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Original Article

Effect of troxerutin and cerebroprotein hydrolysate injection for the treatment of acute cerebral infarction: a multi-center randomized, single-blind and placebo-controlled study

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Abstract: Objective: This study aims to investigate the efficacy and safety of troxerutin and cerebroprotein hydrolysate injection (TCHI) for the treatment of acute cerebral infarction (ACI). Methods: A total of 456 acute cerebral infarction (ACI) patients were enrolled into this study. These patients were divided into two groups: placebo group (n=114), patients were injected with sterile water; experimental group (n=342), patients were treated with TCHI. The basic treatment for all patients was the same. The main outcome measure was the percentage of patients without significant disability (grades 0-2), which were evaluated by the modified Rankin scale at 90 days after onset (PS0-2). The secondary outcome measures were the percentage of patients with NIHSS scores that decreased by ≥ 7 points at seven and 14 days after treatment, compared with the percentage at baseline (PN7), and the percentage that had a Barthel index ≥ 75 at 90 days after onset (PB75). Results: PS0-2, PN7 and PB75 were higher in the experimental group (83.92%, 14.04% and 85.38%, respectively) than in the placebo group (69.3%, 1.75% and 76.32%, respectively); and the differences between these two groups were statistically significant (P<0.05 for all). In addition, 26 cases (7.28%) and 11 cases (9.17%) of adverse reactions (general adverse events) occurred in the experimental and placebo groups, respectively; and the difference was not statistically significant (P>0.05). Conclusion: In the treatment of ACI, TCHI can improve neurological defects, promote functional recovery, and has fewer adverse reactions and good safety. Hence, it is worthy of clinical popularization.

Keywords: Troxerutin, roxerutin and cerebroprotein hydrolysate injection, acute cerebral infarction, curative effect, security

Introduction

At present, ischemic cerebrovascular disease is one of the common diseases that cause harm to the health of the elderly in China, has a high disability and mortality rate, and brings about tremendous mental stress and heavy economic burden to patients and their families.

Effective treatment is the key to improve the degree of infarction and prognosis [1]. At present, the aim of drug treatment is to restore the blood supply of ischemic brain tissues in time, promote the recovery of occluded blood vessels and nerve function, and rescue nerve cells in the ischemic penumbra; thereby improving the clinical symptoms and signs to the highest extent. The degree of clinical recovery of acute ischemic stroke is directly related to the degree of neurologic impairment, and one of the keys to clinical recovery is to promote the recovery of nerves in the ischemic penumbra [2-4]. Troxerutin and cerebrprotein hydrolysate injection (TCHI) is a compound preparation of troxerutin and cerebroprotein hydrolysate, which is developed based on the rational ratio and effective combination of two components. It is a dual-target drug that can protect the blood vessels and nerve cells. This drug can maintain and enhance capillary elasticity, decrease capillary permeability and fragility, resist vascular injury caused by bradykinin and 5-hydroxytryptamine, prevent brain edema caused by increased vascular permeability, improve the energy and substance metabolism of the cerebral nerve, and protect the nerve cells in patients with cerebral infarction [5, 6]. This study aims to investigate the efficacy and safety of TCHI in the treatment of acute cerebral infarction (ACI).

