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REVIEW

Understanding the Relationship Between Vascular Smooth Muscle Cell Function and the Efficacy of Acupuncture in Treating Cerebral Ischemic Stroke: A Preclinical Meta-Analysis and Systematic Review

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Video abstract

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Background: Cerebral blood flow and vascular structures serve as the fundamental components of brain metabolism and circulation. Acupuncture, an alternative and complementary medical approach, has demonstrated efficacy in treating cerebral ischemic stroke (CIS). Nevertheless, the mechanisms underlying the impact of acupuncture on vascular smooth muscle cell (VSMC) function remain uncertain. The objective of this systematic review and meta-analysis is to assess the alterations in VSMC function following acupuncture stimulation in CIS models.

Methods: The databases PubMed, Web of Science, SCOPUS, and EMBASE were queried until November 2022 using a predetermined search strategy. The FORMAT BY SYRCLE guidelines were adhered to, and the risk of bias of the included studies was evaluated using the Risk of Bias tool developed by the Systematic Review Centre for Laboratory Animal Experimentation. The random-effects model was employed to estimate the standardized mean difference (SMD).

Results: Eighteen articles are included in this review. Acupuncture showed significant positive effects on the region cerebral blood flow (SMD=8.15 [95% CI, 4.52 to 11.78]) and neurological deficiency (SMD=−3.75 [95% CI, −5.54 to −1.97]). Descriptive analysis showed a probable mechanism of acupuncture stimulation in CIS rats related to VSMC function. Limitations and publication bias were presented in the studies.

Conclusion: In this systematic review and meta-analysis, our findings indicate that acupuncture stimulation has the potential to improve regional cerebral blood flow and alleviate neurological deficits, possibly by regulating VSMC function. However, it is important to exercise caution when interpreting these results due to the limitations of animal experimental design and methodological quality.

Keywords: acupuncture therapy, vascular smooth muscle cell, vasomotor, cerebral ischemic stroke, systematic review

Introduction

Although the incidence, prevalence, and mortality of stroke have shown a decline in the United States,[1] it remains the second most prevalent cause of mortality and a significant contributor to disability on a global scale.[2] Furthermore, the Global Burden of Disease Study 2019 conducted in China reveals a noteworthy increase of 86.0% in the incidence rate and 32.3% in the mortality rate of stroke between 1990 and 2019.[3] Cerebral ischemic stroke (CIS) is a prevalent

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subtype of stroke characterized by a significant vascular incident that hinders or completely obstructs blood supply to brain tissue, leading to infarction, thromboembolic stroke, or atherosclerotic stroke.[4] The optimal approach for managing CIS involves reestablishing blood flow in the occluded artery, with intravenous alteplase being the sole FDA-approved medication capable of ameliorating acute ischemic stroke-related disability without impacting mortality.[5,6] The increasing global popularity and aging population contribute to a significant rise in the number of individuals affected by CIS, particularly in China. This surge in cases poses a substantial financial burden on both the Chinese public health system and affected families. Consequently, it is crucial to explore affordable and effective treatment options for CIS.[7]

Acupuncture, a widely utilized approach, has shown potential in providing neuroprotective and anti-inflammatory effects for CIS patients.[8,9] In recent years, there has been a disproportionate emphasis on enhancing brain metabolism, particularly in relation to "ischemia reperfusion injury", while neglecting the equal significance of brain circulation. Even when attention is directed towards brain circulation, it primarily revolves around enhancing the structural aspects of blood flow, disregarding the crucial role of vessel function. It is important to recognize that vessels are not merely conduits, but rather integrated organs with vital functions such as vasomotor regulation and intricate secretory processes, which significantly contribute to brain circulation. The mechanism by which vascular smooth muscle cell (VSMC) function influences the efficacy of acupuncture in the treatment of CIS remains uncertain.

Given the limited availability of high-quality evidence pertaining to the efficacy, safety, and underlying mechanism of the treatment under investigation, we chose to focus our research on a permanent CIS model. Our objective was to conduct a preclinical systematic review and meta-analysis of relevant studies in order to assess and compare the VSMC function between the acupuncture group and the model group.

Methods

The review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Table S1).[10] The protocol design followed the FORMAT BY SYRCLE (WWW.SYRCLE.NL) (Table S2). Supplemental Material provides detailed information.

Data Sources and Searches

PubMed, Web of Science, SCOPUS, and EMBASE were chosen as electronic databases from their inception until December 6, 2022. The search terms and basic search strategy were as follows: ("cerebral ischemic" OR "ischemic stroke" OR "brain infarction" OR "apoplexy" OR "brain ischemia" OR "cerebrovascular accident") AND ("animal study" OR "rat" OR "mice" OR "mechanism" OR "preclinical study") AND ("acupuncture" OR "acupuncture therapy" OR "electroacupuncture" OR "acupoints") AND ("vasodilatation" OR "vascular function"). Further details regarding the search strategies can be found in Table S3. No restrictions were imposed on language, publication date, or publication status. Additionally, the reference lists of included studies and relevant reviews were examined to ensure eligibility, while irrelevant studies were excluded.

Inclusion Criteria

(1) Study Design: This study employs randomized controlled trials to assess the function of VSMC in rats with permanent CIS. (2) Animal Selection: The study includes healthy rats or mice of any gender and age, which have been established with a permanent CIS model (compared to the MCAO model, the nylon suture was not removed from the cranial cavity). (3) Intervention Type: The interventions utilized in this study are acupuncture or electroacupuncture. (4) Outcome Measures: The evaluation of the CIS model includes monitoring regional cerebral blood flow (rCBF) using laser-Doppler or Zea Longa scores, assessing neurological deficits (symptoms caused by cerebral infarction, such as motor dysfunction, cognitive decline, sensory disorder, etc) using modified neurological severity score (mNSS) or Longa scores, and examining vascular variables such as the assessed region, technique used, and VSMC function. (5) Language Restriction: There is no restriction on the language used in this study. (6) Publication date restrictions: all publication dates.

