Meta-Analysis of the Effects of Electroacupuncture on Post-Stroke Apoptosis in Animal Models

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Abstract [**Objectives**] To systematically evaluate the effects of electroacupuncture (EA) on post-stroke apoptosis in animal models, focusing on key apoptotic markers (TUNEL-positive cells, caspase-3, Bcl-2/Bax ratio) and exploring potential sources of heterogeneity related to EA parameters and the timing of interventions. [**Methods**] A comprehensive search of PubMed, EMBASE, Web of Science, and the Cochrane Library (from inception to July 2025) was conducted to identify randomized controlled animal studies investigating EA in ischemic stroke models (tMCAO/pMCAO). Data pertaining to apoptotic outcomes were extracted, and the methodological quality was assessed using the CAMARADES checklist. A meta-analysis was conducted using random- or fixed-effects models in Stata 17.0, with subgroup analyses for EA timing (pre- *vs.* post-ischemia) and waveforms (continuous *vs.* disperse). Heterogeneity among studies was quantified via the I^2 statistic. [**Results**] Thirty-two studies were included in the analysis. EA significantly reduced apoptosis, as evidenced by a decrease in TUNEL-positive cells (Hedges' $g = -3.38$, 95% *CI*: -4.09 to -2.67), reduced caspase-3 expression ($g = -2.67$, 95% *CI*: -3.35 to -2.00), and an increased Bcl-2/Bax ratio ($g = 2.60$, 95% *CI*: 1.72 to 3.47). Subgroup analyses showed comparable efficacy between pre- and post-ischemia EA ($p = 0.50$) and revealed a non-significant trend favoring continuous over disperse waveforms ($p = 0.09$). High heterogeneity ($I^2 > 50\%$) was observed, which was attributed to variations in animal models, EA protocols, and outcome assessments. [**Conclusions**] EA demonstrates robust anti-apoptotic effects in stroke models, likely mediated through the PI3K/Akt, NF-κB, and TRPV1 pathways. While both timing and waveforms show promise, standardizing EA protocols and conducting translational clinical trials are essential to optimize neuroprotective applications in stroke rehabilitation.

Key words Electroacupuncture, Stroke, Apoptosis, Meta-analysis

1 Introduction

Ischemic stroke, characterized by the sudden occlusion of cerebral blood vessels, initiates a cascade of pathophysiological events that culminate in extensive neuronal damage and functional impairment[1]. A central feature of this process is the interplay between neuroinflammation and apoptosis, which collectively contribute to the expansion of the ischemic core and the progressive loss of neurons in the penumbra. Following ischemia, the disruption of cerebral blood flow leads to energy depletion, glutamate excitotoxicity, and excessive production of reactive oxygen species (ROS), triggering a robust inflammatory response[2]. Activated microglia and astrocytes release pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), which not only exacerbate vascular permeability and blood-brain barrier breakdown but also directly induce apoptotic signaling pathways. These cytokines activate the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, promoting the expression of pro-apoptotic proteins like Bax and caspase-3, while downregulating anti-apoptotic factors such as Bcl-2. The resulting imbalance shifts the cellular fate toward apoptosis, characterized by hallmark features such as DNA fragmentation, chromatin condensation, and the formation of apoptotic bodies[3]. This apoptotic process is particularly pronounced in the ischemic penumbra, where neurons remain metabolically compromised but viable, making this region a critical target for neuroprotective interventions. Accumulating evidence from preclinical studies highlights that modulating this inflammatory-apoptotic axis can significantly reduce infarct volume and preserve neurological function, underscoring the need for effective strategies to intervene in these interconnected pathways[4].

