Effect of Kv channel blockers on infarct size and neurological severity score in rodent models of brain ischemia: a systematic review and meta-analysis

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Submitted 2025/10/18
Revised 2026/5/09

Accepted 2026/5/09

Abstract

Background: Ischemic stroke is a leading cause of disability with limited approved treatment. Recently, Kv channel blockers have gained attention for their neuroprotective properties. This systematic review evaluates the effects of Kv channel blockers on infarct size and neurological severity score (NSS) in rodent models of ischemic stroke.

Materials and methods: This study adhered to the PRISMA guidelines. In April 2024, a systematic search was carried out across several databases, such as Medline, Embase, PubMed, CINAHL, Cochrane Library, Web of Science, and Scopus. Additionally, Google Scholar was explored to identify further relevant articles. We included all experimental studies that reported the effects of Kv channel blockers on rodent models of stroke. The quality of the included studies was assessed using the CAMARADES critical appraisal tool, and a quantitative synthesis was performed using CMA v.3 software.

Results: Out of 1,211 studies screened, 8 were included in the analysis. Eight of these studies looked at infarct size using histology, magnetic resonance imaging (MRI), and triphenyl tetrazolium chloride (TTC) staining. Six of them looked at NSS on days 4, 7, and 8 after the stroke. The quantitative synthesis indicated substantial disparities between the treatment and vehicle groups in both infarct size (standardized mean difference [SMD]: 1.67; 95% CI: 0.90-2.45; $p < 0.001$) and NSS (SMD: 1.65; 95% CI: 1.25-2.04; $p < 0.001$).

Conclusion: This meta-analysis demonstrated that Kv channel blockers effectively reduce infarct size and improve NSS in preclinical models of ischemic stroke. Findings suggest the Kv channel blockers as potential therapeutic agents and warrant further investigation in clinical studies.

Keywords: Voltage gated potassium channels; Ischemic stroke, Infarct size; Neurological severity score; Rodent

Introduction

Ischemic stroke is caused by the occlusion of brain vessels, reduces blood flow to the brain[1] and is one of the leading causes of death and permanent disabilities.[2,3] Post-stroke disabilities, including but not limited to sensorimotor and balance deficits, hemiplegia, decreased reflexes,

ptosis, visual field defects, aphasia, and apraxia[4,5] have significant socioeconomic burdens and reduces patients quality of life.[6,7] A series of interconnected and complex pathophysiological events, such as oxidative stress,[8] neuroinflammation, and apoptosis,[9-11] contributes to the progression of stroke neuronal injuries, leading to structural damage and neurological deficits.[12-14]

Thrombolysis for ischemic stroke is a key intervention that can reduce disability. Also, tissue plasminogen activator (tPA) within 4.5 hours of stroke onset remains the standard of care for most ischemic stroke patients. However, time and technical limitations reduce their advantages.[15,16] Given the fact there is limited effective treatment for post-stroke disabilities, investigation of novel therapeutic strategies is of great importance.[17,18] Recently, the neuroprotective properties of voltage-gated potassium channel (Kv) blockers have gained attention based on findings from neurological conditions such as multiple sclerosis (MS)[19] and amyotrophic lateral sclerosis (ALS).[20] The Kv channels are a major class of K^+ channels and comprise 12 subfamilies (Kv1–Kv12), which play a pivotal role in neuronal excitability.[21,22] In neurons, Kv channels modulate action potential generation and regulate the resting membrane potential, neuronal excitability, and firing frequency.[23,24] In addition, these channels play an important role in maintaining intracellular K^+ homeostasis. Notably, intracellular K^+ suppresses both K^+ efflux and the activation of apoptotic enzymes. The function of Kv channels is significantly influenced by stroke-related insults. Under stroke conditions, a reduction in intracellular K^+ resulting from enhanced K^+ efflux facilitates the activation of apoptotic pathways.[24-26]

Excitotoxicity is a critical event in ischemic brain injury,[27] resulting in energy depletion, loss of membrane potential, neuronal depolarization, and activation of voltage-gated Ca^{2+} channels. This cascade leads to the accumulation of glutamate in the extracellular space, which increases Ca^{2+} influx into neurons, thereby establishing a vicious cycle in the ischemic brain.[28-30] Furthermore, the accumulation of Ca^{2+} leads to mitochondrial dysfunction, which subsequently enhances the production of reactive oxygen species (ROS). The resulting toxicity and elevated ROS levels further activate microglia.[31] The Kv1.3 channel in microglia,[32] contributes to the secretion of inflammatory mediators in the brain.[33] Both selective and nonselective blockers of the Kv1.3 channel can exert modulatory effects by inhibiting cell activation and reducing the secretion of pro-inflammatory cytokines.[34-36]

Malfunction of Kv channels leads to an increase in infarct volume and neuronal deficits, which are further exacerbated by cerebral ischemia.[37,38] Several preclinical studies have investigated the effects of Kv channel blockers on infarct size and NSS deficits caused by ischemic stroke.[18,37,38] These studies suggest that Kv channel blockers not only can significantly reduce infarct size but also improve and neurological outcomes in part by mitigating oxidative stress, inflammation, and apoptosis.[38-40]

Classically, the determination of infarct size provides an unbiased estimate of the extent of ischemia and the effectiveness of treatments.[41] However, since infarct size alone does not fully reflect functional outcomes, the assessment of neurological deficits is equally important. Additionally, various rodent models have been developed to study ischemic stroke, most of which rely on transient or permanent middle cerebral artery occlusion (MCAO).[42] This model offers a comprehensive understanding of the complex molecular and cellular mechanisms underlying cerebral ischemia, as well as its long-term consequences. In experimental studies, techniques such as histology, magnetic resonance imaging (MRI), as well as tetrazolium chloride (TTC)-based staining are used for the determination of infarct size, while neurological deficits can be assessed by the Bederson Scale or neurological severity scores (NSS).[43-48]

Additionally, systematic reviews and meta-analyses are invaluable in synthesizing data, particularly in research fields involving heterogeneous populations and varying methodologies.[49] This review synthesizes existing research to inform preclinical practices and guide future investigations, addressing the diverse methodologies used in the included studies. By combining data from different studies, quantitative synthesis increases statistical power and gives accurate estimates of how Kv channel blockers affect infarct size and NSS. By identifying gaps in the current literature, the review points out areas requiring further exploration, aiming to optimize and streamline the development of effective therapies for ischemic stroke.

Methods

This review was performed based on the statements of the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement.[50]

Inclusion and exclusion criteria

All experimental study publications that were related to our topic and reported Kv channel blocker effects on rodent models of stroke were included. Review articles, conference papers, letters to the editors, human studies, studies on animals other than rodents, *in vitro* or *ex vivo* studies, studies not evaluating infarct size and NSS and articles published in languages other than English were excluded. The primary outcome measure was infarct size, and the secondary outcome was NSS.

Search strategy

A systematic search was conducted in Medline (via Ovid), PubMed, Embase (www.embase.com), CINAHL (via EBSCO), Cochrane library, Google Scholar, Scopus and Web of Science up to April 2025. Google Scholar was searched for any further related studies. The search was conducted by an information specialist (FSG). The reference lists of the selected studies were checked for any potential related studies. Based on the research question, the main keywords were voltage gated potassium channel, Kv channel, brain ischemia, cerebral ischemia, and ischemic stroke. A combination of subject headings, synonyms, related terms and different spellings was used in the search strategy. The search strategy of PubMed is available in Appendix 1.