Materials and methods

General information

A total of 480 ACI patients, who were 40-75 years old, were enrolled into this study. All subjects provided a signed informed consent, and underwent the screening and inspection items required for this trial. Inclusion criteria: (1) patients that met the diagnosis standard in the "Prevention and Treatment of Cerebrovascular Disease in China" [2]; (2) patients who were diagnosed by cranial CT or MRI; (3) patients with total anterior circulation infarction (TACI) or partial anterior circulation infarction (PACI); (4) patients with National Institutes of Health Stroke Scale (NIHSS) scores ranging between 4-22 points; (5) patients with a disease course of <14 days; (6) patient between 40-75 years old with no limitations for both genders; (7) patients who volunteered to participate in the study, and provided a signed informed consent. Exclusion criteria: (1) patients with progressive stroke, posterior circulation infarcts (POCI), and cerebral hemorrhage; (2) patents with craniocerebral injury, brain tumor, brain parasites and cerebral infarction caused by heart disease; (3) patients with epileptic state or epilepsia gravior; (4) patients in the acute phase, who are receiving thrombolysis; (5) patients complicated with digestive system diseases; (6) patients with allergic constitution and are allergic to the components of the drugs used in this study; (7) patients complicated with uncontrolled hypertension and serious diseases in other important organs; (8) patients with abnormal liver and kidney functions and metabolic disorders; (9) patients currently participating in other clinical trials or administering other drug treatments. Patients who met the criteria were selected using a stratified random sampling method. Randomly assigned codes were simulated by the SAS software. Patients were divided into two groups: placebo group, and experimental group. In this experiment, some of the packaging of drugs was damaged. Hence, these were not randomly assigned. A total of 479 patients were selected for the trial. Among these subjects, 120 patients were assigned to the placebo group, and 359 patients were assigned to the experimental group. After excluding patients who withdrew from the trial, do not meet the inclusion and exclusion criteria, used illicit drugs, and did not take drugs during the required visiting time window, full data set of 114 patients in the placebo group and 342 patients in the experimental group were used for analysis. Differences in baseline demographic characteristics (including gender, age, height and body weight) and vital signs (including body temperature, respiration, heart rate and blood pressure) between these two groups were not statistically significant Supplementary Data 2 (P>0.05).

Method

All patients received basic treatment: During the trial, all patients orally administered aspirin enteric-coated tablets provided by the sponsors (1 tablet/d, 0.1 g/tablet, produced by Bayer Health Care Pharmaceuticals Inc.; national drug approval: No. J20080078, batch NO. BJ10119), and Xueshuantong Injection (1 bottle/day, 5 ml: 175 mg/bottle, produced by Limin Pharm, Livzon Group; national drug approval: No. Z44020284, batch NO. 1212072) was administered. Subjects were divided into

two groups: (1) experimental group, five bottles of TCHI (specifications: 2 ml/bottle, national drug approval: NO. 22026572, produced by Jilin Sihuanpharm batch NO. 20121018) were diluted in 250 ml of normal saline, administered by intravenous instillation, 1 time/day; (2) placebo group, five bottles of sterile water for injection (2 ml/bottle, provided by Jilin Sihuanpharm, batch NO. 20121101) were diluted in 250 ml of normal saline, administered by intravenous instillation, 1 time/day. Treatment duration was 14 days, and follow-up period persisted up to the 90th day after the onset of the disease Supplementary Data 1.

Observation indexes

Efficacy indexes: (1) Modified Rankin scale (MRS): The percentage of subjects without significant disability (grades 0-2) was evaluated at 90 days after the onset by MRS. MRS scores are divided into 0-5 grades: Grade 0 means completely asymptomatic; grade 1 means patients have symptoms, have no obvious dysfunction, and are able to maintain their daily work and life; grade 2 means mild disability, and patients are able to maintain their daily work and life without aids; grade 3 means moderate disability, patients are able to walk upright, but need some help; grade 4 means moderate-severe disability, patients cannot walk alone, and need the help of others to maintain their daily life; grad 5 means severe disability, patients have urinary and fecal incontinence, lay in bed, and are completely dependent on others to maintain their daily life. (2) NIHSS score: The percentage of patients with a NIHSS score that decreased by ≥ 7 points at seven and 14 days after treatment compared with that at baseline was calculated. Evaluation was conducted using the NIHSS (USA), in which the score ranged within 0-35 points. The scale consists of 11 items. The higher the score was, the more serious the condition became. (3) Modified Barthel index (MBI): The percentage of patients with a MBI ≥ 75 at 90 days after onset was calculated. The evaluation was conducted using MBI, in which the total score was 100 points. More than 60 points means good, and patients can basically maintain their daily life; a score of 40-60 points means moderate dysfunction, and patients need the help of others to maintain their daily life; a score of 20-40 points means severe functional disability; a score of <20 points means complete disability, in which patients are completely dependent on others to maintain their daily life.

