

Rationale and Study Design to Assess the Efficacy and Safety of Minocycline in Patients with Moderate to Severe Acute Ischaemic Stroke (EMPHASIS)

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ABSTRACT

Background Inflammation and blood-brain barrier disruption may contribute to the pathogenesis of ischaemic stroke. Minocycline was shown to exert anti-inflammatory effects by attenuating microglial activation and protecting blood-brain barrier in preclinical studies. Previous small-scale clinical studies have suggested that minocycline may have a potential beneficial effect on prognosis in acute ischaemic stroke. However, the efficacy and safety of minocycline in patients with acute ischaemic stroke need to be further confirmed.

Study aims We designed the study, Efficacy and Safety of Minocycline in Patients with Moderate to Severe Acute Ischaemic Stroke (EMPHASIS), to evaluate the effect of minocycline in improving the functional outcome and the drug safety in patients with acute ischaemic stroke.

Methods The EMPHASIS study is a multicentre, randomised, double-blind, placebo-controlled trial aiming to recruit patients with acute ischaemic stroke. Patients who had ischaemic stroke within 72 hours of onset, a National Institutes of Health Stroke Scale score between 4 and 25 and $la \leq 1$ (moderate-to-severe) will be randomly allocated to either minocycline or placebo groups in a 1:1 ratio. Patients will receive minocycline (or placebo) with a loading dose of 200 mg, and subsequent 100 mg every 12 hours for 4 days. All patients will receive routine guideline-based treatment. The primary efficacy outcome is an excellent functional outcome assessed by the proportion of modified Rankin Scale score of 0–1 at 90±7 days. The main safety outcomes include the number of symptomatic intracranial haemorrhage at 24±2 hours and 6±1 days.

Discussion The EMPHASIS trial is the first phase III trial to investigate whether minocycline is effective and safe in improving functional outcome at 90 days in patients with moderate-to-severe acute ischaemic stroke. The data generated may provide valuable evidence of a potential anti-inflammation treatment for ischaemic stroke.

INTRODUCTION AND RATIONAL

Acute ischaemic stroke (AIS) is a major contributor to global mortality and disability, of which the key treatment is vascular recanalisation to improve the perfusion of ischaemic

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Minocycline has an anti-inflammatory effect from attenuating microglial activation and maintaining the integrity of blood-brain barrier in preclinical studies. Previous small-scale clinical studies have also indicated that minocycline may have a positive impact on prognosis in acute ischaemic stroke. Further validation of its efficacy and safety is warranted through a large-scale randomised controlled trial.

WHAT THIS STUDY ADDS

⇒ The Efficacy and Safety of Minocycline in Patients with Moderate to Severe Acute Ischaemic Stroke (EMPHASIS) trial will address the issue of whether minocycline is effective and safe in patients with an acute ischaemic stroke (National Institutes of Health Stroke Scale between 4 and 25 and $la \leq 1$) within the past 72 hours.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ EMPHASIS study will provide valuable evidence for the potential use of minocycline as a complementary therapy to the routine treatment of acute ischaemic stroke.

penumbra and prevent the progression of ischaemic injury.^{1–3}

However, owing to the narrow therapeutic time window, strict patient selection and technical requirements, only a small subset of patients can successfully receive reperfusion treatment and restore cerebral perfusion.^{4 5} There is a pressing necessity to develop novel neuroprotective strategies to further reduce disability in these patients.

Neuroinflammation may be one of the mechanisms initiating brain tissue damage related to AIS. Within a few hours of AIS, injured cells release ‘danger signals’, such as damage-associated molecular patterns and

activate microglia. Activated microglia secrete proinflammatory cytokines, damage the blood-brain barrier (BBB) through the matrix metalloproteinase (MMP) pathway and recruit peripheral immune cells, triggering a cascade of local and global inflammatory reactions in the brain and leading to long-term neurological deficits.^{6–8} Therefore, in patients with AIS, early regulation of neuroinflammation to further improve neuronal survival may be an effective strategy worth exploring in the future.

