



# Minocycline for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials

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## Abstract

**Background** Various randomized-controlled clinical trials (RCTs) have investigated the neuroprotective role of minocycline in acute ischemic stroke (AIS) or acute intracerebral hemorrhage (ICH) patients. We sought to consolidate and investigate the efficacy and safety of minocycline in patients with acute stroke.

**Methods** Literature search spanned through November 30, 2017 across major databases to identify all RCTs that reported following efficacy outcomes among acute stroke patients treated with minocycline vs. placebo: National Institute of Health Stroke Scale (NIHSS), Barthel Index (BI), and modified Rankin Scale (mRS) scores. Additional safety, neuroimaging and biochemical endpoints were extracted. We pooled mean differences (MD) and risk ratios (RR) from RCTs using random-effects models.

**Results** We identified 7 RCTs comprising a total of 426 patients. Of these, additional unpublished data was obtained on contacting corresponding authors of 5 RCTs. In pooled analysis, minocycline demonstrated a favorable trend towards 3-month functional independence (mRS-scores of 0–2) (RR = 1.31; 95% CI 0.98–1.74,  $p = 0.06$ ) and 3-month BI (MD = 6.92; 95% CI – 0.92, 14.75;  $p = 0.08$ ). In AIS subgroup, minocycline was associated with higher rates of 3-month mRS-scores of 0–2 (RR = 1.59; 95% CI 1.19–2.12,  $p = 0.002$ ;  $I^2 = 58%$ ) and 3-month BI (MD = 12.37; 95% CI 5.60, 19.14,  $p = 0.0003$ ;  $I^2 = 47%$ ), whereas reduced the 3-month NIHSS (MD – 2.84; 95% CI – 5.55, – 0.13;  $p = 0.04$ ;  $I^2 = 86%$ ). Minocycline administration was not associated with an increased risk of mortality, recurrent stroke, myocardial infarction and hemorrhagic conversion.

**Conclusions** Although data is limited, minocycline demonstrated efficacy and seems a promising neuroprotective agent in acute stroke patients, especially in AIS subgroup. Further RCTs are needed to evaluate the efficacy and safety of minocycline among ICH patients.

**Keywords** Minocycline · Ischemic stroke · Intracerebral hemorrhage · Recovery

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## Introduction

Minocycline is a semisynthetic second-generation tetracycline that provides neuroprotective effects in various neurological disease processes [1–4]. As a lipophilic agent that readily crosses the blood–brain barrier (BBB), minocycline has demonstrated improved outcomes in focal cerebral ischemia [5, 6], and further suggested reduction in the infarct size and hemorrhagic conversion in ischemic stroke. Minocycline does not affect the fibrinolytic effects of intravenous tissue-plasminogen-activator (tPA) [7], rendering a scope to extend the thrombolytic window in ischemic stroke patients [8]. Although the results seem to be promising, majority of the efficacy data on minocycline has involved animal studies, whereas data from human studies remain scarce.

A small number of phase II RCTs (randomized-controlled clinical trials) have investigated the efficacy of minocycline by assessing clinical recovery after acute ischemic stroke (AIS) without convincing results [9]. Recently, the efficacy and safety of minocycline administration has been analyzed in acute intracerebral hemorrhage (ICH) cohorts [10, 11]. Given the limited data available on the clinical efficacy and safety of minocycline on post-stroke functional outcomes, the objective of our systematic review and meta-analysis was to consolidate the evidence on the utilization, efficacy and safety of minocycline in ischemic and hemorrhagic strokes. We additionally analyzed the role of minocycline in reduction of hematoma and perihematomal edema (PHE) volumes among ICH patients.

## Methods

Our study is reported in accordance to Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines, and was exempt for approval from the Institutional Review Board of our Institution. Our study protocol for inclusion and exclusion criteria was designed a priori, however, was not registered.

## Data sources and searches

All the RCTs that reported and compared the neuroprotective effects of minocycline with placebo were identified by systematic search in Ovid Medline, Ovid Embase, Scopus, ClinicalTrials.gov and Google Scholar databases from database inception to November 30, 2017. The combinations of search strings used to perform our search were “stroke”, “cerebrovascular disease”, “cerebral ischemia”, “hemorrhagic stroke”, “intracerebral hemorrhage”, “neuroprotection” and “minocycline”. The

complete search algorithm used in MEDLINE is available in the online supplement. For any missing or unpublished data, corresponding authors were contacted and individual patient-level data was obtained. We restricted our search to human studies and publications in English language. We performed an additional manual search of conference abstracts and bibliographies of articles meeting study criteria for a comprehensive literature search.