Adverse drug reaction monitoring: (1) Vital signs; (2) Routine blood test (HB, WBC, RBC and PLT), routine urine test (BLD, LEU, GLU and PRO), liver and kidney function (ALT, AST, BUN and Cr), fasting blood glucose, four coagulation tests (TT, PT, APTT, and FIB); (3) 12-lead ECG; (4) Possible adverse events.

Statistics processing

Data were analyzed using SAS 9.13 software. Counting data were evaluated using the R*C chi-square test, and Fisher's exact test. Ranked data were evaluated using rank sum test and CMH test. Except for the superiority test, all statistical tests were conducted using two-sided test. $P \leq 0.05$ was considered statistically significant.

Results

Baseline analysis

The Oxfordshire Community Stroke Project (OCSP) clinical classification: A total of 102 patients (79.82%) and 28 patients (24.56%) were diagnosed with total anterior circulation infarct (TACI) in the experimental group and placebo group, respectively; and 240 patients (70.18%) and 86 patients (75.44%) were diagnosed with PACI in the experimental group and placebo group, respectively. The differences between these two groups were not statistically significant ($P>0.05$), while the differences in the other medical history characteristics between these two groups were not statistically significant ($P>0.05$).

Analysis using MRS in these two groups

Before treatment, the difference in the percentage of patients without significant disability evaluated by MRS in these two groups was not statistically significant ($P>0.05$). At 90 days after onset, the percentage of patients without significant disability was higher in the experimental group (287/342, 83.92%) than in the placebo group (79/114, 69.3%); and the difference was statistically significant ($P<0.05$, **Table 1**).

Analysis using NIHSS between these two groups

The difference in NIHSS scores after seven days of treatment between the two groups was not statistically significant ($P>0.05$). After the

Table 1. Analysis using MRS in these two groups [n (%)]

Items	TACI		PACI	
	Experimental group	Placebo group	Experimental group	Placebo group
Cases	102	28	240	86
Before treatment	39 (38.24)	14 (50.00)	98 (40.83)	35 (40.70)
Drug use 90 d	89 (87.25)$^{\Delta}$	19 (67.66)	198 (82.50)$^{\Delta}$	60 (69.77)

Note: compared with the placebo group, $^{\Delta}P<0.05$.

Table 2. Analysis using NIHSS between these two groups [n (%)]

Groups	Experimental group	Placebo group
Cases	342	114
Drug use 7 d	2 (0.58)	0
Stopping drug	48 (14.04)$^{\Delta}$	2 (1.75)

Note: complied with placebo group, $^{\Delta}P<0.05$.

end of treatment, the percentage of patients, who had scores that decreased by ≥ 7 points, was higher in the experimental group (14.04%) than in the placebo group (1.75%); and the difference was statistically significant ($P<0.05$, **Table 2**).

Analysis using MBI between these two groups

Before treatment, differences in MBI between these two groups were not statistically significant ($P>0.05$). At 90 days after onset, when the treatment was finished, the percentage of patients with an MBI ≥ 75 was higher in the experimental group (85.38%) than in the placebo group (76.32%); and the difference was statistically significant ($P<0.05$, **Table 3**).

Comparison of adverse events during the trial

During the trial process, 26 cases (7.28%) and 11 cases (9.17%) of general adverse events occurred in the experimental and the placebo groups, respectively; and the difference between these two groups was not statistically significant ($P>0.05$). Adverse events in the experimental group were mainly upper respiratory tract infections, which occurred in 11 patients. A few number of patients developed the following symptoms: insomnia, constipation, pneumonia, headache, dizziness, rash, gout, conjunctivitis, abnormal routine urine test results, elevation of aminotransferase and nasal bleeding. Adverse events in the placebo group were mainly upper respiratory tract infections, which occurred in five patients. A few number of patients developed the following symptoms: constipation, pneumonia and abnormal routine urine test results. One serious adverse event (infusion reaction) occurred in the experimental group. The patient recovered after symptomatic treatment.