Exclusion Criteria

Review; not an in vivo animal study; human research; not a permanent CIS model; without a model group;

Study Selection, Extraction and Data Collection

Two investigators (Cao JP and Chen LL) conducted a thorough examination of all abstracts and full texts in accordance with the predetermined inclusion and exclusion criteria. Any discrepancies were resolved through discussion or by seeking input from a third investigator (Du YH). The relevant information from the studies was extracted by the two investigators using a pre-designed Excel sheet, with the following items being collected: Study ID, characteristics of the study design (such as experimental groups, number of animals, randomization), characteristics of the animal model (such as species, gender, age, weight, method of cerebral ischemia induction), and characteristics of the intervention (such as duration of acupuncture treatment).

Assessment Risk of Bias

The methodological quality of the included studies was assessed using the Systematic Review Centre for Laboratory Animal Experimentation's Risk of Bias tool.[11] This tool encompasses items related to selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. Two investigators (Cao JP and Zhang M) independently provided evaluations of "yes", "no", or "unclear" to assess the risk of bias, indicating low, high, or insufficient details for assessment, respectively. Any discrepancies were resolved through discussion.

Data Analysis

The included studies were analyzed using Stata 12.0 software (College Station, Texas, USA) with standardized mean difference (SMD) and 95% confidence intervals (CI). The heterogeneity among the studies was assessed using the I^2 statistics, where $I^2>75\%$ represented high heterogeneity, $50\%<I^2\leq75\%$ represented moderate heterogeneity, and $25\%<I^2\leq50\%$ represented low heterogeneity. The Z score was utilized to assess the overall effect, with a significance level of $P<0.05$ indicating statistical significance. In cases where substantial heterogeneity was observed among the included studies, the random effects model was employed to analyze the pooled data. To assess publication bias, Egger's test and funnel plot analysis were conducted. Additionally, subgroup analysis was performed to examine variations in study characteristics, such as species, weight, reference for model establishment, model assessment, test technique for outcomes, and electroacupuncture parameters. Median values of continuous variables are employed in the analysis. Z score represents the testing for an overall effect and the $P<0.05$ considered as significant. The random effects model was applied to calculating the overall effect size of the set of studies. Egger's test and funnel plot were conducted to evaluate the publication bias and the heterogeneity. And the subgroup analysis was carried out based on the differences of included study's characteristics group by species, weight, the reference for the model establishment, model assessment, the test technique of outcomes and electroacupuncture parameters. The grouping of studies was based on the utilization of median values as cut-off points for continuous variables. In cases where the meta-analysis was not feasible, descriptive analysis was conducted.

Results

Study Selection

The search process is visually represented in Figure 1, which displays the flow diagram of our search results. Initially, a total of 238 articles were obtained from the PubMed, Web of Science, SCOPUS, and EMBASE databases. Subsequently, 86 duplicate articles were eliminated during the screening stage. Following the evaluation of titles and abstracts, an additional 59 articles were excluded. Finally, after a thorough examination of the full-text articles, 75 more articles were excluded based on the predefined inclusion and exclusion criteria. We finally got 18 articles as our included studies.[12–29]

Characteristics of Included Studies

A comprehensive review of the literature was conducted, resulting in the inclusion of a total of 18 studies obtained through database searches (Tables S4 and S5). The publication years of these studies ranged from 2008 to 2022. Among the selected studies, 6 utilized SD rats for model establishment,[15,16,22,25–27] while 12 studies employed Wistar rats.[12–14,17–21,23,25,28,29] One study employed both female and male rats,[13] while the remaining studies exclusively used male rats. Additionally, 6 studies provided information on the age of the rats.[15,16,18,21,26,28]

