Effect and Safety of Naomaili Granules (□□□□□) for the Treatment of Acute Ischemic Stroke: A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial

Xiaogang Li (xgangli2002@163.com)
Peking University Third Hospital
Dongjing Song
The Second Hospital of Harbin
Peiyang Zhou
Xinjiang No. 1 Peoples Hospital
Huagang Wang
Cangzhou People's Hospital
Chengfang Zhou
The Second Affiliated Hospital of University of South China
Bo Hu
Wuhan Union Hospital of China
Wentao Li
Shanghai Municipal Hospital of Traditional Chinese Medicine
Dongdong Yang
The teaching Hospital of Chengdu University of TCM

Research

Keywords: Naomaili Granules, acute Stroke, clinical trial, rankin scale, incidence of cardiovascular events, TCM Syndrome effect

Posted Date: August 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54570/v1

Abstract

Background: It is of vital importance for the treatment and prognosis of Acute Stroke to find effective Chinese medicine that can be combined with western medicine in the acute stage. The purpose of this study is to investigate the effect and safety of Naomaili Granules (□□□□□, NML) for the treatment of acute stroke, hoping to provide a new idea and drug choice for the integrated treatment of Chinese and Western medicine in the acute stage of ischemic stroke, and at the same time to improve the treatment plan in the acute stage of ischemic stroke from the perspective of TCM syndromes.

Methods: A total of 187 patients with acute ischemic stroke were randomly divided into the NML group (93 cases) and the placebo group (94 NML mimics), 1 bag (10g/bag), thrice daily for 20 days. Basic medications during the trial: Aspirin enteric-coated tablets, 1 tablet (0.1g/tablet), once a day. After treatment, the modified Rankin scale, the incidence of cardiovascular events and TCM Syndrome effect were the main efficacy indicators. Meanwhile, adverse events (AEs) were evaluated during the whole clinical trial.

Results: In the FAS 90 days after the onset, the experimental group was 70.00%, and the control group was 45.24%. There was a statistically significant difference between the two groups. The incidence of acute cardio-cerebrovascular events was 1 case (1.08%) in the experimental group and 0 in the control group after 20 days of FAS treatment.

Conclusion: The combined application of NML in the acute stage of ischemic stroke can effectively improve the prognosis of patients, and improve the independent survival ability of patients, and its safety is reliable, providing a new way of thinking and medication choice for the treatment of acute ischemic stroke with integrated traditional Chinese and western medicine.

Trial registration: ChiCTR, ChiCTR2000033619. Registered 7 June 2020 - Retrospectively registered, http://www.chictr.org.cn/showproj.aspx?proj=54619

Background

Cerebrovascular accidents (stroke) are the second leading cause of death and the third leading cause of disability[1]. Stroke occurs when the blood flow to the brain is lost due to blockage or rupture of arteries, resulting in the sudden death of some brain cells due to insufficient oxygen supply[2]. Stroke survivors suffer various degrees of disability, including urinary incontinence, dysarthria, swallowing deficits, dysphasia, and consciousness disorders. Along with ischemic heart disease, Stroke has also become the largest socioeconomic disease burden[3]. Ischemic stroke is one of the most common types, accounting for about 60%-80% of strokes. For acute treatment, modern medicine usually adopts thrombolysis, intravascular intervention, antiplatelet, anticoagulation, defibrillation, neuroprotection and symptomatic treatment for clinical practice[4]. Although it has achieved success in certain treatments, for example,

aspirin is the cornerstone of the prevention and treatment of ischemic stroke recommended by multinational guidelines, some patients may have antiplatelet drug resistance, resulting in ineffective treatment[4–7]. In addition, due to the narrow time window of intravenous thrombolysis, less than 3% of patients can be treated, and stent restenosis and reperfusion injury, etc are also common. Thus, additional treatment strategies are needed to improve poststroke recovery.

Ischemic stroke is a complicated and multi-factor pathogenesis, so it is difficult to improve the cure rate and reduce the disability rate from one aspect and one level of treatment. Traditional Chinese Medicine (TCM) has been historically used for stroke treatment. On the basis of Western medicine treatment, the integrated of TCM combined with evidence-based Chinese medicine and Western medicine in the treatment of ischemic stroke can overlap and merge in the course of practicing process, and give full play to the advantages of their own treatment, so as to better improve the clinical efficacy, reduce adverse reactions and the incidence of sequelae[9]. According to the recommendation of guideline[10], the integrated treatment of traditional Chinese and Western medicine should be carried out as soon as possible after thrombolysis. However, currently oral Chinese patent medicine is mainly used in recovery period, and the use of traditional Chinese medicine for the treatment of ischemic stroke only accounts for a very low proportion of total treatment expenditure globally[11, 12]. Therefore, it is very important to find effective Chinese medicine which can be combined with western medicine in the acute stage for the treatment and prognosis of ischemic stroke.