## Study selection and data extraction

We identified trials that investigated clinical outcomes in patients treated with minocycline and placebo. The inclusion criteria were: (1) randomized controlled design; (2) trials comparing neuroprotective effects of minocycline vs. placebo in patients with acute stroke; (3) availability of clinical outcome data including NIHSS (National Institutes of Health Stroke Scale), mRS (modified Rankin Scale) score, BI (Barthel Index), and mortality; (4) adult patients (> 18 years old).

Two authors (KM and AK) independently reviewed all the retrieved articles. In case of disagreements regarding study inclusion or exclusion, the remaining coauthors were consulted and disagreement was resolved with mutual consensus. The following information were extracted: name of the study, first author and year of publication, mean age, sex distribution, trial name, total number and type of study participants, prevalence of cerebrovascular risk factors, stroke clinical syndrome, clinical outcomes, and follow-up duration. Clinical outcomes (mRS, BI, NIHSS scores) were studied at baseline, day 5–7, 1-month, and 3-month time periods. We further dichotomized the clinical outcomes: mRS 0–1 (favorable functional outcome), mRS 0–2 (functional independence), and mRS 6 (mortality). Our primary objective was to assess the efficacy of minocycline in regards to the clinical recovery of acute stroke patients, and further evaluate among AIS and ICH subgroups by performing sensitivity analyses. Following efficacy outcomes were analyzed: functional independence (mRS 0–2), mean NIHSS and BI scores at 3 months. We also evaluated pooled and individual efficacy outcomes based on different time intervals of clinical assessment. Our secondary objective involved the assessment of safety and neuroprotective role of minocycline among AIS and ICH patients. We additionally assessed neuroimaging and biochemical endpoints in ICH patients by analyzing the reduction of matrix metalloproteinase (MMP)-9 levels, PHE and hematoma volumes. The corresponding authors of 5 RCTs [9–13] were contacted and were requested by KM to contribute unpublished original data regarding primary and secondary outcomes for this meta-analysis.

## Risk of bias assessment

Cochrane risk of bias assessment was used to explore the potential sources of bias among the included RCTs. This scale evaluates the following criteria: (1) Randomized sequence generation, (2) allocation concealment, (3) blinding of participants, personnel and outcome assessors, (4) incomplete outcome data, (5) selective outcome reporting, and (6) other sources of bias. Risk of bias was labeled as high, low or unclear by 2 independent investigators (KM and AK). We assessed the symmetry of funnel plots to analyze the publication bias amongst the involved trials.

## Data synthesis and statistical analysis

We calculated mean difference (MD) or relative risk (RR) and their corresponding 95% confidence interval (CI) of reported outcomes between minocycline and placebo groups for each RCT, and pooled separately using the random-effects model (DerSimonian Laird) [14]. We used inverse variance method to calculate MD for continuous outcomes, and Mantel–Haenszel (M-H) method to assess the difference in risks between the treatment arms for categorical variables. For studies with a zero cell, we used a continuity correction of 0.5 [15]. The RCTs with zero events in both cells were not depicted in the final plots; however, their data was used to obtain the final pooled RR using M-H method. We additionally performed shift analysis based on 3-month mRS-scores, and the effect corresponds to common OR (cOR) in ordinal logistic regression analyses (per 1-grade improvement in mRS-scores). As per the Cochrane Handbook for Systematic Reviews of Interventions [16], we assessed for heterogeneity using Cochran Q and  $I^2$  statistics. For the qualitative interpretation of heterogeneity,  $I^2 > 50\%$  and  $I^2 > 75\%$  indicated substantial and considerable heterogeneity, respectively. Publication bias across individual studies was graphically evaluated using a funnel plot [17], while formal assessment with Egger's test could not be performed due to small number of studies (< 10 RCTs) [18]. We performed equivalent z test for each pooled RR, and a two-tailed  $p$  values < 0.05 was considered statistically significant [14]. Sensitivity analyses were conducted separately for the subgroups of AIS and ICH. All statistical analyses were carried out with Cochrane Collaboration's Review Manager Software Package (RevMan 5.3) and the metafor package of R 3.3.2 (The R Foundation).