Selection process and data extraction

After deduplication, two authors (FSG and EIA) independently screened the retrieved studies for eligibility based on title and abstract, considering the inclusion and exclusion criteria, using Endnote X8. Then, two authors (AEK and SSE) independently screened the full texts to identify eligible studies. The following information was extracted and recorded from the selected articles: The number of animals, animal strain, age, weight, method of stroke induction, treatments or drugs category, dose, administration route, treatment duration, infarct size and NSS and other main outcomes. Extracted data were double-checked by two other authors (EIA and MF). Disagreements between the authors were resolved at all stages in consultation with the third author (SSE).

Quality assessment

Two reviewers (JM and HSP) independently assessed the quality of the selected studies and risk of bias by using a collaborative approach to meta-analysis and review of experimental animal studies (CAMARADES). This checklist has 10 items as follows: Peer-reviewed publication, control of temperature, random allocation to treatment or control, blinded outcome assessment, use of anesthetic without significant intrinsic neuroprotective activity, animal model, sample size

calculation, compliance with animal welfare regulations, and statement of potential conflict of interests.[51]

Statistical analysis

For the meta-analysis of the quantitative data, the third version of the comprehensive meta-analysis (CMA v.3) software was used. The meta-analysis included studies that reported infarct size and NSS after administration of Kv channel blockers in a brain ischemia model. Furthermore, the number of animals, mean, and standard deviation (SD) in each group were used to calculate the effect size in each study. Converting the standard error (SE) values to SD was performed by the following formula:

$SD = SE * \sqrt{n}$

The results were reported with a standardized mean difference (SMD), confidence intervals (CIs) of 95%, and p-value. Subgroup analyses were performed based on infarct size evaluation methods and days of NSS assessment. The I^2 statistic was applied to assess the heterogeneity of the data and stratified to 25%, 50%, or 75%, groups as low, modest, and high, respectively. Because of the high levels of heterogeneity that were confirmed by the I^2 index, random effect model analysis was used in this study. Furthermore, for sensitivity analysis, outlier studies were removed using a stepwise method, and the significance of effects was reassessed until the I^2 statistic reached zero. Additionally, we employed a funnel plot and trim-and-fill analysis to assess publication bias.[52]

Results

Literature search

We identified 2620 studies during the search of bibliographic databases. After deduplication, 1211 studies entered the screening phase. After screening, 1177 studies were excluded based on titles and abstracts. The full texts of 34 articles were reviewed, and 26 articles were removed by reason, and finally, 8 studies met our inclusion criteria and included in the review. Of the excluded studies, 3 involved Kv channel openers,[53-55] one did not involve rodents,[56] and 9 did not use any treatment for evaluation of channels.[57-65] Additionally, 6 were considered cerebrovascular examinations without inducing stroke,[66-71] 3 were conducted in vitro,[72-74] 3 were *ex vivo* investigations,[26,75,76] and one did not evaluate infarct size and NSS.[77] Ultimately, 8 studies for infarct size and 6 studies

for NSS met all inclusion criteria for meta-analysis. The selection process is shown in the PRISMA flow chart (Fig. 1).

General characteristics of the studies and risk of bias

Out of 8 studies, treatments were administered by gavage in 2 studies,[78,79] intraperitoneally in the remaining 6 studies.[18,37-40,80] Mice were used in 4 studies [18,37,79,80] and rats in 3 others.[39,40,78] One study utilized mice and rats together.[38] In one study, female animals were tested,[18] whereas only male animals were used in the remaining studies.[37-40,78-80] Generally, studies used 8-20 week-old rodents, but one study involved the concurrent use of 16- and 80-week-old mice to assess infarct size and NSS.[18] One study used the permanent middle cerebral artery occlusion (pMCAO) model,[78] while the other 7 studies employed the transient middle cerebral artery occlusion (tMCAO) model.[18,37-40,79,80] The studies differed in the types, dosage and duration of administration of treatments. Infarct size in included studies was assessed using histology,[38,78] MRI,[18,39] and TTC [37,40,79,80] methods. Also for NSS evaluation among the 6 included studies, 6,[40] 9,[80] and 15 [18,37-39,79] severity score scale methods were reported (Table 1).

Quality assessment

In the current study, a CAMARADES quality checklist was used to assess the internal and external validity of the selected studies.

Most of the studies (6 of 8) mentioned the control of temperature. All papers, except one, included statements regarding allocation concealment and blinded assessment of outcomes. Every selected article reported random allocation, using anesthetics without neuroprotective activity, appropriate animal models, compliance with animal welfare, and statements of potential conflicts of interest. None of the studies provided details on the methodology of sample size calculation. The results of quality assessments are presented in Table 2.

Results of individual studies

General Kv channel blockers

Dalfampridine

In a crossover and dose-escalation study, no significant change in infarct size was observed following administration of dalfampridine, the extended-release form of 4-Aminopyridine (4-AP)

and a general Kv channel blocker. Sensorimotor function was assessed using forelimb and hindlimb placing tests and the body-swing test. In the double-crossover phase, a high dose (2 mg/kg) significantly improved sensorimotor function compared to the vehicle or a lower dose (0.63 mg/kg). In the dose-escalation phase, the 1 mg/kg dose improved hindlimb function, while the 2 mg/kg dose significantly improved function in both forelimbs and hindlimbs.[78]

Kv2.1 channel blockers

TAT peptides

In one study, infarct size and NSS were significantly reduced in rats treated with TAT-C1aB (6 nmol/g/i.p) at 1 and 6 hours after ischemic stroke. Electrophysiological analysis revealed that TAT-C1aB prevented Kv2.1-mediated current increases and mitigated neuronal damage *in vitro*.[80] Similarly, another study showed that infarct size at 24 hours post-MCAO and NSS over an extended period (42 days post-MCAO) were significantly reduced by the peptide TAT-DP-2 (6 nmol/g/i.p).[81] TAT-DP-2 also demonstrated notable neuroprotective effects by inhibiting Kv2.1-mediated pro-apoptotic potassium currents. Immunohistochemistry further revealed that TAT-DP-2 administration (6 nmol/g/i.p) significantly reduced the density of Kv2.1 clusters in the cortical region.[37]

Kv1.3 channel blockers

PAP-1, ShK-223 and Bergapten

In four studies, the infarct size and NSS were significantly reduced following intraperitoneal administration of PAP-1, a specific Kv1.3 channel blocker.[18,38-40] PAP-1 treatment at 40 mg/kg reduced Kv1.3 channel expression on microglia and enhanced neuronal survival in hippocampal slices. This treatment also decreased pro-inflammatory cytokines (IL-1β and IFN-γ) while increasing the anti-inflammatory cytokine IL-10 in the ischemic region.[38] To further investigate the role of Kv1.3 in ischemic stroke, a study employed genetic deletion of Kv1.3 in young and old, male and female mice. In these animals, PAP-1 (40 mg/kg) reduced infarct size and NSS on day 8 compared to the vehicle group. Additionally, PAP-1 and Kv1.3 deletion both led to decreased levels of Iba-1 and CD3 markers, reflecting reduced activation or proliferation of microglia, monocyte-derived macrophages, and T cell infiltration, contributing to reduced inflammation.[18]

Another study compared two Kv1.3-blocking compounds, ShK-223 and PAP-1, in an ischemic stroke model. While PAP-1 reduced infarct size and NSS following tMCAO, ShK-223 did not. RT-qPCR analysis revealed that PAP-1 reduced inflammatory cytokines (IL-1β, IL-6, TNF-α, COX-2, iNOS, and IFN-γ) in ischemic tissues.[39]

Treatment with PAP-1 for 7 days following MCAO/reperfusion significantly reduced infarct size and improved NSS on days 2 to 7 postoperative. This treatment was associated with decreased expression of cleaved IL-1β and cleaved Caspase-1. Additionally, PAP-1 appeared to promote a shift in microglial phenotype from the pro-inflammatory M1 to the anti-inflammatory M2 state, while also reducing inflammasome activation, contributing to improved outcomes.[40]

In another study, the effects of Bergapten (BG), a Kv1.3 channel blocker, on infarct size and NSS were assessed using TTC and NeuN staining 72 hours post-ischemia. BG treatment significantly reduced infarct size and NSS, with improvements noted in neurobehavioral functions such as rotational performance and grip strength. Furthermore, BG reduced levels of inflammatory cytokines, including IL-1β, IL-6, and TNF-α, indicating its potential anti-inflammatory and neuroprotective effects in ischemic conditions.[79]

Meta-analysis

The findings showed significant heterogeneity among the analyzed publications, as demonstrated by the prediction interval and I2 statistic.

