Therapeutic effects of Buyang Huanwu Tang combined with RT-PA intravenous thrombolysis on stroke of Qi deficiency and blood stasis type and its impact on Keap1-Nrf2/ARE pathway antioxidant stress

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Introduction

Ischemic stroke, often referred to as acute cerebral infarction, represents a prevalent cerebrovascular disorder in clinical practice. This condition is characterized by cerebral atherosclerosis, resulting in the narrowing of vascular lumens, and thrombosis, leading to the occlusion of these lumens. Such occurrences interrupt the essential blood supply to brain tissues, causing local ischemia and hypoxia. Ischemic strokes are notorious for their high rates of disability and mortality, imposing a substantial burden on both society and healthcare resources (1). In Western medicine, standard treatments for ischemic stroke typically involve intravenous thrombolysis, anticoagulation, and strategies to enhance cerebral circulation. Alteplase (rt-PA) is a widely used intravenous thrombolytic drug in clinical practice. It effectively dissolves existing thrombi and prevents the formation of new ones, thereby facilitating the restoration of cerebral tissue blood perfusion. However, the effectiveness of rt-PA intravenous thrombolytic therapy is limited by a therapeutic time window, and outcomes tend to be less favorable when this window is exceeded (2).

Traditional Chinese medicine (TCM) categorizes ischemic stroke as a type of "stroke" and frequently attributes it to evidence of qi deficiency and blood stasis. Qi deficiency, in this context, signifies an inability to promote proper blood circulation. When blood circulation becomes compromised, blood stasis can form within the brain. This impedes the nourishment of the brain's clear orifices by qi and blood, ultimately leading to the development of the disease. The TCM approach to treatment involves reinforcing qi, stimulating blood circulation, eliminating blood stasis, and clearing the channels (3). Buyang Huanwu Tang is a renowned Chinese herbal formula employed to address blood stasis-related conditions, and its clinical efficacy is well-recognized (4). Nonetheless, there remains a need for more in-depth research on its mechanisms of action.

In light of these considerations, this study aims to investigate the therapeutic effect and underlying mechanisms of Buyang Huanwu Tang in combination with rt-PA intravenous thrombolysis for the treatment of ischemic stroke patients presenting with both qi deficiency and blood stasis. This approach promoted neurological function recovery and improved overall treatment outcomes.

Keywords:
Traditional chinese medicine, Buyang Huanwu Tang, alteplase, intravenous thrombolysis, Qi blood stasis type, ischemic stroke

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blood stasis. Our research seeks to shed light on how this TCM approach, designed to invigorate yang and restore balance, complements the conventional rt-PA treatment in enhancing outcomes for individuals with ischemic stroke in the context of qi deficiency and blood stasis. By elucidating the mechanisms at play, we aspire to contribute to a better understanding of the synergistic potential of TCM and Western medicine in the management of ischemic stroke, ultimately offering more effective therapeutic strategies for patients with this challenging condition.

Materials and Methods

Basic information on research subjects

Using a single-centre clinical randomized trial, 117 ischemic stroke patients who implemented thrombolytic therapy in the Department of Neurology of our hospital from January 2019 to December 2021 were randomly grouped, of which 58 patients were treated with rt-PA intravenous thrombolysis (control group), the other 59 patients were treated with rt-PA intravenous thrombolysis + Chinese medicine tonic yang and five soups (combined group), and the basic data of patients in the combined group and the control group were compared, and the two groups were comparable and balanced ($P > 0.05$), as shown in Table 1.

Exclusion criteria: (i) The diagnostic criteria of ischemic stroke patients referred to the criteria in the Chinese Guidelines for Diagnosis and Treatment of Acute Ischemic Stroke 2018 (5), and the diagnostic criteria of traditional Chinese medicine referred to the Guidelines for Clinical Research of New Traditional Chinese Medicines (Trial) (6), which belonged to the type of qi deficiency and blood stasis; (ii) The patients were admitted to the hospital to receive examinations and treatments within 4.5 h after onset of the disease; (iii) The patients' age ranges were 19-79 years old; (iv) Acute ischaemic stroke was diagnosed by cranial CT and MRI; (v) All patients were willing to accept the interventions related to this study; (vi) The study protocol was approved by the Medical Ethics Committee.