Buyang Huanwu decoction is currently the most commonly used classical prescription in TCM clinical treatment of ischemic stroke[13, 14] and many prescriptions are tailored from it. Naomaili granules (NML) is an oral Chinese patent medicine derived from Buyang Huanwu decoction, composing *Leonurus artemisia*, *Panax pseudo-ginseng*, *Astragalus propinquus*, *Curcuma longa* L., *Ligusticum chuanxiong* hort, *Carthamus tinctorius* L., *Salvia miltiorrhiza*, *Paeonia lactiflora*, *Angelica sinensis*, *Paeonia lactiflora*, *Achyranthes bidentate*. Analyzed from the prescription, the active ingredients are mainly leonurine, notoginsenosides and astragaloside[15]. NML have anti-platelet effect[15], promotion of angiogenesis[15], protection against ischemia-reperfusion injury[16, 17], improvement of cerebral circulation[18] and brain protection[17].

In this research, a randomized, double-blind, placebo-controlled multicenter clinical trial was designed, and the effect and safety of western medicine basic therapy (aspirin) combined with NML were investigated in patients with acute stroke. This study aims to provide a new idea and drug choice for the treatment of acute ischemic stroke with integrated traditional Chinese and Western medicine, and to improve the treatment plan of acute ischemic stroke from the perspective of TCM syndromes.

Methods

Diagmostic Criteria

Medical diagnostic standards (refer to "Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2010" issued by the Chinese Medical Association Neurology Branch[4]).

Key points of diagnosis:

(1) Acute onset; (2) Focal neurological deficits, a few are general neurological deficits; (3) Symptoms and signs last more than 24 hours; (4) Exclude non-vascular brain lesions; (5) Brain CT or MRI excludes cerebral hemorrhage and other lesions, responsible ischemic lesion.

TCM syndrome diagnostic criteria

Reference to Wang, etc., 2011[19]. Developing Syndrome Differentiation of Qi Deficiency (QD) and Blood Stasis Syndrome (BSS) in the Acute Phase of Stroke Disease standard.

Inclusion Criteria

(1) Those who meet the medical diagnostic criteria for acute ischemic stroke (belonging to anterior circulation infarction); (2) The syndromes of QD and blood stasis comply with TCM syndrome differentiation, that is, the scores of QD and BSS are ≥ 10 points; (3) Patients whose disease course is 7–14 days after the onset (including 7 days and 14 days); (4) NIHSS scores between 4 ~ 20 points; (5) Age between 40 ~ 80; (6) Patients with first or recurrent stroke have fully recovered before onset (mRS points are 0–1); (7) Signed informed consent.

Exclusion Criteria

Patients with any of the following conditions were excluded: (1) History of cerebral hemorrhage in the past six months; (2) With conscious disturbance in clinical manifestations; (3) Treated with blood vessels (such as thrombolysis, arterial thrombectomy, ultra-early thrombus aspiration, and stent forming) within 6 h after the onset; (4) It is confirmed by examination that brain embolism caused by brain tumor, brain trauma, cerebral parasitic disease, metabolic disorder, rheumatic heart disease, coronary heart disease; (5) TIA, cerebral hemorrhage, subarachnoid hemorrhage, and asymptomatic cerebral infarction; (6) Cannot be administered orally; (7) Oral anticoagulants; (8) Combined with other diseases that affect limb function; (9) Complicated with severe hypertension or diabetes and other diseases; (10) With active ulcer and bleeding tendency; (11) With severe heart and lung diseases and chronic liver and kidney dysfunction; (12) Pregnant or lactating women; (13) Disabled patients; (14) Those who are allergic to the ingredients of this medicine and allergies; (15) Those who are not suitable for inclusion or influence the participation or completion by the investigator; (16) Participating in other clinical trials within 3 months.

Rejection Criteria

Patients with false acceptance, misdiagnosis, no medication, or no inspection records were rejected.

Patients

Eligible patients were recruited from 16 clinical centers located in different regions of China between May 2015 and March 2018, including the Peking University Third Hospital, Huazhong University of Science and Technology Tongji Medical College Affiliated Union Hospital, Affiliated Hospital of Chengdu University of traditional Chinese Medicine, the Second Affiliated Hospital of South China University, Shanghai Chinese Medicine Hospital. Participants were randomly assigned to the NML group or control group in a ratio of 1:1 via central randomization. The study protocol was reviewed and approved by the Ethic Committee of the Peking University Third Hospital (Approval No. ChiCTR2000033619). The study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki.

Interventions

The study period is 20 days, and the follow-up period was 90 days after the onset. During the start-up period, patients were prohibited from taking other CM to activate blood or dissolve stasis. Both NML (batch No. 11-141201, 10 g/bag) and the NML simulation granules (batch No. 11-160301) were manufactured by Nanjing Kefeiping Shenghui Pharmaceutical Co., Ltd. and supplied by Jiangsu Kefeiping Pharmaceutical Co., Ltd., China. As a basic medicine, aspirin enteric-coated tablets (batch No. BJ19533, 0.1 g/tablet) are produced by Bayer HealthCare Manufacturing S.r.l. and supplied by Jiangsu Kefeiping Pharmaceutical Co., Ltd. During the study, thrombolytic drugs, anticoagulants, defibrinators, neuroprotective agents, and other antiplatelet drugs are not allowed to use. Drugs that must be taken in combination with diseases, such as antihypertensive drugs, lipid-lowering drugs, hypoglycemic drugs, or antibiotics, can remain unchanged.