## Results

### Study selection and study characteristics

Systematic search of all the databases yielded a total of 2009 articles. After removing the duplicates, the titles and abstracts

from the remaining 1122 studies were screened and 11 potentially eligible studies were retained for full-text evaluation. After retrieving the full-text version of the aforementioned 11 studies, 2 RCTs [19, 20] were excluded due to lack of comparator groups, whereas remaining 2 studies were duplicates among the 11 potentially eligible studies (Supplemental Table I). After careful evaluation and without disagreements amongst the 2 reviewers, 7 RCTs were included that met the study protocol's inclusion criteria. The detailed study flow chart is presented in Supplemental Figure I. The corresponding authors of 5 RCTs [9–13] were individually contacted who contributed their original unpublished data for clinical and radiological outcomes from their respective RCTs.

Table 1 summarizes the 7 RCTs comprising of 426 patients with a median follow-up of 3 months. Three RCTs were conducted in Asia, two in Australia, and the remaining two RCTs were conducted in North America. The mean values for various clinical outcomes assessed at 3-months were available from 6 RCTs for mRS-scores [9–12, 21, 22], and 5 RCTs each for NIHSS [10, 11, 13, 21, 22] and BI [9, 10, 12, 21, 22] scores. The doses of minocycline varied from 200 mg/day in 4 RCTs, 400 mg/day in 1 RCT and 10 mg/kg/day in 1 RCT with a maximum allowed dosage up to 700 mg/day. The distribution of acute stroke subtypes across the 7 RCTs were AIS ( $n=4$ ), ICH ( $n=2$ ), AIS and ICH ( $n=1$ ). Although majority of the study cohort for Kohler and colleagues [9] comprised of AIS patients, approximately 12% of the cohort involved hemorrhagic strokes and separate clinical outcome data was not available for each subgroup of patients. The baseline characteristics (demographics and vascular risk factors) of the included studies are presented in Supplemental Table II.

### Study quality and publication bias

The individual and pooled assessments of the risk of bias across included RCTs are presented in Supplemental Figure II. Random sequence generation and allocation concealment was low in half of the included trials, while the risk was unclear in the remaining trials. All the RCTs were noted to have a high risk of performance bias due to their open-label design, while only one RCT was noted to have detection-bias. Visual inspection of the funnel plots revealed no evidence of publication bias for the outcomes of 3-month mRS (Supplemental Figure III), 3-month NIHSS score (Supplemental Figure IV), and 3-month BI (Supplemental Figure V).

### Efficacy outcomes

#### Modified Rankin Scale score

In the pooled analysis, minocycline demonstrated a trend towards higher likelihood of functional independence

**Table 1** Study design and characteristics of included studies in our meta-analysis

References	Continent	Patient cohort	No. of patients—minocycline, placebo	Minocycline dose, route	Route, duration of treatment	Minocycline administration from symptom onset, hours	Outcomes
Amiri-Nikpour et al. [13]	Asia	AIS	26, 27	200 mg, once daily	Oral, 5 days	6–24 h	NIHSS
Blacker et al. [12]	Australia	AIS	21, 23	200 mg, twice daily	IV, 5 doses	< 6 h	NIHSS, mRS, BI
Chang et al. [10]	North America	ICH	10, 10	10 mg/kg (max 700 mg/day), once daily	IV, 5 days	< 12 h	NIHSS, mRS, BI
Fouda et al. [11]	North America	ICH	8, 8	400 mg, once daily	IV (day 1)+Oral (day 2–5)	< 24 h	mRS
Kohler et al. [9]	Australia	AIS+ICH	44, 48	100 mg, twice daily	IV, 5 doses	< 24 h	NIHSS, mRS, BI
Lampl et al. [21]	Asia	AIS	74, 77	200 mg, once daily	Oral, 5 days	6–24 h	NIHSS, mRS, BI
Padma Srivastava et al. [22]	Asia	AIS	23, 27	200 mg, once daily	Oral, 5 days	6–24 h	NIHSS, mRS, BI

AIS acute ischemic stroke, ICH intracerebral hemorrhage, NIHSS National Institutes of Health Stroke Scale, mRS modified rankin scale, BI Barthel Index, IV intravenous