Minocycline is a second-generation semisynthetic tetracycline that has been widely used for the treatment of skin and other infections.⁹ When taken orally, it has good pharmacokinetic characteristics, including high bioavailability, a long half-life and high safety for long-term application. Moreover, its high lipophilicity allows it to readily penetrate the BBB and play multiple roles in regulating neuroinflammation: anti-inflammation, anti-apoptosis and anti-oxidation.^{10 11}

In preclinical experiments, minocycline can inhibit factors such as MMP-2 and MMP-9,^{12 13} mitigating ischaemia-reperfusion injury, enhancing neuronal survival and supporting BBB remodelling.¹⁴ It also functions as an anti-inflammatory agent by suppressing the proliferation and activation of microglia^{15 16} as well as shifting proinflammatory microglia towards anti-inflammatory phenotype.¹⁷ Therefore, minocycline may serve as a promising therapeutic agent with multiple targets and mechanisms to treat patients with AIS.

A meta-analysis including four human studies and 14 rodent experiments showed that receiving minocycline may have effect on reducing National Institutes of Health Stroke Scale (NIHSS) score, modified Rankin Scale (mRS) score and infarct volume in acute ischemic stroke.¹⁸ Another systematic review involving 35 animal studies and six clinical trials explored the neuroprotective effect of minocycline against ischaemia-induced damage, and revealed that minocycline may increase the activity of neurons and reduce the infarct volume.¹⁹

Regarding its safety, there is no evidence that minocycline might increase the risk of intracranial haemorrhage in ischaemic stroke, but previous studies contained small sample sizes, which may limit the study conclusion.^{20–22} Besides, the possible effect of minocycline on BBB and platelet-related inflammation might have an effect on the risk of intracranial haemorrhage.

Therefore, we designed this phase III clinical trial to prove the efficacy and safety of minocycline. We hypothesise that minocycline may reduce the excess neuroinflammation during AIS and improve the clinical outcomes.

METHODS

Study design

Efficacy and Safety of Minocycline in Patients with Moderate to Severe Acute Ischaemic Stroke (EMPHASIS) is a prospective, multicentre, randomised, double-blind, placebo-controlled parallel trial. Eligible participants will be randomly allocated to one of the two treatment groups

receiving either minocycline or placebo in a ratio of 1:1, with a follow-up duration of 90 days. The study will be conducted at 58 hospitals in China (online supplemental table 1).

Study population

The study will recruit patients with moderate-to-severe AIS. Participants are eligible if they are aged 18–80 years, suffered AIS in the past 72 hours with an NIHSS score between 4 and 25 and $Ia \leq 1$ and have functional independence assessed by $mRS \leq 1$ before the current attack. The main exclusion criteria for participating in the study include being allergic to tetracycline antibiotics or any component of minocycline, and use of tetracycline antibiotics within one week. The detailed inclusion and exclusion criteria are shown in box 1.

Blinding and randomisation

This study employs a double-blind design. To ensure blinding, the treatment and control drugs are uniformly packaged, with each package bearing a unique random number. Subjects' medications are pre-allocated according to the random code table. Randomisation allocation will be generated by separate statistical teams using a centralised computer. To maintain an approximate balance of allocation in a 1:1 ratio, block randomisation with fixed block sizes is adopted. For participants eligible for randomisation, each subcentre will assign a random number in an ascending sequence. Unblinding is permitted when adverse events occur and disclosing investigational drug information is essential for the participant's treatment.

Intervention

Eligible participants will be randomly allocated into minocycline or placebo groups in a 1:1 ratio. All participants will receive an initial dose of 200 mg within 30 min after randomisation and followed by 100 mg every 12 hours for 4 days (figure 1). Minocycline hydrochloride or placebo capsules will be administered orally or through a nasogastric feeding tube if the participant has dysphagia. All study intervention is based on participants' routine guideline-based treatment.

