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Safety and efficacy of Angong Niuhuang Pills in patients with moderate-to-severe acute ischemic stroke (ANGONG TRIAL): A randomized double-blind placebo-controlled pilot clinical trial

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Abstract

Background: Preclinical studies have indicated that Angong Niuhuang Pills (ANP) reduce cerebral infarct and edema volumes. This study aimed to investigate whether ANP safely reduces cerebral infarct and edema volumes in patients with moderate to severe acute ischemic stroke.

Methods: This randomized, double-blind, placebo-controlled pilot trial included patients with acute ischemic stroke with National Institutes of Health Stroke Scale (NIHSS) scores ranging from 10 to 20 in 17 centers in China between April 2021 and July 2022. Patients were allocated within 36 h after onset via block randomization to receive ANP or placebo (3 g/day for 5 days). The primary outcomes were changes in cerebral infarct and edema volumes after 14 days of treatment. The primary safety outcome was severe adverse events (SAEs) for 90 days.

Results: There were 57 and 60 patients finally included in the ANP and placebo groups, respectively for modified intention-to-treat analysis. The median age was 66.0 years, and the median NIHSS score at baseline was 12.0. The changes in cerebral infarct volume at day 14 were 0.3 mL and 0.4 mL in the ANP and placebo groups, respectively (median difference: −7.1 mL; interquartile range [IQR]: −18.3 to 2.3 mL, $P = 0.30$). The changes in cerebral edema volume of the ANP and placebo groups on day 14 were 11.4 mL and 4.0 mL, respectively (median difference: 3.0 mL, IQR: −1.3 to 9.9 mL, $P = 0.15$). The rates of SAE within 90 days were similar in the ANP (3/57, 5%) and placebo (7/60, 12%) groups ($P = 0.36$). Changes in serum mercury and arsenic concentrations were comparable. In patients with large artery atherosclerosis, ANP reduced the cerebral infarct volume at 14 days (median difference: −12.3 mL; IQR: −27.7 to −0.3 mL, $P = 0.03$).

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Conclusions: ANP showed a similar safety profile to placebo and non-significant tendency to reduce cerebral infarct volume in patients with moderate-to-severe stroke. Further studies are warranted to assess the efficacy of ANP in reducing cerebral infarcts and improving clinical prognosis.
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Keywords: Angong Niuhuang Pill; Acute ischemic stroke; Cerebral infarct; Cerebral edema; Pharmacology

Introduction

Final infarct volume is a strong predictor of neurological outcomes in patients with acute ischemic stroke (AIS).[1,2] A large infarct volume, which is associated with poor outcomes, can be reduced by intravenous thrombolysis and endovascular therapy (EVT), which salvages the penumbra via vessel recanalization.[3,4] The time window for revascularization has been extended from 3 h to 24 h.[5,6] However, previous studies found that early infarct growth continued up to 5 days after stroke,[7,8] and the benefit decreased as the interval between the time of stroke onset and reperfusion therapy grew.[9,10] Previous studies attempted to extend the time window of intravenous thrombolysis, but unfortunately, without success.[11,12] Thus, intravenous thrombolysis is not permitted in patients after 4.5 h of stroke onset, regardless of infarct growth. In clinical practice, over half of the patients with ischemic stroke caused by large-vessel occlusions were found to be disabled or dead at follow-up, despite undergoing EVT.[4] One factor limiting the efficacy of reperfusion is infarct growth, which occurs despite adequate reperfusion after EVT and predicts a poor outcome.[13,14] The underlying mechanism of infarct growth remains unclear. Age, baseline infarct volume, dysglycemia, collateral circulation, incomplete reperfusion status, and onset-to-reperfusion time can influence infarct growth.[13–16] In addition, each extra pass during EVT leads to a 14% increase in post-EVT infarct volume.[14] Moreover, patients without reperfusion therapy have a higher risk of infarct growth.[17] Thus, minimizing infarct growth might ultimately improve clinical outcomes, especially in patients with moderate-to-severe stroke.

Another challenge in patients with a large infarct is cerebral edema, a known cause of secondary injury after stroke onset, which is associated with high morbidity and mortality.[18,19] Decompressive craniectomy can reduce mortality from malignant cerebral edema but shows substantial morbidity.[20,21] The GAMES-RP study revealed that intravenous glyburide has the potential to reduce cerebral edema in patients with large hemispheric strokes but did not improve the functional prognosis.[22] However, no effective drug is currently available to prevent infarct growth or cerebral edema.

Results of preclinical studies suggested that Angong Niuhuang Pills (ANPs), a traditional Chinese patent medicine, including realgar, cinnabaris, Bovis Calculus, artificial Moschus, and powdered buffalo horn extract, decreased infarct volume and cerebral edema, and presented anti-atherosclerosis and cardio-protective effects in clinical and preclinical studies.[23–25] Furthermore, a meta-analysis of randomized controlled trials found that ANP reduced the neurologic deficit score and increased the Glasgow Coma Scale in patients with AIS,[26] with a low risk of adverse events (AEs).[27] Increasing evidence indicates that ANP is a candidate drug for reducing infarct growth and cerebral edema, but its efficacy needs to be verified in a well-designed clinical trial.