Inclusion criteria: (i) patients with advanced cancer pain; (ii) cerebral haemorrhage, ruptured cerebral aneurysm, cerebral infarction, etc.; (iii) HIV-infected patients; (iv) acute myocardial infarction; (v) history of drug abuse, patients with long-term use of analgesic drugs, history of psychiatric disorders, and history of Alzheimer's disease.

Methods

Patients in the control group were treated with intravenous thrombolysis with rt-PA (Boehringer Ingelheim, Germany: specification: 50 mg). The dose of rt-PA was 0.9 mg/kg. First, 10% of the drug was injected intravenously, and the remaining 90% of the drug was injected intravenously within 1 h. After that, the patients' vital signs were closely observed, and if any adverse reaction occurred, the drug was stopped immediately, and the cranial CT was reviewed. After intravenous thrombolysis, patients' vital signs were closely observed, and if adverse reactions occurred, the drug was immediately stopped, and cranial CT was repeated. 24 h later, anticoagulation, neuroprotection, improvement of circulation and other treatments were given.

Patients in the combined group were treated with rt-PA intravenous thrombolysis + Chinese medicine tonifying yang and restoring Wu Tang, after the patients' vital signs were stabilised, they were given tonifying yang and restoring Wu Tang: 120 g of Astragalus membranaceus, 6 g of Angelica sinensis, 6 g of Paoniae lactiflora, 3 g of Dilong, Rhizoma ligustici chuanxiong, safflower, peach kernel, the above medicines were administered once a day in the form of a decoction of water to extract 200 ml of juice, which was warmed up in the morning and evening and taken in the morning to take 100 ml of juice, and the patients with impaired consciousness could be given nasal feeding. Continuous treatment for 8 weeks.

Indicators collected in this study and their evaluation measures

Compare the neurological deficit score (NIHSS), blood rheological indexes (plasma viscosity, whole blood low-cut viscosity, whole blood high-cut viscosity, erythrocyte aggregation index), vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), Keap1, Nrf2, ARE, and NQO1 protein expression levels, and differences in clinical efficacy of the patients in the two groups before and after treatment.

The clinical efficacy of the patients was evaluated at 4 weeks, 8 weeks and 12 weeks of treatment, and the evaluation was graded according to the changes in NIHSS.
Fasting venous blood specimens of 10 ml were drawn from patients in both groups before treatment and after 8 weeks of treatment and were divided into three vacuum blood collection tubes. One blood specimen was tested for plasma viscosity, whole blood low-cut viscosity, whole blood high-cut viscosity, and red blood cell aggregation index by using a South990JS3 blood rheometer. One blood specimen was left at room temperature for half an hour, centrifuged at 3500 r/min for 10 min, and the serum was taken to detect vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) by enzyme-linked immunosorbent assay (ELISA) using a kit from Shanghai Zhenke Biotech Co, Ltd. (Shanghai, Cina), and the instrument was a Thermo Fisher (Waltham, MA, USA) MK3 enzyme marker. One blood specimen was anticoagulated with heparin, and peripheral blood lymphocytes were isolated within 2 h. Peripheral blood single nucleated cells were extracted, washed and resuspended in 1640 culture medium, and then lysed by adding cell lysate and then centrifuged and the supernatant was taken to obtain the total protein. Total protein was obtained by centrifugation of the supernatant. SDS-PAGE electrophoresis was carried out using a concentration gel at 80 V and a separator gel at 120 V. The cells were closed at room temperature with 5% skimmed milk sealing solution for 2 h. The specific primary antibody was added and incubated overnight at 4°C. The cells were washed and incubated with fluorescently labelled sheep's blood lymphocytes. Incubate the fluorescently labelled sheep anti-rabbit secondary antibody for 1h, wash the membrane, wash, and develop by ECL luminescence. QuantityOne software was used to calculate the ratio of the grey value of the target protein bands to the grey value of the inner bands to indicate the protein expression levels of Keap1, Nrf2, ARE and NQO1.

