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REVIEW

The Analgesic Effect of NLRP3 Inflammasome in the Relief of Inflammatory Pain by Electroacupuncture: A Systematic Review and Meta-Analysis of Animal Studies

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Introduction: Inflammatory pain originates from the inflammatory response triggered by tissue injury, accompanied by the release of pro-inflammatory mediators. NLRP3 inflammasome, as a key innate immune receptor, plays a central role in inflammatory pain. Electroacupuncture (EA) is a commonly used analgesic method in clinical practice. Its anti-inflammatory mechanism involves regulating neural-immune interaction, regulating the balance of pro-inflammatory/anti-inflammatory cytokines, and inhibiting the NF-κB pathway. However, whether EA can alleviate inflammatory pain by specifically inhibiting NLRP3 inflammasome activation and its downstream signaling pathways is still unclear.

Purpose: This study aims to evaluate the analgesic effects of electroacupuncture on inflammatory pain, focusing on inflammatory cytokines and NLRP3-related signaling pathways to elucidate the underlying mechanisms.

Methods: Studies that meet the inclusion and exclusion criteria were selected by searching PubMed, EMBASE, Cochrane, and Web of Science databases. To evaluate the quality of each included study using the modified 10-item checklist from the Collaboration for the Advancement of Meta-analysis in Animal Research. After extracting the relevant data, perform a meta-analysis using RevMan software.

Results: A total of 10 studies were included. The results of the meta-analysis indicated that electroacupuncture treatment increased the heat pain threshold [−2.74 (95% CI −3.50 ~ −1.98)] and mechanical pain threshold [−2.89 (95% CI −3.59 ~ −2.20)]. Electroacupuncture reduced the levels of pro-inflammatory cytokines IL-1β, IL-18, IL-6, IL-12, IL-17, TNF-α, and PGE2, and increased the levels of anti-inflammatory cytokines IL-10. This study found that EA mainly regulated NLRP3 related signaling pathway through ROS, P2X7R and other pathways, thereby reducing the expression of NLRP3 inflammasome. Subgroup analysis of CFA-induced inflammatory pain showed that EA reduced inflammatory pain by regulating NLRP3 inflammasome.

Conclusion: Electroacupuncture may alleviate inflammatory pain by modulating inflammatory cytokines and regulating the NLRP3 signaling pathway, which could help reduce the use of analgesic medications.

Keywords: animal model, electroacupuncture, inflammatory pain, meta-analysis, NLRP3

Introduction

Inflammatory pain arises from inflammatory responses, encompassing acute and chronic processes, with its core mechanism involving tissue injury or stimulation triggering the release of pro-inflammatory mediators that activate

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and sensitize nociceptors. The chronic inflammatory pain discussed herein refers to pain lasting over six weeks, closely linked to persistent inflammation.[1] During the inflammatory response, when cells or tissues are damaged, the immune system responds rapidly. Immune cells such as tissue-resident macrophages, neutrophils, and mast cells release numerous pro-inflammatory mediators, including prostaglandins, cytokines, chemokines, proteases, neuropeptides, and growth factors. These mediators act on the peripheral nerve endings of nociceptive neurons, leading to pain sensitization and a strong pain response in the body.[1–3] In turn, these pro-inflammatory mediators trigger the recruitment and activation of macrophages, forming an inflammation response centered around macrophages, which participates in the complex tissue repair process.[4] In cases of immune system disorders, the NOD-like receptor protein 3 (NLRP3) is believed to be closely related to inflammatory pain. NLRP3 is a cytoplasmic immune factor that responds to cellular stress signals and is typically activated during infection or inflammation. Activated under cellular stress, NLRP3 promotes interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α) production via NF-κB signaling, driving pain responses and apoptosis.[5–7]

Electroacupuncture (EA) is a commonly used analgesic method in clinical practice, and its anti-inflammatory mechanism involves multiple levels of regulation. Specifically, EA activates the vagus nerve-adrenal axis to trigger the cholinergic anti-inflammatory pathway, curbing excessive immune activation.[8,9] It modulates cytokine balance by reducing pro-inflammatory factors (IL-1β, TNF-α, IL-6) and upregulating anti-inflammatory factors (IL-10, TGF-β).[10,11] At the molecular level, EA inhibits NF-κB activation by preventing IκBα degradation and nuclear translocation, reducing downstream inflammatory gene expression.[12] Notably, recent studies show EA suppresses NLRP3 inflammasome assembly via AMPK/SIRT1 and Nrf2/HO-1 pathways, reducing IL-1β/IL-18 maturation.[10] These findings provide a new molecular mechanism explanation for the treatment of inflammatory pain with EA.

Currently, the treatment for inflammatory pain primarily involves analgesics such as Non-steroidal Anti-inflammatory Drugs (NSAIDs) and opioids. However, the continuous administration of analgesics can lead to reduced efficacy, and opioids may cause a range of side effects, including constipation, rash, and even addiction.[13–15] Therefore, there is an urgent need for alternative therapeutic methods to alleviate pain and improve patients' quality of life. Numerous studies have found that electroacupuncture plays a significant role in pain relief.[16] Preoperative electroacupuncture can alleviate anxiety, optimize preoperative conditions, and reduce the amount of anesthetic required.[11] The combination of electroacupuncture with conventional analgesics for postoperative pain can reduce the dosage of analgesics needed and minimize side effects, making it an effective pain management strategy.[17,18] In recent years, Sun et al[19] summarized that acupuncture can regulate hypersensitivity and neurotransmitters, reducing the levels of inflammatory factors involved in pain treatment. Additionally, Yuan et al[6] explored the relationship between electroacupuncture and the NLRP3 inflammasome in the treatment of various diseases, identifying multiple pathways through which electroacupuncture can inhibit the NLRP3 inflammasome to treat inflammation-related disorders in different systems. However, the specific analgesic mechanisms and associated signaling pathways remain unclear.

Therefore, this paper aims to investigate the signaling pathways involved in the analgesic effects of electroacupuncture, analyzing the mechanisms of pain relief from the perspective of inflammatory pain. In this review, we conducted a meta-analysis of inflammatory cytokines and NLRP3-related signaling pathways in various animal models of inflammatory pain to determine the impact of electroacupuncture on inflammatory pain and its analgesic mechanisms.

Material and Methods

Searching

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[20] The review has been registered on the PROSPERO database (PROSPERO ID =CRD42024609197). This study included reports written in English regarding the analgesic mechanisms of electroacupuncture (EA) in rodent models of inflammatory pain. We searched PubMed, EMBASE, Cochrane, and Web of Science for randomized controlled animal studies published from January 1, 2000, to January 2025 that investigated the effects of transcutaneous acupoint electrical stimulation/electroacupuncture on inflammatory pain. The search terms included EA-related terms (eg, "transcutaneous electrical acupoint stimulation", "TEAS", or "electroacupuncture") and inflammatory pain-related terms (eg, "inflammatory pain")

Table 1 PubMed Search Terms

Number	Search Terms
#1	"Transcutaneous acupoint electrical stimulation"
#2	"Transcutaneous electrical acupoint stimulation"
#3	"electroacupuncture*"
#4	"teas"
#5	#1 OR #2 OR #3 OR #4
#6	"Mouse (mice)"
#7	"Rat (rats)"
#8	#6 OR #7
#9	"Inflammatory pain"
#10	#5 AND #8 AND #9

(Table 1). Based on the research subjects"rodents" and the intervention "electroacupuncture", we utilized a combination of subject headings and free-text terms for our searches, supplemented by manual searches and reference tracking of included literature, relevant meta-analyses, and reviews. The search strategies and terms applied to the PubMed database serve as an example, with the search methods for other databases detailed in Table S1.

Inclusion/Exclusion Criterions

The inclusion criteria for the study were as follows: (1) Study type: randomized controlled trials (RCTs), with a language restriction to English. (2) Subjects: rodent models of inflammatory pain. (3) Intervention: acupuncture as the primary intervention, limited to transcutaneous acupoint electrical stimulation and electroacupuncture. (4) Primary outcome measure: pain threshold levels to assess the efficacy of acupuncture. (5) Secondary outcome measures: levels of inflammatory cytokines and molecular expression related to the NLRP3 signaling pathway. The exclusion criteria were: (1) Article types: reviews, case reports, crossover studies, letters, editorials, review articles, meta-analyses, and retrospective studies; (2) Studies that did not allow for data extraction or lacked sufficient data; (3) Studies that, after full-text screening, did not meet the methodological and outcome standards were also excluded.