Effect of Kv channel blockers on infarct size after stroke

8 articles were included in the meta-analysis of infarct size evaluation.[18,37-40,78-80] Overall analysis across three subgroups (histology, MRI, and TTC) showed a significant difference between Kv channel blockers and vehicle group (SMD: 1.67; 95% CI: 0.90 to 2.45; $p<0.001$; I2: 77.16). There were 2 articles with 3 outcomes in histological examination,[38,78] (SMD: 1.24; 95% CI: 0.05 to 2.42; $p<0.05$; I2: 75.88), 2 articles with 3 outcomes in MRI evaluation,[18,39] (SMD: 2.03; 95% CI: 0.35 to 3.72; $p<0.05$; I2: 86.42) and 4 articles in TTC assessment,[37,40,79,80] (SMD: 1.97; 95% CI: 0.68 to 3.25; $p<0.01$; I2: 79.69). All subgroup analysis showed a significant difference between the treatment and vehicle groups (Fig. 2).

Effect of Kv channel blockers on NSS after stroke

The analysis of NSS in 6 included studies, showed a significant difference between Kv channel blockers and vehicle groups across 3 subgroups (4, 7, and 8 days after ischemic stroke) (SMD: 1.65; 95% CI: 1.25 to 2.04; p<0.001; I2: 58.81). 3 studies examined NSS on days 4, 7, and 8;[18,38,39] one study examined NSS on days 4 and 7;[40] and 2 studies examined NSS only on day 8.[37,80] The meta-analysis of the subgroups at day 4 (SMD: 1.75; 95% CI: 1.10 to 2.39; p<0.001; I2: 53.13), day 7 (SMD: 1.52; 95% CI: 0.86 to 2.18; p<0.001; I2: 67.46), and day 8 (SMD: 1.68; 95% CI: 0.91 to 2.45; p<0.001; I2: 59.63) indicated significant differences between treatment and vehicle groups (Fig. 3).

Publication bias

The trim-and-fill analysis indicated four and two missing studies in infarct size and NSS outcomes. Before the inclusion of missing studies, the overall efficacy estimated for infarct size and adjusted NSS were 1.6 (95% CI 0.9 to 2.4) and 1.6 (95% CI 1.2 to 2), respectively. After the inclusion of missing studies, the adjusted overall efficacy estimate changed to 2.1 (95% CI 1.2 to 2.9) and 1.3 (95% CI 0.8 to 1.7), respectively (Fig. 4 and 5).

Sensitivity analysis

In the sensitivity analysis of infarct size, three studies those by Iaci et al. (SMD: 0.19; 95% CI: -0.64 to 1.03; p = 0.65), Lee et al. (SMD: 4.81; 95% CI: 2.92 to 6.69; p = 0.000), and Ma et al. (SMD: 4.72; 95% CI: 3.01 to 6.42; p = 0.000) were excluded (Fig. 6) (Table 3). Similarly, for the NSS, six studies by Lee et al. (SMD: 2.66; 95% CI: 1.34 to 3.98; p = 0.000), Ma et al. (SMD: 3.02; 95% CI: 1.73 to 4.30; p = 0.000), Chen et al. (d) (SMD: 2.44; 95% CI: 1.22 to 3.67; p = 0.000), Lee et al. (SMD: 3.53; 95% CI: 2 to 5.06; p = 0.000), Ma et al. (SMD: 2.47; 95% CI: 1.31 to 3.63; p = 0.000), and Lee et al. (SMD: 3.39; 95% CI: 1.89 to 4.88; p = 0.000) were removed. In both analyses, the effect estimates remained significant. No important heterogeneity was observed across the studies (Fig. 7) (Table 4).

Discussion

This systematic review demonstrated the effects of Kv channel blocker administration on infarct size and NSS in rodent models of ischemic stroke. Based on the findings, Kv channel blockers reduce infarct size and improve NSS in these models.

During ischemia, dysfunction of the Na^+/K^+-ATPase in damaged brain leads to membrane depolarization, which promotes the efflux of K^+ and the influx of Na^+ into the cell.[82,83] Disruption of the membrane potential subsequently elevates intracellular Ca^{2+} levels and increases glutamate accumulation in the synaptic cleft, thereby exacerbating excitotoxicity.[28-30]

The accumulation of Ca^{2+} induces mitochondrial dysfunction, which subsequently increases the production of ROS. The resulting cytotoxicity, along with elevated ROS levels, further activates microglia and promotes the release of inflammatory mediators, such as interleukins and TNF-α via Kv1.3 channels.[31,38]

Oxidative stress promotes the release of Zn^{2+} from mitochondria and metallothioneins, which subsequently activates Src and p38 MAPK signaling pathways. Activation of these kinases leads to the phosphorylation of Kv2.1 channels and their translocation to the plasma membrane. The resulting K^+ efflux ultimately contributes to the activation of caspase and promotes the progression of apoptosis (Fig 8).[84,85]

These processes lead to an expansion of infarct size and worsening of neurological deficits. Given that the penumbral region retains substantial regenerative potential, inhibiting Kv channels may help reduce infarct size and neurological deficits following cerebral ischemia.

Studies have shown that pretreatment with Kv channel blockers, such as 4-AP, reduces oxidative stress in animal models of Parkinson's disease and in the hippocampus of diabetic mice by decreasing lipid peroxidation and increasing antioxidant enzyme activity.[86-88] Furthermore, previous studies found that Kv channel blockers may reduce pro-inflammatory cytokines, apoptotic factors, infarct size, and neurological impairment in ischemic stroke.[38,40] Kv channel blockade can also inhibit pro-inflammatory cytokine production by microglia in response to β-amyloid-induced neurotoxicity.[89] Other studies have shown that blocking the Kv1.3 channel reduces levels of inflammatory factors, including IL-1β, IL-6, TNF-α, and iNOS, in mouse models of selective innate immune activation and in a rat model of ischemic stroke.[39,90] Another study demonstrated the strong anti-apoptotic effects of blocking Kv channels on cerebellar granule neurons in rats.[91] Shi et al. showed that administering 4-AP increases BCL-2 levels, decreases Caspase-3 expression, and reduces apoptosis in Parkinsonian mice.[87]

Kv1.3 blockers (e.g., PAP-1 and BG) primarily reduce microglial activation and pro-inflammatory cytokine production, which corresponds to the marked reductions in infarct size reported in several studies.[18,79] In contrast, Kv2.1 blockers (e.g., TAT-C1aB and TAT-DP-2) predominantly target pro-

apoptotic K^+ currents, offering neuroprotection.[37,80] Pal et al. demonstrated that the Kv2.1 channel plays a significant role in the apoptosis of cortical neurons in vitro, with neurons expressing lower levels of Kv2.1 showing heightened resistant to apoptotic stimuli.[92]

Although blockade of Kv channels, especially Kv1.3 and Kv2.1 subtypes, may provide multifaceted neuroprotection in ischemic stroke, some studies present conflicting results. The study by Iaci et al. demonstrated a lack of effect of Kv channel blockade on the measured outcomes. This discrepancy may due to differences in study design, such as the timing of infarct size assessment following cerebral ischemia. Notably, although infarct size was not significantly altered, dalfampridine improved sensorimotor function in this study.[78] This finding suggests that the dose and timing of administration were key factors influencing the ability to produce a measurable change in infarct volume.

