knee joint caused histological changes in the cartilage.^{26,47} Indeed, in our experimental conditions, the MIA dose used produced a clear loss of proteoglycan and chondrocyte degeneration in the cartilage. We also found that EA significantly improved the cartilage degradation. However, EA showed no significant effect on histological changes in the cartilage after CB2 receptor knockout. Furthermore, cannabinoid receptor agonists have been found to protect against collagen-induced arthritis⁴⁸ and to inhibit IL-1-induced

cartilage degradation in vitro.⁴⁹ Thus, these findings suggest that EA improved the cartilage degradation by activating the CB2 receptor and inhibiting the level of IL-1 β .

Conclusion

EA inhibited the expression of IL-1 β through activating the CB2 receptor, thus inhibiting chronic pain in the model of KOA. Our findings provide novel information about the mechanisms through which EA and CB2 receptor activation reduce chronic pain in the KOA mouse model.

Acknowledgments

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Author contributions

ML and QW conceived and designed the experiments. X-CY did most of the experiments and analyzed the data; C-HW, WS, and H-PL helped with the behavior test experiments. HZ and H-CX helped with double-immunofluorescence labeling experiments. L-XL and FG analyzed the data. CFH and JC helped to feed the animals. X-CY, J-JL and ML wrote the manuscript. All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

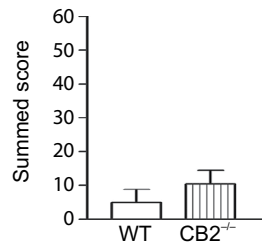


Figure S1 Comparison of the OARSI score in WT mice and CB2^{-/-} mice after saline injection.

Notes: Summary data show the OARSI score of WT mice and CB2^{-/-} mice after saline injection. Data are expressed as mean±SD (n=6 mice in each group).

Abbreviations: OARSI, Osteoarthritis Research Society International; WT, wild-type.

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