Data Extraction

Two researchers (Su and Wang) independently screened the literature and extracted data based on the inclusion and exclusion criteria, with discrepancies resolved through group discussion. The data extraction included: (1) Basic information about the included studies, such as study title, first author, journal of publication, and date; (2) Baseline characteristics of the subjects, including the type of rodent inflammatory pain model, disease condition, sample size, type of acupuncture, and type of specimens; (3) Outcome measures of interest, which included the mean levels of inflammatory cytokines and related inflammatory indicators for each group, along with the standard deviation or standard error of the mean.

Risk of Bias Assessment

Two independent reviewers (Su and Wang) used the risk of bias assessment tool of Systematic Review Center for Laboratory animal Experimentation (SYRCLE)[21] to assess the risk of bias. Raters recorded the selection, performance, detection, attrition, and reporting biases as "Yes", "No", or "Unclear." The results were discussed after the scoring was completed to standardize the application of the tool. Consensus was reached on item 10 "other". In case of disagreement or inconsistency, the third reviewer (Zhang) joined the discussion and resolved.

Quality Assessment

The methodological quality of each included study was assessed by two authors (Su and Wang) using a modified 10-item checklist from the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES):[19,22] publication in a peer-reviewed journal, a statement describing temperature control, random allocation to treatment or control groups, blinding in model establishment, exclusion of hypertensive or diabetic animals, blinded outcome assessment, use of anesthetics without obvious inherent characteristics, sample size calculation, adherence to animal welfare regulations, and declaration of any potential conflicts of interest. The quality scores for each article were recorded, with a maximum possible score of 10. The studies were categorized as high risk (0–3), medium risk (4–6), or low risk (7–10).[23]

Statistical Analysis

All statistical analyses were performed using RevMan version 5.4 (Foundation for Statistical Computing, Vienna, Austria). In this study, the cytokine levels, relevant signaling molecule levels, and pain threshold levels were considered continuous data for the disease group and the EA group. Differences between the disease and EA groups were calculated by comparing studies that used units with different cytokine levels. A fixed-effect model was used to estimate the standardized mean difference (SMD) for the 10 studies that treated the disease pain model as a variable and analyzed its correlation with the outcomes. This study conducted a meta-analysis on pain thresholds, each cytokine subgroup, and NLRP3 upstream and downstream indicators. A p-value of <0.05 was considered statistically significant for the 95% confidence interval (CI). The assessment of study heterogeneity was conducted using Chi-square statistics and I^2 statistics.

Results

Study Inclusion

According to our search strategy, a total of 527 articles were extracted from the aforementioned databases, with an additional 63 articles identified through supplementary searches. We initially excluded 231 duplicate articles, followed by the exclusion of 150 irrelevant articles based on title and abstract. The remaining 209 studies underwent full-text screening, of which 33 were not written in English. Among the 176 studies remaining, 156 were excluded because their focus did not relate to the analgesic mechanisms of the NLRP3 inflammasome, and 10 were excluded due to the absence of clear pain assessment indicators. Ultimately, 10 articles were included in the study (Figure 1).

Risk of Bias Assessment

The results of the risk of bias assessment based on the SYRCLE tool are shown in Figure 2. Of note, none of the studies were properly blinded to the EA intervention received by each animal during the experimental period. Only one study[24] showed that outcome assessors were blinded when conducting the analysis of experimental results. Importantly, none of the studies had selective outcome reporting and no other clear issues that could have contributed to a high risk of bias emerged.

Quality Assessment

The quality assessment of the included studies is summarized in Table 2. The quality scores of the included studies ranged from 5 to 8 points (out of a maximum of 10): five studies received a score of 8, four studies scored 7, and one scored 5. None of the ten studies used hypertensive or diabetic animals, or violated animal welfare regulations. All ten studies employed anesthetics without inherent properties and were peer-reviewed. Eight studies included statements regarding temperature control. Eight studies randomized subjects into treatment or control groups, and all studies conducted sample size calculations. Eight studies declared no potential conflicts of interest related to the research. None of the ten studies employed blinding in model establishment, and none of them conducted blinded outcome assessments. Most of the scores were 7 or 8, indicating a relatively low risk, but the blinding element was overlooked.

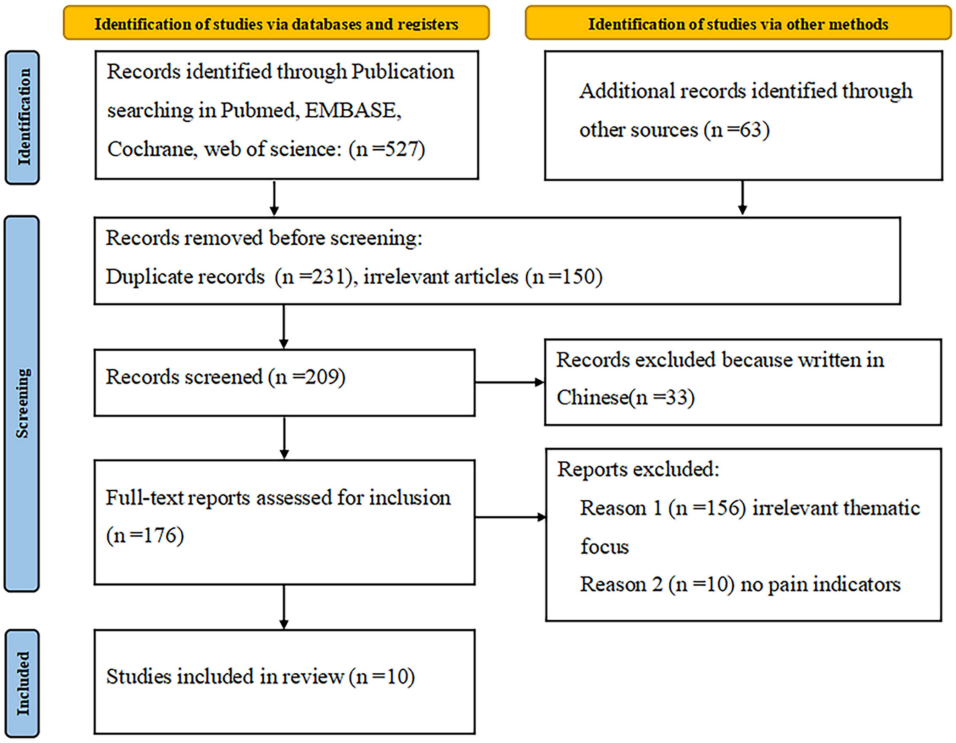

Figure 1 Flow diagram of PRISMA (2020) for included studies. Adapted from Page MJ, Moher D, Bossuyt PM et al PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. *BMJ*. 2021: 372:n160. Creative Commons.[20]

Study Characteristics

The characteristics of the included studies are summarized in Table 3. All ten studies involved rats, mice, or guinea pigs. Four studies used complete Freund's adjuvant (CFA) -induced inflammatory pain models. In addition, one study used a colitis model, three studies used arthritis models such as gouty-arthritis and knee osteoarthritis (KOA), one study used a chronic postischemic pain (CPIP) model, and one study used a migraine model induced by inflammatory decoction. Electroacupuncture was used in all the 10 studies. Samples were collected to detect the levels of cytokines and NLRP3 related indicators, including serum and inflammatory site tissue.

Overall Efficacy of Acupuncture Analgesia

The types of inflammatory pain included were four CFA-induced inflammatory pain studies, one chronic pelvic pain syndrome study, one study using a colitis model, three inflammatory arthritis studies (one gouty-arthritis, two knee osteoarthritis), and two neuroinflammatory studies (one chronic postischemic pain and one migraine). The evaluation indicators for analgesic efficacy included two types: (1) Thermal stimulation pain threshold indicator: paw withdrawal latency (PWL) (Figure 3a); (2) Mechanical stimulation pain threshold indicator: paw withdrawal threshold (PWT) and paw withdrawal frequency (PWF) (Figure 3b).

