Supplementary Material

The exclusion criteria of ANGONG trial.

(1) Not suitable for taking this medicine according to the judgement of traditional Chinese medical doctor;

(2) Suffering from intracranial tumors, encephalitis, subarachnoid hemorrhage, and other brain organic diseases;

(3) Patients with hemorrhagic transformation after cerebral infarct;

(4) Received or planned to receive endovascular treatment, including thrombectomy, ultra-early thrombus aspiration, and stenting;

(5) Received or planned to receive decompression craniectomy;

(6) With mRS (modified Rankin Scale) score >1 before onset of this episode;

(7) Participants who cannot tolerate the MRI(magnetic resonance imaging) scans, such as using a pacemaker or automatic defibrillator or having claustrophobia;

(8) With thrombocytopenia ($<100 \times 10^9$/L), hematologic diseases or other systemic bleeding tendency;

(9) With alanine transaminase or aspartate aminotransferase >1.5 times than normal upper limit or creatinine >1.5 times than normal upper limit;

(10) Allergic to ingredients of Angong Niuhuang pill;

(11) Received Angong Niuhuang pill within 1 month before stroke onset;

(12) Plan to become pregnant within 3 months after stroke onset, or women of childbearing age with negative pregnancy test but refuse to accept contraception; during pregnancy or lactation;

(13) Had participated in other clinical trials within 30 days before randomization or currently involved in other clinical trials;

(14) Participants with a life expectancy less than 3 months;

(15) Incapable to finish follow-ups due to mental illness, cognitive or emotional disorders;

(16) Participants are not eligible for this clinical trial as evaluated by the investigators.

Supplementary Table 1. The parameters of MRI in the ANGONG TRIAL.

	TR/TE (ms)	FOV (mm)	Voxel (mm)	Number of slices	Time	Note
DWI	9000/98	240	1.8 × 1.8 × 2.0	50	2 min/53 s	isotropic voxel scan
FLAIR	9000/96		1.9 × 1.9 × 2.0	50		–

(1) MRI equipment requires 3.0T. (2) The line connecting anterior commissure and splenium of corpus callosum as the baseline. (3) The cross-sectional DWI scan covers the whole brain. DWI: Diffusion weighted imaging; FOV: Field of view; FLAIR: Fluid attenuated inversion recovery; MRI: magnetic resonance imaging; TE: Echo time; TR: Repetition time.

Supplementary Table 2. Key baseline data between non-FAS and FAS groups.

	Non-FAS (n=3)	FAS (n=117)	*P*-value
Age (years)	73 (58-74)	66 (58-73)	0.47
Female	1 (33)	40 (34)	0.98
Hypertension	2 (67)	71 (61)	0.83
Diabetes mellitus	2 (67)	23 (20)	0.05
NIHSS score at baseline	17 (12-18)	12 (11-15)	0.12

FAS: Full analysis set; NIHSS: National Institutes of Health Stroke Scale. Data are shown as n (%), or median (range).

Supplementary Table 3. Concomitant treatments in FAS.

	ANP (n = 57)	Placebo (n = 60)	*P*-value
Antiplatelet therapy	54 (90)	57 (95)	0.30
Antihypertensive therapy	31 (52)	29 (48)	0.72
Antidiabetic therapy	11 (18)	14 (23)	0.50
Lipid-lowering therapy	54 (90)	56 (93)	0.51
Mannitol/glycerin fructose therapy	10 (17)	6 (10)	0.28

Data were presented as n (%). ANP: Angong Niuhuang Pill; FAS: Full analysis set.

Supplementary Table 4: Efficacy outcomes based on per-protocol analysis.