**Statistical analysis**

Data were analysed using the statistical software Statistical Package for Social Science (SPSS) 21.0 (IBM, Armonk, NY, USA), Keap1, Nrf2, ARE and NQO1 proteins belonged to the category of measurement data in this study, and the statistical description was described using the mean ± standard deviation (X±s), and the statistical inference method used was the independent samples t-test; comorbidities, clinical efficacy, etc. belonged to the category of count data, and the statistical description was described by rate (%), and the statistical inference method of the above count data was adopted by the x² test or Mann-WhitneyU test; the difference was considered to be statistically significant at P<0.05.

**Results**

**Comparative analysis of patients' neurological function recovery**

Before treatment, there was no significant difference in the degree of neurological deficit (NIHSS score) between the patients in the combined group and the control group (P>0.05), and after active treatment, the NIHSS scores of the combined group were lower than those of the control group at 4, 8, and 12 weeks of treatment, and the neurological recovery of the patients in the combined group was better (P<0.05); Table 2, Figure 1.

**Analysis of the improvement of blood rheology**

The blood rheological indexes of patients before and after treatment were tested and analysed, and the baseline results of plasma viscosity, whole blood low-cut viscosity, whole blood high-cut viscosity and erythrocyte aggregation index determination of the patients in the combined group and the control group were not significantly different (P>0.05), and the study group was lower than the control group. The plasma viscosity, whole blood low-cut viscosity, and whole blood high-cut viscosity levels of the two groups of patients were measured again after 8 weeks of treatment, and the study group was lower than the control group, and the improvement of blood rheology of the patients in the study group was more significant (P<0.05); Table 3, Figure 2.

**Analysis of changes in serum MMP-9, VEGF before and after treatment**

Comparison of serum MMP-9 and VEGF levels between patients in the combined group and the control group before treatment showed no significant difference (P>0.05), and after 8 weeks of treatment, the above in-

### Table 2. Comparison of changes in NIHSS scores of patients in the two groups (X±s, points).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before treatment</th>
<th>2 weeks of treatment</th>
<th>4 weeks of treatment</th>
<th>8 weeks of treatment</th>
<th>12 weeks of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>59</td>
<td>16.86±2.94</td>
<td>14.77±2.20*</td>
<td>12.86±1.85*</td>
<td>10.50±1.48*</td>
<td>8.98±1.30*</td>
</tr>
<tr>
<td>Control</td>
<td>58</td>
<td>17.40±3.11</td>
<td>15.41±2.76*</td>
<td>14.48±2.20*</td>
<td>12.71±1.73*</td>
<td>9.78±1.56*</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>-0.965</td>
<td>-1.388</td>
<td>-4.314</td>
<td>-7.429</td>
<td>-3.015</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.336</td>
<td>0.168</td>
<td>0.000</td>
<td>0.000</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Note: Comparison with this group before treatment *P<0.05.
Dexes of the two groups were reviewed again, and the combined group had a higher value of serum MMP-9 measurement than the control group, and the level of VEGF was lower than that of the control group, and the intergroup differences of the indexes were statistically significant ($P<0.05$); Table 4, Figure 3.

Comparison of Keap1-Nrf2/ARE pathway oxidative stress indicators between the two groups of patients

Before treatment, there was no statistically significant difference in the protein expression levels of Keap1, Nrf2, ARE and NQO1 between the patients in the combined group and the control group ($P>0.05$), and when re-examined after 8 weeks of treatment, the combined group showed a higher expression of serum Nrf2 and NQO1 proteins than that of the control group, and the difference between the groups of the above indicators was significant ($P<0.05$); Table 5, Figure 4.

Comparison of the clinical efficacy of the two groups of patients

After the clinical efficacy evaluation of the two groups of patients after treatment, the overall efficacy distribution of the patients in the combined group was better than that of the control group, and the difference was statistically significant ($P<0.05$); Table 6, Figure 5.
Ischemic stroke is the leading cause of disability among Chinese residents, and the Report on Nutrition and Chronic Disease Status of Chinese Residents (2020) (8) pointed out that the number of deaths due to cerebrovascular diseases accounted for 80.7% of the total number of deaths in China in 2019, with the incidence rate of ischemic stroke being about 276.75/10,000, with males being higher than females. Western medical treatment of ischaemic stroke is based on thrombolysis, anticoagulation, improvement of circulation, and nutrition of nerves. Intravenous thrombolytic therapy can rapidly promote vascular recanalisation, but its overall efficacy is unsatisfactory due to the deficiencies of narrowed time windows, haemorrhagic transformation, and ischemia-reperfusion injury (9).