The ANGONG TRIAL was designed as a pilot study to explore the safety and efficacy of ANP in patients with moderate-to-severe AIS. Based on infarct growth dynamics and the current time window for reperfusion therapy, we set the time of ANP prescription to 36 h after stroke onset. We hypothesized that ANP would safely reduce infarct growth and cerebral edema 14 days after enrollment.

Methods

Study design and participants

The ANGONG TRIAL was a randomized, double-blind, placebo-controlled, multicenter trial conducted in 17 Chinese hospitals. The steering committee designed and supervised the trial and analysis. This study was conducted in accordance with the Guidelines for Good Clinical Practice in China and the *Declaration of Helsinki*. This trial was approved by the Ethics Committee of Peking Union Medical College Hospital (No. JS-2371), and written informed consent was obtained from all participants. The study was registered at clinicaltrials.gov, No. NCT04475328.

All consecutive patients with AIS aged 40–80 years visiting any center in our study within 36 h from onset were screened for eligibility according to the inclusion and exclusion criteria. The inclusion criteria were: (1) an age of 40–80 years, (2) diagnosis of AIS, (3) diagnosis of acute cerebral infarction of the internal carotid artery system, (4) a National Institutes of Health Stroke Scale (NIHSS) score ranging from 10 to 20, (5) a time from symptom onset to randomization within 36 h, and (6) provision of informed consent. Patients were excluded if they were found not suitable for taking ANP after the dialectical process by a traditional Chinese medical doctor, received or planned to receive EVT, if they had hemorrhagic transformation after AIS, or received ANP within one month before stroke onset. The full exclusion criteria are presented in Supplementary Material, http://links.lww.com/CM9/B997.

An independent clinical event adjudication committee masked to the trial group assignments was established to assess the primary and secondary outcomes. In addition, a data and safety monitoring board oversaw the trial and regularly monitored safety events.

Randomization and masking

Eligible participants were randomly assigned (1:1) to receive either ANP or placebo (3 g/pill, 1 pill/day for 5

days) using a central stratified block randomization method. Patients were assigned random serial numbers based on their time of enrolment and were provided with the corresponding medicines, which were blind-covered beforehand. The principal investigators, site investigators, physicians, patients, and outcome personnel were blinded to the treatment. For both groups, the pill colors, shapes, and packaging were identical.

Procedures

Clinical procedures

Treatments were orally or nasally administered to the participants immediately after randomization. The dosing schedule was based on clinical practice and previous clinical studies of ANP.[26] Concomitant treatments were administered according to the guidelines from the Chinese Society of Neurology.[28] Antiplatelet therapy and statins were used as a standard of care based on standard guidelines.[28]

Demographic data, signs and symptoms, medical history, laboratory test results, NIHSS scores, and modified Rankin Scale (mRS) scores were collected at baseline. The NIHSS and mRS scores were assessed on days 14 and 90 by a trained senior neurologist using a standardized questionnaire via site visits or telephone. The Glasgow Coma Scale (GCS) was assessed at baseline and on days 7 and 14. Electrocardiography (ECG) was performed at baseline and on day 7. Blood samples were obtained at baseline and on days 2 and 7. To detect the concentrations of mercury and arsenic, blood samples on days 2 and 7 were centrifuged and stored at –20°C for centralized analysis.

Image procedures

Brain MRI (3.0T), including T1W, diffusion weighted imaging (DWI), apparent diffusion coefficient (ADC), and fluid attenuated inversion recovery (FLAIR) series, was performed prior to study enrollment and randomization. Follow-up brain magnetic resonance imagings (MRIs) were conducted on days 14 and 90 with the same MRI machine used at baseline. The imaging parameters are listed in Supplementary Table 1, http://links.lww.com/CM9/B997.

All images were centrally processed using AccuBrain2.0® (Brainnow Medical Technology Ltd., Shenzhen, China). AccuBrain2.0® can automatically segment and quantify brain structures and regions with high accuracy.[29] Given the T1W MRI data, brain structures including the hippocampus, lateral ventricle, and amygdala, with three major brain tissues (white matter, grey matter, and cerebrospinal fluid) were automatically segmented by experienced radiologists. The absolute (mL) and relative volumes (% normalized by intracranial volume [ICV]) of the brain structures and regions were recorded. The cortical regions were measured with "atrophy ratios", which were defined as the ratio of the volume of cerebrospinal fluid (CSF) to the volume of parenchyma in each lobe. After aligning each subject's FLAIR and DWI MRI scans, brain infarcts were segmented by a trained expert in semi-automatic mode. Hyperintensities with correspondences in both T2-FLAIR and DWI MRI were identified as infarcts. In our study, cerebral infarctions were detected using DWI. Cerebral edema was defined as a region with hyperintensity on T2-FLAIR but not on DWI. The absolute volumes (mL) of the cerebral infarct and cerebral edema were recorded.

Outcomes

The primary outcomes were changes in cerebral infarct and edema volumes that changed after 14 days of treatment. The secondary efficacy outcomes were (1) changes in cerebral infarct and cerebral edema volume after 90 days, (2) changes in the NIHSS score after 14 days and 90 days, (3) the proportion of patients with an mRS score of 0–2 at days 14 and 90; and (4) changes in GCS after 7 days and 14 days.

