

doi:10.3969/j.issn.1673-5374.2013.34.005 [http://www.nrronline.org; http://www.sjzsyj.org]

Zhou DS, Li M, Hu H, Chen Y, Yang Y, Zhong J, Liu LJ. *Huoxue Rongluo Tablet reduces matrix metalloproteinase-9 expression in infarcted brain tissue. Neural Regen Res. 2013;8(34):3216-3224.*

Huoxue Rongluo Tablet reduces matrix metalloproteinase-9 expression in infarcted brain tissue

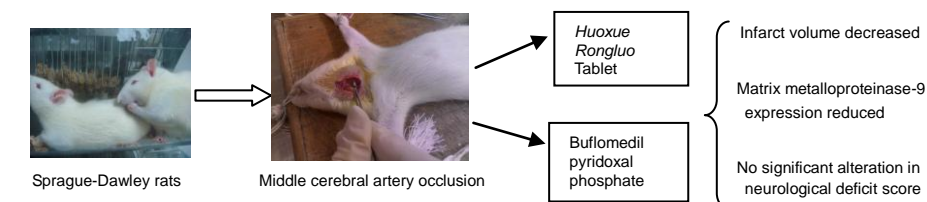
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Graphical Abstract



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Abstract

Huoxue Rongluo Tablet was made of tall gastrodis tuber, dahurian angelica root, honeysuckle stem, grassleaf sweetflag rhizome, common flowering quince fruit, figwort root, red peony root and peach seed at a ratio of 3:2:6:2:3:3:3:3. *Huoxue Rongluo Tablet* is a well-established and common prescription for the treatment of cerebral infarction. In this study, a rat model of cerebral ischemia was established and the animals were intragastrically administered *Huoxue Rongluo Tablet*. This treatment reduced infarct volume, decreased matrix metalloproteinase-9 expression, and improved neurological function. Moreover, the effects of *Huoxue Rongluo Tablet* were better than those of buflomedil pyridoxal phosphate. These results indicate that *Huoxue Rongluo Tablet* is effective in treating cerebral infarction by regulating matrix metalloproteinase-9 protein expression.

Key Words

neural regeneration; traditional Chinese medicine; *Huoxue Rongluo Tablet*; cerebral infarction; neuroprotection; matrix metalloproteinase-9; buflomedil pyridoxal phosphate; grants-supported paper; neuroregeneration

Received: 2013-05-21

Accepted: 2013-11-15

(N201303015)

Funding: This study was supported by the Scientific Research Project of Traditional Chinese Medicine of Hunan Province Chinese Medicine Research Administration Bureau in China, No. 201136.

Author contributions: Zhou DS obtained the funding, participated in study concept and design, provided technical data, was in charge of manuscript authorization and served as principle investigator. Li M and Liu LJ performed the experiments. Hu H provided advice on techniques on data collection. Li M, Hu H, Chen Y, Yang Y and Zhong J participated in data and statistical analyses. All authors approved the final version of the paper.

Conflicts of interest: None declared.

Ethical approval: This study was approved by the Animal Ethics Committee, the First Hospital of Hunan University of Chinese Medicine, China.

Author statements: The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

INTRODUCTION

Matrix metalloproteinases are involved in the pathophysiological process of cerebral infarction. Abnormal expression of matrix metalloproteinase-2 and matrix metalloproteinase-9 induces brain injury and damage to the blood-brain barrier. Local increases in MMP-9 expression in the infarct and hemorrhagic regions is strongly associated with altered blood-brain barrier permeability^[1], poor neurological function, increase in infarct area, and hemorrhagic transformation^[2-3]. The underlying mechanisms may include release of cerebral ischemia-induced cytokines and inflammatory mediators, which elicit an increase in interleukin- β and tumor necrosis factor- α levels, and the activation of mitogen-activated protein kinase and nuclear factor- κ B signaling. These factors, in turn, enhance matrix metalloproteinase-9 mRNA and protein expression. Numerous studies have demonstrated that matrix metalloproteinase-9 is downstream of the toll-like receptor 4-nuclear factor- κ B transduction pathway, and produces cerebral edema and blood-brain barrier damage, which directly results in brain injury^[4-8]. Infiltration of peripheral blood neutrophils highly expressing matrix metalloproteinase-9 into ischemic tissue is associated with basement membrane degradation and impaired blood-brain barrier integrity. Furthermore, matrix metalloproteinase-9 plays an important role in the occurrence, development and hemorrhagic transformation of cerebral infarction^[9-10]. Another study showed that matrix metalloproteinase-9 expression is strongly associated with stability of the atherosclerotic plaque, and that expression of the protease is increased in plaques that are easily disrupted^[11]. Liu *et al*^[12] demonstrated that reduced matrix metalloproteinase-9 expression and degradation of laminin and fibronectin is likely involved in the neuroprotection conferred by ischemic postconditioning on cerebral ischemia/reperfusion injury. Matrix metalloproteinase-9 can degrade and remodel the extracellular matrix^[13]. Matrix metalloproteinase-9 expression is strongly associated with instability of the plaque and the