Rodents and humans share many fundamental aspects of neuroanatomy and cerebrovascular physiology, making rodents a widely accepted preclinical model for ischemic stroke research.[93] Specifically, Kv channels are highly conserved across mammals, and their roles in neuronal function, providing a strong mechanistic rationale for potential translational relevance.[94,95] Although there are some limitations in fully translating rodent model outcomes to clinical applications, these models are still considered an irreplaceable foundation for clinical studies.[96,97]

From a methodological point of view, among the included studies, treatments were administered either via oral gavage or intraperitoneal injection. The animal models consisted of mice and rats, with the exception of one study that used both sexes. Most studies, except for one, utilized male animals. Additionally, the majority of studies employed young rodents. Experimental stroke models included both transient and permanent MCAO. Variations were observed in the type, dosage, and duration of treatment administration. Infarct size was quantified using histological analysis, MRI, or TTC staining. For neurological evaluation, all studies used the NSS test.

We accounted for the potential presence of unpublished studies by estimating their effects. The results were subsequently adjusted using the trim-and-fill method. This adjustment did not significantly increase or decrease the overall outcome values. Moreover, sensitivity analysis, performed by omitting outlier studies, indicated that the overall effect of treatment on infarct size and NSS score remained significant.

Limitations

The observed heterogeneity in outcomes among the included studies, along with the limited number of articles, suggests potential variability in the results. In this study, we examined most of the relevant variables that may affect the outcomes and increase the risk of heterogeneity. The age and sex factors were not the influencing factors, as most rodents were adults, and the majority were male. Instead, the main source of heterogeneity was identified as the evaluation techniques used for infarct size and time of assessment in NSS, and subsequent subgroup analyses were conducted based on these factors. Additionally, the diversity of drug types and dosages, ischemic stroke models, experimental designs, Kv channel subtypes, and the lack of reported sample size calculations may contribute to publication bias or inconsistency. For the reduction of these factors' effect on outcomes, a random effects model was used to account for inter-study variability. Publication bias was assessed using funnel plots and trim and fill analysis. Furthermore, we performed sensitivity analysis to verify the stability and reliability of the findings.

However, it is essential to note that the results should be interpreted with caution due to methodological limitations. Future studies should aim to address these limitations to minimize risk factors, enhance the study's statistical power, and improve methodological consistency.

Conclusions

Kv channel blockers have demonstrated the ability to reduce infarct size and improve NSS in rodent models of ischemic stroke. This suggests their potential as promising candidates to improve outcomes in ischemic stroke patients. However, to establish their definitive efficacy, high-quality experimental studies and clinical trials are needed.

Declarations

Abbreviations: 4-AP: 4-Aminopyridine; 4-HNE: 4-Hydroxynonenal; ALS: Amyotrophic lateral sclerosis; BDNF: Brain-derived neurotrophic factor; BG: Bergapten; Caspase-1: Cysteine-aspartic acid protease-1; CAMARADES: Collaborative approach to meta-analysis and review of experimental animal studies; CBR1: Carbonyl reductase 1; CD: Cluster of differentiation; CIs: Confidence intervals; COX-2: Cyclooxygenase-2; Iba1: Ionized calcium-binding adaptor molecule 1; IC: Intracerebral; IFN: Interferon; IL: Interleukin; iNOS: Inducible nitric oxide synthase; IP: Intraperitoneal; KCa3.1: Calcium-activated potassium channels; Kv: Potassium voltage-gated channel; MRI: Magnetic resonance imaging; MS: Multiple sclerosis; NF-κB:

Nuclear factor kappa B; NLRP3: NLR family pyrin domain containing 3; NSS: Neurological severity score; P2X4: P2X purinoceptor 4; P2X7: P2X purinoceptor 7; PAP-1: Phenoxy Alkyl Psoralen-1; pMCAO: Permanent middle cerebral artery occlusion; PO: Oral administration; PRISMA: Preferred reporting items for systematic review and meta-analyses; SD: Standard devotion; S-D: Sprague-Dawley; SE: Standard error; SMD: Standardized mean difference; ShK-223: Stichodactyla toxin-223; TAT-C1aB: Peptide targeting Kv2.1 channels; TAT-DP-2: Trans activator of transcription–linked derivative of DP-2; TEA: Tetraethylammonium; TGF-β1: Tumour growth factor beta 1; tMCAO: Transient middle cerebral artery occlusion; TNF-α: Tumor necrosis factor alpha; TTC: Triphenyl tetrazolium chloride.

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: Not applicable.

Competing Interests: The author states no conflict of interest.

Funding: The research was supported by the Neurosciences Research Center, Tabriz University of Medical Sciences (grant number 71620).

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Acknowledgements: The research was approved and supported by the Neurosciences research center, Tabriz University of Medical Sciences.

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Figure legends

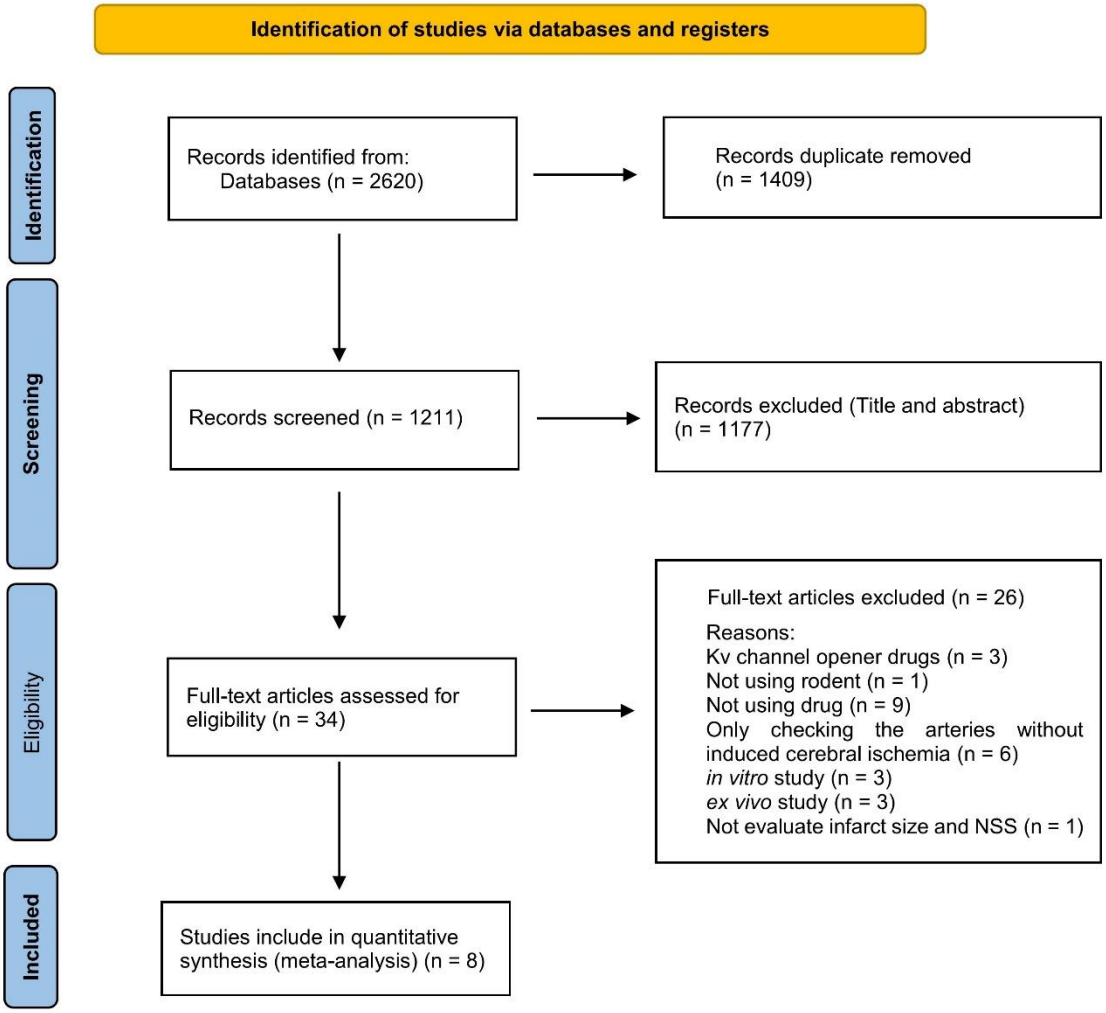

Fig. 1. PRISMA flow diagram illustrating the number of studies included and excluded at each stage of the systematic review process.