Primary Outcome

The experimental results are mainly evaluated by the following three methods:

Evaluation criteria of improved Rankin scale

(1) The t-test/rank and test were used to compare the changes in the Rankin Scale (mRS) scores before and after treatment; (2) mRS ≤ 2 are relatively independent, and mRS > 2 or more points are obvious disability or poor prognosis. The ratio of relative independence can be compared using Fisher variable probability method/χ^2 test.

Evaluation criteria for the incidence of cardiovascular and cerebrovascular events

The Fisher prediction probability method/χ^2 test was used to compare the incidence of cardio-cerebral vascular events (myocardial infarction, cerebral infarction, TIA, etc.) 20 days after the onset of the two groups of patients.

Analysis of efficacy indicators of TCM syndromes

The ischemic stroke QD and BSS dialectical scale was used for evaluation according to the "General Principles of Clinical Research of New Chinese Medicines". The t test/Wilcoxon rank sum test was used to compare the scores and reduction rates of the syndromes of QD and blood stasis before and after the two groups of tests ((pre-treatment score-post-treatment score)Pre-treatment score × 100%).

Secondary Outcomes

Barthel index efficacy evaluation criteria

(1) The t-test/rank sum test was used to compare the changes in the Barthel index (BI) before and after treatment; (2) ≥ 60 are relatively independent, and < 60 means obvious disability or poor prognosis. Fisher's exact probability method/χ^2 test was used to compare the relative independence of the two groups.

National Institutes of Health Stroke Scale (NIHSS) evaluation criteria

(1) The t-test/Wilcoxon rank sum test was used to compare the changes in NIHSS scores before and after treatment; (2) After the treatment, the NIHSS score ≤ 1 is classified as good clinical recovery; otherwise, the clinical recovery is poor, and Fisher exact probability method/χ^2 test is used to compare the good clinical recovery ratio between the two groups.

Safety evaluation

The safety indices observed included: (1) General physical examination items, such as body temperature, heart rate, breathing, blood pressure, symptomatic cerebral hemorrhage (bleeding at the infarct site), etc.; (2) Blood, urine, stool routine, electrocardiogram and liver function (ALT, AST, TBIL, γ-GT, ALP), renal function (BUN, Cr), coagulation (PT, APTT), FIB, TT, etc.; (3) The incidence of possible adverse events and adverse reactions.

Randomization and Blinding

Randomized design

Stratified block randomization was used. With the help of SAS v9.3 statistical software, given the number of seeds, the random arrangement of subjects (i.e. random coding table) is generated. The corresponding drug number shall be pasted on the conspicuous position of the external package of the drug by the personnel unrelated to the clinical observation, supervision, statistical analysis, etc. of this clinical trial according to the formed processing code. The divided test kits are sent to each test center together.

Blindness

(1) Blind method design: This experiment uses secondary blinding; (2) Management and preservation of blind bottom: The random coding table is established by the clinical trial data management and statistical unit. The blind bottom is sealed in duplicate and submitted to the sponsor and the lead unit for

proper storage. The entire drug coding process is written by the blind editor into a file form, that is, a blind edit record, which is saved as one of the documents of the clinical trial. Emergency blind envelopes are kept by the project leaders of each clinical trial unit. No one may unpack it without permission; (3) Emergency blind-breaking: If serious adverse events occur during the test, emergency blind-breaking can be performed. Emergency blindness must be decided by the main investigator of the research center, and the reason, time, and place of blindness should be recorded in detail and signed. After the blindness is broken, the team leader unit and the clinical monitor shall be notified in time. Case data should be kept intact.

Sample Size Estimation

The primary outcome measure was the poor prognosis rate (defined as mRS > 2). According to the relevant literature, it is assumed that the adverse outcome rate of the treatment group through NML and conventional treatment is 20% lower than that of the control group alone. The type I error is set to 0.05 and the control is 80%. According to the 1:1 parallel control design of the experimental group and the control group, the minimum sample size of each group was 93 cases. In consideration of the reasons such as the drop off of the subjects during the clinical trial (assuming 20% drop off rate), the total sample size of the clinical trial of NML in the treatment of acute stroke was designed as 240 cases, 120 cases in the trial group and 120 cases in the control group, which were undertaken by each sub center.

Statistical Analyses

(1) For quantitative data, the data are described by case number, mean, standard deviation, minimum, median, maximum, upper quartile (Q1), lower quartile (Q3), 95% confidence interval (95% CI). Statistical analysis of the data between the two groups or within the group before and after treatment, using t-test or paired t-test; (2) For qualitative data, use frequency tables, percentages or composition ratios to describe the data. Statistical analysis before and after treatment, using χ^2 test, Fisher exact probability method, Wilcoxon rank sum test or Wilcoxon symbol rank sum test; comparison of two classification indicators and rank indicators; (3) All hypothesis tests use a two-sided test, taking $\alpha = 0.05$. All statistical calculations were performed using SAS 9.3 statistical analysis software.