Thermal Nociception: Overall Effect

A total of 76 animals (disease group n=38, EA group n=38) from eight studies were included in the pain sensitivity analysis. The final measurements of PWL from each experiment were aggregated. The standardized mean difference (SMD) between the disease group and the EA group for PWL was calculated as a measure of effect size. Electroacupuncture treatment resulted in an increased thermal stimulation pain threshold (SMD −2.74, 95% confidence interval [CI] −3.50~-1.98, P = 0.0001, heterogeneity X^2=22.80, I^2=82%, Figure 3a).

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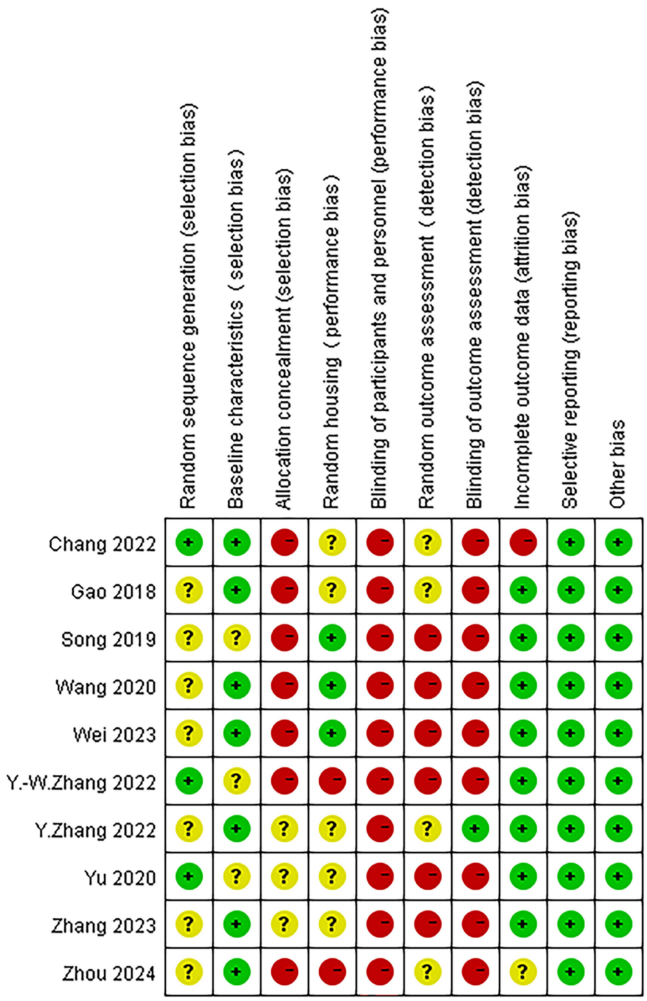


Figure 2 Risk of bias assessment using the SYRCLE tool.

Mechanical Nociception: Overall Effect

A total of 100 animals were included across ten studies (disease group n=50, EA group n=50). The overall effect size for the mechanical withdrawal threshold (PWT) was SMD −2.89 (95% CI −3.59 ~ −2.20; P < 0.0001, heterogeneity

Table 2 Quality Evaluation Form

	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Total
Chang et al, 2022[25]	√	√			√	√			√		5
Gao et al, 2018[26]	√	√			√	√		√	√	√	7
Song et al,2019[27]	√	√			√	√	√	√	√	√	8
Wang et al, 2020[28]	√	√			√	√	√	√	√	√	8
Wei et al, 2023[29]	√				√	√	√	√	√	√	7
Yu et al, 2020[3]	√	√			√	√	√	√	√	√	8
Y. Zhang et al, 2022[24]	√				√	√	√	√	√	√	7
Y.-W. Zhang et al, 2022[30]	√	√			√	√	√	√	√		7
Zhang et al, 2023[31]	√	√			√	√	√	√	√	√	8
Zhou et al, 2024[32]	√	√			√	√	√	√	√	√	8

Notes: Q1, sample size calculation; Q2, random allocation to treatment or control; Q3, blinded building of model; Q4, blinded assessment of outcome; Q5, use of animals without hypertension or diabetes; Q6, use of anesthetic without marked intrinsic properties; Q7, statements describing control of temperature; Q8, compliance with animal welfare regulations; Q9, publication in a peer-reviewed journal; Q10, declared any potential conflict of interest.

Table 3 Fundamental Characteristics and Electroacupuncture Intervention

	Animal Species	Age (Weight)	Disease Model	Pain Assessment	Specimen	Cytokines	Indicator Protein	Intervention	Acupuncture point	Frequency and Intensity	Intervention Time	The Control Group
Chang,2022[25]	SD rats	3 months (200 ± 20 g)	CPPS (CFA)	PWL	Prostate	IL-1β, PGE2, TNF-α	P2X7R, NLRP3, caspase-1, IL-18	EA	CV4, CV3, BL35, SP6	2/100 Hz	/	No needling
Gao,2018[26]	1. SD rats 2. (CB2-KO) C57BL/6 mice	1. Adult (180–200 g) (/)	IP(CFA)	PWL, PWT	Skin	IL-1β	ASC, caspase-1	EA	GB30, GB34	2Hz; 1mA	30min	Sham (placebo)-needling
Song,2019[27]	C57BL/6 mice	6-8 weeks (/)	Colitis (DSS-induced)	/	Colon, serum	IL-6, IL-12, IL-10, IL-1β, IL-6, IL-12, IL-10, IL-17, TNFα,	iNOS, CD206, Arg-1, FIZZ1, NLRP3, Nrf2, HO-1	EA	ST36	/	30min	Sham (placebo)-needling
Wang,2020[28]	Hartley guinea pigs	17 months (/)	OA	PWT	Serum, cartilage	IL-1β, TNF-α	NLRP3, caspase-1, MMP13	EA	Ex-LE4, ST35	2Hz	30min	Sham (placebo)-needling
Wei,2023[29]	C57BL/6 mice	8 weeks (20–25 g)	Gout arthritis (MSU crystals-induced)	PWL, PWT	Ankle	IL-1β, IL-18	NLRP3, caspase-1, ASC, 4-HNE	EA	ST36, BL60	2/100Hz; 0.5mA	30min	Sham (placebo)-needling
Yu,2020[3]	C57BL/6 mice	Adult (/)	IP(CFA)	PWL, PWT	Paw	IL-10, IL-1β, TNF-α	NLRP3	EA	ST36, SP6	2/100Hz; 2mA	20min	No needling
Y. Zhang, 2022[24]	C57BL/6 mice	Adult (20–25 g)	IP(CFA)	PWT, PWL	Dorsal horn of the spinal	/	NLRP3, RIP3	EA	GB30, ST36	2Hz, 2/ 100Hz, 100Hz; 2mA	30min	Sham (placebo)-needling
Y.W. Zhang,2022[30]	SD rats	3-4 months (/)	CPIP	PWT	Lumbar spinal cord	IL-1β	NLRP3, ASC	EA	ST36, BL60	2/100Hz; 0.5–1.5mA	30min	Sham (placebo)-needling
Zhang,2023[31]	SD rats	/ (250–300 g)	KOA (MIA-induced)	PWT	Knee joint	IL-1β	NLRP3, ASC, caspase-1, GSDMD, GSDMD+N-terminus, MMP13	EA	ST35, Ex-LE4, SP9, GB34	2Hz; 1mA	20min	No needling
Zhou,2024[32]	SD rats	Adult (200–250 g)	Migraine (IS)	PWT	TNC	IL-1β	c-Fos, Iba1-1, P2X4R, IL-1β, NLRP3, Caspase-1	EA	GB20, GB34	2/15 Hz; 0.5–1mA	20 min	Sham (placebo)-needling

Abbreviations: CPPS, chronic pelvic pain syndrome; CB2-KO, CB2 receptor knockout mice; PWL, paw thermal withdrawal latencies; PWT, paw withdrawal mechanical threshold; IP, Inflammatory pain; DSS, dextran sulfate sodium; FM, Fibromyalgia; ICS, intermittent cold stress; mPFC, medial frontal cortex; SSC, primary somatosensory cortex; OA, Osteoarthritis; MSU, monosodium urate; LDH, lumber disc herniation; SDH, lumbar spinal dorsal horn; CPIP, Chronic post-ischemia pain; KOA, knee osteoarthritis; MIA, monosodium iodoacetate; IS, inflammatory soup; TNC, trigeminal nucleus caudalis; CV4, Guanyuan acupoint; CV3, Zhongji acupoint; BL35, bilateral Huiyang acupoint; SP6, Sanyinjiao acupoint; GB30, Huantiao acupoint; GB34, Yanglingquan acupoint; ST36, Zusanli acupoint; Ex-LE4, Neixiyan acupoint; ST35, Dubi acupoint; BL60, Kunlun acupoint; SP9, Yinlingquan acupoint; GB20, Fengchi acupoint.