The primary safety outcome was the proportion of severe adverse event (SAE) occurred within 90 days. The other safety outcomes included (1) the proportion of SAE within 7 days; (2) changes in routine blood examination, biochemical test, and mercury and arsenic concentrations after 7 days; (3) changes in electrocardiogram after 7 days; (4) proportions of AEs within days 7 and 90; (5) proportions of all-cause mortality within 7 days and 90 days; (6) proportions of combined vascular events (ischemic stroke, hemorrhagic stroke, myocardial infarction, or vascular death) within 7 days and 90 days. SAE was classified into (1) death, (2) life-threatening events, (3) hospitalization (initial or prolonged), (4) significant or persistent disability/damage, (5) congenital anomaly/birth defect, and (6) other serious medical events determined by the investigator.

Statistical analysis

Before the start of the study, the sample size was set based on our hypothesis that ANP would reduce the cerebral infarct and edema volume at follow-up.[25,26] ANP decreased the infarct volume ratio from 20% to 2% in mice with middle cerebral artery occlusion.[25] Due to the lack of data on ANP regarding the prevention of cerebral infarct growth in humans, the study was designed to consecutively enroll 120 eligible participants with moderate-to-severe AIS.

A full analysis set (FAS) was established based on intention-to-treat analysis (ITT). This set included participants who had undergone randomization and received the study drug. Thus, modified ITT was adopted in our analysis.

The FAS was the primary population used for efficacy analysis. The per-protocol set (PPS) included all participants who completed the treatment specified in the protocol or who did not seriously violate the protocol. The PPS was the secondary analysis population used to assess efficacy. The safety set (SS) included participants who received at least one dose of the treatment. Baseline data were presented according to treatment assignment, with descriptive statistics as appropriate.

Efficacy analysis of the primary outcome between treatment groups was conducted on the FAS and PPS using the Wilcoxon rank-sum test to compare the median change in response across treatment groups, and the Hodges–Lehmann method was used to calculate the median difference (MD) and confidence intervals (CIs). A similar approach was used for the secondary outcomes of changes in NIHSS and GCS scores. Logistic regression, with the trial centers set as a random effect, odds ratios (ORs), and 95% CIs were reported for mRS scores of 0–2. A Kaplan–Meier curve was constructed to compare the treatment groups for all-cause mortality at 90 days. Moreover, we assessed the efficacy of ANP in pre-specified subgroups.

As large artery atherosclerosis (LAA) is thought to be the main cause of moderate-to-severe stroke, a sensitivity analysis of the primary and secondary outcomes was conducted in patients diagnosed with LAA, thus providing clues for further efficacy assessment of ANP.

All safety analyses were conducted on SS and are presented as frequencies and percentages of safety events by treatment group. The Wilcoxon rank-sum test (for changes in blood tests and concentrations of mercury and arsenic), χ^2 test, or Fisher's exact test was used to compare treatment groups.

Missing values were deleted in the primary efficacy analysis. All tests were two-sided, and *P*-values <0.05 were considered statistically significance. All statistical analyses were conducted using the SAS software (version 9.4; SAS Institute, Cary, North Carolina, U.S.).

Results

Between April 2021 and July 2022, a total of 120 patients were enrolled. Sixty participants were randomly assigned to each of the ANP and placebo groups. In the ANP group, three patients did not receive the study drug, while in the placebo group, 60 patients received the study drug. Thus, 117 participants remained in the FAS for the intention-to-treat analysis. Nine and eight additional patients failed to undergo primary outcome assessment in ANP group and placebo group, respectively. Moreover, three patients in the placebo group did not meet the inclusion criteria after randomization, and one patient in the ANP group received trial-prohibited drugs. Finally, 96 were included in the per-protocol analysis [Figure 1]. Age, sex, hypertension, diabetes mellitus, and NIHSS score at baseline were comparable between FAS and non-FAS groups [Supplementary Table 2, http://links.lww.com/CM9/B997]. Demographic, clinical, and radiological characteristics and laboratory test results at baseline were comparable between the two treatment groups [Table 1]. The median age of the patients was 66.0 years (interquartile range [IQR]: 58.0–73.0 years), and 34% (40/117) were female. The median NIHSS score was 12.0 (IQR: 11.0–15.0). In the intention-to-treat analysis, the median time from symptom onset