Results

Baseline Characteristics of Patients

A total of 187 patients were enrolled from 16 clinical centers in this study, 93 patients in medication group, 94 patients in control group. The demographic data (age, gender, weight, height, BMI) and vital signs (systolic pressure, diastolic pressure, resting heart rate, respiration, body temperature) of the two groups of subjects were statistically analyzed. There was no significant difference on patients' demographic profiles between the two groups (P > 0.05, Table 1).

Table 1
Baseline Characteristics of Patients in Both Groups (± s)

Characteristics	NML (93 cases)	Control (94 cases)
Age (Year)	61.47 ± 9.74	62.35 ± 10.63
Sex (Male/female, case)	57/36	51/43
Height (cm)	167.72 ± 7.72	166.70 ± 6.92
Weight (kg)	68.85 ± 10.80	68.57 ± 9.24
Body mass index (kg/m^2)	24.40 ± 2.95	24.64 ± 2.67
Body temperature (℃)	36.46 ± 0.20	36.50 ± 0.24
Pulse (Beat per min)	73.78 ± 8.48	71.74 ± 7.80
Systolic blood pressure (mmHg)	136.27 ± 10.71	138.00 ± 11.01
Diastolic blood pressure (mmHg)	83.83 ± 8.07	84.03 ± 8.22
Breath (per min)	18.08 ± 1.50	18.31 ± 1.73
Previous medication history [Case (%)]	71 (76.34)	65 (69.15)
Previous treatment [Case (%)]	4 (4.3)	5 (5.32)
Previous rehabilitation [Case (%)]	15 (16.13)	11 (11.70)
Drug allergy history [Case (%)]	3 (3.23)	2 (2.13)
Other current illnesses and medications [Case (%)]	64 (68.82)	72 (76.6)

Comparisons of main efficacy indicators between Two Groups

Improved Rankin's relative independence rate

In the FAS 90 days after the onset, the experimental group was 70.00%, and the control group was 45.24%. There was a statistically significant difference between the two groups ($P < 0.05$), the test group is better than the control group.

Table 2
Baseline of efficacy indicators

Observation index	A(n = 93)	B(n = 94)	Statistics	*P* value
Rankin total score				
n (Missing)	93(0)	94(0)	$\chi^2 = 1.5430$	0.2142
0–2 point n(%)	43(46.24)	52(55.32)		
3–5 point n(%)	50(53.76)	42(44.68)		

Incidence of acute cardio-cerebral vascular events

(1) The incidence of acute cardio-cerebrovascular events was 1 case (1.08%) in the experimental group and 0 in the control group after 20 days of FAS treatment; (2) The incidence of myocardial infarction was 0 in the experimental group and the control group; (3) Cerebral infarction incidence: 1 case (1.08%) in the experimental group and 0 cases in the control group; (4) The number of visits to the FAS test group was 0 in the TIA group (Table 3).

Table 3
Incidence of acute cardiovascular and cerebrovascular events

Observation index	A(n = 93)	B(n = 94)	Statistics	*P* value
Acute cardiovascular and cerebrovascular events				
n (Missing)	93(0)	94(0)	Fisher	0.4973
positive n (%)	1(0.08)	0(0.00)		
Incidence of myocardial infarction				
n (Missing)	93(0)	94(0)	-	-
positive n (%)	0(0.00)	0(0.00)		
Incidence of cerebral infarction				
n (Missing)	93(0)	94(0)	Fisher	0.4973
positive n (%)	1(1.08)	0(0.00)		
Incidence of TIA				
n (Missing)	93(0)	94(0)	-	-
positive n (%)	0(0.00)	0(0.00)		

TCM Syndrome Effect

(1) The score of QD and BSS after 20 days of medication was 36.08% ± 28.53% in the FAS test group and 25.03% ± 26.56% in the control group. The difference was statistically significant ($P < 0.05$); (2) The score

of BSS score after 20 days of medication was 23.23% ± 35.67% in the FAS test group and 16.86% ± 34.78% in the control group. The difference was not statistically significant ($P > 0.05$); (3) The score of QD syndrome after 20 days of medication was 35.93% ± 31.09% in the FAS test group and 20.80% ± 30.12% in the control group. There was a significant difference between the two groups ($P < 0.05$) (Table 4).