Electroacupuncture (EA), a modern adaptation of traditional acupuncture that combines needle insertion with electrical stimulation, has emerged as a promising neuroprotective modality in stroke research[5]. Over the past decade, numerous animal studies have demonstrated that EA exerts multifaceted effects on ischemic brain injury, with a growing focus on its ability to regulate apoptosis[6-7]. In rodent models of middle cerebral artery occlusion (MCAO), EA has been shown to reduce infarct volume, improve neurological deficits, and mitigate neuronal loss—outcomes closely linked to its effects on apoptotic mechanisms. Mechanistically, EA interventions at specific acupoints—such as Baihui (GV20), Zusanli (ST36), and Quchi (LI11)—have been associated with the downregulation of pro-apoptotic markers, including caspase-3, caspase-9, and Bax, while upregulating Bcl-2 and Bcl-xL[8-9]. These effects are thought to be mediated through the modulation of key signaling pathways, such as the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, which inhibits apoptotic cascades by phosphorylating downstream targets like Bad and forkhead box O (FoxO) transcription factors. Additionally, EA has been shown to suppress the activation of NF-κB, thereby reducing the release of pro-inflammatory cytokines and indirectly alleviating cytokine-induced apoptosis[10]. Furthermore, recent studies indicate that EA may regulate transient receptor potential vanilloid 1 (TRPV1) channels, which are involved in calcium in-

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flux and mitochondrial dysfunction—both critical steps in the apoptotic process[11]. The diversity of these mechanisms, coupled with variations in EA parameters (*e. g.*, frequency, intensity, duration) and animal models, underscores the complexity of EA's effects while also highlighting its potential as a targeted intervention for post-stroke apoptosis.

Despite the growing body of preclinical evidence supporting EA's anti-apoptotic effects in stroke[10-12], several critical gaps remain. Existing studies exhibit substantial heterogeneity in terms of experimental design, including the type of ischemic model used (transient *vs.* permanent MCAO), animal species (Sprague-Dawley rats *vs.* C57BL/6 mice), EA stimulation parameters (*e. g.*, 2 Hz *vs.* 2/15 Hz frequency, 1 mA *vs.* 2 mA intensity), and the timing of intervention (pre- *vs.* post-ischemia). This variability complicates the synthesis of results and hinders the formulation of consistent conclusions regarding the magnitude and mechanisms underlying EA's anti-apoptotic efficacy. Moreover, although individual studies have reported reductions in apoptotic markers such as TUNEL-positive cells and caspase activity, no comprehensive meta-analysis has systematically quantified these effects across diverse animal models or examined the influence of study-specific factors on the outcomes. The present meta-analysis aims to address these limitations by aggregating data from randomized controlled animal studies investigating EA's effects on post-stroke apoptosis. Specifically, this analysis seeks to: i) quantify the overall impact of EA on key apoptotic markers (*e. g.*, TUNEL-positive cells, caspase-3, Bcl-2/Bax ratio) and ii) assess the heterogeneity among studies and identify potential sources, including EA parameters and intervention timing. By providing a rigorous synthesis of preclinical evidence, this meta-analysis aims to inform the design of future animal studies and facilitate the translation of EA-based interventions into clinical practice for stroke rehabilitation.

2 Methods

2.1 Study eligibility criteria Included studies met the following criteria. (i) Study design: randomized controlled trials (RCTs) were conducted to investigate the effects of EA on ischemic stroke in animal models. (ii) Animal models: rodents (rats or mice) with experimentally induced ischemic stroke were employed, including transient middle cerebral artery occlusion (tMCAO) or permanent middle cerebral artery occlusion (pMCAO). Animals of any age, gender, or strain were included. (iii) Intervention: the experimental group received EA treatment, defined as electrical stimulation applied to specific acupoints with detailed parameters (frequency, intensity, duration, and acupoint selection). (iv) Control group: animals received either sham EA (needle insertion without electrical stimulation), no treatment, or standard care (*e. g.*, anesthesia alone). (v) Outcome measures: studies reported at least one apoptosis-related outcome, including TUNEL-positive cell count, caspase-3 activity/expression, Bcl-2 expression, Bax expression, or the Bcl-2/Bax ratio. (vi) Language: studies published in English.

Exclusion criteria were: (i) clinical studies involving human subjects; (ii) *in vitro* or *in vivo* studies; (iii) studies focusing on hemorrhagic stroke; (iv) reviews, case reports, or conference abstracts; (v) studies without a control group or with incomplete outcome data; and (vi) duplicate publications.