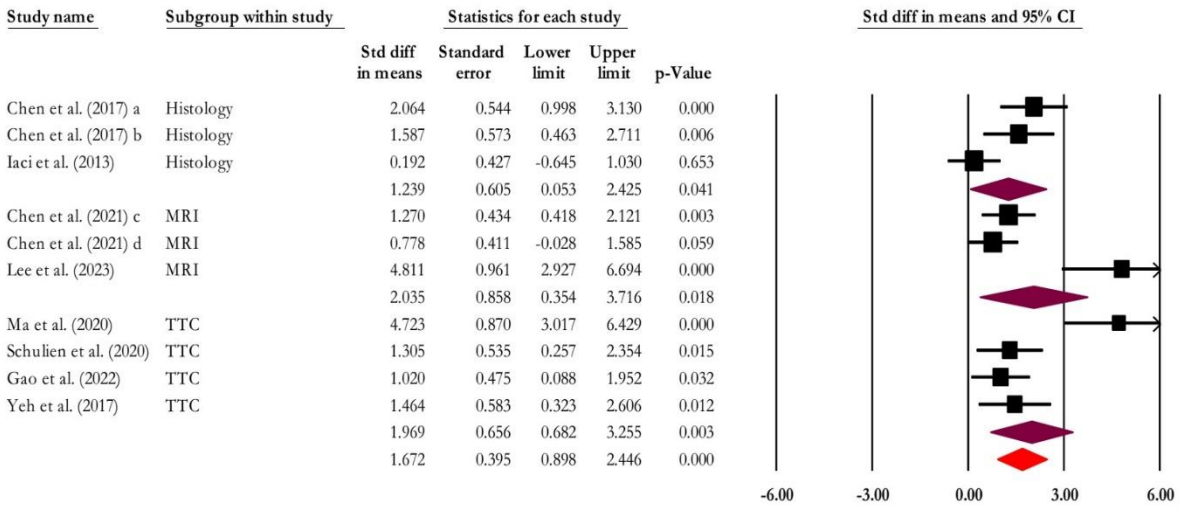

Fig. 2. Forest plot of standardized mean difference (SMD) for the effect of Kv channel blockers on infarct size after ischemic stroke. The red diamond shows the overall pooled effect. The purple diamonds show pooled effects in each subgroup. Black squares indicate the SMD in each study. Horizontal lines indicate a 95% confidence interval (CI). Letters in the study name field represent 2 studies with different outcomes (a: mice, b: rat, c: 16 weeks, and d: 80 weeks).

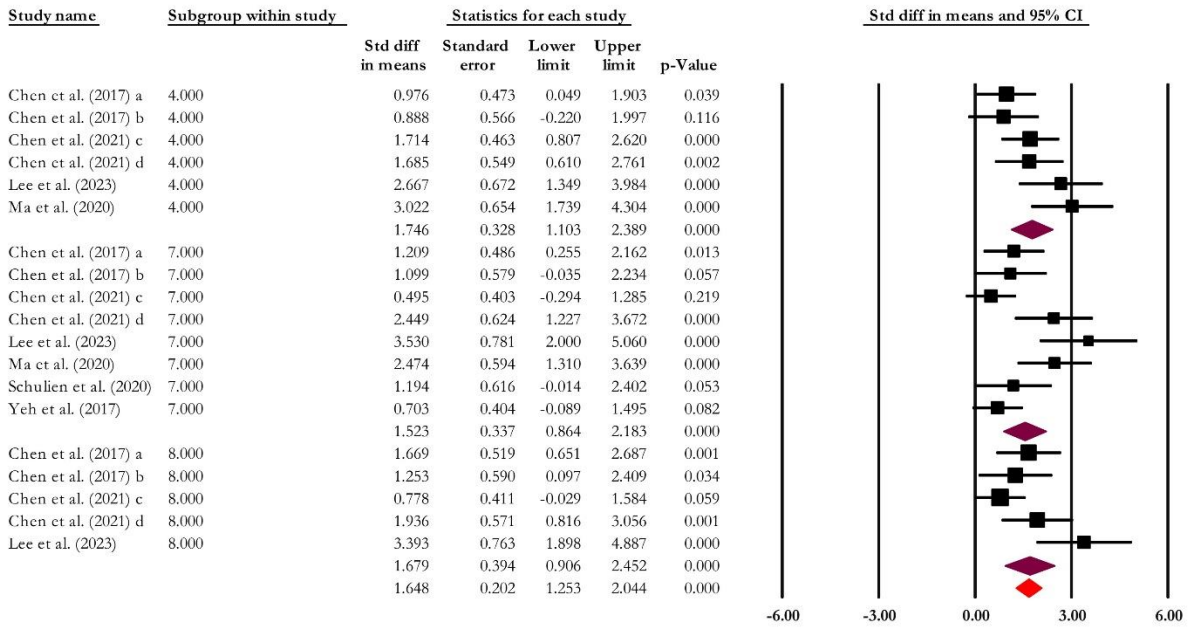

Fig. 3. Forest plot of standardized mean difference (SMD) for the effect of Kv channel blockers on NSS after stroke ischemic. The red diamond shows the overall pooled effect. The purple

diamonds show pooled effects in each subgroup. Black squares indicate the SMD in each study. Horizontal lines indicate a 95% confidence interval (CI). Letters in the study name field represent 2 studies with different outcomes (a: mice, b: rat, c: 16 weeks, and d: 80 weeks).