X^2=31.11, I^2=81%, Figure 3b). Thus, electroacupuncture treatment significantly increased the mechanical pain threshold. Both PWL and PWT serve as acute indicators of thermal and mechanical nociceptive thresholds, both of which can be effectively alleviated through acupuncture. A shorter PWL indicates greater sensitivity to thermal pain.

Effects of Electroacupuncture on NLRP3 and Cellular Inflammatory Cytokines After Inflammatory Pain

Figures 3 and 4 display the cytokines and NLRP3-related activation pathways analyzed in this study. Given the distinct characteristics of these factors, we conducted a meta-analysis and categorized cytokines and signaling factors into three sections: (1) pro-inflammatory/anti-inflammatory cytokines; (2) molecules related to NLRP3 activation signaling pathways; (3) M1/M2 macrophages.

Pro-Inflammatory/Anti-Inflammatory Cytokines

Based on the cytokines measured in the literature, the pro-inflammatory cytokines analyzed in this study included IL-1β, IL-6, IL-12, IL-17, IL-18, TNF-α, and PGE2, while the anti-inflammatory cytokines included IL-10. The results showed that pro-inflammatory cytokine levels were elevated in the disease group, and acupuncture significantly reduced these levels (n = 125, SMD 2.76 [95% CI 2.34 ~ 3.18]; P < 0.00001; heterogeneity X^2 = 59.96, I^2=70%, Figure 4a). Conversely, anti-inflammatory cytokines exhibited an upward trend after acupuncture treatment in the disease models (n = 13, SMD −5.66 [95% CI −8.00 ~ −3.32]; P =0.001; heterogeneity X^2 =10.20, I^2 =90%, Figure 4b). The subgroup analysis showed that EA could significantly reduce the expression of pro-inflammatory cytokines in inflammatory pain and arthritis pain induced by CFA (Figures S1 and S2). However, there were relatively few studies on anti-inflammatory cytokines in the included literature, so no analysis was conducted in this regard.

NLRP3 Activation-Related Signaling Pathway Molecules

This study mainly explores the NLRP3-related signaling pathway of EA analgesia from the following perspectives: 1) NLRP3-(Caspase-1)/IL-1β signal pathway; 2) P2X7/NLRP3 signaling pathway; 3) ROS oxidative stress signaling pathways and antioxidant pathways (Nrf2/HO-1 signaling pathway).

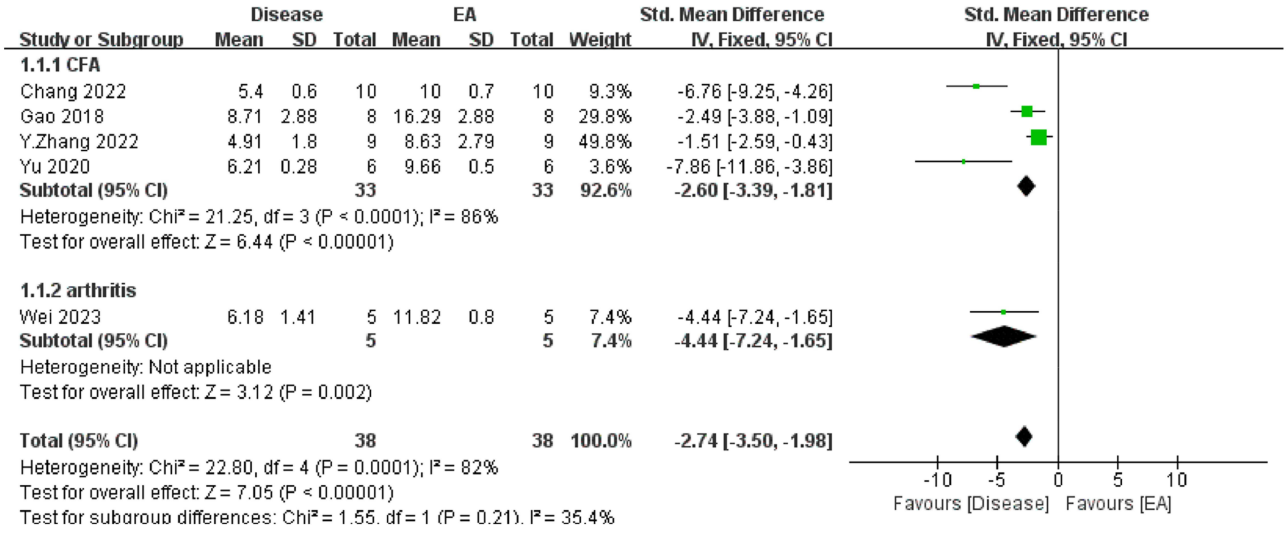
NLRP3-(Caspase-1)/IL-1βsignal pathway: The results indicated that NLRP3, IL-1β, Caspase-1, and other related signaling molecule levels were elevated in the disease group, while acupuncture reduced these levels (n = 165, SMD 2.50 [95% CI 2.15 ~2.85]; P < 0.00001; heterogeneity X^2 = 77.43, I^2=64%, Figure 5). Therefore, electroacupuncture may alleviate inflammatory pain by modulating the NLRP3-(Caspase-1)/IL-1β signal pathway.

Subgroup analysis showed that EA was involved in the regulation of NLRP3-(Caspase-1)/IL-1β signaling pathway in CFA-induced inflammatory pain (n = 59, SMD 1.99 [95% CI 1.48 ~2.49]; P = 0.04; heterogeneity X^2 = 17.43, I^2=48%, Figure S2a), and that EA was also involved in the regulation of NLRP3 in arthritic pain (n = 59, SMD 3.47 [95% CI 2.75 ~4.18]; P = 0.02; heterogeneity X^2 = 21.10, I^2=53%, Figure S2b).

P2X7R/NLRP3 signaling pathway: The results showed elevated levels of P2X7R and NLRP3 signaling molecules in the disease group, while electroacupuncture reduced these levels (n = 181, SMD 2.60 [95% CI 2.25 ~2.94]; P < 0.00001; heterogeneity X^2 = 91.72, I^2=67%, Figure S3). Thus, electroacupuncture may influence changes in related inflammatory factors through the P2X7R/NLRP3 signaling pathway.

ROS oxidative stress signaling pathway and antioxidant pathway (Nrf2/HO-1 signaling pathway): This analysis differentiated between antioxidant molecules and oxidative products. The antioxidant substances included GSH, SOD, Nrf2, and HO-1, while the oxidative products included H2O2 and 4-HNE. The results indicated that electroacupuncture enhanced the levels of antioxidant pathways and related substances compared to the disease group, although the result was not statistically significant (P=0.69) (Figure S4a). Electroacupuncture also reduced levels of oxidative substances, but this too was not statistically significant (P=0.13) (Figure S4b).