Discussion

Cerebral infarction is one of the most common cardiovascular and cerebrovascular diseases in clinic, accounting for approximately 70% of the cases of all cardiovascular and cerebrovascular diseases. This disease presents a serious threat to human health and life safety. Cerebral infarction has a high disability rate and mortality rate, and its mortality rate ranks first place in all kinds of cardiovascular and cerebrovascular diseases. The incidence of cerebral infarction gradually increases with the increase in life expectancy and the accelerated speed of population aging. In early treatment, cerebral perfusion in the ischemic region should be restored as soon as possible, in order to improve microcirculation, promote the establishment of collateral circulation, increase cerebral blood flow, rescue nerve cells in the ischemic penumbra region, improve neurological function, and reduce the injury of reperfusion; thus, improving clinical symptoms and signs [2]. In July 2001, the US National Institutes of Neurological Disorders and Stroke (NINDS) called for a stroke research group, and proposed the concept of the neurovascular unit. Its structural foundation includes neurons, the blood brain barrier (BBB, including endothelial cells, basement membrane, foot processes of astrocytes, and pericytes), microglical cells and the extracellular matrix, which maintains the integrity of brain tissues. This concept places stroke in correlation with integrated tissue reaction [7-10].

Troxerutin is a good drug for the treatment of ischemic cerebrovascular disease, which can inhibit platelet aggregation, reduce blood viscosity and improve microcirculation [11, 12]. Cerebroprotein hydrolysate contains a large number of active peptides, a variety of amino acids, nucleic acids and other substances. This has the ability to improve the metabolism of brain tissues, help brain cells resist ischemia

Table 3. Analysis using MBI between these two groups [n (%)]

Groups	Cases	Stopping drug	Drug use 90 d
Experimental group	342	261 (76.76)$^{\Delta}$	292 (85.38)$^{\Delta}$
Placebo group	114	71 (62.28)	87 (76.32)

Note: complied with the placebo group, $^{\Delta}P<0.05$.

and hypoxia, improve the microcirculation, and participate in the repair of nerve cell damage [6]. When the compound of troxerutin and cerebroprotein hydrolysate is used for the treatment of cerebral infarction, results have revealed that the active substances of cerebroprotein hydrolysate passed through the blood-CSF barrier under the action of troxerutin, which could significantly improve hypercoagulability in patients with cerebral infarction, increase cerebral blood flow and dilate cerebral vessel. This would improve the microcirculation, increase the blood supply in the cerebral ischemic area, promote the repair of brain tissues in the infarct area, and reduce nerve damage [12]. Therefore, these two components have a positive synergistic effect. A study also revealed that troxerutin and cerebroprotein hydrolysate could both act on the nervous and vascular systems; thus, acting as an ion channel gate that expands the blood vessels and stabilizes the cell membrane. Furthermore, it contains 16 free amino acids and a large number of active low molecular polypeptides. Due to the small molecular weights, the various kinds of neuropeptides and neurotrophic factors it contains can pass through the BBB, promote synapse formation in nerve cells, and induce neuronal differentiation [13]. Troxerutin can inhibit cyclin-dependent kinase-1 expression in cells, enhance the dephosphorylation of protein phosphatase-1, deactivate α, MEK/ERK1/2/C/EBPβ, as well as reduce capillary permeability, improve vascular defense capability, and intensify the activity of acetylcholinesterase and adenylyl cyclase. These would lead to the effective maintenance of the integrity of the mitochondrion in brain cells, improve the metabolism in the brain, accelerate the repair of neurons, and improve cognitive impairment [14]. Therefore, troxerutin and cerebroprotein hydrolysate have a specific synergetic protective effect on nerves and blood vessels in cerebrovascula injury. Through troxerutin and cerebroprotein hydrolysate treatment in rats with cerebral contusion and laceration, Baiyun Liu of Tiantan Hospital found that troxerutin and cerebroprotein hydrolysate could effectively prevent and improve the damage to BBB, reduce cerebral edema, protect neurons and glial cells, and reduce the destruction of the neuronal microenvironment [9]. In brief, previous studies revealed that troxerutin and cerebroprotein hydrolysate could protect and repair nerves, blood vessels and BBB. Combined with the concept of the neurovascular unit, the author speculates that the protective effect of troxerutin and cerebroprotein hydrolysate on cerebral vessels may be related to its protective effect on the neurovascular unit. However, more details on its mechanism is required in future studies.