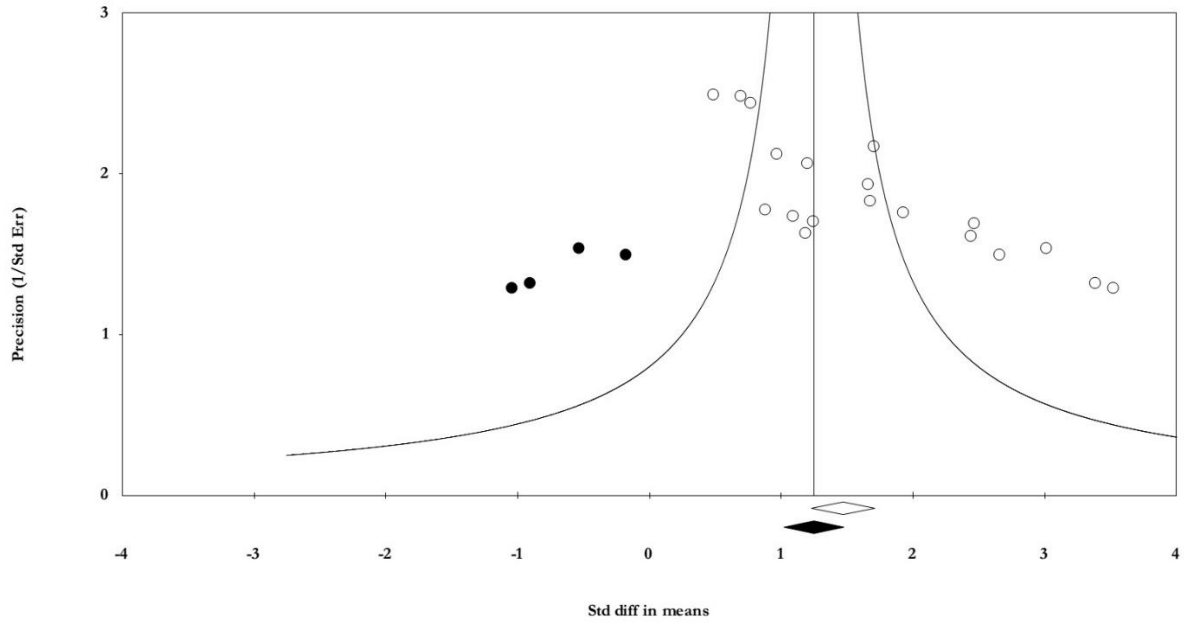

Fig. 4. Funnel plot for the meta-analysis of studies of standard error by the standardized difference in mean. Trim and Fill analysis revealed the presence of 4 “missing studies” (black dots) for infarct size.

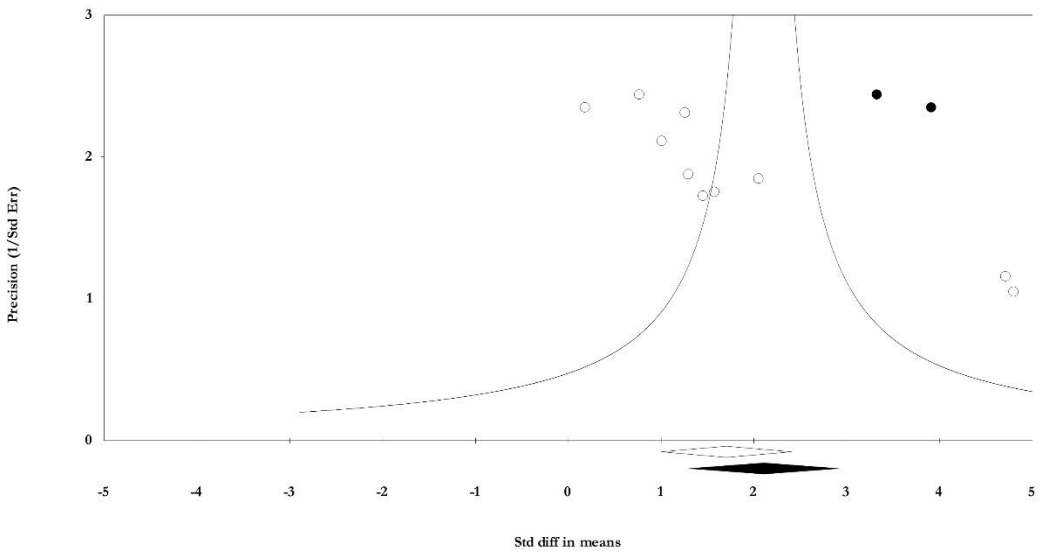

Fig. 5. Funnel plot for the meta-analysis of studies of standard error by the standardized difference in mean. Trim and Fill analysis revealed the presence of 2 “missing studies” (black dots) for NSS.

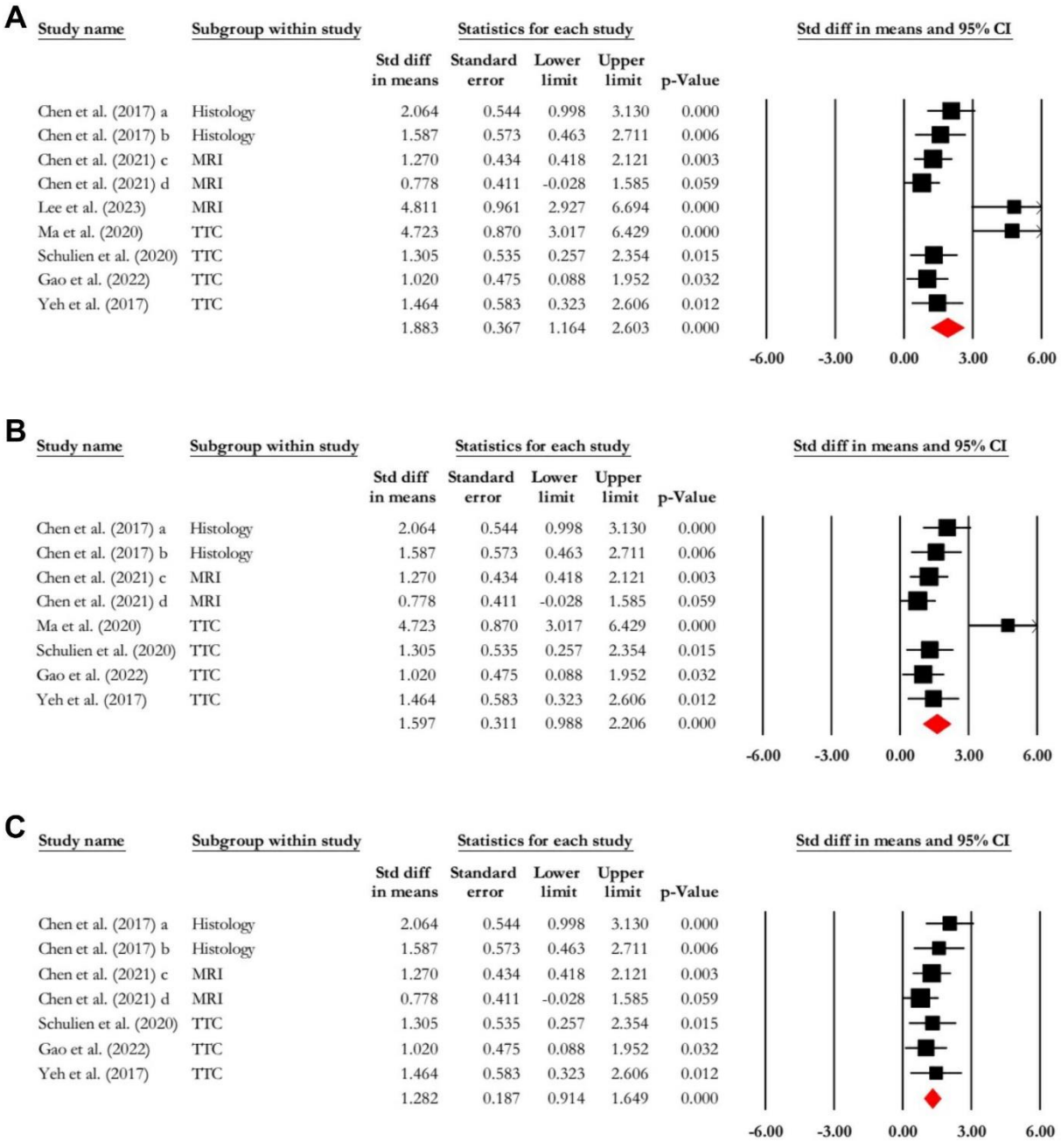

Fig. 6. Sensitivity analysis by stepwise removing the outlier studies on infarct size outcome. The overall effect by removing Iaci et al. (A), Lee et al. (B) and Ma et al. (C) studies.