2.2 Search strategy A comprehensive literature search was conducted across four electronic databases: PubMed, EMBASE, Web of Science, and the Cochrane Library. The search period spanned from the inception of each database to July 2025. The search strategy combined medical subject headings (MeSH) and free-text terms related to EA, ischemic stroke, apoptosis, and animal models. Key search terms included: ("electroacupuncture" OR "electro-acupuncture") AND ("ischemic stroke" OR "cerebral infarction" OR "middle cerebral artery occlusion") AND ("apoptosis" OR "cell death" OR "TUNEL" OR "caspase" OR "Bcl-2" OR "Bax") AND ("animal" OR "rats" OR "mice" OR "rodents"). Reference lists of retrieved articles and relevant systematic reviews were manually screened to identify additional eligible studies.

2.3 Data extraction and quality assessment Two reviewers independently extracted data using a standardized form, with discrepancies resolved by a third reviewer. Extracted information included: (i) study characteristics: first author, publication year, and country; (ii) animal details: species, strain, gender, weight, and sample size; (iii) ischemic model: type (tMCAO/pMCAO), ischemia duration, and reperfusion time; (iv) EA parameters: EA waveforms (continuous wave *vs.* disperse wave) and timing of intervention (pre- *vs.* post-ischemia); (v) control group intervention; and (vi) outcome measures: type of apoptotic marker, mean values, standard deviations (SD), and sample sizes for both EA and control groups.

Methodological quality was assessed using the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist, which evaluates 10 criteria: sample size calculation, random sequence generation, blinded induction, blinded outcome assessment, use of anesthetics without neuroprotective effects, appropriate animal model, temperature control, publication in peer-reviewed journal, compliance with animal welfare laws, and declaration of conflicts of interest. Each criterion was scored as "yes" (1 point) or "no/unclear" (0 point), with a maximum score of 10. Studies with a score $\geqslant 5$ were considered to have moderate methodological quality.

2.4 Statistical analysis Meta-analysis was performed using Stata 17.0 software. Continuous outcomes (*e. g.*, TUNEL-positive cells, caspase-3 expression) were synthesized using mean differences (MD) with 95% confidence intervals (*CIs*). Heterogeneity among studies was assessed using the I^2 statistic, where $I^2 > 50\%$ indicated substantial heterogeneity, prompting the use of a random-effects model; otherwise, a fixed-effects model was applied. Sensitivity analyses were conducted by sequentially excluding each study to evaluate the robustness of the results. Subgroup analyses were performed to explore potential sources of heterogeneity, in-

cluding: (i) intervention timing (pre-ischemia *vs.* post-ischemia) and (ii) EA waveforms (continuous wave *vs.* disperse wave). Publication bias was assessed using funnel plots and Egger's test, with $P<0.05$ indicating significant bias.

3 Results and analysis

3.1 TUNEL-positive cells This forest plot presents the meta-analysis results of the effects of EA on post-stroke apoptosis (assessed by TUNEL assay) in stroke animal models. A total of 27 studies were included[1,5,6,8-9,12-31]. Hedges' g with 95% confidence interval (CI) was calculated for each study. The overall effect size showed that EA treatment in the intervention group (EA-treated stroke model) had a significant inhibitory effect on post-stroke apoptosis compared to the control group. The overall Hedges' g was -3.38 (95% CI: -4.09 to -2.67), with heterogeneity values of $\tau^2=2.54$, $I^2=76.85\%$, and $H^2=4.32$, indicating moderate to high heterogeneity among the included studies. The test of $\theta=0$ (Q test) resulted in $Q(26)=98.09$, $p=0.00$, suggesting significant heterogeneity. The overall effect test (z-test) yielded $z=-9.34$, $p=0.00$, indicating that EA significantly reduced post-stroke apoptosis (TUNEL-assessed) in the stroke model. Each study's effect size (represented by triangles) and the overall effect (represented by a diamond) were visualized, showing that EA consistently reduced apoptosis across most studies, contributing to the significant overall negative effect size (Fig. 1).