Table 4
Curative effect of TCM Syndrome

Observation index	A (n = 93)	B (n = 94)	Statistics	*P* value
Stroke QD Blood Stasis total score				
n (Missing)	87 (6)	86 (8)	Z = 2.3116	0.0208
Mean ± SD	25.62 ± 15.47	28.80 ± 12.03		
95% CI	(22.32, 28.92)	(26.22, 31.38)		
Med (Q1, Q3)	22.00 (15.00, 34.00)	27.00 (20.00, 37.00)		
R (Min, Max)	70 (3,73)	65 (7,72)		
Total blood stasis score				
n (Missing)	76 (17)	78 (16)	Z=-1.1887	0.2346
Mean ± SD	15.14 ± 9.12	15.35 ± 6.32		
95% CI	(13.06, 17.23)	(13.92, 16.77)		
Med (Q1, Q3)	13.00 (9.00, 18.50)	14.00 (10.00, 20.00)		
R (Min, Max)	42 (4, 46)	26 (4, 30)		
Total QD score				
n (Missing)	83 (10)	84 (10)	Z=-2.6393	0.0083
Mean ± SD	12.99 ± 8.43	15.24 ± 6.80		
95% CI	(11.15, 14.83)	(13.76, 16.71)		
Med (Q1, Q3)	11.00 (7.00, 17.00)	15.00 (11.00, 17.50)		
R (Min, Max)	40 (2, 42)	37 (5, 42)		
Comparisons of secondary efficacy indicators between Two Groups				
1. Barthel index changes: After 20 days of treatment, the test group in the FAS was 12.26 points, and the control group was 7.93 points. There was a statistically significant difference between the two groups (P < 0.05).				
2. Change in NIHSS score: 90 days after the onset, the experimental group was 4.55 points in the FAS group and 3.14 points in the control group. There was a statistically significant difference between the two groups in FAS (P < 0.05).				
3. The clinical recovery rate of NIHSS is good: the experimental group was 11.83% in the FAS group after 20 days of treatment, and the control group was 1.06%. There was a statistically significant difference between the two groups in FAS (P < 0.05).				

Medication compliance

There was no significant difference in medication compliance between the two groups (P > 0.05).

Safety Evaluation

A total of 19 adverse events occurred in this study, 8 cases in the test group, and 11 cases in the control group. There was no significant difference between the two groups (P > 0.05); There were 3 cases of adverse reactions, 3 cases of the test group, and 0 in the control group. There was no significant difference between the two groups (P > 0.05). Among them, 3 cases were serious adverse events in the test group, and 0 in the control group. There was no significant difference between the two groups (P > 0.05).

Discussion

In recent years, the incidence of cerebrovascular diseases in China has been on the rise. According to statistics, the mortality rate in 2014 was higher than that of cardiovascular, tumor and other diseases. Acute ischemic stroke (AIS) is a group of clinical syndromes, which is caused by various reasons, such as blood supply disorder of brain tissue, ischemic anoxic necrosis and neurological dysfunction, accounting for 60% − 80% of stroke. According to TCM, the basic pathogenesis is the disorder of Qi and blood, which is committed to the brain and the brain's gods are not used.

In this study, western medicine curative effect evaluation system and TCM syndrome change were simultaneously used to evaluate the efficacy of patients. It reflects the advantages of integrated traditional Chinese and western medicine treatment, and makes up for the deficiency of previous studies that only do western medicine curative effect judgment but lack the effectiveness evaluation of traditional Chinese medicine syndromes[20–22]. The research results of Huang Yan et al. [23] showed that wind syndrome, phlegm syndrome, BSS and QD syndrome were the main syndromes of ischemic stroke within 30 days after the onset of ischemic stroke. The combination of two syndromes was dominant in 4-10d and 11-30d, and the combination of BSS and QD syndrome appears most frequently. The second is the combination of phlegm syndrome and QD syndrome, among which QD syndrome is the root cause of ischemic stroke; The research results of Zhang Teng et al. [24] showed that among the patients with clear consciousness in the acute stage of ischemic stroke, those with QD had more severe neurological impairment in the acute stage and convalescence stage, and the long-term prognosis was worse. Therefore, it is beneficial to improve the long-term prognosis of patients to pay attention to the treatment of QD syndrome in acute stage. In this experiment, the syndrome of QD and blood stasis were evaluated, and QD and blood stasis were analyzed respectively. The final results showed that there was statistical difference in the score reduction rate of QD and BSS and QD syndrome after treatment. At 90 days after onset, there was a statistically significant difference in NIHSS score between the two groups, it suggests that NML can significantly improve the acute QD syndrome and play an important role in the long-term prognosis.

Inflammatory response is one of the important mechanisms in many complex factors of ischemic stroke, which mainly occurs in the central nervous system and its surrounding tissues. Its pathogenesis is

closely related to immune cells and their secreted inflammatory mediators. The abnormal cellular energy metabolism, the activation of ion channels and the release of oxygen free radicals caused by ischemia-reperfusion can all lead to systemic inflammatory response[25–28]. The immune inflammatory response induced by ischemia plays a major role, while ischemia itself plays a secondary role [29]. After cerebral ischemia, the production of inflammatory mediators, the destruction of blood-brain barrier, the activation and infiltration of inflammatory cells can all induce and aggravate the inflammatory reactions, which leads to a series of complex pathophysiological processes and brain injury[30–32]. NML can inhibit the release of tumor necrosis factor (TNF-α), interleukin (IL-6) and other inflammatory factors, reduce the level of adhesion molecule (ICAM-1), thus reducing leukocyte adhesion ,so it has an obvious anti-inflammatory effect[33].