(defined as mRS-scores of 0–2) at 3 months (6 RCTs, 370 patients; RR = 1.31; 95% CI 0.98–1.74,  $p = 0.06$ ;  $p$  for Cochran Q statistic = 0.002,  $I^2 = 73\%$ ; Fig. 1). Minocycline was independently associated with increased mRS 0–2 in AIS patients (3 RCTs, 242 patients; RR = 1.59; 95% CI 1.19–2.12,  $p = 0.002$ ;  $p$  for Cochran Q statistic = 0.09,  $I^2 = 58\%$ ), whereas, no such association was observed in ICH patients (2 RCTs, 36 patients; RR = 1.01; 95% CI 0.76–1.35,  $p = 0.93$ ;  $p$  for Cochran Q statistic = 0.40,  $I^2 = 0\%$ ) or combined AIS and ICH patients (1 RCT, 92 patients; RR = 0.96; 95% CI 0.72–1.28,  $p = 0.77$ ). No association was noted for mean 3-month mRS in the pooled analysis (6 RCTs, MD = -0.27; 95% CI -0.89, 0.34;  $p = 0.38$ ; Supplemental Figure VI) or in either subgroup, however, considerable heterogeneity was observed with an  $I^2 = 80\%$  ( $P$  for Cochran Q statistic = 0.0002). Minocycline, in comparison to placebo, was not associated with improvement in 3-month mRS-scores (shift analysis from 6 RCTs; cOR: 1.64; 95% CI 0.49–5.45;  $p = 0.42$ ;  $p$  for Cochran Q statistic < 0.001,  $I^2 = 87\%$ ; Supplemental Figure VII).

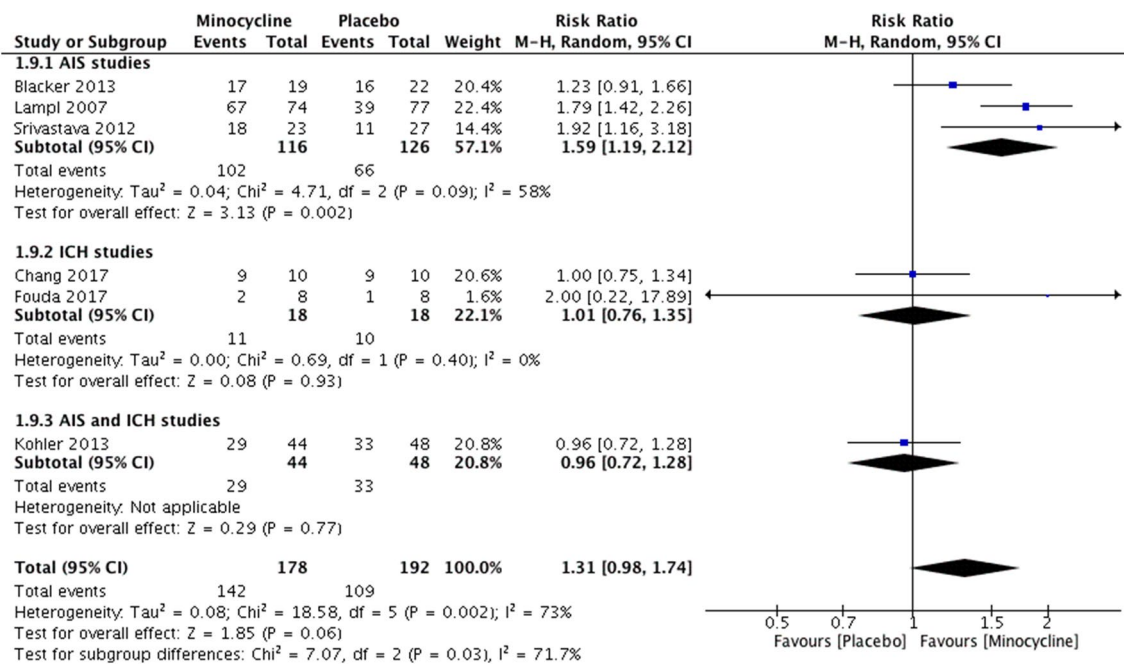
With regards to different time intervals, there was no association of minocycline with 5–7 day mRS (3 RCTs; MD = -0.68; 95% CI -1.78, 0.42,  $p = 0.23$ ;  $p$  for Cochran Q statistic < 0.0001,  $I^2 = 90\%$ ; Supplemental Figure VIII), 30-day mRS (5 RCTs; MD = -0.36; 95% CI -1.20, 0.48,  $p = 0.40$ ;  $p$  for Cochran Q statistic < 0.0001,  $I^2 = 89\%$ ; Supplemental Figure IX), and 3-month favorable functional outcome (defined as mRS-scores of 0–1) (6 RCTs; RR = 1.29; 95% CI 0.71–2.35,  $p = 0.40$ ;  $p$  for Cochran Q statistic < 0.0001,  $I^2 = 83\%$ ; Supplemental Figure X).