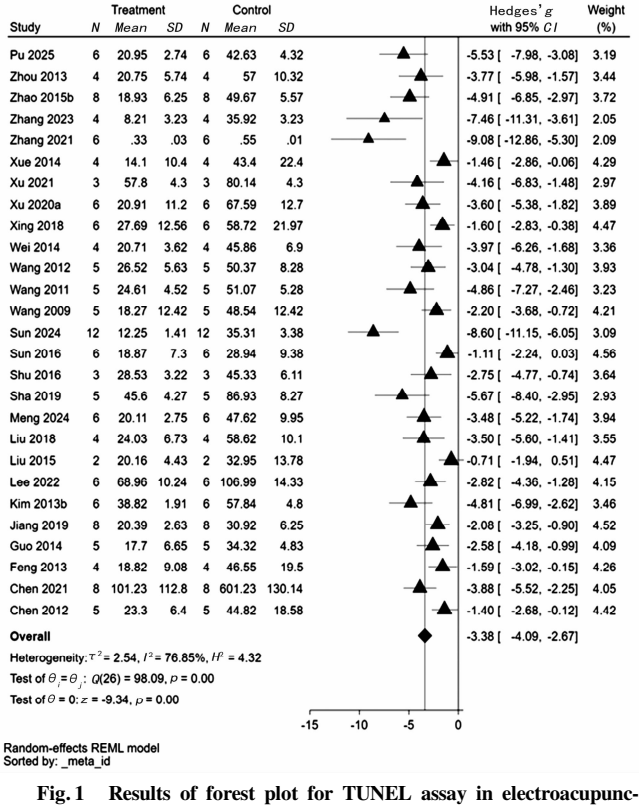

Fig. 1 Results of forest plot for TUNEL assay in electroacupuncture-treated stroke model

3.2 Caspase-3 expression This forest plot illustrates the meta-analysis results of EA's effects on post-stroke apoptosis, as measured by Caspase-3 expression, in stroke-modeled animals. A total of 15 studies were included[5-6,8,14,16-18,24,27,29,31-34]. For each study, Hedges' g with 95% confidence interval (CI) was calculated. The overall Hedges' g was -2.67 (95% CI: -3.35 to -2.00), indicating that EA in the treatment group (stroke-modeled animals receiving EA) significantly reduced Caspase-3 expression related to post-stroke apoptosis compared to the control group. Heterogeneity analysis showed $\tau^2=0.92$, $I^2=56.49\%$, and $H^2=2.30$, suggesting moderate heterogeneity among the studies. The test of homogeneity (Q test) yielded $Q(14)=42.02$, $p=0.00$, confirming significant heterogeneity. The overall effect test (z-test) resulted in $z=-7.73$, $p=0.00$, demonstrating that the overall effect of EA on reducing Caspase-3 expression-associated post-stroke apoptosis in stroke animal models was statistically significant. Each study's effect size, represented by triangles, and the overall effect, represented by a diamond, are shown in Fig. 2. Most studies indicated that EA decreased Caspase-3 expression, contributing to a significant overall negative effect size.

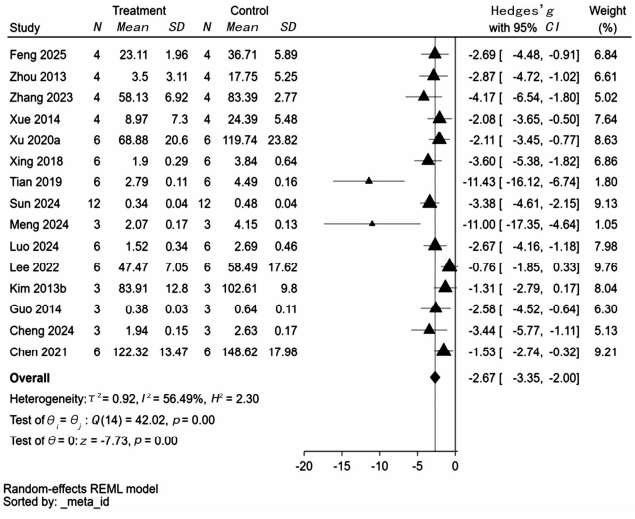

Fig. 2 Electroacupuncture effects on Caspase-3 expression in stroke animal models