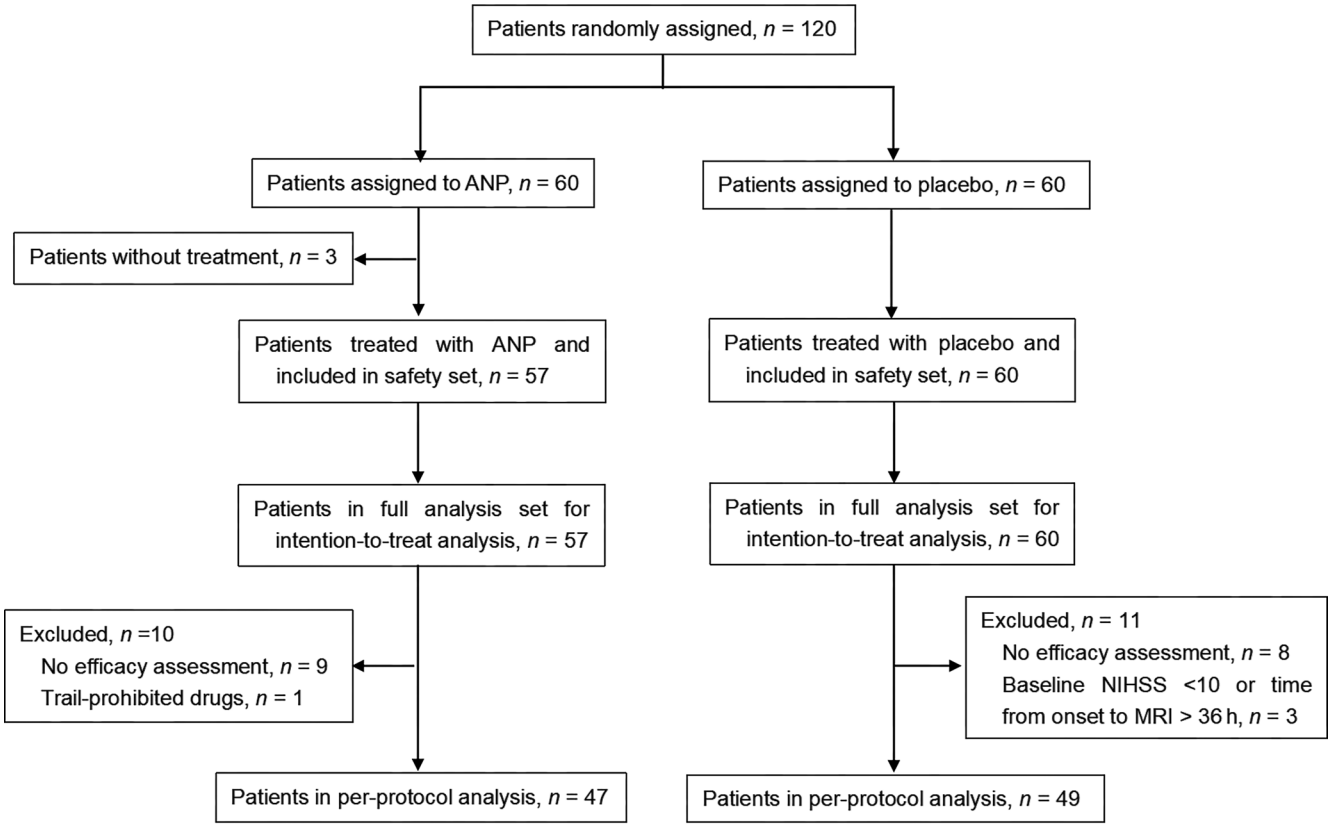

Figure 1: Flow chart of ANGONG TRIAL. ANGONG TRIAL: Safety and efficacy of Angong Niuhuang Pills in patients with moderate-to-severe acute ischemic stroke; ANP: Angong Niuhuang Pills; MRI: Magnetic resonance imaging; NIHSS: National Institutes of Health Stroke Scale.

Table 1: Demographic, clinical, and radiological characteristics of ANGONG TRIAL.