According to the theory of Chinese medicine, vegetative weakness, old age and disease or prolonged illness can lead to the decline of visceral functions, the dark depletion of qi and blood, resulting in qi and blood deficiency, the inability to transport blood, and the lack of smooth blood flow, resulting in blood stasis and blood stasis within the body. Zhang’s medical passages - the blood of the door” in the cloud: "cover the gas and blood, two-dimensional attachment, the gas is not the blood, then scattered and no system, the blood is not the gas, then congealed and do not flow" (10). The use of Astragalus as the principal drug in tonifying the Yang and returning the five soups is good at tonifying the vital energy, so that when the Qi is strong, the blood will flow, and the blood stasis will be removed and the complexes will be cleared. Angelica sinensis tail as the subject drug, good at activating blood circulation, nourishing blood and zamp. Adjuvant red peony activates Blood and disperses Blood stasis, nourishes Blood and astringes Yin; chuanxiong moves Qi and activates Blood, resolves Blood stasis and relieves pain; peach kernel and safflower activates Blood and removes Blood stasis, promotes menstruation and relieves pain, which can activate Blood without injuring the body; and dilong promotes menstruation and activates collaterals, to carry out the strength of the medicine. All the drugs together play the efficacy of benefiting qi and activating blood, resolving blood stasis and opening the channels (11).

In this study, we found that the overall distribution of efficacy of patients in the combined group was better than that of the control group, and the NIHSS scores of the combined group were lower than those of the control group at 4, 8, and 12 weeks of treatment. This result suggests that Chinese medicine tonifying yang and returning five soups combined with rt-PA intravenous thrombolysis for the treatment of patients with ischaemic stroke with qi deficiency and blood stasis can promote the recovery of patients' neurological function and have better efficacy. This is due to the fact that the saponin-like components

Table 5. Comparison of Keap1-Nrf2/ARE pathway oxidative stress indexes between the two groups (±s, relative expression).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Keap1 Before treatment</th>
<th>8 weeks of treatment</th>
<th>t</th>
<th>P</th>
<th>Nrf2 Before treatment</th>
<th>8 weeks of treatment</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>59</td>
<td>1.03±0.24</td>
<td>0.33±0.09</td>
<td>20.819</td>
<td>0.000</td>
<td>0.41±0.18</td>
<td>1.33±0.34</td>
<td>-18.335</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>58</td>
<td>1.12±0.29</td>
<td>0.48±0.12</td>
<td>15.549</td>
<td>0.000</td>
<td>0.45±0.19</td>
<td>1.12±0.37</td>
<td>-12.351</td>
<td>0.000</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>-1.830</td>
<td>-7.658</td>
<td></td>
<td></td>
<td>-1.169</td>
<td>3.197</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.070</td>
<td>0.000</td>
<td></td>
<td></td>
<td>0.245</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Comparison of the clinical efficacy of patients in the two groups (n (%)).

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Basically cured</th>
<th>Significantly cured</th>
<th>Effective</th>
<th>Ineffective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td>59</td>
<td>24(40.68)</td>
<td>22(37.29)</td>
<td>11(18.64)</td>
<td>2(3.39)</td>
</tr>
<tr>
<td>Control</td>
<td>58</td>
<td>14(24.14)</td>
<td>19(32.76)</td>
<td>20(34.48)</td>
<td>5(8.62)</td>
</tr>
<tr>
<td>Z</td>
<td></td>
<td>-2.534</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. Comparison of the clinical efficacy of patients in the two groups.
contained in Astragalus in tonifying yang and returning five soups can improve the activity of superoxide dismu-
tase and scavenge oxygen free radicals. Reduce seconda-
ry oxidative stress (12). The volatile oil components and
ferulic acid monomer contained in Angelica sinensis can
inhibit platelet aggregation, reduce erythrocyte adhesion
activity, and reduce the release of thromboxane to dilate
blood vessels and improve local microcirculation (13). Chuanxiong xiongeline contained in Rhizoma Ligustici
Chuanxiong has pharmacological effects such as reducing
calcium overload, scavenging oxygen free radicals, and
antiplatelet aggregation (14). The earthworm hormone
contained in Dillun can inhibit platelet aggregation and
thrombosis, and dissolve formed thrombus, and have phar-
caceutical effects such as anti-inflammatory, lipid regu-
lation, and enhancement of immunity (15).