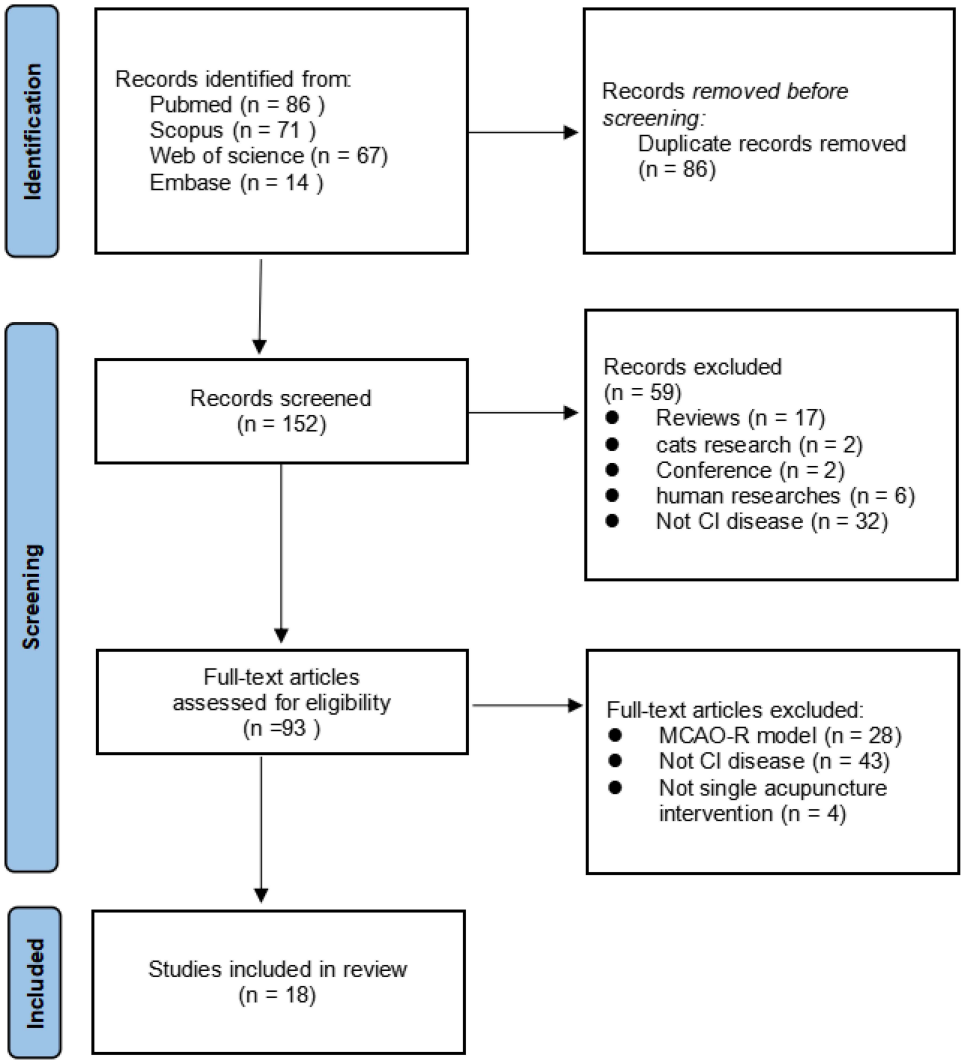

Figure 1 Flow-chart illustrating the literature search strategy and the different phases of study eligibility assessment.

In terms of surgical modeling methods, two types were identified: one study employed bilateral common carotid artery occlusion,[16] while the remaining studies utilized middle cerebral artery occlusion. Three studies were conducted to measure the volume of cerebral infarct, with 2 studies utilizing 2,3,5-Triphenyltetrazolium Chloride,[13,24] and 1 study employing magnetic resonance imaging for evaluation.[26] Additionally, 7 studies examined the rCBF,[14,18,19,21,24,27,28] while 2 studies assessed the micro-vessel density in the cerebral ischemic region.[15,27] The weight of the included rats ranged from 190 gram (g) to 300 g, and from the picture, we can find that the majority of the weight ranged from 190 g and 200 g (Figure 2A). The model assessment involved the utilization of five different methods to determine the success of the established model. Specifically, 5 studies employed the Zea Longa scores,[15,18,19,24,26] where scores ranging from 1 to 3 were considered indicative of a successful model.[30] Additionally, 4 studies deemed a decrease in rCBF to 20–30% of the baseline values,[14,17,26,29] as measured by laser Doppler flowmetry, as a successful model. Three studies utilized the modified neurological severity score,[23,27,28] which ranged from 2 to 18, to assess model success. One study employed the Zausinger scores,[20,31] with scores of 1 to 3 indicating a successful model. Furthermore, 1 study considered the presence of the Homer sign (+) as indicative of a successful model.[13] Lastly, 4 studies did not specify the criteria for determining model success (Figure 2B).[12,16,21,22] In terms of the model reference, 14 studies cited the Longa EZ model,[12,14,15,17–25,27,28] while 1 study cited de la Torre,[16,32] 1 study cited Chen ST,[13,33] 1 study cited Xiao ZY,[26,34] and 1 study did not provide detailed information regarding their model establishment (Figure 2C).[29] Regarding the frequency

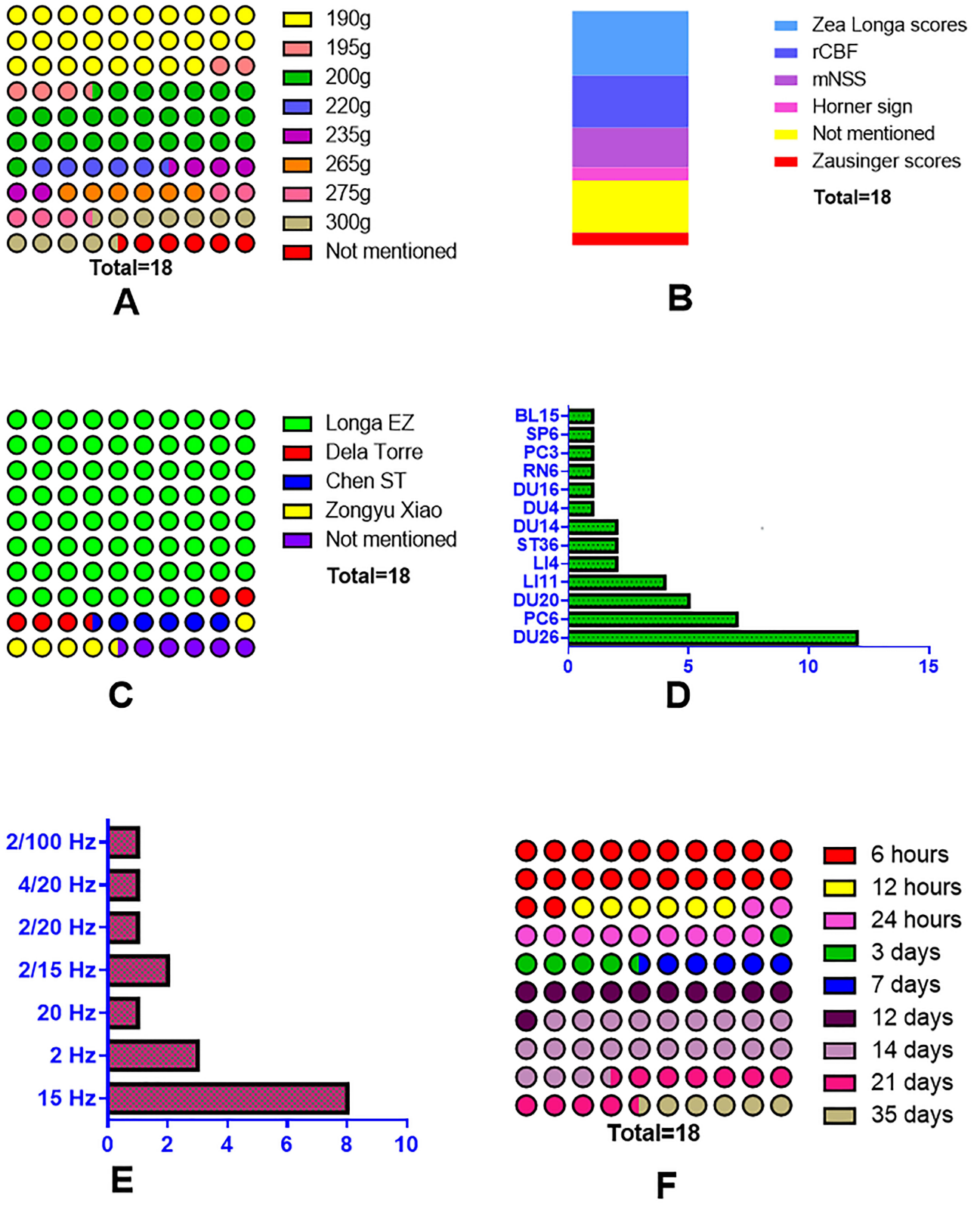

Figure 2 Study characteristic. (**A**) The average weight of rats, (**B**) Model assessment, (**C**) Model reference, (**D**) Frequency of acupoint use, (**E**) The frequency of electroacupuncture, (**F**) Course of treatment.