The primary therapeutic principle for ischemic stroke is to restore blood flow and reperfusion to the ischemic brain tissue as soon as possible to regain blood oxygen supply. However, when cerebrovascular recanalization occurs, ischemia reperfusion injury will follow[34]. Ischemia-reperfusion injury is a complicated pathological process, which is closely related to many factors, It mainly includes energy metabolism disorder, oxidative stress, Ca^{2+} overload, excessive synthesis of NO, cell apoptosis, etc[35]. Some studies have shown that in the process of ischemic stroke, due to the damage of blood-brain barrier, ferritin and free iron ions exudate and accumulate in the endothelial cells and penumbra, causing iron dependent oxidative stress, producing a large amount of superoxide (ROS), which leads to neuronal apoptosis and injury[36–38]. In addition, 24 hours after the onset of acute ischemic stroke, MDA content in the serum was significantly increased and SOD activity was significantly reduced, indicating that oxidative stress injury is one of the important pathogenesis mechanisms in the acute stage, and may be related to the prognosis of patients[39]. NML can significantly reduce the area of cerebral infarction, reduce the score of cerebral histopathology, reduce the content of H_2O_2 and MDA in brain tissue, and improve the ability of anti-superoxide anion free radical and anti-hydroxyl free radical. In addition, the 6 g/kg dose group can significantly reduce the neurological damage, decrease the brain water content, increase the GSH content in brain tissue, and increase the GSH-Px and SOD activity in rats with cerebral ischemia-reperfusion[16]. This suggests that its anti-oxidative stress injury may be the main mechanism to improve the neurological function and prognosis of patients.

At present, angiogenesis is one of the main strategies for the treatment of ischemic stroke at functional recovery stage, a series of studies suggest that endogenous VEGF, EPC, etc. are closely related to the recovery after injury of ischemic stroke[40, 41]. VEGF and its receptor changes are important factors affecting endogenous angiogenesis after stroke, and are also one of the most important neurotrophic factors affecting neurogenesis after stroke[42]. Recent research reports[15] show that promoting angiogenesis may be a potential target for the treatment of ischemic stroke, and may promote the improvement of symptoms and prognosis of patients. The pharmacological study of NML showed that it could significantly promote angiogenesis by increasing the expression of VEGFR1, it suggested that the potential therapeutic mechanism of improving the function and prognosis of patients may be related to its role in promoting angiogenesis, and play a positive role in its efficacy.

According to the results of improved Rankin independence rate, the difference between the two groups was statistically significant 90 days after the onset of the disease, 70.00% in the trial group and 45.24% in the control group. It is suggested that NML has significant effect on the improvement of limb activity in the long term after acute ischemic stroke. This indicated the effectiveness of the treatment, which may be closely related to the above mechanisms.

Although this study proved that cerebral pulsation has better clinical treatment advantages, in terms of the incidence of acute cardiovascular and cerebrovascular events. Owing to our study strictly limited the combination of drugs in acute phase and prohibited the use of other drugs with similar effects, as a result, there are some difficulties in enrolling patients into the group, resulting in a small sample size. Therefore, there was no significant difference between the two groups in the study of incidence of acute cardiovascular and cerebrovascular events. In future studies, the study sample size can be increased and the follow-up period can be extended to further confirm its effectiveness on long-term cardiovascular and cerebrovascular events.

Conclusion

The comprehensive analysis of this clinical trial shows that the combined treatment of NML in the acute phase of stroke can effectively improve the prognosis of patients, improve the independent survival ability of patients, and its safety is reliable and worthy of clinical promotion. This clinical trial provides new drug options for the treatment and improvement of prognosis of acute ischemic stroke, and provides research design and data reference. Meanwhile, it provides new ideas for the integrated treatment of Chinese and western medicine for acute ischemic stroke.

Abbreviations

TCM: Traditional Chinese Medicine; AEs: Adverse events; FAS: Full Analysis Set; SD: Standard Deviation; BI: Barthel index; Med: Median; Q1: Upper Quartile; Q3: Lower Quartile; GCP: Good Clinical Practice; ALT: Alanine Aminotransferase; AST: Aspartate aminotransferase; TBIL: Total Bilirubin; ALP: Akaline Phosphatase; Cr: Creatinine; BUN: Blood urea nitrogen; APTT: Activated Partial Thromboplastin Time; PT: Prothrombin Time; FIB: Fibrinogen; NIHSS: National Institutes of Health Stroke Scale; BMI: Body Mass Index; AIS: Acute ischemic stroke; QD: Qi Deficiency; BSS: Blood Stasis Syndrome; VEGF: Vascular endothelial growth factor.

Declarations

Authors' Contributions

XGL drafted the manuscript and implemented the research plan. XGL, DJS and PYZ conceived and designed this trial. XGL provided a critical contribution to the organization and cooperation of this multi-center task. DJS, PYZ, HGW, CFZ, BH, WTL, DDY participated in the enrollment of the patients. DJS, PYZ,

HGW, CFZ, BH, WTL, DDY recruited the patients and collected data. All authors read and approved the final manuscript.

Data sharing statement

All relevant data are within the paper.