Clinically, troxerutin and cerebroprotein hydrolysate have been widely used in the treatment of ACI and neurological dysfunction caused by ACI, which have achieved good curative effects. In order to further evaluate the curative efficacy and safety of troxerutin and cerebroprotein hydrolysate in clinical applications, a clinical trial has been carried out in a number of hospitals, taking Beijing Hospital as the lead unit. Based on this basic treatment, this study took the placebo as control, and investigated the efficacy and safety of TCHI in the treatment of ACI. These results revealed that the improvement of the Rankin score and Barthel index in cerebral infarction patients in the TCHI group was superior to that in the placebo group, suggesting that TCHI can significantly improve the viability and the prognosis of ACI patients. The improvement of nerve function in the TCHI group was significantly better than in the placebo group. The reason may possibly relate to that troxerutin improves the cerebral circulation and protects nerve cells, and cerebroprotein hydrolysate enhances the ability of brain cells to resist hypoxia and improves glucose utilization levels in brain tissue; thus, promoting the recovery of neurological function [15, 16]. Combined with the results of basic researches, the author hypothesizes that the therapeutic effect of troxerutin and cerebroprotein hydrolysate in decreasing the degree of disability, as well as improving the activities of daily living and nerve function in ACI patients, may possibly relate to its protective effect on the neurovascular unit. The safety of TCHI is good, and the difference in safety between

TCHI and placebo was not statistically significant.

In summary, in the treatment of ACI, TCHI can improve neurological defects, and promote functional recovery; thus, improving the quality of life of patients. Furthermore, this drug has few adverse reactions and good safety.

Disclosure of conflict of interest

None.

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Supplementary Data 1. Provide a flow chart for patients recruitment and follow up
Reply:

Experimental flow

Before being enrolled into the groups (-3~0 days)
*Asking medical history, physical examination
*Signing consent inform
*Measuring vital signs
*Finishing testing items: Blood routine examination (WBC, PLT, RBC, HB); urine routine (LEU, BLD, PRO, GLU); hepatorenal function (ALT, AST, BUN, Cr); fasting blood glucose; coagulation four indices (TT, PT, APTT, FIB); urine pregnancy test; 12-leads ECG; skull CT
*Finishing the score: NIHSS, ADL, improved Rankin
*Evaluating examination results and enrolling the patients with inclusive creteria
*Distributing drugs

7-days treatment later (8±1 days):
*Measuring vital signs
*Finishing the score: NIHSS
*Asking adverse event
*Asking companied treatment
*Follow-up appointments

After finishing the treatment (15±1 days):
*Measuring vital signs
*Finishing testing items: Blood routine examination (WBC, PLT, RBC, HB); urine routine (LEU, BLD, PRO, GLU); hepatorenal function (ALT, AST, BUN, Cr); fasting blood glucose; coagulation four indices (TT, PT, APTT, FIB); urine pregnancy test; 12-leads ECG; skull CT
*Finishing the score: NIHSS, ADL
*Asking adverse event
*Asking companied treatment
*Reclying drugs and packaging
*Follow-up appointments

Follow-up (90 days after onset)
*Asking adverse event
*Finishing the score: NIHSS, ADL, improved Rankin
*Experiment over