Variables	ANP ($n = 57$)	Placebo ($n = 60$)	Statistical values	*P* values
Age (years)	66.0 (57.0–72.0)	66.0 (58.0–74.0)	0.14	0.71
Female	19 (33)	21 (35)	0.04	0.85
Ethnic minorities	1 (2)	1 (2)	0.00	0.97
Education, middle school and above	18 (32)	13 (22)	1.47	0.22
Medical history				
Hypertension	34 (60)	37 (62)	0.05	0.82
Diabetes mellitus	9 (16)	14 (23)	1.05	0.30
Hyperlipidaemia	3 (5)	5 (8)	–	0.72
Ischaemic stroke	15 (26)	13 (22)	0.35	0.56
Transient anemia attack	2 (4)	2 (3)	–	>0.99
Cerebral hemorrhage	1 (2)	0	–	0.49
Coronary artery disease	6 (11)	4 (7)	–	0.52
Atrial fibrillation	9 (16)	9 (15)	0.01	0.91
Smoking, ever	22 (39)	22 (37)	0.05	0.83
Drinking, ever	20 (35)	20 (33)	0.04	0.84
Family history of stroke	1 (2)	3 (5)	0.93	0.33
Past medication history				
Antiplatelet agent	10 (18)	14 (23)	0.60	0.44
Anticoagulation agent	2 (4)	3 (5)	–	>0.99
Lipid-lowering agent	9 (16)	13 (22)	0.66	0.42
Antihypertensive agent	23 (40)	23 (38)	0.05	0.82
Antidiabetic agent	6 (11)	11 (18)	1.43	0.23
Agent containing ingredient of ANP	2 (4)	1 (2)	–	>0.99
Body mass index at baseline (kg/m^2)	24.3 (22.1–26.1)	24.2 (22.2–26.6)	0.01	0.93
Baseline systolic blood pressure (mmHg)	153 (136–166)	148 (134–166)	0.34	0.56
Baseline diastolic blood pressure (mmHg)	86 (80– 99)	86 (78–95)	0.55	0.46
NIHSS score at baseline	13 (11– 15)	12 (10–15)	1.61	0.20
Glasgow coma scale at baseline	13 (11– 14)	13 (11–15)	0.63	0.43
mRS score before symptom onset			–	0.67
0	54 (95)	58 (97)		
1	3 (5)	2 (3)		
Baseline DWI infarction volume (mL)	41.4 (12.2–101.7)*	32.1 (9.5–88.8)	0.74	0.39
Baseline cerebral edema volume (mL)	7.4 (3.9–16.1)†	4.3 (1.8–15.5)	0.99	0.32
TOAST classification‡			–	0.72
Large artery atherosclerosis	36 (67)	40 (69)		
Cardioembolism	8 (15)	9 (16)		
Small artery	8 (15)	8 (14)		
Other	2 (4)	0 (0)		
Unknown	0 (0)	1 (2)		
Intravenous rtPA after onset	14 (25)	14 (23)	0.02	0.88
Laboratory test				
White blood cell (10^9/L)	7.9 (6.5–9.2)§	8.0 (7.1–9.7)	0.24	0.63
Neutrophil (10^9/L)	5.8 (4.8–7.1)§	6.3 (4.6–8.0)	0.43	0.51
Hemoglobin (g/L)	140 (133– 152)§	145 (129–150)	0.00	0.99
Platelet (10^9/L)	211 (175– 255)§	199 (166– 249)	0.86	0.35
Alanine transaminase (U/L)	17 (13– 22)‖	18 (13–25)	0.33	0.56
Creatinine (mmol/L)	70 (56–76)¶	69 (59–89)**	2.14	0.14
Fasting blood glucose (mmol/L)	6.0 (5.4–7.7)§	6.6 (5.7–7.3)	0.20	0.66
Na (mmol/L)	140 (138–142)	140 (138–142)††	0.17	0.68
PT (s)	12 (11–13)‡‡	12 (101–13)§§	0.50	0.48
APTT (s)	27 (26–30)‖‖	28 (25–30)¶¶	0.03	0.87
LDL-C (mmol/L)	2.9 (2.3–3.6)***	2.7 (2.3–3.5)†††	0.14	0.71
High sensitivity C reactive protein (mg/L)	4.9 (2.2–17.0)	6.1 (2.7–11.3)	0.01	0.94
Symptom onset to study drug (h)	27 (18–33)	27 (21–33)	0.96	0.33
Symptom onset to baseline MRI (h)	24(12–30)	24 (12– 33)	0.39	0.53

Data are presented as median (IQR) or *n* (%). Statistical values for data shown as *n* (%) are χ^2; for data shown as median (IQR) are *Z* values; – for not applicable. *56 patients were analyzed. †55 patients were analyzed. ‡54 patients were analyzed. §56 patients were analyzed. ‖55 patients were analyzed. ¶57 patients were analyzed. **59 patients were analyzed. ††59 patients were analyzed. ‡‡53 patients were analyzed. §§59 patients were analyzed. ‖‖53 patients were analyzed. ¶¶58 patients were analyzed. ***55 patients were analyzed. †††57 patients were analyzed. ANGONG TRIAL: Safety and efficacy of Angong Niuhuang Pills in patients with moderate-to-severe acute ischemic stroke; ANP: Angong Niuhuang Pill; APTT: Activated partial thromboplastin time; DWI: Diffusion weighted imaging; GCS: Glasgow coma scale; IQR: Interquartile range; LAA: Large artery atherosclerosis; LDL-C: Low-density lipoprotein cholesterol; MRI: Magnetic resonance imaging; mRS: Modified rankin scale; NIHSS: National Institutes of Health Stroke Scale; PT: Prothrombin time; rtPA: Recombinant tissue plasminogen activator; TOAST: Trial of Org 10172 in Acute Stroke Treatment.

to drug administration was 27 h in the ANP group and 27 h in the placebo group [Table 1]. The rates of intravenous recombinant tissue plasminogen activator (rtPA) administration after symptom onset were similar between the two groups (25% [14/57] *vs*. 23% [14/60]). Concomitant treatments were comparable between the ANP and placebo groups [Supplementary Table 3, http://links.lww.com/CM9/B997].

In the intention-to-treat analysis, the changes in cerebral infarct volume at 14 days were 0.3 mL in the ANP group and 0.4 mL in the placebo group (MD: −7.1 mL, IQR: −18.3 to 2.3 mL, $P = 0.30$). The changes in cerebral edema volume of ANP and placebo groups were 11.4 mL and 4.0 mL, respectively (MD: 3.0 mL, IQR: −1.3 to 9.9 mL, $P = 0.15$). There were no differences in secondary efficacy outcomes between the ANP and placebo groups [Table 2].

The per-protocol analysis included 96 participants, 47 of whom were allocated to the ANP group and 49 to the placebo group. The change in cerebral infarct volume in the ANP group was similar to that in the placebo group (0.3 mL *vs*. 0.7 mL, MD: −8.2 mL, IQR: −20.3 to 2.3 mL, $P = 0.18$). In addition, the change in cerebral edema volume at day 14 was 9.3 mL in the ANP group and 4.4 mL in the placebo group; however, the difference was not statistically significant (2.6 [−1.8 to 9.6], $P = 0.25$). A mRS score of 0–2 was achieved in 22% (9/41) of the patients in the placebo group and 38% (15/40) in the ANP group (OR: 2.1, 95% CI: [0.8–5.7], $P = 0.13$). Other secondary outcomes were comparable between the two treatment groups [Supplementary Table 4, http://links.lww.com/CM9/B997].