3.3 Bcl-2/Bax ratio This forest plot presents the meta-analysis results of EA's effect on the Bcl-2/Bax ratio, a key indicator of post-stroke apoptosis, in stroke-modeled animals. Five studies were included[9,17,19,34]. For each study, Hedges' g with 95% confidence interval (CI) was calculated. The overall Hedges' g was 2.60 (95% CI: 1.72 to 3.47), showing that EA in the treatment group (stroke-modeled animals receiving EA) significantly increased the Bcl-2/Bax ratio compared to the control group, which implies an inhibitory effect on post-stroke apoptosis. Heterogeneity analysis revealed $\tau^2=0.00$, $I^2=0.00\%$, and $H^2=1.00$, indicating no significant heterogeneity among the studies. However, the test of homogeneity (Q test) yielded $Q(4)=12.48$, $p=0.01$, suggesting the presence of slight but statistically significant differ-

ences in study effects. The overall effect test (z-test) resulted in $z = 5.83$, $p = 0.00$, demonstrating that EA significantly increased the Bcl-2/Bax ratio (and thus reducing apoptosis) in stroke animal models. Each study's effect size, represented by triangles, and the overall effect, represented by a diamond, are visualized in Fig. 3. Most studies showed that EA elevated the Bcl-2/Bax ratio, contributing to a significant overall positive effect size.

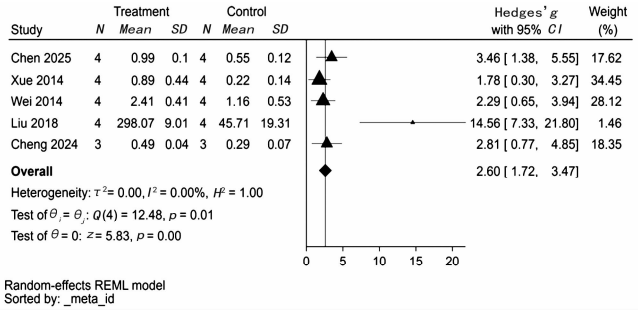

Fig. 3 Electroacupuncture effects on Bcl-2/Bax ratio in stroke animal models

3.4 Subgroup analyses

3.4.1 Intervention timing. This forest plot presents subgroup analyses of EA's effects on post-stroke apoptosis (assessed by TUNEL assay) in stroke-modeled animals, stratified by the timing of EA administration: after MCAO and before MCAO. For the "after MCAO" subgroup, 16 studies were included. The overall Hedges' g was -3.16 (95% CI: -4.05 to -2.27), with heterogeneity values of $\tau^2 = 2.27$, $I^2 = 74.50\%$, $H^2 = 3.92$, and a significant test of heterogeneity [$Q(15) = 52.27$, $p = 0.00$]. The "before MCAO" subgroup contained 11 studies, showing an overall Hedges' g of -3.67 (95% CI: -4.85 to -2.50), with heterogeneity values of $\tau^2 = 3.03$, $I^2 = 79.63\%$, $H^2 = 4.91$, and a significant heterogeneity test [$Q(10) = 44.23$, $p = 0.00$]. Both subgroups demonstrated significant reductions in TUNEL-detected apoptosis with EA, as indicated by z-test values (-6.97 for treatment after MCAO; -6.11 for treatment before MCAO) and $p = 0.00$. The test for group differences [$Q(1) = 0.46$, $p = 0.50$] suggested no statistically significant difference in effect sizes between the two timing subgroups (Fig. 4). Overall, EA, whether applied before or after MCAO, effectively mitigates post-stroke apoptosis in animal models, with comparable efficacy between the two timing strategies.