(a)



(b)

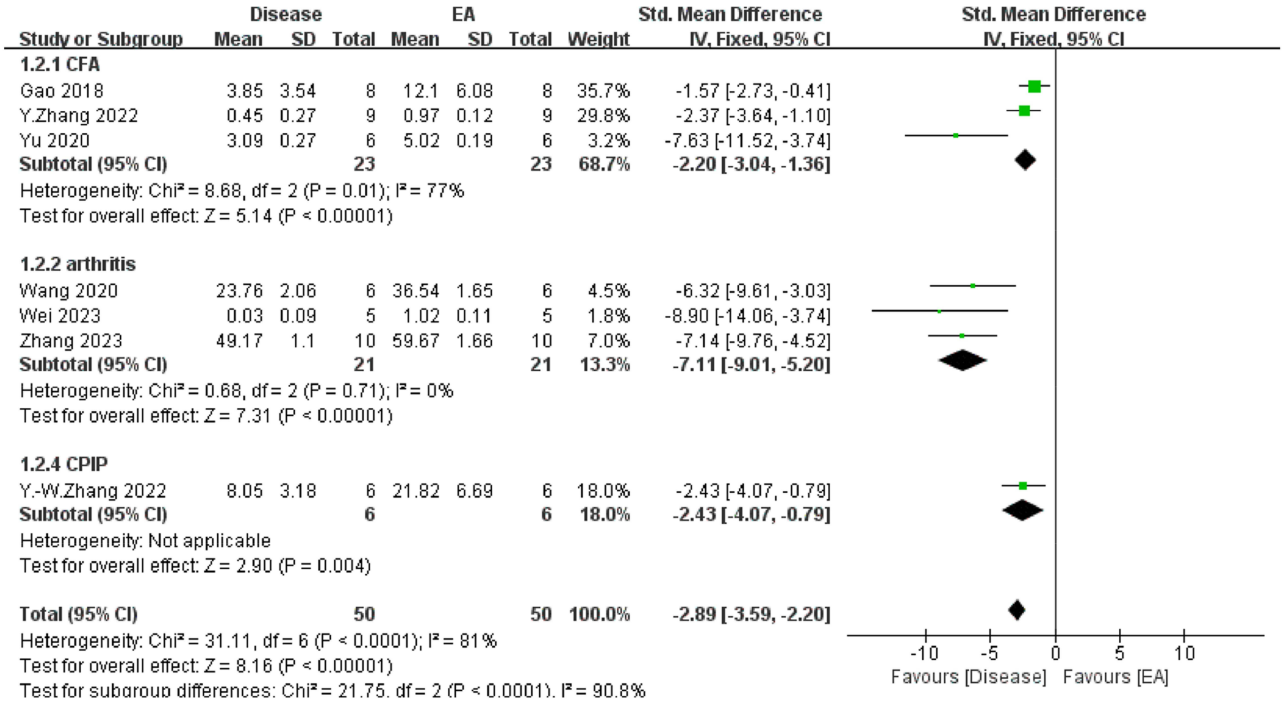


Figure 3 (**a**) Thermal Nociception of PWL; (**b**) Mechanical Nociception of PWT.

M1/M2 Macrophages

To elucidate how electroacupuncture affects M1 and M2 macrophage/microglial cell polarization, we analyzed the expression of M1-related markers (iNOS, IL-1β, and IL-6) and M2-related markers (IL-10, Arg-1, and CD206). The results showed that compared to the disease group, the electroacupuncture group exhibited reduced expression of M1 macrophage markers (SMD 0.64[95% CI 0.61 ~ 0.66]; P< 0.00001; heterogeneity X^2 = 440.00, I^2=98%, Figure S5a). Simultaneously, compared to the disease group, the electroacupuncture group showed an increase in the expression of M2 macrophage markers (SMD −3.36 [95% CI −4.63 ~ −2.08]; P = 0.001; heterogeneity X^2 = 15.58, I^2=81%, Figure S5b).

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Discussion

Numerous studies indicate that electroacupuncture (EA) is closely associated with the alleviation of inflammatory pain. EA blocks pain by activating or inhibiting various bioactive chemicals through peripheral, spinal, and supraspinal mechanisms.[33–36] In this review, we analyzed 10 studies involving electroacupuncture in rodent models of inflammatory pain, primarily focusing on peripheral mechanisms. By studying the inflammatory pain models induced by CFA, inflammatory soup, acute colitis, inflammatory arthritis and chronic postischemic syndrome, this review demonstrates that EA exerts significant anti-inflammatory and analgesic effects across diverse inflammatory pain models. The therapeutic mechanisms involve multi-target synergistic actions, including modulation of inflammatory cytokine networks and suppression of NLRP3 inflammasome activation via upstream pathways, specifically P2X7R-ATP signaling and ROS production.

In inflammatory pain models, the analgesic mechanisms induced by EA are associated with peripheral and central sensitization inhibition. EA suppresses the release of pain-related inflammatory mediators resulting from tissue damage, decreases the expression of nociceptive receptors in peripheral neurons, and inhibits the phosphorylation of cellular signaling pathways, all of which contribute to the desensitization of peripheral pain perception. Additionally, EA promotes the release of peripheral opioid peptides, cannabinoids, and adenosine to mitigate inflammatory pain. Our study similarly found that in the group treated with electroacupuncture, both the thermal pain threshold (PWL) and mechanical pain threshold (PWT) increased, indicating that electroacupuncture can alleviate inflammatory pain. To be positive, most of the included studies used a strict sham acupuncture control group. These controls typically used the same animal fixation and needling procedures as in the true EA group, but no electrical stimulation was applied in the sham EA group. This control design helps to exclude the influence of simple stationary stress and non-specific electrical stimulation, so as to more accurately evaluate the physiological effects of specific EA stimulation.

EA inhibits the activation of excitatory spinal neurons, including those induced by MAPK phosphorylation, as well as the activation of glial cells and the release of inflammatory mediators (such as inflammatory cytokines, chemokines, and ATP), thus suppressing central sensitization.[37,38] Inflammatory cytokines can be categorized based on their functions: pro-inflammatory cytokines promote inflammation, while cytokines involved in tissue repair are considered anti-inflammatory. Based on the included literature, analyses of inflammatory cytokines detected in the brain, colon, joints, or serum/plasma samples from animal models revealed that electroacupuncture reduced levels of pro-inflammatory cytokines, including IL-1β, IL-6, IL-12, IL-17, IL-18, TNF-α, and PGE2. Conversely, anti-inflammatory cytokines, such as IL-10, exhibited an opposing trend. It is worth noting that prostaglandin E2 (PGE2), as an important lipid mediator, has significant pleiotropic and complex effects. PGE2 acts through four distinct G protein-coupled receptors (EP1-EP4), which mediate signals that produce either pro-inflammatory or anti-inflammatory/pro-resolution effects.[39] Despite this complexity, Chang et al[25] stated in their study that PGE2 mainly plays a pro-inflammatory role and is classified as an inflammatory factor. This finding is consistent with our results. Therefore, to clarify whether PGE2 exerts anti-inflammatory or pro-inflammatory effects following electroacupuncture, changes in its levels must be analyzed comprehensively in conjunction with the specific receptor subtypes involved and the local tissue microenvironment. Furthermore, Xie et al[36] discovered that EA alleviates the inflammatory response in mice with chronic inflammatory pain by modulating the quantities of macrophages, neutrophils, and Treg cells, promoting M2 polarization of local macrophages, and inhibiting the release of pro-inflammatory cytokines. M1 microglial cells are considered primarily destructive, while M2 microglial cells are regarded as neuroprotective.[40] Among the various microglial cell phenotypes, M1 polarization is characterized by the release of pro-inflammatory cytokines, such as TNF-α and IL-6.[41] Our findings suggest that following electroacupuncture treatment, the expression of M1 cell markers in activated microglia or macrophages decreased, resulting in reduced M1 polarization and pro-inflammatory cytokine release, while M2 polarization increased, providing neuroprotective effects. Thus, our results suggest that electroacupuncture may improve inflammation by inducing anti-inflammatory cytokines and macrophages while reducing pro-inflammatory cytokines.