Blood flow retardation is an important risk factor for
thrombus formation, which is conducive to platelet adhe-
sion and aggregation, causing local microcirculation obst-
tacles and aggravating ischemic and hypoxic injury of
brain tissue (16). In this study, it was found that when re-
examined after 8 weeks of treatment, the plasma viscosity,
whole blood low-cut viscosity and whole blood high-cut
viscosity of the combined group were lower than those of
the control group. This result suggests that Chinese me-
dicine tonifying yang and returning five soups combined
with rt-PA intravenous thrombolysis can better improve
blood rheology in the treatment of patients with ischaemic
stroke with qi deficiency and blood stasis. This is related
to the pharmacological effects of blood-stasis-activating
herbs such as Angelica sinensis tail, radix peony, Rhizoma
ligustici chuanxiong, peach kernel, safflower, and dirong,
which diaphragm blood stasis, diaphragm blood stasis-ac-
tivating herbs, diaphragm blood stasis-activating herbs,
diaphragm blood stasis-activating herbs, diaphragm blood
stasis-activating herbs, etc.

VEGF can promote vascular neo-vascularisation, and
after the occurrence of ischemic stroke, local brain tissue
undergoes ischemic and hypoxic injury, which stimulates
the high expression of VEGF, which promotes vascular
neo-vascularisation and rescues ischemic hemidiasphragm
cells after binding specifically to the receptor (17). MMP-9
degrades matrix proteins, which leads to the destabilisa-
tion, rupture, and dislodgement of atherosclerotic plaques,
which aggravates cerebral tissue ischemia (18). Cerebral
ischemia-reperfusion injury is a multi-linked and multi-
response pathological process, which is associated with
free radical damage, oxidative stress, calcium overload,
apoptosis, etc. (19). The Keap1-Nrf2/ARE signalling path-
way can exert anti-oxidative stress damage, anti-apopto-
sis and anti-inflammatory effects by activating the expres-
sion of downstream factors, which has an important role
in the pathological course of ischemic stroke (20). Nrf2
can synergistically induce the expression of phase II de-
toxification enzymes, encoding antioxidant proteins, with
antioxidant response element ARE, to protect cellular de-
fences. NQO1 regulates antioxidant enzyme activity and
attenuates oxidative stress damage (21). In this study, we
found that the serum MMP-9 assay, Nrf2, and NQO1 pro-
tein expression in the combined group was higher than that
in the control group after treatment, and the VEGF level
was lower than that in the control group. This result sug-
gests that Chinese medicine tonifying yang and restoring
five soups combined with rt-PA intravenous thrombolysis
in the treatment of patients with ischaemic stroke with qi
deficiency and blood stasis can effectively regulate the
degree of brain damage induced by oxidative stress in the
Keap1-Nrf2/ARE pathway. This is an important mecha-
nism for its improvement of neurological function, which
is related to the antioxidant effects of astragalus polysac-
charide and saponin components.

In conclusion, the treatment of qi deficiency and blood
stasis type ischemic stroke patients with intravenous
thrombolysis of rt-PA combined with tonifying yang and
restoring five soups of traditional Chinese medicine can
effectively regulate the degree of cerebral damage caused
by oxidative stress reaction of the Keap1-Nrf2/ARE path-
way, promote the recovery of patients' neurological
function, and enhance the therapeutic effect.

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nous thrombolysis through the Keap1-Nrf2/ARE signal
pathway (Project No.: 2022ZB165)

Conflicts of interest
The authors declared no conflict of interest.

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