3.4.2 EA wave. This forest plot presents subgroup analyses of the meta-analysis exploring the effects of EA waveforms (continuous wave *vs.* disperse wave) on post-stroke apoptosis, as measured by TUNEL assay, in stroke-modeled animals. The continuous wave subgroup included 5 studies. The overall Hedges' g was -4.72 (95% CI: -6.48 to -2.97), with heterogeneity values of $\tau^2 = 2.58$, $I^2 = 87.90\%$, and $H^2 = 3.12$. The test of heterogeneity [$Q(4) = 11.32$, $p = 0.02$] indicated significant heterogeneity, and the overall effect test ($z = -5.27$, $p = 0.00$) showed that continuous wave EA significantly reduced TUNEL-detected apoptosis. For the disperse wave subgroup, 22 studies were analyzed. The overall Hedges' g was -3.10 (95% CI: -3.84 to -2.35), with heterogeneity values of $\tau^2 = 2.30$, $I^2 = 76.34\%$, and $H^2 = 4.23$. The test of heterogeneity [$Q(21) = 76.72$, $p = 0.00$] was significant, and the overall effect test ($z = -8.12$, $p = 0.00$) demonstrated that disperse wave EA also effectively decreased post-stroke apoptosis. The test for group differences [$Q(1) = 2.79$, $p = 0.09$] suggested a trend, though not statistically significant, toward different effect sizes between the two waveform subgroups (Fig. 5). Overall, both continuous and disperse wave EA can mitigate post-stroke apoptosis in animal models, with potential differences in efficacy that warrant further exploration.

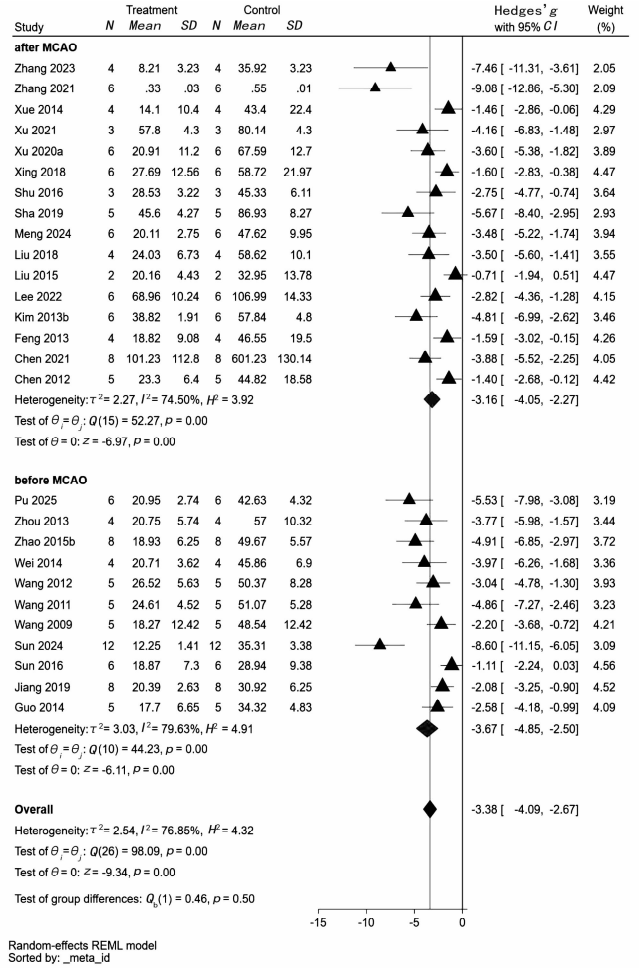

Fig. 4 Electroacupuncture timing (pre- *vs.* post-MCAO) on TUNEL-detected apoptosis in stroke animal models

4 Discussion

This meta-analysis aimed to comprehensively evaluate the effects of EA on post-stroke apoptosis in animal models. Our primary finding was that EA significantly reduced apoptosis in stroke-induced animals, as indicated by multiple apoptosis-related markers, including TUNEL, Caspase-3 expression, and the Bcl-2/Bax ratio.

The significant anti-apoptotic effect of EA observed in our study aligns with findings from previous basic research[10]. For example, studies on the neuroprotective mechanisms of acupuncture have shown that it can modulate various signaling pathways related to cell survival and death. The reduction in Caspase-3 expression after EA treatment observed in our analysis supports the view that EA may inhibit the activation of the apoptotic cascade[34]. Caspase-3 is a key executor of apoptosis, and its down-regulation implies a potential blockade of the apoptotic process. Additionally, the increase in the Bcl-2/Bax ratio further supports the anti-apoptotic effect of EA. Bcl-2 is an anti-apoptotic protein, whereas Bax is a pro-apoptotic one. An elevated Bcl-2/Bax ratio shifts the balance toward cell survival, suggesting that EA may promote the survival of neurons in the post-stroke environment[17].