	ANP (n = 47)	Placebo (n = 49)	Measurement of effect size	Effect size (95% CI)	*P*-value
Primary outcome					
Change in cerebral infarct volume at 14 days (mL)	0.3 (−19.4 to 8.3)	0.7 (−8.9 to 18.4)	Median difference	−8.2 (−20.3 to 2.3)	0.18
Change in cerebral edema volume at 14 days (mL)	9.6 (1.3–23.7)	4.4 (0.4–18.6)*	Median difference	2.6 (−1.8 to 9.6)	0.25
Secondary outcome					
Change in cerebral infarct volume at 90 days (mL)	−5.1 (−31.8 to 2.6)†	−1.9 (−19.0 to 11.1)‡	Median difference	3.9 (−4.6 to 23.3)	0.35
Change in cerebral edema volume at 90 days (mL)	2.9 (−0.9 to 17.1)§	6.3 (0.6–15.4)‖	Median difference	0.9 (−3.9 to 5.7)	0.60
mRS 0–2 at 14 days	6/47 (13)	8/49 (16)	OR	0.8 (0.2–2.4)	0.62
mRS 0–2 at 90 days	15/40 (38)	9/41 (22)	OR	2.1 (0.8–5.7)	0.13
Change in NIHSS score at 14 days	−2.0 (−5.0 to −1.0)	−3.0 (−6.0 to −1.0)	Median difference	1.00 (−1.0 to 2.0)	0.25
Change in NIHSS score at 90 days	−7.0 (−9.5 to −3.0)¶	−6.0 (−7.5 to −4.5)**	Median difference	0 (−2.0 to 1.0)	0.59
Change in GCS score at 7 days	0 (0–1.0)	0 (0–1.0)	Median difference	0 (0–0)	0.18
Change in GCS score at 14 days	0 (0–2.0)	0 (0–1.0)	Median difference	0 (0–1.0)	0.14

Data were presented as median (IQR) and n (%). ANP: Angong Niuhuang Pill; CI: Confidence interval; GCS: Glasgow coma scale; mRS: Modified Rankin Scale; NIHSS: National Institutes of Health Stroke Scale; OR: Odds ratio.

[*]48 patients were analyzed.

[†]35 patients were analyzed.

[‡]31 patients were analyzed.

[§]34 patients were analyzed.

[‖]31 patients were analyzed.

[¶]40 patients were analyzed.

[**]40 patients were analyzed.

Supplementary Table 5: Subgroup analysis in FAS.

	ANP (n = 57) *	Placebo (n = 60) †	*P*-value
Change in cerebral infarct volume at 14 days (mL)			
Age			
≤65 (years)	-7.2 (-24.6 to 5.4)	-1.1 (-14.5 to 13.0)	0.55
>65 (years)	1.3 (-12.9 to 9.7)	2.8 (-3.3 to 21.2)	0.30
Sex			
Male	0.3 (-19.4 to 8.3)	0.2 (-11.3 to 18.4)	0.25
Female	0.1 (-12.9 to 4.6)	0.7 (-5.2 -13.0)	0.64
History of ischemic stroke			
No	-0.2 (-15.5 to 6.9)	0.9 (-4.7 to 16.2)	0.18
Yes	2.8 (-24.6 to 8.3)	-2.5 (-10.8 to 18.5)	0.90
Baseline NIHSS score			
<15	0.3 (-12.9 to 5.4)	-0.3 (-12.3 to 10.5)	0.87
≥15	-3.2(-53.2 to 15.4)	13.3 (-4.2 to 32.9)	0.08
Baseline DWI infarct volume (mL)			
<33.8	1.4 (-0.3 to 6.9)	1.2 (-1.5 to 13.1)	0.91
≥33.8	-12.8 (-56.5 to 9.7)	-3.2 (-23.5 to 29.7)	0.12
TOAST classification			
LAA	-1.0 (-19.4 to 8.3)	1.2 (-5.2 to 21.2)	0.05
Cardioembolism	0.8 (-50.7 to 89.3)	-12.3 (50.5 to 102.9)	0.95
Small artery	0.3 (-6.0 to 0.6)	0.2 (-1.7 to 2.8)	0.73
Other	45.7 (34.6 – 56.8)	——	——
Unknown	——	1.1 (1.1–1.1)	——
Intravenous rtPA			
No	-0.2 (-24.6 to 4.9)	1.2 (-6.8 to 19.8)	0.11
Yes	1.4 (-6.8 to 9.7)	-2.1 (-8.2 to 3.3)	0.42
Antiplatelet therapy			