### NIHSS score

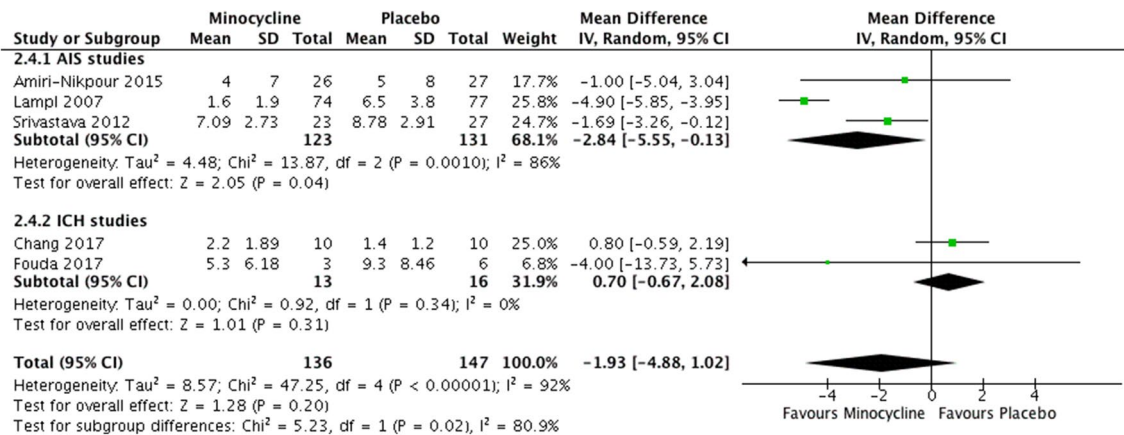
Although no association was observed with mean 3-month NIHSS score (5 RCTs, 283 patients, MD = -1.93; 95% CI -4.88, 1.02;  $p = 0.20$ ;  $p$  for Cochran Q statistic < 0.0001,  $I^2 = 92\%$ ; Fig. 2), minocycline was associated with a reduction in 3-month NIHSS-score in AIS subgroup (3 RCTs, 254 patients; MD = -2.84; 95% CI -5.55, -0.13,  $p = 0.04$ ;  $p$  for Cochran Q statistic = 0.001,  $I^2 = 86\%$ ), whereas no association was observed in ICH subgroup (2 RCTs, 29 patients; MD = 0.70; 95% CI -0.67, 2.08,  $p = 0.31$ ;  $p$  for Cochran Q statistic = 0.34,  $I^2 = 0\%$ ). No significant association was observed with minocycline for 5–7 day NIHSS score (7 RCTs; MD = -0.74; 95% CI -1.85, 0.37,  $p = 0.19$ ;  $p$  for Cochran Q statistic = 0.26,  $I^2 = 22\%$ ; Supplemental Figure XI) and 30-day NIHSS score (4 RCTs; MD = -1.09; 95% CI -5.16, 2.99,  $p = 0.60$ ;  $p$  for Cochran Q statistic < 0.0001,  $I^2 = 92\%$ ; Supplemental Figure XII).

### Barthel Index score

Minocycline was associated with a higher likelihood of improved 3-month BI amongst AIS patients (3 RCTs, 242 patients; MD = 12.37; 95% CI 5.60, 19.14,  $p = 0.0003$ ;  $p$  for Cochran Q statistic = 0.15,  $I^2 = 47\%$ ; Fig. 3); however, there was no beneficial effect in the ICH (1 RCT, 20 patients; MD = -1.00; 95% CI -11.51, 9.51,  $p = 0.85$ ) or combined AIS and ICH (1 RCT, 92 patients; MD = -1.62; 95% CI -12.69, 9.45,  $p = 0.77$ ) subgroups. Additionally,



**Fig. 1** Forest plot showing the association of minocycline (compared to placebo) with 3-month functional independence (defined as mRS-scores of 0–2) in the subgroups of AIS and ICH