Subgroup analysis of the primary outcomes in the FAS showed that ANP significantly reduced infarct growth in ischemic stroke with LAA [Supplementary Table 5, http://links.lww.com/CM9/B997]. To further assess the efficacy of ANP, we conducted sensitivity analyses in patients with LAA. In FAS, the change in cerebral infarct volume at 14 days was −1.0 mL in the ANP group and 1.2 mL in the placebo group (MD: −12.3 mL, IQR: −27.7 to −0.3 mL; $P = 0.03$), as shown in Table 3. The per-protocol analysis also showed that the change in cerebral infarct volume at day 14 in the ANP group was significantly lower than that in the placebo group (MD: −13.6 mL, IQR: −29.8 to −0.4 mL), with a *P*-value of 0.04 [Supplementary Table 6, http://links.lww.com/CM9/B997]. There were no differences in the changes of cerebral edema volume or secondary efficacy outcomes between the ANP and placebo groups.

The rate of SAE at 90 days was similar between the ANP and placebo groups (5% [3/57] *vs*. 12% [7/60], respectively; $P = 0.36$) [Table 4]. By 90 days, 3 (5%) of 57 patients died in the ANP group compared to 2 (3%) of 60 patients in the placebo group ($P = 0.95$). The Kaplan–Meier curve for all-cause mortality did not indicate a significant difference between the ANP and placebo groups [Supplementary Figure 1, http://links.lww.com/CM9/B997]. In addition, the changes in the concentrations of mercury, arsenic and biochemical indicators on day 7 were similar between the two treatment groups

Table 2: Efficacy outcomes based on intention-to-treat analysis of ANGONG TRIAL.

Variables	ANP ($n = 57$)	Placebo ($n = 60$)	Measurement of effect size	Effect size (95% CI)	*P* values
Primary outcome					
Change in cerebral infarction volume at 14 days (mL)	0.3 (−17.4 to 7.6)[*]	0.4 (−7.0 to 16.2)[†]	MD	−7.1 (−18.3 to 2.3)	0.30
Change in cerebral edema volume at 14 days (mL)	11.4 (1.3–23.7)[‡]	4.0 (0.4–18.5)[§]	MD	3.0 (−1.3 to 9.9)	0.15
Secondary outcome					
Change in cerebral infarction volume at 90 days (mL)	−5.3 (−32.7 to 2.6)[‖]	−2.3 (−19.0 to 10.4)[¶]	MD	3.9 (−5.5 to 23.1)	0.54
Change in cerebral edema volume at 90 days (mL)	2.7 (−0.9 to 17.1)[**]	3.1 (0.4–14.1)[††]	MD	0.6 (−3.9 to 4.7)	0.89
mRS 0–2 at 14 days	7/54 (13)	10/57 (18)	OR	0.7 (0.3–2.0)	0.50
mRS 0–2 at 90 days	17/45 (38)	12/49 (24)	OR	1.9 (0.8–4.6)	0.16
Change in NIHSS score at 14 days	−2.5 (−5.0 to −1.0)[‡‡]	−3.0 (−6.0 to −1.0)[§§]	MD	0 (−1.0 to 1.0)	0.91
Change in NIHSS score at 90 days	−6.0 (−9.0 to −4.0)[‖‖]	−6.0 (−7.0 to −4.0)[¶¶]	MD	−1.0 (−2.0 to 1.0)	0.46
Change in Glasgow coma score at 7 days	0 (0–1.0)[***]	0 (0–1.0)[†††]	MD	0	0.05
Change in Glasgow coma score at 14 days	0 (0–1.0)[‡‡‡]	0 (0–1.0)[§§§]	MD	0 (0–1.0)	0.05

Data were presented as median (IQR) or *n* (%). [*]48 patients were analyzed. [†]52 patients were analyzed. [‡]48 patients were analyzed. [§]51 patients were analyzed. [‖]38 patients were analyzed. [¶]35 patients were analyzed. [**]37 patients were analyzed. [††]35 patients were analyzed. [‡‡]54 patients were analyzed. [§§]57 patients were analyzed. [‖‖]45 patients were analyzed. [¶¶]48 patients were analyzed. [***]54 patients were analyzed. [†††]58 patients were analyzed. [‡‡‡]54 patients were analyzed. [§§§]57 patients were analyzed. ANGONG TRIAL: Safety and efficacy of Angong Niuhuang Pills in patients with moderate-to- severe acute ischemic stroke; ANP: Angong Niuhuang Pill; CI: Confidence interval; GCS: Glasgow coma scale; IQR: Interquartile range; MD: Median difference; mRS: Modified rankin scale; NIHSS: National Institutes of Health Stroke Scale; OR: Odds ratio.

Table 3: Sensitivity analysis in patients with LAA based on intention-to-treat analysis.