No	0.1 (-17.4 to 7.6)	0.1 (-12.3 to 13.6)	0.41
Yes	3.6 (-26.3 to 10.4)	5.9 (-0.5 to 18.4)	0.38
Antihypertensive therapy			
No	0.1 (-13.1 to 4.9)	0.0 (-8.9 to 13.6)	0.53
Yes	1.1 (-24.6 to 11.9)	3.1 (-5.2 to 21.2)	0.23
Antidiabetic therapy			
No	0.3 (-19.4 to 8.3)	0.1 (-5.2 to 13.1)	0.46
Yes	-5.3 (-12.9 to 5.4)	3.4 (-8.9 to 30.6)	0.23
Lipid-lowering therapy			
No	-0.2 (-21.9 to 5.7)	0.2 (-8.9 to 21.2)	0.14
Yes	5.1 (-2.7 to 52.3)	1.2 (-1.8 to 14.1)	0.61
Mannitol/glycerin fructose therapy			
No	0.3 (-19.4 to 8.3)	0.2 (-8.9 to 18.4)	0.20
Yes	4.6 (4.6–4.6)	5.9 (5.9–5.9)	0.26
Change in cerebral edema volume at 14 days (mL)			
	ANP (n = 57) ‡	**Placebo (n = 60)** §	***P*-value**
Age			
≤65 (years)	7.0 (1.3–19.0)	4.0 (0.6–18.6)	0.55
>65 (years)	14.3 (1.7–23.7)	3.8 (0.3–15.5)	0.20
Sex			
Male	11.4 (1.3–25.2)	3.3 (-0.3 to 17.6)	0.11
Female	9.9 (0.6–22.0)	5.2 (2.0–19.3)	>0.99
History of ischemic stroke			
No	13.5 (1.3–25.2)	3.5 (0.6–18.1)	0.1870
Yes	3.1 (1.3–19.0)	7.0 (-1.5 to 20.2)	>0.99
Baseline NIHSS score			
<15	7.3 (0.6–20.7)	2.5 (-0.3 to 11.9)	0.10
≥15	17.9 (3.5–30.5)	17.4 (4.0 –39.8)	0.75

Baseline DWI infarct volume (mL)			
<33.8	2.6 (1.2–16.9)	2.7 (0.7–15.5)	0.83
≥33.8	20.7 (3.5–30.5)	6.3 (-2.6 to 24.0)	0.11
TOAST classification			
LAA	9.3 (1.3–23.6)	3.8 (-0.4 to 16.5)	0.12
Cardioembolism	23.7 (13.7–102.0)	32.9 (13.9–48.1)	0.75
Small artery	1.2 (-1.2 to 2.3)	2.2 (0.9–5.3)	0.30
Other	23.7 (16.8–30.5)	——	——
Unknown	——	0.3 (0.3–0.3)	——
Intravenous rtPA			
No	13.6 (1.3–23.7)	3.3 (-0.3 to 18.5)	0.11
Yes	9.3 (1.2–22.0)	6.3 (1.9–20.0)	>0.99
Antiplatelet therapy			
No	8.8 (1.3–23.7)	4.4 (0.3–18.6)	0.34
Yes	13.6 (2.4–57.5)	3.3 (0.7–14.6)	0.22
Antihypertensive therapy			
No	7.0 (0.3–25.2)	4.6 (0.6–18.6)	0.64
Yes	13.7 (3.5–23.6)	3.6 (0.4–11.9)	0.11
Antidiabetic therapy			
No	13.5 (1.3–25.2)	3.8 (0.4–16.5)	0.15
Yes	3.9 (1.3–22.0)	4.9 (0.6–25.2)	>0.99
Lipid-lowering therapy			
No	8.8 (1.3–24.4)	4.4 (0.3–20.2)	0.38
Yes	13.6 (2.4–22.2)	3.3 (0.7–13.9)	0.22
Mannitol/glycerin fructose therapy			
No	9.3 (1.3–23.7)	3.8 (0.4–18.5)	0.21
Yes	18.6 (18.6–18.6)	7.0 (7.0–7.0)	——

Data were presented as median (IQR).

ANP: Angong Niuhuang Pill; DWI: Diffusion weighted imaging; FAS: Full analysis set; LAA: Large artery atherosclerosis; NIHSS: National Institutes of Health Stroke Scale; rtPA: Recombinant tissue plasminogen activator.

[*]48 patients were analyzed.