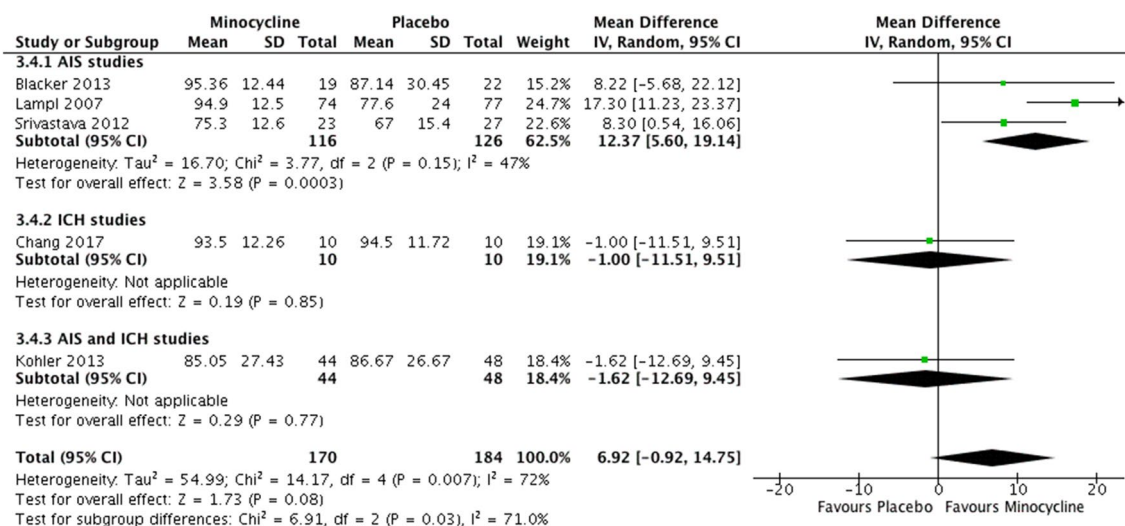
**Fig. 2** Forest plot showing the association of minocycline (compared to placebo) with 3-month NIHSS score in the subgroups of AIS and ICH

a trend towards significance was noted for a higher likelihood of mean 3-month BI in pooled analysis (5 RCTs, 354 patients; MD = 6.92; 95% CI -0.92, 14.75;  $p = 0.08$ ;  $p$  for Cochran Q statistic = 0.007,  $I^2 = 72\%$ ). No significant association was observed with minocycline for 5–7 day BI (3 RCTs; MD = 11.26; 95% CI -3.97, 26.49,  $p = 0.15$ ;  $p$  for Cochran Q statistic = 0.004,  $I^2 = 82\%$ ; Supplemental Figure XIII), and 30-day BI (5 RCTs; MD = 5.45; 95% CI -6.49, 17.40,  $p = 0.37$ ;  $p$  for Cochran Q statistic = 0.0004,  $I^2 = 80\%$ ; Supplemental Figure XIV).

**Safety outcomes**

**Mortality**

In the pooled analysis, minocycline administration did not increase the risk of 3-month mortality (7 RCTs involving 426 patients; RR: 0.70; 95% CI 0.29–1.72,  $p = 0.44$ ; Supplemental Figure XV) after using the continuity correction (Supplemental Table III). There was no evidence of heterogeneity, with an  $I^2 = 10\%$  ( $p$  for Cochran Q statistic = 0.34).



**Fig. 3** Forest plot showing the association of minocycline (compared to placebo) with 3-month BI in the subgroups of AIS and ICH

### Recurrent stroke, myocardial infarction and hemorrhagic conversion

After using the continuity correction, no significant association of minocycline was observed for recurrent stroke or myocardial infarction (5 RCTs; RR = 0.51; 95% CI 0.20–1.31;  $p = 0.16$ ;  $p$  for Cochran Q statistic = 0.95,  $I^2 = 0\%$ ; Supplemental Figure XVI) and hemorrhagic transformation (4 RCTs; RR = 0.84; 95% CI 0.13–5.33;  $p = 0.85$ ;  $p$  for Cochran Q statistic = 0.25,  $I^2 = 23\%$ ; Supplemental Figure XVII).