Follow-up visits and data collection

All study patients are hospitalised. We will visit participants at baseline, 24 ± 2 hours, 6 ± 1 days and 90 ± 7 days.

At baseline, the study will record details of the presenting stroke, medical history, medication use, physical examination, mRS before stroke onset, NIHSS score, laboratory biomarkers including high-sensitivity C-reactive protein (hs-CRP) level. 6 mL of venous blood will be collected for the assessment of inflammation and BBB-related biomarkers. The neuroimaging data used to diagnose ischaemic stroke will also be retrieved.

At 24 ± 2 hours and 6 ± 1 days, researchers will evaluate the NIHSS score and re-collect the blood sample. hs-CRP will be repeatedly assessed at 6 ± 1 days.

At 90 ± 7 days, the mRS score will be evaluated by a trained local investigator by telephone or in-person

Box 1 Inclusion and exclusion criteria of Efficacy and Safety of Minocycline in Patients with Moderate to Severe Acute Ischaemic Stroke trial

Inclusion criteria

1. Aged between 18 and 80 years.
2. Having acute ischaemic stroke (diagnosed by CT or MRI) in the past 72 hours.
3. Having 4≤NIHSS score ≤25, and the level of consciousness level (la)≤1.
4. The first stroke, or mRS score ≤1 prior to this episode.
5. Informed consent signed by the patient or their legal representative.

Exclusion criteria

1. Having a history of pseudomembranous colitis or antibiotic-related colitis.
2. Being allergic to tetracycline antibiotics or any component of the study drug.
3. Being tolerant to tetracyclines.
4. Taking tetracycline antibiotics in the past 7 days.
5. Having a community-acquired bacterial infection, for example, urinary tract infection or pneumonia.
6. Having a history of intracranial haemorrhage in the past 3 months, for example, parenchymal haemorrhage, intraventricular haemorrhage, subarachnoid haemorrhage, subdural or external haematoma.
7. Having brain tumours or vascular malformations.
8. Having large vessel occlusion with uncommon or undetermined aetiology, for example, vasculitis and arterial dissection.
9. Having severe renal insufficiency (blood creatinine >3.0 mg/dL (265.2 µmol/L), or glomerular filtration rate <30 mL/min/1.73 m², or receiving dialysis), or having severe hepatic insufficiency (serum ALT or AST >three times of the upper limit of normal).
10. Having bleeding tendency, including receiving heparin in the past 48 hours with APTT≥35 s, receiving oral warfarin with INR>2, having platelet count <100×10⁹/L, or having inherited bleeding disorders.
11. Receiving tretinoin, androgen or antiandrogen treatment (eg, anabolic steroids, spironolactone) in the past 3 months.
12. Having a history of intracranial or spinal surgery in the past 3 months; having a history of surgery or severe trauma in 1 month.
13. Pregnant, likely pregnant or breastfeeding.
14. Life expectancy less than 6 months.
15. Participated in any other clinical trial in the past 3 months.
16. Having any conditions that, in the opinion of the clinician may represent difficulties for the subjects' full participation in the study, such as unable to follow the study procedures due to physical, cognitive, emotional or mental disorders.

ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate transaminase; INR, international normalised ratio; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale.

interview. The process of evaluation should be recorded and the mRS score is centrally corrected by clinical inspectors to ensure the accuracy and consistency. Other variables include EuroQol Group 5-Dimension (EQ-5D) questionnaire score and adverse events (table 1).

All adverse events will be recorded including the date and possible causes. Participants are encouraged to contact their local centre to report any symptom

occurring during the follow-up visits. Collected and verified data will be transmitted using the electronic data capture system.

Study outcomes

Efficacy outcome

The primary outcome is an excellent functional outcome measured by the proportion of the mRS score of 0–1 at 90±7 days. Secondary outcomes include mRS distribution at 90±7 days; (2) changes in NIHSS score at 24±2 hours and 6±1 days compared with baseline score; (3) changes in hs-CRP level at 6±1 days compared with baseline level; (4) early neurological deterioration at 24±2 hours and 6±1 days (5) recurrent stroke (including ischaemic and haemorrhagic stroke) at 90±7 days; (6) recurrent ischaemic stroke at 90±7 days; (7) composite vascular events (stroke, myocardial infarction and vascular death) at 90±7 days; (8) quality of life (EQ-5D) score at 90±7 days.