[†]52 patients were analyzed.

[‡]48 patients were analyzed.

[§]51 patients were analyzed.

Supplementary Table 6: Sensitivity analysis in patients with LAA based on per-protocol analysis.

	ANP (n = 31)	Placebo (n = 34)	Measurement of effect size	Effect size (95% CI)	*P*-value
Primary outcome					
Change in cerebral infarct volume at 14 days (mL)	−1.0 (−19.4 to 8.3)	2.1 (−8.9 to 23.8)	Median difference	−13.6 (−29.8 to −0.4)	0.04
Change in cerebral edema volume at 14 days (mL)	9.3 (1.3–23.6)	4.0 (−0.6 to 17.6)*	Median difference	4.5 (−1.7 to 11.5)	0.16
Secondary outcome					
Change in cerebral infarct volume at 90 days (mL)	−9.0 (−54.2 to 2.0)†	−3.2 (−7.6 to 11.1)‡	Median difference	16.0 (−2.0 to 42.2)	0.09
Change in cerebral edema volume at 90 days (mL)	7.0 (−0.7 to 18.2)§	3.1 (0.3–12.6)¶	Median difference	2.31(−5.3 to 13.7)	0.50
mRS 0–2 at 14 days	5 (16)	5 (15)	OR	1.1 (0.3–4.3)	0.87
mRS 0–2 at 90 days	10 (40)‖	5 (19)**	OR	2.9 (0.8–10.3)	0.09
Change in NIHSS score at 14 days	−2.0 (−4.0 to −1.0)	−3.0 (−6.0 to 0)	Median difference	1.0 (−1.0 to 2.0)	0.30
Change in NIHSS score at 90 days	−7.0 (−9.0 to −3.0)††	−6.0 (−7.0 to −4.0)‡‡	Median difference	0 (−3.0 to 2.0)	0.66
Change in GCS score at 7 days	0 (0–1.0)	0 (0–1.0)	Median difference	0 (0–1.0)	0.23
Change in GCS score at 14 days	0 (0–2.0)	0 (0–1.0)	Median difference	0 (0–1.0)	0.19

Data were presented as median (IQR) and n (%).

ANP: Angong Niuhuang Pill; CI: Confidence interval; GCS: Glasgow coma scale; LAA: Large artery atherosclerosis; mRS: Modified Rankin Scale; NIHSS: National Institutes of Health Stroke Scale; OR: Odds ratio.

*33 patients were analyzed.

†21 patients were analyzed.

‡21 patients were analyzed.

§20 patients were analyzed.

‖25 patients were analyzed.

¶21 patients were analyzed.

**27 patients were analyzed.

††25 patients were analyzed.

‡‡26 patients were analyzed.

Supplementary Table 7: Safety outcomes for laboratory tests.

	ANP	Placebo	*P*-value
Change in laboratory tests at 7 days			
White blood cell (10^9/L)	−1.1 (−2.1 to 0.6)	−0.7 (−2.3 to 0.8)	0.49
Neutrophil (10^9/L)	−1.0 (−2.4 to 0.5)	−0.4 (−2.4 to 0.8)	0.35
Hemoglobin (g/L)	−6.0 (−13.5 to 2.5)	−8.0 (−13.0 to 1.0)	0.70
Platelet (10^9/L)	5.0 (−18.0 to 26.0)	0.5 (−19.0 to 37.0)	0.59
Alanine transaminase (U/L)	3.0 (−2.0 to 10.1)*	2.6 (−3.8 to 13.9)†	0.91
Creatinine (mmol/L)	−3.3 (−9.0 to 6.7)‡	−7.0 (−14.0 to −1.0)§	0.08
Fasting blood glucose (mmol/L)	−0.3 (−0.8 to 0.5)¶	−0.3 (−1.1 to 0.8)‖	0.63
Na (mmol/L)	−0.7 (−3.7 to 3.0)**	0 (−2.2 to 1.9)††	0.88
PT (s)	0.01 (−0.3 to 0.6)‡‡	0.2 (−0.2 to 0.6)§§	0.27
APTT (s)	−0.2 (−1.2 to 1.8)¶¶	0.7 (−2.1 to 3.6)‖‖	0.46
LDL-C (mmol/L)	−0.2 (−0.4 to −0.1)***	−0.2 (−0.3 to −0.1)†††	0.70

Data were presented as median (IQR).