### Neuroimaging and biochemical endpoints in ICH patients

Although the number of patients included in 2 RCTs involving ICH patients was low ( $n = 36$  patients), we sought to assess the neuroprotective effect of minocycline among ICH patients by analyzing serum biomarker MMP-9 levels, and neuroimaging metrics including PHE and ICH volumes. Minocycline was not associated with a reduction of MMP-9 at days 1–3 (2 RCTs; MD 28.87; 95% CI -57.81, 115.55,  $p = 0.51$ ;  $p$  for Cochran Q statistic = 1.00,  $I^2 = 0\%$ ; Supplemental Figure XVIII), PHE volume at days 1–3 (2 RCTs; MD 5.45; 95% CI -6.12, 17.02,  $p = 0.36$ ;  $p$  for Cochran Q statistic = 0.74,  $I^2 = 0\%$ ; Supplemental Figure XIX), PHE volume at days 5–11 (2 RCTs; MD 10.11; 95% CI -5.47, 25.68,  $p = 0.20$ ;  $p$  for Cochran Q statistic = 0.98,  $I^2 = 0\%$ ; Supplemental Figure XX), ICH volume at days 1–3 (2 RCTs; MD 3.00; 95% CI -8.43, 14.43,  $p = 0.61$ ;  $p$  for Cochran Q statistic = 0.99,  $I^2 = 0\%$ ; Supplemental Figure XXI) and ICH volume at days 5–11 (2 RCTs; MD -2.94; 95% CI -15.66, 9.78,  $p = 0.65$ ;  $p$  for Cochran Q statistic = 0.70,  $I^2 = 0\%$ ; Supplemental Figure XXII).

### Subgroup analyses

We compared the RCTs for their reported clinical outcomes based on the route of minocycline administration. In the subgroup analysis of RCTs comparing oral and intravenous minocycline administration, oral route was significantly associated with a higher likelihood of 3-month functional independence (3 RCTs involving 217 patients; RR = 1.81; 95% CI 1.47–2.24,  $p < 0.001$ ;  $p$  for Cochran Q statistic = 0.96,  $I^2 = 0\%$ ; Supplemental Figure XXIII). Similarly, oral route was associated with higher clinical improvement for mean 3-month NIHSS score (4 RCTs involving 263 patients; MD = -2.93; 95% CI -5.45, -0.42,  $p = 0.02$ ;  $p$  for Cochran Q statistic = 0.003,  $I^2 = 78\%$ ; Supplemental Figure XXIV) and mean 3-month BI (2 RCTs involving 201 patients; MD = 13.14; 95% CI 4.34–21.93,  $p = 0.003$ ;  $p$  for Cochran Q statistic = 0.07,  $I^2 = 69\%$ ; Supplemental Figure XXV).

### Discussion

To the best of our knowledge, this is the first comprehensive systematic review and meta-analysis to evaluate the efficacy and safety profiles of minocycline in comparison to placebo involving acute stroke patients. Among 7 RCTs involving 426 patients, minocycline suggested a trend towards improved 3-month functional independence and BI scores. This association was significantly noted in our sensitivity analyses involving AIS patients for 3-month functional independence, BI and NIHSS scores. Additionally, minocycline was safe in both ischemic and hemorrhagic stroke patients, and was not related to increased mortality, recurrent stroke or adverse events. Finally, we observed no association of

minocycline with any of the neuroimaging outcomes including hemorrhagic conversion, PHE, and hematoma volume.

Prior human studies have investigated the efficacy of minocycline in patients with ischemic stroke. However, the results were conflicting to assess the efficacy and further stratify its neuroprotective role. Kohler and colleagues [9] performed a multicenter RCT to assess the efficacy and safety of minocycline in both ischemic and hemorrhagic stroke patients. Although the trial demonstrated that intravenous administration of minocycline is safe, its efficacy could not be established, as the study was underpowered to analyze the treatment effect of minocycline. On the contrary, three additional RCTs [13, 21, 22] have demonstrated the efficacy of minocycline among ischemic stroke patients by demonstrating the improvement in NIHSS score [13, 21, 22], mRS score [21, 22] and BI [21, 22]. Our detailed analyses highlights the net clinical benefit of minocycline in AIS patients for early stroke recovery. With the significant clinical benefit observed with minocycline in AIS subgroup, it is reasonable to anticipate that minocycline could be routinely used for clinical recovery of AIS patients in future. Although the results are reassuring, the included trials were observed to have inherent heterogeneities amongst the comparator groups, especially in relation to the dosing, route of delivery, and therapeutic window for minocycline. At this stage, our results likely serve for the design of future adequately powered phase III RCTs that will eventually establish potential efficacy and neuroprotective role of minocycline in AIS patients.