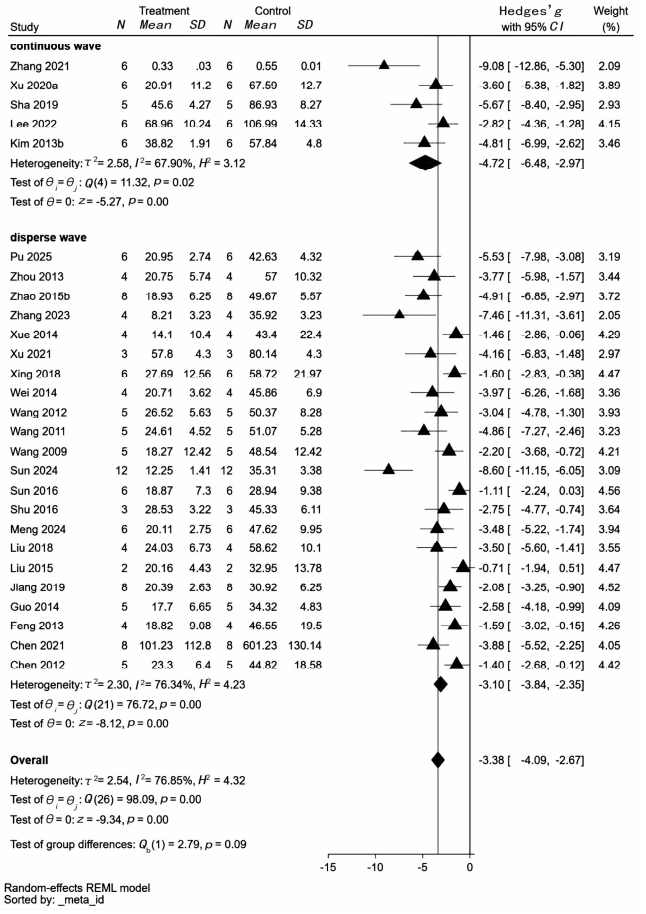

Fig. 5 Effects of electroacupuncture waveforms (continuous *vs.* disperse) on TUNEL-assessed apoptosis in stroke animal models

However, there are also some differences compared to several existing studies. Some previous research has reported inconsistent results regarding the optimal timing or waveform of EA[13]. In our subgroup analysis, although EA administered both before and after MCAO showed anti-apoptotic effects, the difference in their effectiveness was not statistically significant. This may be due to the relatively small number of studies in each subgroup, which limits the the power to detect subtle differences. Regarding the waveform, both continuous and disperse wave EA reduced apoptosis, but the test for group differences showed a non-significant trend toward different effect sizes. This could be attributed to variations in experimental conditions across studies, such as differences in the intensity, frequency, and duration of EA, as well as the species and strain of experimental animals.

The results of this meta-analysis have important implications for stroke research. Firstly, at the basic research level, they provide further evidence supporting the potential neuroprotective mechanisms of EA, which may help in the development of new therapeutic strategies for stroke. Secondly, from a translational perspective, if these findings can be replicated in clinical trials, EA could serve as a promising adjunctive therapy for stroke patients. It may help reduce neuronal apoptosis, promote neurological recovery, and improve the overall prognosis for these patients.

Mechanistically, the anti-apoptotic effects of EA are likely mediated through interconnected pathways. The PI3K/Akt pathway, frequently implicated in EA studies, is a plausible candidate: activation of Akt phosphorylates Bad, preventing its translocation to the mitochondria and thus inhibiting the Bcl-2/Bax imbalance[23]. EA may also suppress NF-κB activation, thereby reducing the release of pro-inflammatory cytokines and indirectly limiting cytokine-induced apoptosis. Furthermore, modulation of TRPV1 channels—shown in recent studies to regulate calcium influx and mitochondrial function—could contribute to EA's effects, as calcium overload is a key trigger of apoptotic cascades[11]. The interplay between these pathways warrants further investigation to identify the primary targets for optimizing EA protocols.