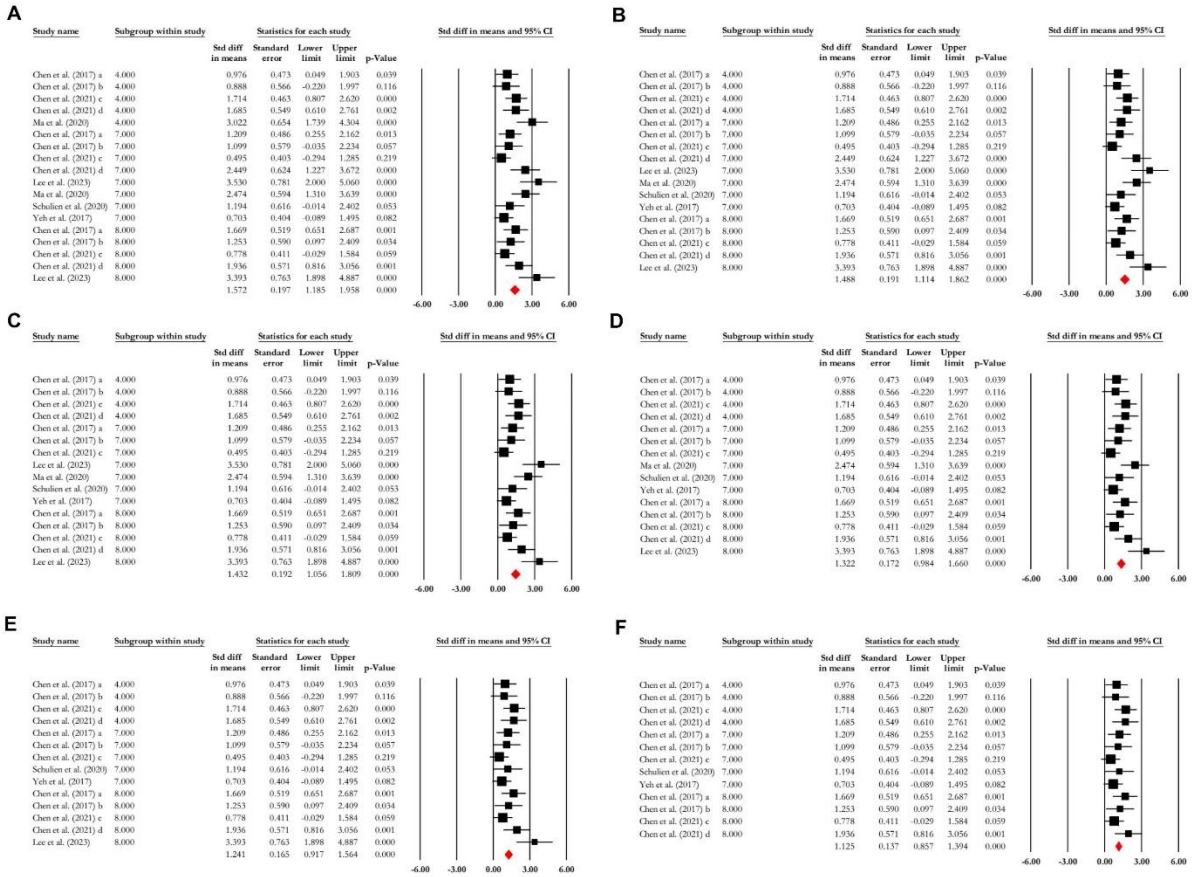

Fig. 7. Sensitivity analysis by stepwise removing the outlier studies on NSS outcome. The overall effect by removing Lee et al. (A), Ma et al. (B), Chen et al. (d) (C), Lee et al. (D), Ma et al. (E) and Lee et al. (F) studies.

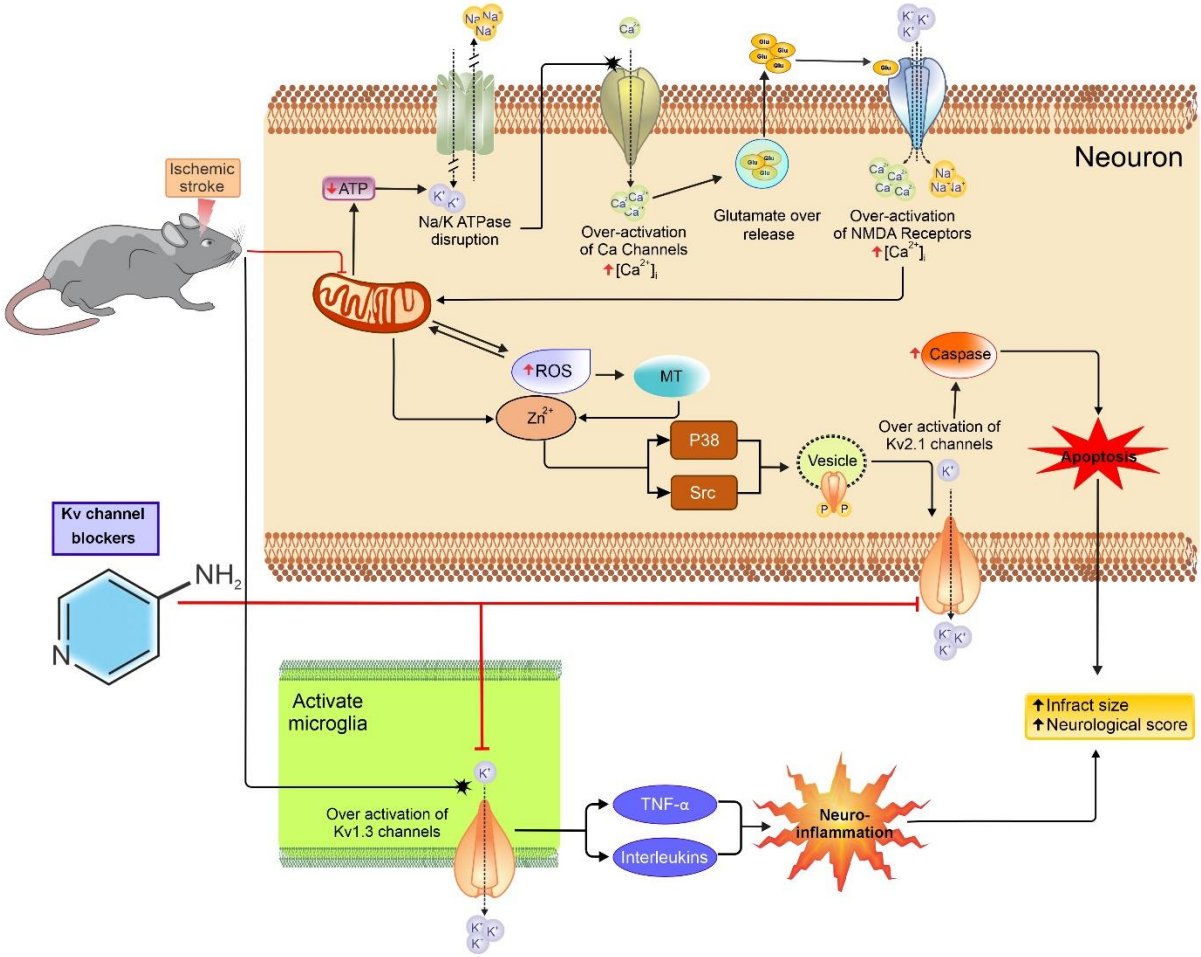

Fig. 8. Proposed modulatory mechanisms of Kv channel blockers in post-stroke brain.

Table 1: Characteristics of the included studies in the systematic review and meta-analysis.