This review highlights that the analgesic mechanisms of electroacupuncture are associated with signaling pathways related to the NLRP3 inflammasome. The NOD-like receptor protein 3 (NLRP3) is a protein closely linked to inflammatory responses, typically activated during infection or inflammation, leading to the formation of the NLRP3

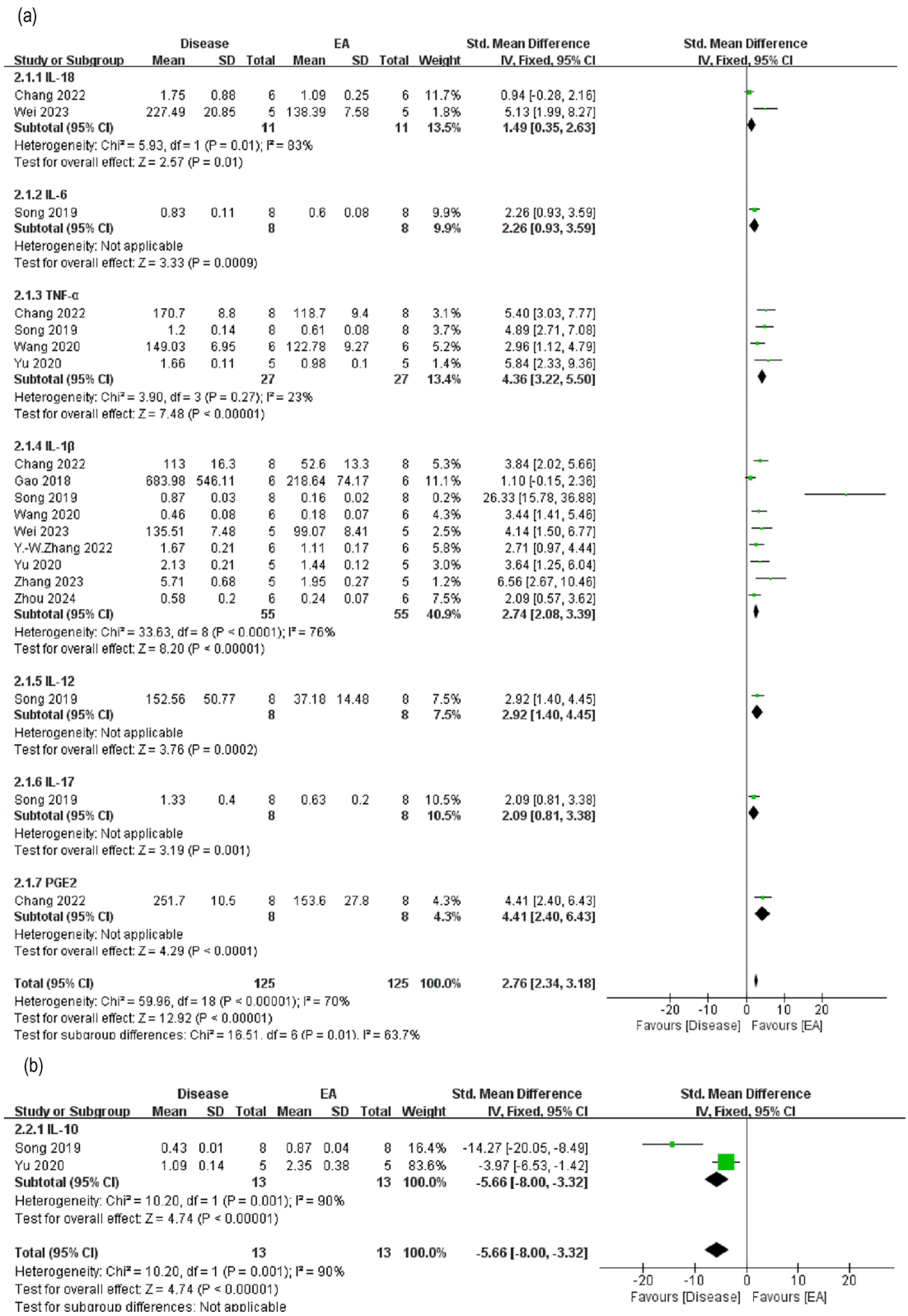

Figure 4 (**a**) Pro-inflammatory cytokines; (**b**) Anti-inflammatory cytokines.

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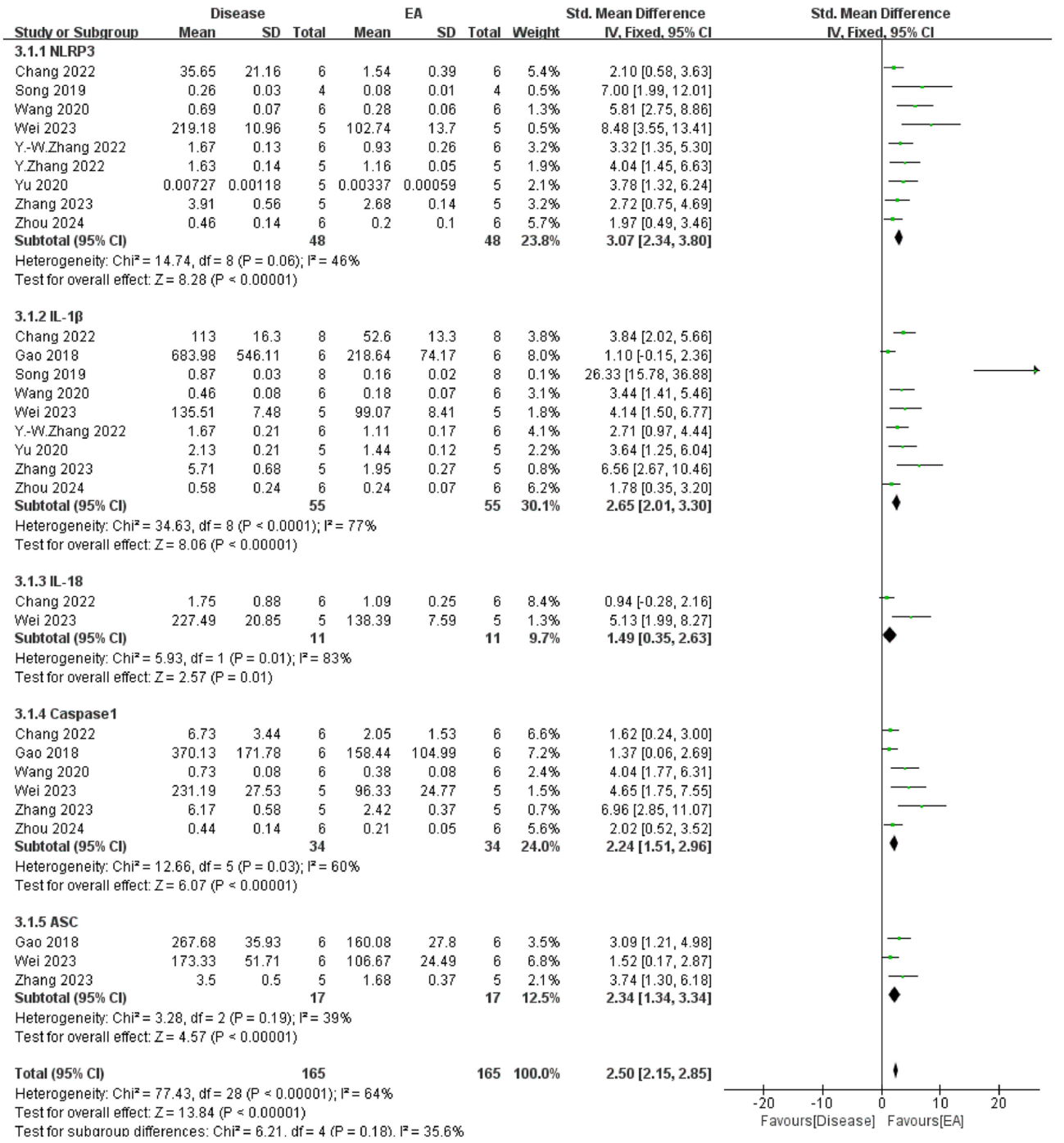

Figure 5 NLRP3-(Caspase-1)/IL-1βsignal pathway.