Despite these significant findings, our study has several limitations. A major limitation is the high heterogeneity among the included studies. The heterogeneity metrics (such as I^2 and H^2 values) for different outcomes and subgroups were relatively high in many cases. This could be attributed to multiple factors. For example, the included studies varied in terms of the animal species and strains used, with some using rats and others using mice. Different species may exhibit varying physiological responses to stroke and EA. Additionally, the experimental procedures for inducing stroke (*e. g.*, the method and duration of MCAO), the parameters of EA (including frequency, intensity, duration, and treatment schedule), and the time points for assessing apoptosis also differed among studies. To address this heterogeneity, we conducted subgroup analyses based on factors like EA timing and waveform. However, the high heterogeneity still limits the precision of our pooled estimates and the generalizability of the results.

Furthermore, most of the included studies were conducted using animal models, and there is a significant gap between animal experiments and clinical applications. The physiological and pathological characteristics of animals may not fully replicate those of human stroke patients. Therefore, the translation of these findings to the clinical setting requires further validation through well-de-

signed clinical trials.

For future research, it is recommended to conduct more high-quality animal studies using standardized experimental procedures. This includes using a single species or strain of animals, clearly defining the stroke induction method, standardizing the EA parameters, and improving the blinding and randomization methods. In addition, large-scale, multi-center clinical trials should be carried out to confirm the effectiveness of EA in reducing post-stroke apoptosis in humans. These clinical trials should also focus on optimizing the EA treatment protocol by determining the optimal timing, frequency, and duration of treatment. Moreover, further exploration into the underlying molecular mechanisms of EA's anti-apoptotic effects is necessary. This could involve studying the regulation of other apoptosis-related genes and proteins, as well as the roles of neurotransmitters and signaling pathways in the EA-mediated neuroprotection.

In conclusion, this meta-analysis provides evidence for the anti-apoptotic effects of EA in post-stroke animal models. However, due to the limitations mentioned above, further research is warranted to fully elucidate the potential of EA as a therapeutic option for stroke. Our study highlights the need for future research to bridge the gap between basic research and clinical practice and to optimize the application of EA in stroke treatment.

5 Conclusions

EA exerts significant anti-apoptotic effects in animal models of ischemic stroke, as demonstrated by a reduction in TUNEL-positive cells and caspase-3 levels, and an increase in Bcl-2 expression and the Bcl-2/Bax ratio. These effects are most pronounced in transient ischemic models, where mixed-frequency EA administered post-ischemia yields the greatest benefits. The findings underscore EA's potential as a neuroprotective strategy and provide a foundation for future clinical trials to refine its application in stroke rehabilitation.

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Table 4 Toxicological study of the small molecule inhibitor

Dose//mg/kg	Toxicity manifestation	Main observations	Assessment
0	None	No abnormalities	Non-toxic
10	Mild	Decreased appetite, mild weight loss	Safe
25	Moderate	Significantly decreased appetite, marked weight loss	Safe
50	Severe	Extreme weakness, rapid weight loss, shortness of breath	Not recommended

5 Conclusion

This study conducted an in-depth exploration of the synthetic process for PD-L1 small molecule inhibitors, focusing on optimizing reaction conditions and synthetic routes, and exploring efficient and green synthetic pathways, thereby providing a potential new solution for tumor immunotherapy. Through the optimization of pharmacophore design, the affinity and selectivity of the molecules for PD-L1 were successfully improved, significantly enhancing inhibitory activity. Preclinical studies demonstrated that the synthesized inhibitors exhibit good antitumor efficacy and safety both *in vitro* and in animal models, supporting their potential for application in future clinical trials. Looking ahead, with the continuous advancement of synthetic technologies and the application of precision design methods, PD-L1 small molecule inhibitors are expected to become an important weapon in the field of tumor immunotherapy.

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