of acupoint use, a total of 13 acupoints were utilized across the included studies. The four most commonly used acupoints were DU26 (12 occurrences), PC6 (7 occurrences), DU20 (5 occurrences), and LI11 (4 occurrences) (Figure 2D). As for the frequency of electroacupuncture, with the exception of 1 study which employed manual acupuncture,[13] the remaining studies utilized electroacupuncture (EA). In 5 studies, the application of distant-dense wave was utilized, with EA frequencies of 2/15 Hz,[18,19] 2/20 Hz,[24] 4/20 Hz,[27] and 2/100 Hz.[26] The remaining studies employed continuous wave, with EA frequencies of 2 Hz,[16,21,22] 20 Hz,[15] and 15 Hz (Figure 2E).[12,14,17,20,23,25,28,29] Regarding the course of treatment, 18 included studies provided specific timeframes for each treatment, typically lasting between 20 and 30 minutes. Additionally, the overall duration of the treatment primarily spanned 6 hours and 14 days following the modeling process (Figure 2F).

Risk of Bias Assessment

The quality and reporting of the 18 included studies were evaluated using the SYRCLE's RoB tool (Table S6). In general, the studies exhibited suboptimal quality. All of the studies demonstrated satisfactory performance in terms of baseline characteristics, selective outcome reporting, free of contamination, free of the unit of analysis errors and design-specific risks of bias absence. However, the studies did not provide evidence of allocation concealment, performance and detection blinding, incomplete outcome data, and free of inappropriate influence of funders. Random allocation was mentioned in all studies, with half of them describing the randomization method as a random number table.[16,18–20,25–29] Two studies mentioned the random housing,[15,23] 2 studies mentioned random outcome assessment,[26,27] and 1 study mentioned new animals added to replace drop-outs.[26]

Meta-Analysis, Heterogeneity and Risk for Publication Bias

rCBF

A meta-analysis utilizing a random-effects model was performed, incorporating a total of 6 studies.[18,19,21,24,27,28] The findings of this analysis indicated a significant positive effect of acupuncture on the improvement of rCBF (SMD=8.15, [95% CI 4.52 to 11.78]; $P<0.001$, $I^2=90.5\%$; Figure S1A), while also highlighting a notable degree of heterogeneity among the included studies. To further investigate potential publication bias, an Egger's test (Figure S1B) and funnel plot (Figure S1C) were conducted, both of which suggested the presence of such bias.

Neurological Deficiency

Additionally, the meta-analysis, which encompassed 7 studies,[14,18,19,21,26–28] demonstrated a clear amelioration of neurological deficiency in rats with CIS (SMD=−3.75, [95% CI −5.54 to −1.97]; $P<0.001$, $I^2=89.6\%$; Figure S2A). The high level of heterogeneity among the studies was quantified with an I^2 value of 89.6%. Furthermore, the P value obtained from the Egger's test (Figure S2B), along with the asymmetrical distribution observed in the funnel plot (Figure S2C), provided evidence of publication bias.

Subgroup Analysis

Considering the various testing methodologies employed, a subgroup analysis was conducted on the BDNF. The collective findings from the included studies indicated a significant enhancement in BDNF expression through acupuncture (SMD=1.19, [95% CI −0.29 to 2.67]; $P<0.001$, $I^2=86.9\%$; Figure S3A). Two studies utilized immunohistochemistry (IHC) to measure grey value and positive cell count, both of which exhibited minimal heterogeneity ($I^2=0\%$); however, the observed effect did not reach statistical significance ($P=0.621$, $P=0.689$ respectively). Conversely, 1 study employed ELISA and qRT-PCR to assess BDNF expression. The Egger's test (Figure S3B) and funnel plot (Figure S3C) did not reveal any indication of publication bias.

The subgroup analysis was conducted to examine the impact of acupuncture on VEGF, as divided by various testing methods. The findings indicated a general effect of acupuncture stimulation in CIS rats, leading to an increase in VEGF expression (SMD=4.47, [95% CI 2.54 to 6.39]; $P<0.001$, $I^2=93.8\%$) (Figure S4A). Notably, there was a tendency for a reduction in heterogeneity in the expression of grey value as measured by IHC ($I^2=76.9\%$), which reached statistical significance ($P=0.037$). However, no statistical significance was observed in the expression of AOD and the number of

positive cells as measured by IHC (P=0.788, P=0.267 respectively). Additionally, the P value of Egger's test (Figure S4B) and the asymmetrical distribution of the funnel plot (Figure S4C) indicated a publication bias.