Supplementary Data 1. Experimental flow chart

	Before enrollment	7 days after drug use	Drug use over	Follow-up
	0 day	8±1 days	15±1 days	90 days after onset
Asking medical history	●			
Physical examination	●			
Signing consent inform	●			
Vital signs	●	●	●	
Blood routine examination	●		●	
Hepatorenal function	●		●	
Urine routine	●		●	
Coagulation four indices	●		●	
12-leads ECG	●		●	
Urine pregnancy test	●			
Skull CT/MRI	●			

Radomiazed enrollment into groups	●				
NIHSS	●	●	●		
Improved Rankin	●			●	
ADL	●		●	●	
Distribution of drugs	●				
Reclying drugs and packaging			●		
Asking adverse event		●	●	●	
Asking companied treatment		●	●		
Evaluating compliance				●	

Supplementary Data 2. Provide demographic information and clinic feature in a table with comparison
Reply:

Items		Placebo group	Experimental group	*Statitical method*	*Statistics*	*P value*
Sex (cases)	Male	69 (60.53)	224 (65.5)	R*C chi-square	2.086	0.352
	Female	45 (39.47)	118 (34.5)			
Age (years old)	Mean±SD	63.044±8.124	61.377±8.743	ANOVA	2.753	0.065
Height (m)	Mean±SD	165.911±7.852	166.365±7.561	ANOVA	0.492	0.611
Weight (kg)	Mean±SD	65.411±10.369	65.771±10.814	ANOVA	0.059	0.943
Temperature (°C)	Mean±SD	36.426±0.28	36.458±0.32	ANOVA	0.411	0.663
Heart rate (times/minute)	Mean±SD	74.132±8.783	73.608±8.997	ANOVA	0.154	0.857
Respiration (times/minute)	Mean±SD	19.096±1.667	18.848±1.416	ANOVA	2.542	0.08
Systemic blood pressure (mmHg)	Mean±SD	138.474±16.682	136.927±16.739	ANOVA	1.335	0.264
Diastolic blood pressure (mmHg)	Mean±SD	81.447±10.983	82.24±10.279	ANOVA	0.548	0.578
Cosure of disease (days)	Mean±SD	4.886±4.111	4.614±3.636	ANOVA	0.28	0.756
History of drug allergy (cases)	No	110 (96.49)	334 (97.66)	R*C chi-square	0.456	0.796
	Yes	4 (3.51)	8 (2.34)			
Tretament history (cases)	No	110 (96.49)	334 (97.66)	R*C chi-square	0.456	0.796
	Yes	4 (3.51)	8 (2.34)			
OCSP clinical classification (cases)	TACI	28 (24.56)	102 (29.82)	R*C chi-square	1.173	0.556
	PACI	86 (75.44)	240 (70.18)			
Other diease and drug use (cases)	No	42 (36.84)	142 (41.52)	R*C chi-square	1.251	0.535
	Yes	72 (63.16)	200 (58.48)			
TACI (cases)	No obvious physical disabilities	14 (50)	39 (38.24)	R*C chi-square	1.38	0.502
	No obvious physical disabilities	14 (50)	63 (61.76)			
PACI (cases)	No obvious physical disabilities	14 (50)	39 (38.24)	R*C chi-square	1.38	0.502
	No obvious physical disabilities	14 (50)	63 (61.76)			

Comparison and analysis of percentage of social demography, vital signs before drug use, charateristics related with medical history, no obvious disabilities before drug use indicates: Compared between items, there is no significcant difference (P>0.05). Note: Social demography: sex, age (years old), distribution of age bracket, height (m), weight (kg) vital signs before drug use: Temperature (°C), heart rate (times/minute), respiration (times/minute), systolic blood pressure (mmHg), diastolic blood pressure (mmHg) charateristics related with medical history: Cosure of disease (days), History of drug allergy (cases), History of drug allergy, Tretament history, OCSP clinical classification, OCSP clinical classification Percentage of no obvious disabilities before drug use: TACI, PACI (OCSP clinical classification: TACI = Total anterior circulation infarction, PACI = Part anterior circulation infarction).