Variables	ANP ($n = 36$)	Placebo ($n = 40$)	Measurement of effect size	Effect size (95% CI)	*P* values
Primary outcome					
Change in cerebral infarction volume at 14 days (mL)	–1.0 (–19.4 to 8.3)*	1.2 (–5.2 to 21.2)†	MD	–12.3 (–27.7 to –0.3)	0.03
Change in cerebral edema volume at 14 days (mL)	9.3 (1.3 to 23.6)‡	3.8 (–0.4 to 16.5)§	MD	4.8 (–1.1 to 11.5)	0.10
Secondary outcome					
Change in cerebral infarction volume at 90 days (mL)	–9.0 (–56.5 to 2.6)‖	–3.9 (–15.4 to 10.4)¶	MD	–14.3 (–40.1 to 3.9)	0.09
Change in cerebral edema volume at 90 days (mL)	7.0 (–0.5 to 18.6)**	1.1 (0.1–10.2)††	MD	3.0 (–3.3 to 13.9)	0.10
mRS 0–2 at 14 days	5/36 (14)	7/39 (18)	OR	0.7 (0.2–2.6)	0.63
mRS 0–2 at 90 days	11/28 (39)	8/33 (24)	OR	2.0 (0.7–6.1)	0.21
Change in NIHSS score at 14 days	–2.0 (–4.0 to –1.00)	–3.0 (–6.0 to 0)‡‡	MD	0 (–1.0 to 2.0)	0.60
Change in NIHSS score at 90 days	–6.0 (–9.0 to –3.5)§§	–5.5 (–7.0 to –4.0)‖‖	MD	–1.0 (–2.0 to 1.0)	0.63
Change in Glasgow coma score at 7 days	0 (0–1.0)	0 (0–1.0)	MD	0	0.18
Change in Glasgow coma score at 14 days	0 (0–1.0)	0 (0–1.0)¶¶	MD	0 (0–1.0)	0.09

Data were presented as median (IQR) or *n* (%). *31 patients were analyzed. †37 patients were analyzed. ‡31 patients were analyzed. §36 patients were analyzed. ‖23 patients were analyzed. ¶25 patients were analyzed. **22 patients were analyzed. ††25 patients were analyzed. ‡‡39 patients were analyzed. §§28 patients were analyzed. ‖‖32 patients were analyzed. ¶¶39 patients were analyzed. ANP: Angong Niuhuang Pill; CI: Confidence interval; GCS: Glasgow coma scale; IQR: Interquartile range; LAA: Large artery atherosclerosis; MD: Median difference; mRS: Modified rankin scale; NIHSS: National Institutes of Health Stroke Scale; OR: Odds ratio.

Table 4: Safety outcomes of ANGONG TRIAL.

Variables	ANP ($n = 57$)	Placebo ($n = 60$)	Statistical values	*P* values
SAE up to day 90	3 (5)	7 (12)	0.82	0.36
SAE up to day 7	0	4 (7)	2.17	0.14
AE up to day 90	14 (25)	12 (20)	0.35	0.55
AE up to day 7	10 (18)	7 (12)	0.81	0.36
All-cause deaths up to day 90	3 (5)	2 (3)	0	0.95
All-cause deaths up to day 7	0	0	–	–
Combined vascular events to day 90	1 (2)	3 (5)	0.21	0.65
Combined vascular events to day 7	0	1 (2)	–	>0.99
Abnormal ECGs after baseline	26 (55)	25 (58)	0.07	0.79
Change in concentration of mercury (mg/L)	0*	0†	1.31	0.25
Change in concentration of arsenic (mg/L)	0 (–1.1 to 0.3)‡	0 (–0.7 to 1.0)§	0.01	0.94

Data were presented as median (IQR) or *n* (%); statistical values for data shown as *n* (%) are χ^2; for data shown as median (interquartile range) are *Z*. *54 patients were analyzed; †57 patients were analyzed; ‡54 patients were analyzed; §57 patients were analyzed. AE: Adverse event; ANGONG TRIAL: Safety and efficacy of Angong Niuhuang Pills in patients with moderate-to-severe acute ischemic stroke; ANP: Angong Niuhuang Pill; ECG: Electrocardiograph; SAE: Severe adverse event; –: Not applicable.

[Table 4 and Supplementary Table 7, http://links.lww.com/CM9/B997]. There were no significant differences in the daily body temperature within 7 days of enrollment between the ANP and placebo groups (data not shown).

Discussion

The ANGONG TRIAL showed that ANP was safe in patients with acute moderate-to-severe ischemic stroke but was negative for primary and secondary outcomes. However, we found that ANP showed the potential to reduce infarct growth and improve the 90-day functional outcomes. Moreover, ANP significantly reduced infarct growth in ischemic stroke patients with LAA. This is a study to demonstrate the potential effect of ANP in reducing cerebral infarct volume in patients with acute moderate-to-severe ischemic stroke.

Previous studies reported that large infarct volume and infarct growth both predicted poor clinical outcomes.[7,30] Final infarct volume but not recanalization was independently associated with outcome.[30] Penumbra salvage volume also mediated the improved functional outcome after recanalization.[31] Infarct growth might be a potential therapeutic target in patients with AIS.[32] However, the infarct volume still increased from 14.8 mL at baseline to

37.5 mL at 24 h after EVT.[15] A randomized study assessed the efficacy of intravenous glyburide on large hemispheric infarct, but non-significantly increased proportion of the good outcome (mRS 0–4, 17% *vs*. 14%).[22] In our study, the baseline DWI infarct volume was >25 mL, similar to the lesion volume in patients receiving EVT.[15] A larger baseline infarct volume was assumed to indicate higher infarct growth.[33] We found ANP to be a potential candidate for reducing the infarct volume in patients with moderate-to-severe ischemic stroke, especially in those with LAA.