In the study, various model references were utilized for CIS rats. Subgroup analysis was conducted based on the specific model reference employed. Three studies utilized Zea Longa scores (SMD=6.08, [95% CI 3.36 to 8.79]; P<=0.026, I^2=72.6%), while 2 studies employed mNSS (SMD=16.01, [95% CI 9.55 to 22.47] P=0.098, I^2=63.4%), both of which effectively mitigated heterogeneity to a moderate extent (Figure S5A). The study encompassed two species of rats. Subgroup analysis was performed based on the different species, namely Wistar rats (SMD=6.94, [95% CI 2.77 to 11.12]; P<0.001, I^2=87.8%) and SD rats (SMD=11.47, [95% CI −3.33 to 26.26]; P<0.001, I^2=96.3%), both of which exhibited high levels of heterogeneity (Figure S5B). The CIS rats were categorized into two weight types, namely 190–220g and 220–300g. Subgroup analysis was conducted according to weight, revealing those rats weighing 190–220g (SMD=8.77, [95% CI 4.74 to 12.80]; P=0.030, I^2=71.4%) demonstrated a moderate reduction in heterogeneity. The rats weighing 220–300g (SMD=7.58, [95% CI 1.82 to 13.34]; P<0.001, I^2=94.4%) remain a high heterogeneity (Figure S5C). Additionally, two EA waveforms are utilized in the treatment of CIS rats. Subgroup analysis was conducted based on the EA waveforms, namely distant-dense wave (SMD=9.06, [95% CI 4.42 to 13.70]; P<0.001, I^2=90.5%) and continuous wave (SMD=6.94, [95% CI −3.54 to 17.42]; P<0.001, I^2=92.0%), both of which exhibit significant heterogeneity (Figure S5D).

The CIS rat model encompasses two model assessments. Subgroup analysis was performed according to the different model assessments. One study employed rCBF, one study did not specify, and the remaining studies employed mNSS (SMD=−2.25, [95% CI −3.91 to −0.60]; P=0.057, I^2=72.5%), resulting in a reduction of heterogeneity to a moderate level (Figure S6A). The neurological deficiency of CIS rats was evaluated using two types of assessments. Subgroup analysis was conducted based on the varying Zea Longa scores (SMD=−4.22, [95% CI −8.24 to −0.21]; P<0.001, I^2=93.8%) and mNSS scores (SMD=−3.50, [95% CI −5.84 to −1.35]; P<0.001, I^2=87.6%), both of which exhibited high heterogeneity (Figure S6B). The study included two species of rats, Wistar rats (SMD= −3.16, [95% CI −5.26 to −1.07]; P<0.001, I^2=89.5%) and SD rats (SMD=−5.41, [95% CI −10.22 to −0.60]; P<0.001, I^2=91.8%), and subgroup analysis was performed according to the different species, revealing high heterogeneity in both (Figure S6C). The CIS rats were categorized into two weight types, 190–220g (SMD=−3.36, [95% CI −5.99 to −0.73]; P<0.001, I^2=92.1%) and 220–300g (SMD=−4.32, [95% CI −7.00 to −1.65]; P<0.001, I^2=86.0%), and subgroup analysis based on weight demonstrated that both groups exhibited high heterogeneity (Figure S6D). In the study, two different model references were utilized for CIS rats. Subgroup analysis was conducted based on the chosen model reference. One study employed Zongyu Xiao's reference, while the remaining studies utilized Zea Longa scores (SMD=−3.12, [95% CI −4.77 to −1.47]; P<0.001, I^2=87.4%). Despite this, a significant level of heterogeneity persisted (Figure S6E). Additionally, two distinct EA waveforms were employed for treating the CIS rats. Subgroup analysis was performed based on the EA waveforms. The distant-dense wave (SMD=−3.83, [95% CI −6.31 to −1.36]; P<0.001, I^2=91.0%) and continuous wave (SMD=−3.71, [95% CI −7.09 to −0.34]; P<0.001, I^2=91.7%) both exhibited high levels of heterogeneity (Figure S6F).

Descriptive Analysis

Descriptive analysis was employed to present the findings of the study, which are not amenable to synthesis. In comparison to the control group, the effects of acupuncture intervention on CIS rats were determined as follows: 2 studies demonstrated that acupuncture effectively reduces the levels of inositol triphosphate (IP3) and diacylglycerol (DAG).[12,28] Additionally, 1 study revealed a significant increase in the proliferation of vascular endothelial cells (VECs) following acupuncture.[14] Furthermore, another study suggested that acupuncture enhances the expression of miR-494-3p in CIS rats.[21] Two studies have found that acupuncture has a significant impact on the expression of protein kinase C (PKC).[17,20] Additionally, 1 study has indicated that acupuncture can greatly elevate the levels of serum insulin and blood glucose.[18] One study has demonstrated an ameliorating effect on the expression of cytochrome P450 epoxygenase (CYP2C11) following acupuncture stimulation.[19] One study has suggested that acupuncture can significantly enhance the expression of Apelin and APJ.[23] One study has shown an increased effect of acupuncture on the expression of vascular endothelial growth factor receptor 2 (VEGFR2) and basic fibroblast growth factor (bFGF).[24] One study revealed a significant increase in the levels of soluble guanylate cyclase (sGC), cyclic guanosine monophosphate (cGMP), and protein kinase G (PKG) in the acupuncture group.[25] Another study demonstrated an enhanced impact on glial fibrillary acidic protein (GFAP), neuronal nucleus antigen (NeuN), β-catenin, and a reduction in Axin2

following acupuncture stimulation.[26] Additionally, a separate study indicated a notable decrease in the expression of G-protein subtype (Gq), calmodulin (CaM), and the concentration of intracellular Ca^{2+} in the acupuncture group.[28] Lastly, a study discovered that acupuncture has the potential to diminish the expression of myosin light chain (MLCK), myosin-ATPase activity, MLC phosphorylation, and three subunits of myosin light chain phosphatase (MLCP) (MYPT1, PP1c-δ, M20).[29]

Discussion

A systematic review and meta-analysis were conducted to evaluate the impact of acupuncture on VSMC function in the permanent CIS model. The primary outcome of this meta-analysis revealed that acupuncture stimulation effectively improves rCBF and neurological deficits in CIS rats. Additionally, the meta-analysis demonstrated that acupuncture stimulation enhances the expression of VEGF and BDNF in CIS rats. Our descriptive analysis provides valuable evidence suggesting that acupuncture stimulation may promote the phenotypic transformation of VSMCs in the CIS model (Figure 3).