Ethical approval and consent to participate

The protocol was carried out according to GCP guidelines and in accordance with the principles of the Declaration of Helsinki. This study had been approved by the Medical Scientific Research Ethics Committee of the Third Hospital of Peking University on 20 April 2015 (ref: 2015BL-082). All participants had signed written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

Acknowledgements

Not applicable

Author details

[1]Department of Neurology, Peking University Third Hospital, 49 Huayuan North Road, Beijing, China [2]Department of Neurology, The Second Hospital of Harbin, 38 Weixing Road, Harbin, China [3]Department of Neurology, Xiangyang No.1 Peoples Hospital, 15 Jiefang Road, Xiangyang, China [4]Department of Neurology, Cangzhou People's Hospital, 7 Qingchi Avenue, Cangzhou, China [5]Department of Neurology, The Second Affiliated Hospital of University of South China, 35 Jiefang Avenue, Hengyang, China

[6]Department of Neurology, Wuhan Union Hospital of China, 1277 Jiefang Avenue, Wuhan, China [7]Department of Neurology, Shanghai Municipal Hospital of Traditional Chinese Medicine, 274 Zhijiang Middle Road, Shanghai, China [8]Department of Neurology, The Teaching Hospital of Chengdu University of TCM, 39 Shierqiao Road, Chengdu, China

References

1. Johnson W, Onuma O, Owolabi M, Sachdev S. Stroke: a global response is needed. Bull World Health Organ. 2016;94(9):634.
2. Owolabi MO, Akarolo-Anthony S, Akinyemi R, Arnett D, Gebregziabher M, Jenkins C, et al. Members of the H3Africa Consortium. The burden of stroke in Africa: a glance at the present and a glimpse into the future. Cardiovasc J Afr. 2015;26:27–38.
3. Guidelines for the prevention of stroke. in patients with stroke or transient ischemic attack: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2011;42:227–76.
4. Guidelines for diagnosis. and treatment of acute ischemic stroke in China 2010. Chinese J Med Fron. 2010;2:50–9.
5. Guidelines for the. Acute Treatment of Patients with Ischemic Stroke. ANA/ASA, 2018.
6. Guidelines for Management of Ischemic Cerebrovascular Diseases. ESO, 2008.
7. Altman R, Luciardi HL, Muntaner J, Herrera RN. The antithrombotic profile of aspirin. Aspirin resistance, or simply failure? Thromb J. 2004;2:1.
8. Wahlgren N, Ahmed N, Davalos A, Hacke W, Lees K. Symptmtomatic intracerebral hemorrhgge, mortality and independence at 3 months for patients treated 3-4.5 h after stroke onset in the sits thrombolysis register (SITS-ISTR). Cerebmvasc Dis. 2008;25(Suppl2):3–4.
9. Guo XD. Advances in Traditional Chinese and Western medicine treatment of Ischemic Stroke. The Journal of Medical Theory Practice. 2012;25(1):31–3.
10. Guidelines for the. Diagnosis and Treatment of Cerebral Infarction in China by Integrated Traditional Chinese and Western Medicine 2017. Chinese Journal of Integrated Traditional Western Medicine. 2018;38(2):146–144.
11. Hung YC, Cheng YC, Muo CH, Chiu HE, Liu CT, Hu WL. Adjuvant Chinese Herbal Products for Preventing Ischemic Stroke in Patients with Atrial Fibrillation. PLoS One. 2016;11(7):e0159333.
12. Peng W, Lauche R, Ferguson C, Frawley J, Adams J, Sibbritt D. Efficacy of Chinese herbal medicine for stroke modifiable risk factors: a systematic review. Chin Med. 2017;12(1):1–29.
13. Chang CC, Lee YC, Lin CC, Chang CH, Chiu CD, Chou LW, et al. Characteristics of traditional Chinese medicine usage in patients with stroke in Taiwan: A nationwide population-based study. J Ethnopharmacol. 2016;186:311–21.
14. Luo YH, Ge JW, Liu L. Clinical Research Progress of Buyang Huanwu Decoction in the Treatment of Ischemic Stroke. Chinese Journal of Integrative Medicine on Cardio-/Cerebrovascuiar Disease.

2016;14(3):278–81.