Exploratory outcomes include changes in the levels of venous neuroinflammation and thrombo-inflammation indicators at 24±2 hours and 6±1 days compared with baseline levels; cerebral haemodynamic function at 6±1 days and 90±7 days as well as changes in the levels of venous intestinal flora metabolites (plasma TMAO [trimethylamine N-oxide] and its precursors, etc) at 6±1 days compared with baseline levels.

Safety outcomes

The main safety outcome includes symptomatic intracranial haemorrhage at 24±2 hours and 6±1 days, including both spontaneous and treatment-related. Other safety outcomes include any bleeding event classified by Global Utilisation of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries Criteria,²³ vascular death, all-cause death, investigator-reported adverse events/serious adverse events at 90±7 days and antibiotic-associated diarrhoea, enteritis and constipation at 6±1 days.

The detailed definitions of study outcomes mentioned above are shown in online supplemental table 2.

Sample size estimate

We calculate the proportion of mRS of 0–1 at 3 months after the onset of initial acute ischaemic stroke in the China National Stroke Registry III. The result shows 60% of patients could reach an mRS 0–1 score at 3 months and we use it as the estimate of event rate in the control group. According to previous studies,²⁴ we hypothesize that participants who receive minocycline therapy will increase 8% of the excellent functional outcome. Considering a drop-out rate of 10%, a total of 1672 patients (836 per group) are required to give 90% power to detect the difference of the proportion of mRS 0–1 between the minocycline group and the control group, under a two-tailed significance level of 0.05.

Statistical analyses

In this study, intention-to-treat analysis (ITT) will be used to compare study outcomes between treatment groups.

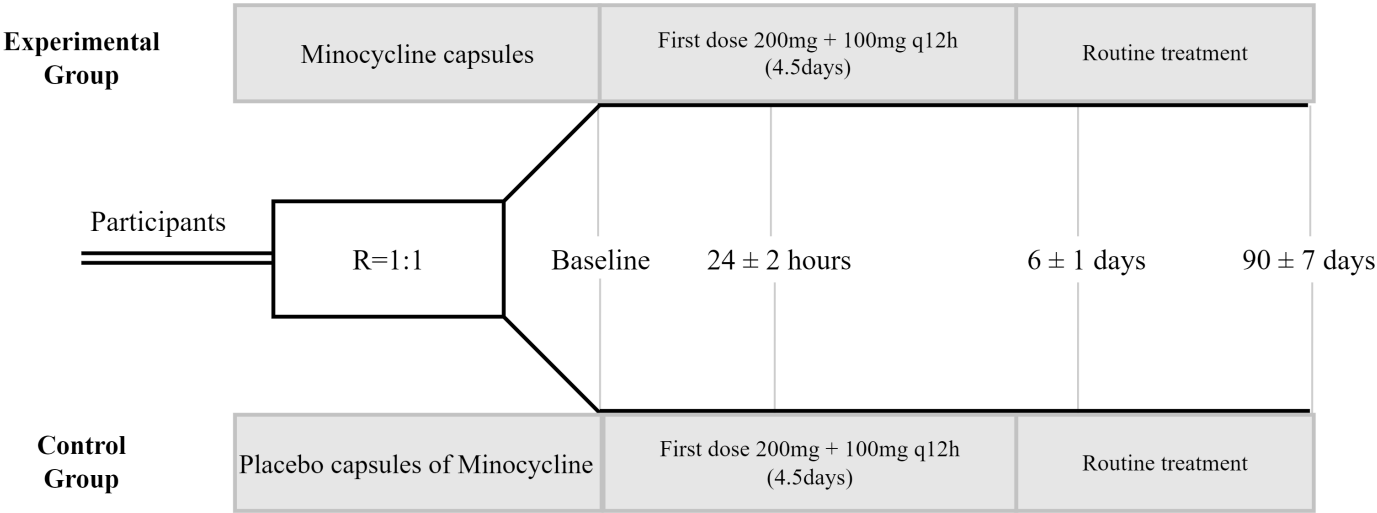


Figure 1 The flow chart of Efficacy and Safety of Minocycline in Patients with Moderate to Severe Acute Ischaemic Stroke trial.