Minocycline is a semisynthetic antibiotic with additional neuroprotective properties. Apart from inhibition of MMP-9 levels, data suggests that minocycline has other pleiotropic properties including anti-inflammation, anti-oxidation, anti-apoptosis, vascular protection, and reduction of harmful bleeding effects from IV tPA [7, 8]. Increase in MMP-9 levels has been associated with the disruption of BBB [23], poor functional recovery [24], worsening of hematoma [25] and perihematomal edema [26] volumes in patients with hemorrhagic strokes. Switzer et al. have previously assessed the levels of MMP-9 in AIS patients receiving IV tPA, and corroborated these findings with a trend for reduced MMP-9 levels within an hour of minocycline infusion [19]. These findings underscore the potency of minocycline as an adjunctive therapy with IV tPA, with a potential to expand the narrow thrombolytic window period in ischemic stroke patients [7, 8].

Recently, few studies have investigated the clinical efficacy and safety of minocycline administration in ICH patients. Chang et al. [10] conducted a pilot RCT for the assessment of MMP-9 inhibition and safety profile of minocycline in patients with acute spontaneous ICH. Although the trial was underpowered to assess for functional recovery or radiological improvement based

on PHE and ICH volumes, the authors noted a trend towards reduced MMP-9 levels, especially after days 3–5 of minocycline administration. A similar trial assessed the pharmacokinetic and safety profile of minocycline in ICH patients [11]. However, due to limitations in sample size and incomplete enrollment, no clinical benefit was detected with minocycline in any of the primary and secondary clinical, neuroimaging and biochemical endpoints. Additionally, these RCTs primarily targeted to assess the safety of minocycline and accordingly included lower hematoma volumes (mean 14.8 ml and 20.1 ml, respectively). This might have introduced type II error since minocycline would require substantial perihematomal volumes to demonstrate neuroprotection via MMP inhibition [27]. However, these RCTs coupled with the present meta-analysis highlight the safety of intravenous minocycline at doses as high as 10 mg/kg/day among patients with acute ICH. Future phase II RCTs will provide additional insights regarding the potential benefit of minocycline in hematoma expansion, PHE and functional improvement. Further, the safety of minocycline in ICH would permit its use in the prehospital setting, facilitating ultra-early treatment in the field by paramedics, potentially salvaging the penumbra for thrombolytic or endovascular treatment, and increasing the likelihood of functional recovery.

Our study has certain limitations that need to be acknowledged. First, the design of all included RCTs was open-label and this may have resulted in performance bias. Second, majority of the included RCTs were small and underpowered, and this could have introduced random error in our analyses. Third, heterogeneity in the majority of the included trials could have imposed further limitations. We attempted to address these methodological shortcomings by conducting sensitivity analyses separately in ischemic and hemorrhagic stroke patients. Additionally, we used random-effects model to assess the heterogeneity in our analyses. Fourth, the included RCTs evaluated heterogeneous doses and routes for minocycline administration that could have affected the pharmacokinetic profile, and thus may account for the heterogeneity documented in our meta-analysis. Finally, the variable therapeutic time window ranging between 6 and 24 h following symptom onset may represent another source of heterogeneity across the included studies.

In conclusion, our systematic review and meta-analysis consolidates all available clinical data on minocycline investigated in acute stroke patients. Although the data is limited, our results suggest minocycline as an efficacious and a safe agent in acute stroke patients. Our study underscores minocycline as a promising neuroprotective agent in AIS subgroup. Future adequately powered RCTs are warranted to establish the potential efficacy of minocycline separately in AIS and ICH subtypes.

**Author contributions** KM: Study concept and design, acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content. JJC: Acquisition and interpretation of data, critical revision of the manuscript for important intellectual content. AK: Analysis and interpretation, critical revision of the manuscript for important intellectual content. DB: Acquisition and interpretation of data, critical revision of the manuscript for important intellectual content. JAS: Acquisition and interpretation of data, critical revision of the manuscript for important intellectual content. NG: Acquisition and interpretation of data, critical revision of the manuscript for important intellectual content. AVH: Analysis and interpretation, critical revision of the manuscript for important intellectual content. VP: Analysis and interpretation, critical revision of the manuscript for important intellectual content. AVA: Acquisition and interpretation of data, critical revision of the manuscript for important intellectual content. GT: Study concept and design, study supervision, critical revision of the manuscript for important intellectual content.

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### Compliance with ethical standards

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