rCBF

rCBF is a direct indicator of the condition of regional cerebral tissue. In this literature review, one study employed a laser confocal microscope, while the remaining studies utilized laser Doppler flowmetry to measure rCBF. The meta-analysis demonstrated a positive impact of rCBF on the efficacy of acupuncture treatment, and the substantial heterogeneity observed could be mitigated to a moderate level through subgroup analysis based on model reference. Furthermore, it is noteworthy that the substantial heterogeneity observed can be mitigated to a moderate level within the subgroup of rats weighing between 190

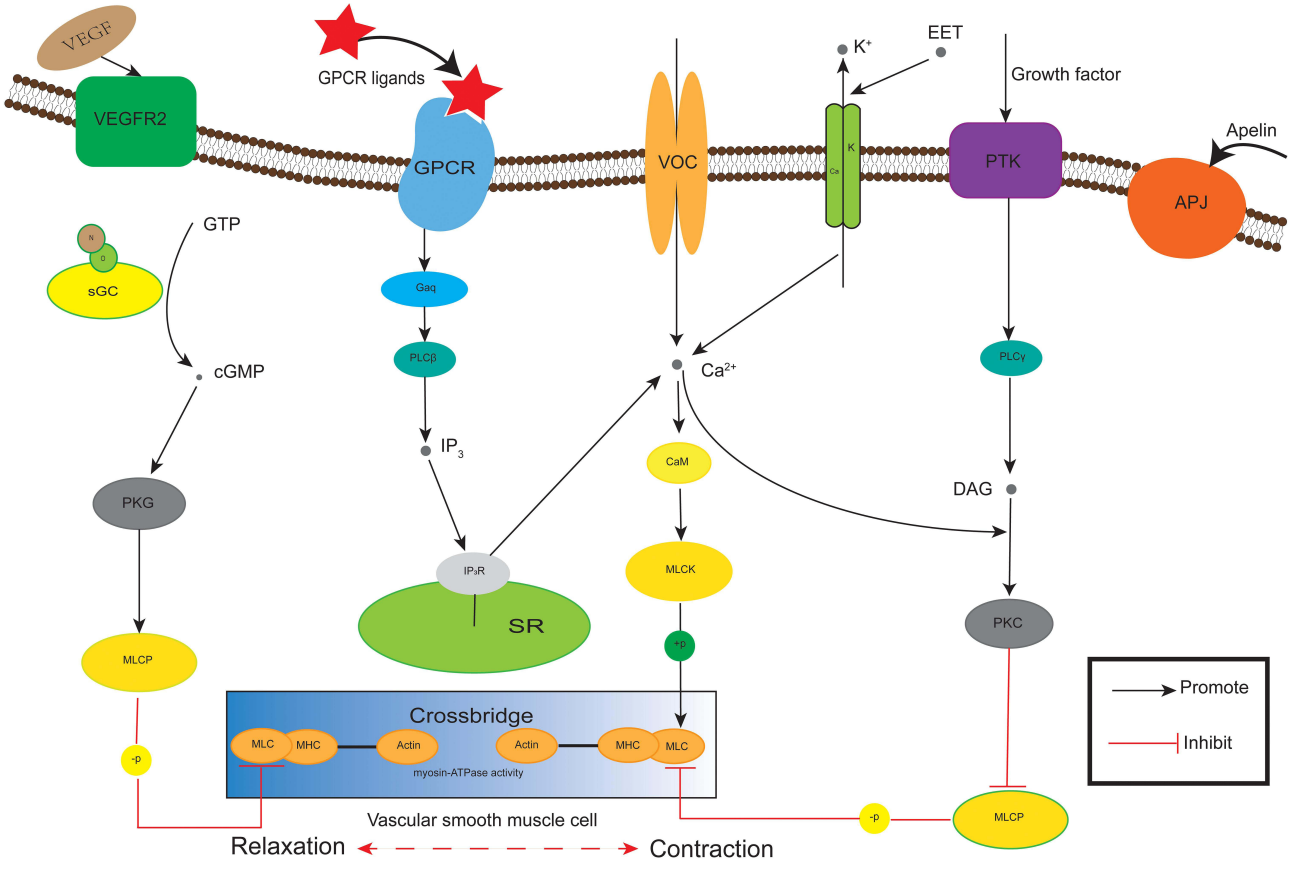

Figure 3 The mechanism of vascular smooth muscle cell contraction.

Abbreviations: VEGF, Vascular endothelial growth factor; VEGFR2, Vascular endothelial growth factor receptor 2; GTP, Guanosine triphosphate; sGC, Soluble guanylate cyclase; cGMP, Cyclic guanosine monophosphate; PKG, Protein kinase G; MLCP, Myosin light chain phosphorylation; GPCR, G protein-coupled receptors; Gαq, G-protein subtype; PLC, Phospholipase C; IP3, Inositol 1,4,5-trisphosphate; SR, Sarcoplasmic reticulum; MLC, Myosin light chain; MHC, Myosin heavy chain; CaM, Calmodulin; MLCK, Myosin light chain kinase; DAG, Diacylglycerol; PKC, Protein kinase C.

and 220 g. Conversely, the presence of significant heterogeneity in rats weighing above 220 g prompts us to consider the selection of rat weight in future investigations.

Neurological Deficiency

The meta-analysis revealed a favorable impact on the enhancement of neurological deficiency. The inclusion of a subgroup focused on model reference effectively mitigated the high heterogeneity, while the subgroup utilizing the mNSS scale exhibited a moderate level of heterogeneity. The evaluation of motor function and reflexes in a closed head injury rat model employed the NSS,[35] which was subsequently modified as ΔNSS to indicate the disparity between NSS at 1 hour and any other time.[36,37] The mNSS encompasses a battery of assessments including motor, sensory, reflex, and balance tests. Each test is assigned a score of 1 point if the individual is unable to perform it or if a reflex is absent. Consequently, a higher score on the mNSS indicates a greater severity of injury.[38,39]