For efficacy outcomes, Chi-squared test and generalized linear model will be used to evaluate the proportion of mRS 0–1 (the primary outcome) between minocycline and control groups in 90 days. Shift analysis of mRS score using an ordinal logistic regression model will also be analyzed. Kaplan-Meier curves and log-rank tests will be used to compare the cumulative risks of vascular events between two treatment groups at 90 days. Cox-proportional hazards regression and generalized linear model will be used to compare different secondary and exploratory efficacy outcomes, as well as the subgroup analyses (below). Confounding factors will be included in the multivariate models based on the result of univariate analysis, previous literature and clinical knowledge. To compare safety outcomes and AE/SAE between the two treatment groups, both Chi-squared test (or Cochran-Mantel-Haenszel test) and survival analysis will be used. A two-sided *p* value of <0.05 will be deemed statistically significant and all analyses will be performed using SAS software V.9.4 (SAS Institute).

Subgroup analyses will evaluate the different effects of minocycline according to age, gender, concomitant diseases (such as hypertension, hyperglycaemia, hyperlipidaemia, previous ischaemic stroke), smoking, drinking, aetiological classification and disease severity, type of therapy, time-to-treatment, the level of inflammatory or BBB-related biomarkers, cerebral haemodynamics, whether combining with cerebral small vessel diseases, intracranial and extracranial vessel status and other neuroimaging features of research interest.

Data safety monitoring and data organisation

The steering committee will provide scientific guidance and ensure the quality of the research. The executive committee will collect available blinded data, assess the trial progress and provide appropriate guidance for the study. The Data and Safety Monitoring Board (DSMB), consisted of independent academic experts and

statisticians not involved in the trial, will monitor the trial progress periodically to ensure that the trial meets the standards of ethics and patient safety. DSMB charter will be certified by its members and the executive committee before enrolment. The clinical event adjudication committee, consisting of independent experts, will adjudicate outcome events uniformly (online supplemental table 3).

Patient data will be recorded and archived in the case record form (CRF). All data will be transmitted to the electronic CRF (eCRF, <https://www.91trial.com>) with password required for access. The data transfer between eCRF and CRF database will use an encrypted connection. The clinical investigators will check the completeness of eCRF, adherence to the study protocol and the consistency between eCRF and the original CRF data set. The principal investigators of all subcentres should be certified in Good Clinical Practice, and they are responsible for the data quality of their centres.

DISCUSSION

Neuroprotection following AIS represents a promising therapeutic approach yet requires additional investigation due to inconsistent findings observed in previous clinical trials.²⁵ The EMPHASIS trial is a multicentre, randomised, double-blind, placebo-controlled trial addressing the question of whether minocycline can enhance the functional prognosis of patients with AIS compared with placebo.

The information from prior clinical studies investigating the application of minocycline in patients with AIS is presented in online supplemental table 4. The first clinical study investigating minocycline in AIS randomly assigned 152 patients within 6–24 hours after onset to receive either oral minocycline or placebo at a dosage of 200 mg for 5 days. The results indicated a significant enhancement in outcomes