In ANP group, 52 patients were analyzed for White blood cell, Neutrophil, Hemoglobin, and Platelet.

In placebo group, 54 patients were analyzed for White blood cell, Neutrophil, Hemoglobin, and Platelet.

ANP: Angong Niuhuang Pill; APTT: Activated partial thromboplastin time; PT: Prothrombin time.

*50 patients were analyzed.

†56 patients were analyzed.

‡51 patients were analyzed.

§53 patients were analyzed.

‖53 patients were analyzed.

¶48 patients were analyzed.

**51 patients were analyzed.

††56 patients were analyzed.

‡‡50 patients were analyzed.

§§54 patients were analyzed.

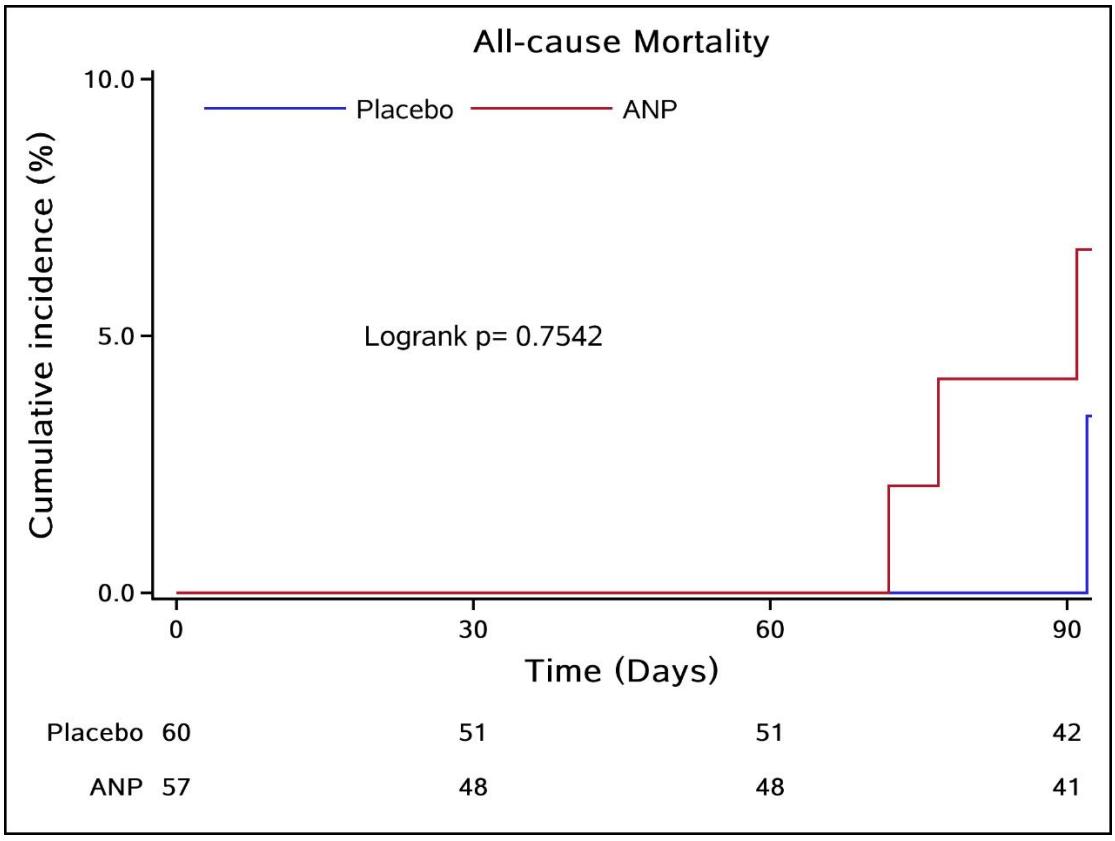
‖‖53 patients were analyzed.

¶¶50 patients were analyzed.

***48 patients were analyzed.

†††48 patients were analyzed.

Supplementary Figure 1: Cumulative incidence of death for each treatment group.



ANP: Angong Niuhuang Pill.