Probable Mechanism of Pro-Phenotypic Transformation of VSMC via Acupuncture

Descriptive analysis was employed to ascertain the underlying mechanisms of acupuncture in treating CIS rats with respect to VSMC function. It is widely acknowledged that myosin, comprising myosin heavy chain (MHC), 17-kD myosin light chain (MLC17), and 20-kD myosin light chain (MLC20), serves as the fundamental protein component of the thick filament. The MHC exhibits ATPase activity, which is involved in filament sliding. The MLC20 functions as the primary regulatory domain of myosin, facilitating phosphorylation and dephosphorylation through MLCK and MLCP, respectively. The relaxation and contraction of VSMC can be influenced either directly by vasoactive substances or indirectly through the endothelium. Regardless, it is crucial to enhance the activity of MLCP, reduce the activity of MLCK, and decrease the concentration of intracellular Ca^{2+} for VSMC relaxation.[40,41]

Calcium-Dependent Mechanism

In our literature review, a study demonstrated that acupuncture stimulation in CIS rats resulted in a vasodilatory effect.[29] This effect was potentially achieved by reducing the expression of MLCK, MLC phosphorylation, and myosin ATPase activity in the tested cerebral arteries (Willis ring, anterior, middle, and posterior) and their respective branches. Additionally, an elevation in intracellular Ca^{2+} concentration, either through influx in voltage-gated Ca^{2+} channels or release from the endoplasmic reticulum, facilitated the formation of a Ca^{2+}/CaM complex by binding to CaM. Then, the activation of MLCK by the Ca^{2+}/CaM complex initiates a cascade of events, including the phosphorylation of MLC20, the activation of myosin ATPase activity on the cross-bridge of the thick filament, and ultimately leading to the contraction of VSMC. In one study discussed in this review,[28] it was found that acupuncture can downregulate the concentration of intracellular Ca^{2+} and the expression of CaM in CIS rats, potentially exerting a relaxation effect on VSMC through the MCLK pathway. Additionally, the PI system, also known as the double messenger system, which consists of the DAG/PKC and IP3/Ca^{2+} signaling pathways, is closely associated with vasoconstriction. Gq functions as a molecular switch that is capable of activating phospholipase C (PLC), resulting in the production of DAG and IP3.[42] IP3 facilitates the release of Ca^{2+} from intracellular stores by binding to receptors on the endoplasmic reticulum, thereby increasing the concentration of intracellular Ca^{2+}. This, in turn, activates PKC along with DAG.[43] PKC, a group of serine/threonine kinases, not only induces VSMC contraction through calcium-dependent mechanisms but also enhances VSMC contraction by directly inhibiting MLCP activity or phosphorylating CPI-17.[44,45] In our review, we observed a positive impact of acupuncture on the downregulation of Gq, DAG, IP3, and PKC expression in a CIS model, leading to a decrease in intracellular Ca^{2+} concentration. An example of a G-protein-coupled receptor upstream of Gq is APJ. The apelin-APJ receptor signaling system is widely expressed in brain tissues and blood vessels,[46,47] yet the vasomotor actions of this system in cerebral arteries remain unclear. Our review indicates a prompt effect on the expression of apelin and APJ protein after the acupuncture stimulate in CIS rats of cerebral arteries.[23] A recent discovery has indicated that the potential mechanism for inhibiting the relaxation of cerebral arteries induced by NO may be associated with the activation of APJ receptors and the inhibition of BKCa channels.[48] This finding contrasts with previous studies conducted on peripheral arteries. Further research is needed to address the discrepancies between these studies. Additionally, CYP2C11 has the ability to convert arachidonic acid into epoxyeicosatrienoic acids, which can directly stimulate the opening of calcium-activated potassium channels on the surface of

VSMC. Consequently, this inhibits the contraction of VSMC.[49] Our review showed that acupuncture can increase the expression of CYP2C11 which can provide a protective effect on CIS model.[50]

Calcium-Independent Mechanism

In our review, 1 study demonstrated a vasodilatory effect of acupuncture stimulation in CIS rats,[29] potentially achieved by upregulating the expression of three subunits of MLCP (MYPT1, PP1c-δ, M20). Consequently, MLCP dephosphorylates MLC, thereby inhibiting the cross-bridge movement between actin and myosin.[51] MYPT1 plays a crucial role in binding and activating PP1c, which subsequently targets myosin. The inhibition of MLCP involves two major signaling pathways: phosphorylation of MYPT1 at Thr696 and Thr853 via the G12/13/RhoA/Rho-kinase pathway.[52,53] The primary signaling pathway responsible for inducing smooth muscle relaxation is the NO/cGMP/PKG pathway. Nitric oxide acts as a vasodilator and activates the sGC by binding to its specific receptor.[54] This activation leads to the conversion of guanosine triphosphate to cGMP, subsequently activating PKG, phosphodiesterases, and cGMP-dependent ion channels. These processes result in various effects, including muscle relaxation, bronchi and blood vessels dilation, and inhibition of platelet aggregation.[55,56] It is widely recognized that PKG has the capability to directly phosphorylate MYPT-1 in VSMC, offering a promising avenue for regulating MLC phosphorylation.[57,58] Within the context of this review, a study indicates that acupuncture has the ability to reduce the levels of sGC, cGMP, and PKG in rats with CIS, thereby suggesting a potential mechanism by which MYPT-1 may be phosphorylated through PKG.[25]