Table 1 Assessment schedule

	Screening and enrolment	Treatment and follow-up		
	Visit 1	Visit 2	Visit 3	Visit 4
Time	Baseline	24±2 hours after randomisation	6±1 days after randomisation	90±7 days after randomisation
Informed consent	x			
Inclusion/exclusion criteria	x			
Demographics	x			
Medical history	x			
SARS-CoV-2 infection	x		x	x
Physical examination	x	x	x	
Swallowing function evaluation	x	x		
NIHSS	x	x	x	
mRS	x			x
EQ-5D				x
Reperfusion therapy evaluation		x		
hs-CRP	x		x	
Routine laboratory test	x*	x†		
Venous blood sample‡	x	x	x	
Cerebral haemodynamic function (single-centre)	x		x	x
Outcome event		x§	x	x¶
ECG	x			
Primary diagnosis	x			
Final diagnosis			x	
Randomisation	x			
Compliance		x	x	
Drug dispense/retrieve	x	x	x	
AE/SAE		x	x	x
Concomitant medication	x	x	x	x

*Routine blood counts, liver function, renal function, coagulation function and pregnancy test (for women of childbearing age) must be completed during screening.

†Results of routine blood counts, biochemical panels (including liver function, renal function, blood lipids, creatine kinase), glycosylated haemoglobin, homocysteine and coagulation function test should be recorded if completed in clinical.

‡Venous blood samples will be collected, followed by the separation, packaging and freezing of plasma for storage.

§The assessment of outcome events at 24±2 hours and 6±1 days after randomisation included early neurological deterioration, stroke (ischaemic stroke and haemorrhagic stroke), myocardial infarction, death, symptomatic intracranial haemorrhage, any bleeding event, antibiotics-associated diarrhoea, enteritis and constipation.

¶The assessment of outcome events at 90±7 days after randomisation included stroke (ischaemic and haemorrhagic strokes), myocardial infarction, death and any bleeding event.

AE, adverse event; EQ-5D, EuroQoL-5 Dimension; hs-CRP, high-sensitivity C-reactive protein; mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; SAE, serious adverse event.

for patients treated with minocycline compared with those receiving the placebo.²⁶ Similar favourable effects were also observed in subsequent trials, indicating that timely and short-term administration of minocycline within a therapeutic window of 24 hours after AIS onset and lasting 3–5 days could be helpful in reducing neurological deficits.^{24 27 28} However, a pilot study suggested that commencing five doses of intravenous minocycline within 24 hours following

stroke onset was safe but lacked efficacy.²⁹ Previous clinical trials assessing minocycline in AIS exhibited substantial heterogeneity and were conducted with open-label or non-randomised study design, which were underpowered to reliably ascertain the treatment effect of minocycline.

Several clinical studies on minocycline recruit patients with stroke who underwent reperfusion therapy, for example, the Effects of Minocycline on

Patients With Ischaemic Stroke Undergoing Intravenous Thrombectomy (NCT05487417), Minocycline for Acute Ischaemic Stroke Undergoing Endovascular Treatment Due to Basilar Artery Occlusion (NCT05512910) and Minocycline Efficacy in Improving Neurological Outcome of Patients Who Undergo Endovascular Revascularisation for Acute Ischaemic Stroke (NCT05367362). These studies support the study rationale that minocycline may have an effect on anti-neuroinflammation and improve the clinical outcomes combined with reperfusion therapy of ischaemic stroke as a neuroprotectant. However, evidences have demonstrated that neuroinflammation plays an important role in all phases of ischaemic stroke.^{7 8 30} Several clinical studies thus designed to recruit participants with ischaemic stroke including both the ones receiving reperfusion therapy and others with medication, for instance, Perth Iv Minocycline Stroke Study (ACTRN12612000237886), Neuroprotection With Minocycline Therapy for Acute Stroke Recovery Trial (NCT00930020) and the ongoing open-label clinical trial 'Maimonides Minocycline in Stroke Study (NCT06107725)' which explores the efficacy of minocycline in acute stroke within 24 hours. In order to examine the effect of minocycline in the whole stage of ischaemic stroke and improve the study's generalisability, we designed this study to include patients with ischaemic stroke irrespective of their treatment. In this phase-III clinical trial, we could verify the efficacy and safety of minocycline on all patents with ischaemic stroke and further compare the anti-neuroinflammatory effect of minocycline between participants who have medication therapy and those with futile recanalisation.