inflammasome and the induction of apoptosis. The three main components of the NLRP3 inflammasome are NLRP3, caspase-1, and ASC. NLRP3 captures danger signals and recruits downstream molecules, while caspase-1 mediates the maturation of IL-1β and IL-18, as well as the processing of Gasdermin D (GSDMD) to facilitate the release of cytokines and pyroptosis. ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain, acts as a bridge between NLRP3 and caspase-1.[5] In recent years, Yuan et al[6] discovered that the NLRP3 inflammasome is part of the analgesic mechanism of electroacupuncture. The activation of the NLRP3 inflammasome involves multiple factors,

including P2X receptor activation on the cell membrane, ROS production, mitochondrial dysfunction, and endoplasmic reticulum stress, all of which trigger NLRP3 activation, exacerbating inflammatory responses and fibrosis.[42]

Based on the included studies, we found that electroacupuncture could suppress the expression of NLRP3 and pro-inflammatory cytokines through several key signaling pathways, thereby alleviating inflammatory pain.[6] Firstly, NLRP3-(Caspase-1)/ IL-1β signaling pathway plays a crucial role. When inflammation occurs, NLRP3 oligomerizes and activates caspase-1 in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs, such as ATP, HMGB1, DNA, etc). ASC (apoptosis-associated speck-like protein),[43] NEK-7 (a serine/threonine kinase involved in mitosis, NIMA-associated kinase),[44] and caspase-1 form the NLRP3 inflammasome. EA can affect NLRP3, reduce the signal transduction of this pathway, inhibit the production of NLRP3 inflammasome, and inhibit the production of GSDMD-N, thereby alleviating pyroptosis. Secondly, regarding the purinergic pathway P2X7R-ATP, ATP stimulation promotes the influx of Ca^{2+} and Na^{+} via the P2X7 receptor (a ligand-gated ion channel of the purinergic receptor family) and coordinates with the two-pore domain K^{+} channel 2 (TWIK2) to induce NLRP3 inflammasome activation, resulting in the generation of caspase-1 and IL-1β.[45] Electroacupuncture can directly modulate the levels of extracellular ATP (eATP), reverse the elevated expression of its receptor (P2X7) and inhibit NLRP3 inflammasome activation. This is achieved as electroacupuncture promotes eATP hydrolysis by regulating nucleotide enzymes and prevents eATP from binding to P2X7, thereby alleviating pain.[34] P2X4R belongs to the P2X receptor family, but P2X4R has a higher sensitivity to ATP and is closely related to the activation of NLRP3 inflammasome in neuroinflammation.[46–48]

The third aspect involves the reactive oxygen species (ROS) oxidative stress signaling pathway and antioxidant pathway (Nrf2-ARE signaling pathway). The signaling molecules in this pathway are divided into antioxidant molecules and oxidative products. During cellular stress, mitochondria produce ROS as a byproduct of oxidative phosphorylation, activating the NLRP3 inflammasome.[49] Nrf2 (nuclear factor E2-related factor 2) regulates antioxidant genes, limiting ROS levels and inhibiting NLRP3 activation.[50] Previous studies have shown that electroacupuncture alleviates cellular stress and inflammatory responses by increasing antioxidant signaling molecule levels and inhibiting oxidative product generation.[29,49,51,52] However, the limited number of studies included in this review does not allow for a definitive conclusion regarding the relationship between electroacupuncture and the ROS oxidative pathway, necessitating further research.

According to the findings of the included studies, cannabinoid type 2 receptor (CB2R) activation can inhibit the activation of NLRP3 inflammasome. Ke et al[53] previous study showed that electroacupuncture promoted AMPK phosphorylation by activating CB2R. This subsequently activated the AMPK-SIRT1-NLRP3 pathway while inhibiting the activation of both NF-κB and the NLRP3 inflammasome. AMPK is an AMP-activated protein kinase that plays a crucial role in maintaining homeostasis and energy balance in the intracellular environment, while SIRT1 is an NAD+ -dependent deacetylase involved in a variety of regulatory processes, including cell differentiation, apoptosis, and oxidative stress.[24] Previous studies have shown that overexpression of constitutively activated mutant AMPK significantly enhances SIRT1 expression and activity in macrophages, accompanied by reductions in NF-κB acetylation levels, transcriptional activity, and proinflammatory gene expression. The anti-inflammatory effect of AMPK is dependent on SIRT1 and negatively regulates NF-κB and its downstream inflammatory cascade, affecting the activation of NLRP3 inflammasome.[54,55]

According to the included studies, there is a close relationship between RIP3 (Receptor-Interacting Protein Kinase 3) and NLRP3 inflammasome, which is mainly reflected in the regulation of cell death and inflammatory response. RIP3-mediated necroptosis leads to cell membrane rupture and release of DAMPs.[56] These DAMPs can act as activation signals for the NLRP3 inflammasome to promote its assembly and activation. The activation of RIP3 can also lead to mitochondrial dysfunction and the production of a large number of reactive oxygen species (ROS),[57] which is an important signal for the activation of NLRP3 inflammasome. In addition, RIP3 may indirectly affect the expression of NLRP3 by regulating the NF-κB signaling pathway.[58] The specific signal processing is shown in Figure 6.

The neurobiological effects of electroacupuncture are highly complex. When investigating inflammation, chronic pain, and neurological disorders, experimental variables including subject age, species specificity, acupoint localization, stimulation frequency, retention duration, and electroacupuncture course require systematic consideration. Firstly, many

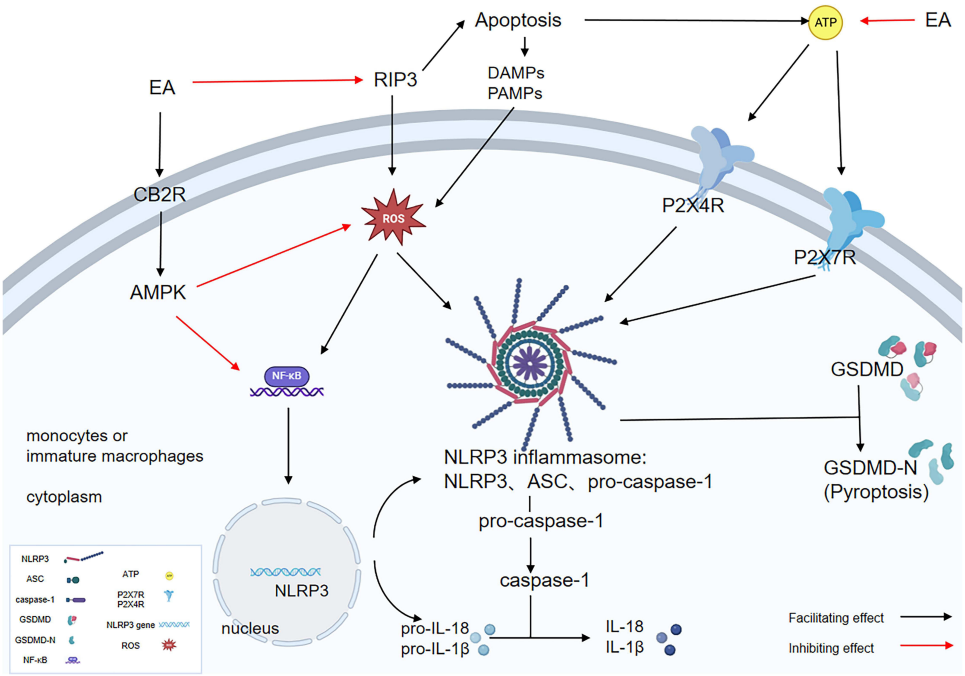

Figure 6 Signal path diagram. The precise activation steps of the NLRP3 inflammasome and inflammatory response are as follows: RIP3 promotes apoptosis to produce DAMPs, PAMPs, and ATP, participates in oxidative pathways in proinflammatory cells, induces NF-κB activation and translocation into the nucleus, leading to the synthesis of NLRP3, IL-1β precursor, and IL-18 precursor. NLRP3 oligomerizes with ASC in the cytoplasm. NLRP3 inflammasome activates and clears caspase-1 precursor to form caspase-1, which produces active IL-1β, IL-18 and GSDMD to initiate pyroptosis and promote inflammatory response. EA can act on CB2R to promote the phosphorylation of AMPK in the spinal cord, activate AMPK pathway, increase the level of antioxidant signaling molecules, inhibit the formation of oxidative products, thereby inhibiting the activation of NF-κB and reducing the formation of NLRP3 inflammasome and subsequent inflammatory response. EA can inhibit the expression of RIP3 to regulate cell apoptosis and reduce ROS production. In addition, EA can directly regulate the level of eATP and reverse the increase in the expression of its receptor (P2X7).