Other Factors

This study revealed that acupuncture has a notable impact on the proliferation of VEC in rats with CIS. The VEC, located in the inner layer of blood vessels, are separated by connective tissue and an internal elastic membrane from VSMC. VEC play a crucial role in vascular biology, including regulating vascular tone, promoting smooth muscle growth, and facilitating Ca^{2+} influx into VSMC.[59,60] Dysfunction of VEC can potentially affect the phenotypic transformation of VSMC.[61] However, the specific mechanism by which VECs regulate VSMC reactivity remains unclear. In recent decades, non-coding RNA molecules, particularly miRNAs, have garnered significant attention.[62] Numerous studies have explored the role of miRNAs in VSMC phenotypic transformation, revealing different channels of influence. For instance, certain miRNAs, such as miR-21,[63] and miR-22,[64,65] have been found to have a prompt effect on VSMC phenotypic transformation. Conversely, other miRNAs, like miR-1 and miR-10a,[66,67] have been shown to inhibit VSMC phenotypic transformation. Our review included one study which suggested that acupuncture may enhance the expression of miR-494-3p in CIS rats.[21] Further research is needed to elucidate the specific impact on VSMC phenotypic transformation, with the aim of developing a miRNA-based biologic therapy for CIS. bFGF is a multifunctional polypeptide that has been shown to protect neurons by preventing an increase in intracellular Ca^{2+} concentration and improving rCBF through vasodilation or opening of closed microvessels.[68,69] The present review demonstrates that acupuncture has an evident impact on the expression of bFGF, leading to a potential decrease in intracellular Ca^{2+} concentration and an increase in rCBF in CIS rats.[24] Additionally, King and Johnson reported that insulin exerts diverse effects on vascular cells,[70] including VSMC and VEC, which can facilitate vasodilation through the stimulation of NO release from VEC via the phosphoinositide (PI)-3 kinase/Akt signaling pathway when insulin receptors on VSMC are activated.[71,72] The activation of NO/cGMP/PKG and its subsequent relaxation effect on VSMC is also observed. Our review demonstrates an increased effect on serum insulin in CIS rats following acupuncture stimulation.[18] The precise mechanism by which acupuncture affects brain insulin, derived from brain cell synthesis and plasma crossing the blood-brain barrier, and its role in VSMC, requires further investigation. The Wnt/β-catenin signaling pathway is crucial for growth control, encompassing differentiation, self-repair, and regeneration.[73] A recent study conducted by Vervloet and Cozzolino demonstrated that the activation of the Wnt/β-catenin signaling pathway can induce a transformation of VSMC into osteoblast-like cells.[74] In addition to the well-known Wnt/β-catenin pathway, there are also other pathways involved, such as the Wnt/planar cell polarity pathway and the Wnt/Ca^{2+} dependent pathway. Activation of the latter pathway not only increases the intracellular concentration of calcium ions, but also activates PKC, both of which contribute to the contraction of VSMC.[75] In our review, the included study investigated the impact of acupuncture stimulation on the Wnt/β-catenin pathway. The findings demonstrated that acupuncture stimulation led to the activation of the Wnt/β-catenin signaling pathway, as evidenced by an increase in β-catenin levels and a decrease in Axin2 levels in the brain tissue of CIS rats. Additionally, acupuncture was found to have a protective effect on the neurovascular unit.[26] The

discovery of VEGF in 1983 revealed its ability to specifically bind to VEC and promote the proliferation of new blood vessels. Subsequent research conducted over the course of six years further confirmed its role in enhancing vascular permeability and stimulating VSMC proliferation.[76,77] There are three distinct types of VEGFRs, namely VEGFR-1, VEGFR-2, and VEGFR-3. Among these, VEGFR-2 and VEGFR-1 exhibit high levels of expression on the surface of VECs.[78] VEGFR-1 exerts a dual effect on endothelial cells. On the one hand, it possesses a stronger affinity for VEGF and thereby inhibits the binding of VEGFR-2, consequently impeding VEC proliferation. On the other hand, under specific circumstances, VEGFR-1 can stimulate VEC proliferation. Furthermore, the interaction between VEGFR-2 and PLCγ leads to the activation of DAG and IP3, ultimately resulting in PKC expression and an elevation in intracellular Ca^{2+} concentration.[79] This meta-analysis demonstrated a significant enhancement in VEGF levels following acupuncture treatment in the CIS model. While the impact of VEGF on cardiac contractility has been documented,[80] there remains a lack of investigation into the associated pathways. The VEGF/VEGFR pathway encompasses a multifaceted process involving proliferation, differentiation, and migration. Further research is warranted to elucidate the precise mechanisms involved.[81] BDNF, a highly expressed brain-derived neurotrophic factor in animal brains, exerts a crucial influence on neuronal survival, differentiation, growth, and synaptic plasticity.[82,83] The literature has extensively documented the advantageous impact of BDNF on the CIS.[84] Nevertheless, the specific mechanism linking BDNF to VSMC in this context remains elusive. This meta-analysis further demonstrated a favorable influence of acupuncture-induced BDNF stimulation in CIS rats. The neuroprotective function of acupuncture and its associated BDNF signaling pathways have been investigated, with a particular focus on exploring the mechanism involving VSMC in greater depth.[85]

Strengths and Limitations of This Review

To the best of our knowledge,[86,87] this study represents the inaugural preclinical systematic review and meta-analysis aimed at summarizing the impact of acupuncture treatment on VSMC function in a CIS model. Through a comprehensive analysis of the included studies, this investigation has unveiled certain favorable effects (such as regional cerebral blood flow and neurological deficiency) and potential mechanisms underlying VSMC function subsequent to acupuncture stimulation.

There are several limitations present in this review. Firstly, the included studies were all conducted in China, this is a potential limitation which may lead to the heterogeneity of this study. Furthermore, the quality of the included studies was deemed low based on the SYRCLE's RoB tool, and moderate-to-high heterogeneity was observed among them. These limitations may be attributed to various factors within the included studies, such as the assessment and reference models, acupoints, detection area, and methods utilized. Additionally, it is widely acknowledged that there exists a disparity in terms of sex regarding CIS incidence, treatment benefit, and complication rate.[88] The study primarily focused on male rats, prompting the need for future neuroscience community research to include both male and female rats. Lastly, the understanding of the comprehensive mechanisms underlying acupuncture is limited, as there are other signaling pathways (such as RhoA/ROCK, ERK, and P38MAPK pathways) involved in vasomotion mechanisms.[89–91] While the findings of this review should be interpreted cautiously, they offer insights into the consideration of vascular smooth muscle cell function in future studies on CIS.

Conclusions

Based on a comprehensive systematic review and meta-analysis, our findings indicate a noteworthy and favorable impact of acupuncture stimulation on rCBF and neurological impairment. The underlying mechanism appears to be intricately linked to the function of VSMCs. Nevertheless, it is imperative to exercise caution when interpreting these results, as they are subject to the constraints imposed by the experimental design and methodological quality of animal studies.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, 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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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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