Our study has several strengths. First, the sample size surpasses that of previous studies, enhancing the precision of minocycline treatment effect analysis. Second, the trial employs a multicentre, double-blind and block-randomised design to mitigate potential confounders and prevent selection biases. Third, considering that microglial activation peaks at 1 week and shows minimal activation before 72 hours post-stroke in positron emission tomography scans,^{6 31} we extend the enrolment window to 72 hours, potentially broadening the population that may benefit from treatment. Lastly, in addition to assessing neurological function and life independence, we also longitudinally collect venous blood samples to monitor inflammatory biomarkers at baseline, 24 hours and 6 days providing valuable insight into dynamic post-stroke inflammatory response.

SUMMARY AND CONCLUSION

EMPHASIS is the first prospective, multicentre, randomised, double-blind, placebo-controlled trial with the largest sample size to date to explore the effect of minocycline in patients with AIS. This trial

aims to generate novel evidence elucidating the efficacy and safety of minocycline in patients with AIS.

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Contributors YLW had full access to all the study data and takes responsibility for the integrity and accuracy of the data. YL and LG contributed equally to this work as first authors. YLW and LG proposed the study conception and study design. YL and LG prepared the draft of the manuscript. YL, QQY and MYZ conduct the study and collect data. BSQ conducted the animal experiments and provided theoretical support on this study. HQ, XJZ, XLL and YCW help conduct the clinical trial and patient management. YSP and LYW prepare the statistical analysis plan and conduct statistical analyses. DYJ supports study conduction and data management. SCJ, PA, PMB, LPL, XQZ and YJW supervised the study design and edited the article. None of the authors received payment for writing the article. All authors approve the submission of this version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Institutional Review Board of Beijing Tiantan Hospital (KY2023-007-04), and all other participating study centres granted their approval for this trial. Before enrolment, participants provided informed consent to participate in the study.