Cerebral edema often occurs as a complication of large infarcts and results in neurological deterioration. Swelling is a strong predictor of poor outcomes in non-lacunar stroke.[18] Reperfusion therapy, osmotic drugs, and intravenous glyburide have been used to reduce edema volume,[17,22,34] but their efficacy was poor. For patients with very large core volumes (>130 mL), EVT might increase the edema volume.[35] Reducing the volume of cerebral edema is another challenge for neurologists, as no effective therapy is recommended by current guidelines.[34] Our study did not confirm the conjecture of previous studies that ANP may reduce cerebral edema.[26] Further large-sample studies are needed to determine whether ANP reduces cerebral edema.

The ANGONG TRIAL included patients with baseline NIHSS scores between 10 and 20, indicating a higher risk of disability and mortality.[36,37] The rates of excellent outcome (mRS 0–1) were inversely associated with NIHSS score in those with or without alteplase.[10] Although EVT was beneficial to patients with LAA,[4] the real-world utilization rates of alteplase and EVT were only 10.2% and 4.9%, respectively.[38] The majority of patients with acute moderate-to-severe stroke did not receive reperfusion therapy and were at high risk of disability. More than half of the patients with NIHSS scores >10 were probably functionally dependent. In addition to alteplase and EVT, our study found that ANP could non-significantly improve the clinical outcome. In patients with LAA, ANP increased the rate of mRS 0–2 at 90 days from 24% to 39%, possibly because of the reduced volume of cerebral infarct.[39] Because of the correlation between infarct volume and functional prognosis, we speculated that ANP might improve the outcome of acute moderate-to-severe stroke, but further randomized controlled trials are warranted to confirm this result.

Exposure to ANP often raises safety concerns regarding heavy metals and their nephrotoxic damage, as realgar and cinnabar in ANP, contain 90% As_2S_3 and 96% HgS, respectively.[26,40] Previous studies reported low rates of AEs; however, the quality of these studies was poor.[26,41] In our study, the concentration of mercury and arsenic at day 7 did not increase in the ANP group. The frequency of SAE was lower in the ANP group than that in the placebo group. In addition, a systematic review reported that 85.2% of AEs were associated with original diseases, and two SAEs (2/49, 4.1%) occurred in children with an overdose of ANP.[27] Another predisposing factor for SAE was the use of ANP against the indications of traditional Chinese medicine (TCM).[27] We speculated that the exclusion of cases with contraindications to TCM may be one of the reasons for the low rate of SAE in our study.

Our study had several strengths. This randomized double-blind placebo-controlled study included patients with acute moderate-to-severe ischemic stroke. ANP was prescribed within 36 h of onset. The primary endpoints–cerebral infarct and edema volumes–were measured by an independent group of radiologists blinded to the treatment. Efficacy analysis was performed for both the FAS and PPS. To verify the safety issues of heavy metals, the concentrations of mercury and arsenic were tested centrally at baseline and day 7, though the safety of ANP has been reported in an animal study.[39] Before the trial initiation, we noticed that the main cause of ischemic stroke in patients with a NIHSS score of 10 or more was likely to be LAA,[42] and efficacy and safety analyses were also scheduled in patients with LAA. In addition, as a TCM, ANP has been widely used in clinical practice for more than one century in patients with unconsciousness due to heat blockage, based on the theory of TCM.[25,26,40] We, on the other hand, addressed the safety issue of ANP by the methodology of a randomized controlled trial,[26,41] and thus provided safety data for the following large-sample clinical study of ANP.

Our study had several limitations. Considering the exploratory nature of this pilot study, the sample size was set at 120. Power and type I errors were not calculated before trial initiation, and the rate of dropout was relatively high. In addition, we would like to remind investigators to focus on patient adherence when designing studies for severe ischemic stroke. The results of our study should be interpreted with caution. For the neurologists, radiological endpoints other than clinical outcomes were chosen as the primary outcomes, limiting the clinical significance of this study. However, cerebral infarct growth was also an acceptable intermediate indicator associated with a 90-day mRS score.[7,32] ANP also showed the potential to improve neurological function. Further studies are needed to confirm the efficacy of ANP in improving clinical outcomes. Collateral status and recanalization status were important factors influencing cerebral infarction and edema volume but were not included in our analysis.[17,33] Finally, we noticed that ANP might inhibit infarct growth 24 h after onset, but its effect on infarct volume increase within 24 h after onset was unclear.

In conclusion, treatment with ANP was safe but failed to significantly reduce cerebral infarct and edema volume. However, in patients with LAA, ANP marginally reduced the infarct volume. ANP also showed a tendency to reduce the volume of cerebral infarcts and increase the rate of functional independence, providing foundation for the following researches. These findings suggested the potential of ANP to reduce cerebral infarct volume improve clinical prognosis, and provided a scientific basis for further trials in patients with acute moderate-to-severe stroke.

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Conflicts of interest

Lin Shi is the director of BrainNow Medical Technology Limited. Yishan Luo is now employed by BrainNow Medical Technology Limited. Other authors declare no competing interests.

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