studies have demonstrated that aging significantly impacts inflammatory pain via immune system, nervous system, and pain perception changes.[59,60] This review mostly included adult rodents, with only one aged guinea pig study, limiting age-stratified analyses. Future studies should explore age-related pain mechanisms by experiment or meta-analysis. Secondly, interspecies and interstrain variations may influence experimental outcomes. In our review, 10 studies included SD rats (n=5), C57BL/6 mice (n=5), and guinea pigs (n=1). We only performed subgroup analyses for CFA-induced pain and arthritis-related pain, with unaddressed interspecies/strain variations due to relatively small other disease types. Guinea pigs have human-like complement immunity.[61] SD rats are attracted to the movement of a social stimulus, while C57BL/6 mice avoid them,[62] affecting pain assessments. These variations may impact result consistency. Thirdly, the different electroacupuncture points have specific effects. ST36 (Zusanli), GB34 (Yanglingquan), LI4 (Hegu), and GV20 (Baihui) are the commonly used acupuncture points for treating chronic pain. ST36 significantly alleviates inflammatory pain by inhibiting the release of pro-inflammatory factors via NF-κB;[63] GB34 reduces astrocyte activation and reduces the expression of pro-inflammatory factors;[64] GV20 modulates the central nervous immune axis.[65] When these acupuncture points are used in combination (such as ST36 + GB30, LI4 + PC6), they exert synergistic effects beyond single-point stimulation.[66–68] Specifically, such combinations can activate the endogenous cannabinoid system, downregulate pro-inflammatory cytokine levels (such as IL-6), and modulate key signal transduction pathways. Through a multi-level neuro-immune regulatory network, these combined interventions significantly enhance anti-inflammatory and analgesic efficacy, yielding more pronounced therapeutic effects compared to stimulation of individual acupoints alone. Notably, different models use site-specific acupuncture points, and interactions between stimulation patterns (single/paired, unilateral/bilateral, combinations) and frequency parameters generate unique neurophysiological response patterns. Fourthly, electroacupuncture parameters like frequency, needle retention time, and treatment course are key for NLRP3 inflammasome-mediated anti-inflammatory and analgesic effects. High-frequency (100 Hz) electroacupuncture has a more significant therapeutic effect than low-frequency (2 Hz) or mixed frequencies (2/100 Hz), enhancing NLRP3 inflammasome inhibition activation via neuro-immune suppression pathways.[27] Single-session needle retention mostly

lasts 20–30 minutes, consistent with clinical practice,[69] but the biological differences between these durations are unclear. Treatment courses vary widely (3–56 days) with intervals from hours to 2 days, causing heterogeneity. Whether retention duration and course statistically impact NLRP3 expression and cumulative dose-dependent efficacy needs further verification. Despite variability, electroacupuncture exerts systemic neuro-immune effects. Future studies should focus on optimizing age-specific models and standardizing stimulation parameters. Additionally, incorporating dynamic monitoring of molecular biomarkers like NLRP3 inflammasome activity will help establish definitive dose-response relationships, enhancing the reproducibility and clinical translatability of electroacupuncture research.

This study has several limitations: Firstly, the heterogeneity of the included studies poses challenges. Diverse pain models (eg, CFA-induced arthritis, visceral inflammation) with distinct pathophysiologies and pain mechanisms, variations in experimental subjects (species, strains, and age), and inconsistencies in EA stimulation parameters (frequency, treatment duration, and acupoint selection) may introduce confounding factors. These factors can affect pain thresholds and responses to stimulation, thereby limiting comparability across studies. Secondly, currently, there is limited literature regarding how electroacupuncture achieves analgesic effects through NLRP3 inflammasome modulation. Then, since this study encompasses the overall effects of electroacupuncture on inflammatory pain, cytokines and related inflammatory signaling pathways were not analyzed by specific disease subgroups. Nonetheless, this study is significant as it is the first to evaluate the efficacy of acupuncture on inflammatory pain through the analysis of inflammatory factors and NLRP3 signaling pathways. To address the limitations outlined, future research should focus on standardizing EA parameters, including frequency, treatment duration, and acupoint selection, to reduce heterogeneity. Additionally, comparing efficacy across different pain models (eg, CFA-induced arthritis vs visceral inflammation) and elucidating how specific protocols modulate key pathways (eg, the P2X7R-ROS-NLRP3 axis) will enhance mechanistic understanding. Conducting disease-subgroup specific analyses, enabled by expanded study cohorts, will also improve comparability and clinical translatability.

Conclusion

In summary, this review indicates that electroacupuncture may improve inflammatory pain by regulating inflammatory cytokines and modulating NLRP3 signaling pathways. In alleviating inflammatory pain, electroacupuncture regulates pro-inflammatory/ anti-inflammatory cytokines, the balance of M1/M2 macrophages, and NLRP3-related signaling pathways. These findings provide a theoretical basis for optimizing EA analgesia in clinical settings, such as managing postoperative pain, and highlight its potential to reduce reliance on analgesic medications, guiding the development of evidence-based EA therapies with implications for clinical translation and public health.

Abbreviations

NLRP3, NOD-like receptor protein 3; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-alpha; NSAIDs, Non-steroidal Anti-inflammatory Drugs; EA, electroacupuncture; TEAS, transcutaneous electrical acupoint stimulation; RCTs, randomized controlled trials; CAMARADES, Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies; SMD, standardized mean difference; CI, confidence interval; CFA, complete Freund's adjuvant; KOA, knee osteoarthritis; CPIP, chronic postischemic pain; CPPS, chronic pelvic pain syndrome, CB2-KO, CB2 receptor knockout mice; PWL, paw thermal withdrawal latencies; PWT, paw withdrawal mechanical threshold; IP, Inflammatory pain; DSS, dextran sulfate sodium; FM, Fibromyalgia; ICS, intermittent cold stress; mPFC, medial frontal cortex; SSC, primary somatosensory cortex; OA, Osteoarthritis; MSU, monosodium urate; LDH, lumber disc herniation; SDH, lumbar spinal dorsal horn; MIA, monosodium iodoacetate; IS, inflammatory soup; TNC, trigeminal nucleus caudalis; CV4, Guanyuan acupoint; CV3, Zhongji acupoint; BL35, bilateral Huiyang acupoint; SP6, Sanyinjiao acupoint; GB30, Huantiao acupoint; GB34, Yanglingquan acupoint; ST36, Zusanli acupoint; Ex-LE4, Neixiyan acupoint; ST35, Dubi acupoint; BL60, Kunlun acupoint; SP9, Yinlingquan acupoint; GB20, Fengchi acupoint; GSDMD, Gasdermin D; PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; ASC, apoptosis-associated speck-like protein; NEK-7, NIMA-associated kinase; TWIK2, two-pore domain K+ channel 2; eATP, extracellular ATP; P2X7R, purinergic 2X7 receptor; P2X4R, purinergic 2×4 receptor; ROS, reactive oxygen species; Nrf2,

nuclear factor E2-related factor 2; CB2R, cannabinoid type 2 receptor; NF-κB, nuclear factor kappa-B; RIP3, Receptor-Interacting Protein Kinase 3; AMPK, Adenosine 5′-monophosphate (AMP)-activated protein kinase.

Author Contributions

RS S: Writing - Original Draft, Writing - Review & Editing, Investigation, Conceptualization. ZF W: Writing - Original Draft, Validation, Investigation. GD Z: Formal analysis, Data Curation. YH T and HN Z: Data Curation, Investigation. HB C: Visualization, Validation. JY L: Writing - Review & Editing, Funding acquisition. ZC S: Writing - Review & Editing, Supervision. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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