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REFERENCES

- Feigin VL, Stark BA, Johnson CO, *et al.* Global, regional, and national burden of stroke and its risk factors, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol* 2021;20:795–820.
- Widimsky P, Snyder K, Sulzenko J, *et al.* Acute ischaemic stroke: recent advances in reperfusion treatment. *Eur Heart J* 2023;44:1205–15.
- Yang N, Lee H, Wu C. Intravenous thrombolysis for acute ischemic stroke: From alteplase to tenecteplase. *Brain Circ* 2023;9:61–3.
- Ye Q, Zhai F, Chao B, *et al.* Rates of intravenous thrombolysis and endovascular therapy for acute ischaemic stroke in China between 2019 and 2020. *Lancet Reg Health West Pac* 2022;21:100406.
- Kan Y, Li S, Zhang B, *et al.* No-reflow phenomenon following stroke recanalization therapy: Clinical assessment advances: A narrative review. *Brain Circ* 2023;9:214–21.
- Shi K, Tian DC, Li ZG, *et al.* Global brain inflammation in stroke. *Lancet Neurol* 2019;18:1058–66.
- DeLong JH, Ohashi SN, O'Connor KC, *et al.* Inflammatory Responses After Ischemic Stroke. *Semin Immunopathol* 2022;44:625–48.
- Shichita T, Ooboshi H, Yoshimura A. Neuroimmune mechanisms and therapies mediating post-ischaemic brain injury and repair. *Nat Rev Neurosci* 2023;24:299–312.
- C AJ. Minocycline. *Ann Intern Med* 1976;85:482–7.
- Yong VW, Wells J, Giuliani F, *et al.* The promise of minocycline in neurology. *Lancet Neurol* 2004;3:744–51.
- Chen S-D, Yin J-H, Hwang C-S, *et al.* Anti-apoptotic and anti-oxidative mechanisms of minocycline against sphingomyelinase/ceramide neurotoxicity: implication in Alzheimer's disease and cerebral ischemia. *Free Radic Res* 2012;46:940–50.
- Ohshima S, Fujimoto S, Petrov A, *et al.* Effect of an antimicrobial agent on atherosclerotic plaques: assessment of metalloproteinase activity by molecular imaging. *J Am Coll Cardiol* 2010;55:1240–9.
- Machado LS, Kozak A, Ergul A, *et al.* Delayed minocycline inhibits ischemia-activated matrix metalloproteinases 2 and 9 after experimental stroke. *BMC Neurosci* 2006;7:56:7.
- Yang Y, Salayandia VM, Thompson JF, *et al.* Attenuation of acute stroke injury in rat brain by minocycline promotes blood–brain barrier remodeling and alternative microglia/macrophage activation during recovery. *J Neuroinflammation* 2015;12:26.
- Tikka T, Fiebich BL, Goldsteins G, *et al.* Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 2001;21:2580–8.
- Scott G, Zetterberg H, Jolly A, *et al.* Minocycline reduces chronic microglial activation after brain trauma but increases neurodegeneration. *Brain (Bacau)* 2018;141:459–71.
- Lu Y, Zhou M, Li Y, *et al.* Minocycline promotes functional recovery in ischemic stroke by modulating microglia polarization through STAT1/STAT6 pathways. *Biochem Pharmacol* 2021;186:114464.
- Sheng Z, Liu Y, Li H, *et al.* Efficacy of Minocycline in Acute Ischemic Stroke: A Systematic Review and Meta-Analysis of Rodent and Clinical Studies. *Front Neurol* 2018;9:1103.
- Naderi Y, Panahi Y, Barreto GE, *et al.* Neuroprotective effects of minocycline on focal cerebral ischemia injury: a systematic review. *Neural Regen Res* 2020;15:773–82.
- Kohler E, Prentice DA, Bates TR, *et al.* Intravenous Minocycline in Acute Stroke. *Stroke* 2013;44:2493–9.
- Malhotra K, Chang JJ, Khunger A, *et al.* Minocycline for acute stroke treatment: a systematic review and meta-analysis of randomized clinical trials. *J Neurol* 2018;265:1871–9.
- Fagan SC, Waller JL, Nichols FT, *et al.* Minocycline to improve neurologic outcome in stroke (MINOS): a dose-finding study. *Stroke* 2010;41:2283–7.
- investigators G. An International Randomized Trial Comparing Four Thrombolytic Strategies for Acute Myocardial Infarction. *N Engl J Med* 1993;329:673–82.
- Padma Srivastava MV, Bhasin A, Bhatia R, *et al.* Efficacy of minocycline in acute ischemic stroke: a single-blinded, placebo-controlled trial. *Neurol India* 2012;60:23–8.
- Eren F, Yilmaz SE. Neuroprotective approach in acute ischemic stroke. *Brain Circ* 2022;8:172–9.
- Lampl Y, Boaz M, Gilad R, *et al.* Minocycline treatment in acute stroke: an open-label, evaluator-blinded study. *Neurology (Ecricon)* 2007;69:1404–10.
- Switzer JA, Hess DC, Ergul A, *et al.* Matrix metalloproteinase-9 in an exploratory trial of intravenous minocycline for acute ischemic stroke. *Stroke* 2011;42:2633–5.
- Amiri-Nikpour MR, Nazarbaghi S, Hamdi-Holasou M, *et al.* An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: gender-dependent effect. *Acta Neurol Scand* 2015;131:45–50.
- Kohler E, Prentice DA, Bates TR, *et al.* Intravenous minocycline in acute stroke: a randomized, controlled pilot study and meta-analysis. *Stroke* 2013;44:2493–9.
- Qin C, Yang S, Chu Y-H, *et al.* Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther* 2022;7:215.
- Price CJS, Wang D, Menon DK, *et al.* Intrinsic activated microglia map to the peri-infarct zone in the subacute phase of ischemic stroke. *Stroke* 2006;37:1749–53.