15. Su M, Lou YJ, Jiang YQ, Guo SY, Xu YQ, Qin YL. Study on the effects of Naomaili granule on angilgenesis and prevention of platelet aggregation thrombosis in zebrafish. Chin Med J Res Prac. 2019;33(5):24–9.
16. Ren Y, Su M, Zheng J, Qin YL, Chen T, Ji H. Protective effects of Naomaili granules on cerebal ischemia-reperfusion in rats and its mechanism. J Chin Pharm Univ. 2015;46(1):100–4.
17. Su M, Chen QY, Duan XB, Teng DY, Chen T, Zhen MQ. Protective effects of Naomaili granules on experimental cerebral thrombosis in rodents. Chin J Clin Pharm Therap. 2016;21(1):1–5.
18. Zhang M, Hui WW, Gao P, Huang YX. Effects of Naomaili Granules on Vascular Endothelial Function and Short-term Prognosis in Patients with Cardioembolic Stroke. Chin J Integr Med Cardio-/Cerebrovascuiar Dis. 2016;14(19):2305–7.
19. Gao Y, Ma B, Liu Q, Wang YY. Establishment and methodology of diagnostic scale of syndrome elements of ischemic stroke. Proceedings of the first international academic forum of internal medicine of TCM 2011;133–139.
20. Han SY, Hong ZY, Xie YH, Zhao Y, Xu X. Therapeutic effect of Chinese herbal medicines for post stroke recovery: A traditional and network meta-analysis. Medicine. 2017;96(49):e8830.
21. Li JG, Wang LQ, Yang XY, Chen Z, Lai LY, Xu H, et al. Chinese herbal medicine Dengzhan Xixin injection for acute ischemic stroke: A systematic review and meta-analysis of randomised controlled trials. Complementary Therapies in Medicine. 2017;34:74–85.
22. Xie HJ, Wang YQ, Li XL, Liu S. A Meta-analysis of Efficacy and Safety of Aspirin Combined with Blood-Activating and Stasis-resolving Drugs in the treatment of Elderly Ischemic Stroke. China Medicine. 2015;10(6):823–7.
23. Huang Y, Zhao M, Cai YF. Distribution Characteristics and Regularity of TCM Syndrome Elements in 4077 Patients with Ischemic Stroke. Chinese Journal of Basic Medicine in Traditional Chinese Medicine. 2011;17(7):759–61.
24. Zhang J, Nie ZL, Zhang T. Study on relationship between QD in acute phase of ischemic stroke and degree of neurologic impairment and prognosis. China Journal of Traditional Chinese Medicine Pharmacy. 2019;34(10):4856–60.
25. Oto J, Suzue A, Inui D, Fukuta Y, Hosotsubo K, Torii M. Plasma prninf1ammatory and anti-inflammatory cytokine and catecholamine concentrations as predictors of neurological outcome in acute stroke patients. J Anesth. 2008;22(3):207–12.
26. Anrather J, Iadecola C. Inflammation and Stroke: An Overview. Neurotherapeutics. 2016;13:661–70.
27. Emsley HC, Smith CJ, Tyrrell PJ, Hopkins SJ. Hopkins. Inflammation in Acute Ischemic Stroke and its Relevance to Stroke Critical Care. Neurocrit Care. 2008;9:125–38.
28. Jin R, Yang G, Li G. Inflammatory mechanisms in ischemic stroke: role of inflammatory cells. J Leukoc Biol. 2010;87(5):779–89.

29. Tobin MK, Bonds JA, Minshall RD, Pelligrino DA, Testai FD, Lazarov O. Neurogenesis and inflammation after ischemic stroke: what is known and where we go from here. J Cereb Blood Flow Metab. 2014;34(10):1573–84.
30. Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Moghaddam HF. Pathogenic mechanisms following ischemic stroke. Neurol Sci. 2017;38:1167–86.
31. Jin R, Liu L, Li G. Role of inflammation and its mediators in acute ischemic stroke. J Cardiovasc Transl Res. 2013;6(5):834–51.
32. Galea J, Brough D. The role of inflammation and interleukin-1 in acute cerebrovascular disease. J Inflamm Res. 2013;6:121–8.
33. Xu Z. Clinical Study of Naomaili Granules on Alleviating Inflammatory Reaction in Acute Cerebral Infarction. Strait Pharm J. 2015;27(4):131–2.
34. Eltzschig HK, Eckle T. Ischemia and reperfusion from mechanism to translation. Nat Med. 2011;17(11):1391–401.
35. Turley KR, Toledo-Pereyra LH, Kothari RU. Molecular mechanisms in the pathogenesis and treatment of acute ischemic stroke. J Invest Surg. 2005;18(4):207–18.
36. Carbonell T, Rama R, Iron. Oxidative Stress and Early Neurological Deterioration in Ischemic Stroke. Curr Med Chem. 2007;14(8):857–74.
37. Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Int J Stroke. 2009;4:461–70.
38. Rodrigo R, Fernández-Gajardo R, Gutiérrez R, Manuel Matamala J, Carrasco R, Miranda-Merchak A, et al. Oxidative Stress and Pathophysiology of Ischemic Stroke: Novel Therapeutic Opportunities. Cns Neurol Disord-Dr. 2013;12(5):698–714.
39. Milanlioglu A, Aslan M, Ozkol H, Çilingir V, Aydın MN, Karadas S. Serum antioxidant enzymes activities and oxidative stress levels in patients with acute ischemic stroke: in uence on neurological status and outcome. Wien Klin Wochenschr. 2016;128(5–6):169–74.
40. Arenillas JF, Sobrino T, Castillo J, Dávalos A. The role of angiogenesis in damage and recovery from ischemic stroke. Curr Treat Options Cardiovasc Med. 2007;9(3):205–12.
41. Ergul A, Alhusban A, Fagan SC. Angiogenesis: a harmonized target for recovery after stroke. Stroke. 2012;43(8):2270–4.
42. Lafuente JV, Ortuzar N, Bengoetxea H, Bulnes S, Argandona EG. Vascular endothelial growth factor and other angioglioneurins: key molecules in brain development and restoration. Int Rev Neurobiol. 2012;102:317–46.