Study (APA citation)	Animal strain	Age or weight	Number of animals	Model and method of induction	Type of drug and dose	Treatment duration	Administration rout	Outcome assessment scales	Target channels	Main findings
Chen, et al (2017)	Male C57BL/6J mice and male Wistar rats	12 weeks mice and 200-230 g rats	30 (mice) and 16 (rats)	tMCAO 60 min in mice and 90 min in rat	PAP-1 (10 or 40 mg/kg)	Every 12 h until day-8	IP	Electrophysiology, infarct size, NSS, (CD11b, Iba1, iNOS, Kv1.3, CD68, IFN-c, IL-1b, IL-6, IL-10, TNF-a, BDNF, TGF-b1) levels	Kv1.3	PAP-1 reduced the secondary inflammatory damage in brain ischemia by targeting the M1-like functions of microglia
Chen, et al (2021)	Female C57BL/6J mice	16 or 80 weeks	180	tMCAO	PAP-1 (40 mg/kg)	Every 12 h until day-8	IP	Infarct size, NSS, (CD3, Iba1, Kv1.3) levels	Kv1.3	PAP-1 introduced as a potential adjunctive treatment for reperfusion therapy after brain ischemia
Iaci, et al (2013)	Male S-D rats	16-20 weeks / 300-350 g	67	pMCAO	Dalfampridine (0.63, 2 mg/kg), then (0.5, 1.0, 2.0 mg/kg)	Every 12 h in days 30-32, 44-46 and 58-60, then every 12 h in days 56-65	PO	Infarct size, forelimb and hindlimb placing, body-swing tests	Kv	Dalfampridine did not significantly reduce infarct size after ischemic stroke, but it was able to improve the sensorimotor function.
Lee, et al (2023)	Male Wistar IGS rats	160 - 180 g	56	tMCAO	ShK-223 (100 mg/kg) and PAP-1 (40 mg/kg)	ShK-223 (once daily) and PAP-1 (twice daily) until day-8	IP	Infarct size, NSS, (IL-1β, IL-6, TNF-α, COX-2, iNOS, P2X4, P2X7, Kv1.3, KCa3.1, IFN-γ, CD68) levels	Kv1.3	PAP-1 decreased inflammation in the penumbra and improved stroke outcomes

Study (APA citation)	Animal strain	Age or weight	Number of animals	Model and method of induction	Type of drug and dose	Treatment duration	Administration rout	Outcome assessment scales	Target channels	Main findings
Ma, et al (2020)	Male S-D rats	280 - 320 g	52	tMCAO	PAP-1 (40 mg/kg)	Every 12 h (7 days)	IP	Infarct area, NSS, (NLRP3, IL-1β, Caspase-1, CD16, CD206) levels	Kv1.3	PAP-1 protects brain against ischemia possibly via switching microglia phenotype from M1 to M2 and inhibiting release of IL-1β
Schulien, et al (2020)	Male mice	28-30 g	17	tMCAO	TAT-DP-2 (6 nmol/g)	1 and 6 h following the reperfusion	IP	Electrophysiology, infarct size, NSS, Kv2.1 level	Kv2.1	TAT-DP-2 blocks pro-apoptotic Kv2.1 potassium currents following injury and improves neurological function following ischemia
Gao, et al (2022)	Male C57BL/6J mice	8 weeks / 20-25 g	20	tMCAO	BG (10 mg/kg) PAP-1 (10 mg/kg)	30 min, 24 h and 48 h after their awakening	PO	Infarct size, NSS, grip strength test, rotation test, (CBR1, CD68, Iba1, NF-κB, 4-HNE, IL-1β, IL-6, TNF-α) levels	Kv1.3	BG effects on cerebral ischemia by targeting Kv1.3 channels (amelioration of inflammatory responses and behavioral dysfunction)
Yeh, et al (2017)	Male mice	8-10 weeks / 24-29 g	36	tMCAO	TAT-C1aB (6 nmol/g)	1 and 6 h after the initiation of reperfusion	IP	Electrophysiology, infarct size, NSS	Kv2.1	TAT-C1aB by targeting the pro-apoptotic function of the Kv2.1 channel reduces cerebral ischemia-induced injury and improves neurological outcome

4-AP: 4-Aminopyridine; 4-HNE: 4-Hydroxynonenal; BDNF: Brain-derived neurotrophic factor; BG: Bergapten; Caspase-1: Cysteine-aspartic acid protease-1; CBR1: Carbonyl reductase 1; CD: Cluster of differentiation; COX-2: Cyclooxygenase-2; Iba1: Ionized calcium-binding adaptor molecule 1; IC: Intracerebral; IFN: Interferon; IL: Interleukin; iNOS: Inducible nitric oxide synthase; IP: Intraperitoneal; KCa3.1: Calcium-activated potassium channels; Kv: Potassium voltage-gated channel; NF-κB: Nuclear factor kappa B; NLRP3: NLR family pyrin domain containing 3; NSS: Neurological severity score; P2X4: P2X purinoceptor 4; P2X7: P2X purinoceptor 7; PAP-1: Phenoxy Alkyl Psoralen-1; pMCAO: Permanent middle cerebral artery occlusion; PO: Oral administration; S-D: Sprague-Dawley; ShK-223: Stichodactyla toxin-223; TAT-C1aB: Peptide targeting Kv2.1 channels; TAT-DP-2: Trans activator of transcription–linked derivative of DP-2; TEA: Tetraethylammonium; TGF-β1: Tumour growth factor beta 1; tMCAO: Transient middle cerebral artery occlusion; TNF-α: Tumor necrosis factor alpha.

Table 2: Quality assessment of included studies based on CAMAREDES checklist. Bold numbers are the overall scores of the studies.

Author, years	**(1)**	**(2)**	**(3)**	**(4)**	**(5)**	**(6)**	**(7)**	**(8)**	**(9)**	**(10)**	**Score**
(Chen, et al 2017)	X	–	X	X	X	X	X	–	X	X	**8**
(Chen, et al 2021)	X	X	X	X	X	X	X	–	X	X	**9**
(Lee, et al 2023)	X	X	X	X	X	X	X	–	X	X	**9**
(Ma, et al 2020)	X	X	X	X	X	X	X	–	X	X	**9**
(Schulien, et al 2020)	X	–	X	X	X	X	X	–	X	X	**8**
(Gao, et al 2022)	X	X	X	X	X	X	X	–	X	X	**9**
(Yeh, et al 2017)	X	X	X	–	–	X	X	–	X	X	**7**
(Iaci, et al 2013)	X	X	X	X	X	X	X	–	X	X	**9**

Studies fulfilling the criteria of: (1) peer-reviewed publication; (2) control of temperature; (3) random allocation to treatment or control; (4) allocation concealment; (5) blinded assessment of outcome; (6) use of anesthetic without significant intrinsic neuroprotective activity; (7) appropriate animal model; (8) sample size calculation; (9) compliance with animal welfare regulations; and (10) statement of potential conflict of interests.

Table 3: Summary of sensitivity analysis on infarct size outcome.

Stepwise removed studies	**SMD**	**Confidence interval**	***p* value**	**Heterogeneity (I^2)**
All studies (without removing any study)	1.67	0.90 - 2.45	0.0001	77.16%
Iaci et al.	1.88	1.16 - 2.60	0.0001	74.28%
Lee et al.	1.59	0.99 - 2.206	0.0001	63.52%
Ma et al.	1.28	0.91 - 1.64	0.0001	0%

Table 4: Summary of sensitivity analysis on NSS outcome

Stepwise removed studies	**SMD**	**Confidence interval**	***p* value**	**Heterogeneity (I^2)**
All studies (without removing any study)	1.65	1.25, 2.04	0.0001	58.81%
Lee et al.	1.57	1.18 - 1.95	0.0001	58.40%
Ma et al.	1.48	1.11 - 1.86	0.0001	53.91%
Chen et al. (d)	1.43	1.05 - 1.80	0.0001	52.50%
Lee et al.	1.32	0.98 - 1.66	0.0001	40.05%
Ma et al.	1.24	0.91 - 1.56	0.0001	31.41%
Lee et al.	1.12	0.85 - 1.39	0.0001	0%