clinical manifestation of atherosclerosis^[13]. Guyton^[14] showed that matrix metalloproteinase-9 degrades matrix, promotes smooth muscle cell migration and proliferation, activates heparinase, and contributes to the transformation of smooth muscle cells from a contractile to a synthetic phenotype during atherosclerotic plaque formation. Matrix metalloproteinase-9 exerts a profound effect by interfering with the normal structure and function of the extracellular matrix^[14]. Matrix metalloproteinase inhibitors play an important role in the treatment of stroke and in the repair of brain tissue, and siRNA liposomes that inhibit matrix metalloproteinase-9 expression can be used to treat cerebral ischemia^[5, 15-17]. In normal brain tissues, matrix metalloproteinase-9 is in a dynamic balance with matrix metalloproteinase inhibitor, which is a natural enzyme inhibitor that can suppress matrix metalloproteinase-9 expression. If the balance is perturbed, matrix metalloproteinase-9 expression increases, which disrupts tight junctions among capillary endothelial cells, a rapid degradation of perlecan, a large alteration in microvascular integrity, a dissociation of other components in the microvascular matrix, and various other pathological processes, ultimately resulting in cerebral edema^[18-19]. Cerebral infarction and hemorrhagic transformation could be earlier diagnosed by measuring matrix metalloproteinase-9 levels, which would help determine prognosis and guide effective therapy^[4].

Reducing matrix metalloproteinase-9 content, function and activity may be a promising new therapeutic strategy for cerebral infarction. Perivascular neutrophil infiltration is a key source of matrix metalloproteinase-9^[9]. Hao *et al*^[20] found that matrix metalloproteinase-9 expression decreases and brain tissue injury is alleviated in the ischemic penumbra after progesterone treatment. Li *et al*^[21] demonstrated that astragaloside A is strongly associated with blood-brain barrier permeability, and that the anti-edema effect of astragaloside A is possibly associated with matrix metalloproteinase-9 regulation. Lan *et al*^[22] suggested

that *Xiao-Xu-Ming* Decoction alleviates blood-brain barrier damage and ischemic brain injury possibly by inhibiting matrix metalloproteinase-9, matrix metalloproteinase-2 and vascular endothelial growth factor expression. Cojocarui *et al* [10] showed that matrix metalloproteinase-9 activity is related to early acute ischemic stroke, and that high matrix metalloproteinase-9 expression contributes to the inflammatory process in acute stroke. Therefore, matrix metalloproteinase-9 and neutrophils may serve as new therapeutic targets for cerebral infarction after ischemic stroke.

Huoxue Rongluo Tablet, an established prescription, was made of tall gastrodis tuber, dahurian angelica root, honeysuckle stem, grassleaf sweetflag rhizome, common flowering quince fruit, figwort root, red peony root and peach seed at a proportion of 3:2:6:2:3:3:3:3. *Huoxue Rongluo* Tablet exhibits a therapeutic effect in the treatment of cerebral infarction in the clinic [23] and in the treatment of vascular dementia [24]. Modern pharmacological studies revealed that *Huoxue Rongluo* Tablet alters blood flow and suppresses blood coagulation [25]. Buflomedil pyridoxal phosphate is a salt of pyridoxal phosphate and buflomedil. Pyridoxal phosphate is the active form of vitamin B6, and can decrease homocysteine levels. Buflomedil is a vasoactive drug used to treat the symptoms of peripheral arterial disease, and can suppress vascular alpha receptor activity, inhibit platelet aggregation, improve erythrocyte deformability, reduce oxygen consumption, and improve hemodynamics in ischemic peripheral or cerebrovascular tissues [26]. A clinical study demonstrated that the effectiveness of buflomedil pyridoxal phosphate was significant in patients with insufficient blood supply, and that it improved prognosis [27].

In this study, we used buflomedil pyridoxal phosphate as a positive control. We evaluated neurological score and matrix metalloproteinase-9 protein expression, and investigated the effect of *Huoxue Rongluo* Tablet on cerebral infarction.

RESULTS

Quantitative analysis of experimental animals

A total of 192 rats were included in this study. Of these, 48 rats served as sham surgery group, and 144 rats were used for the model of cerebral ischemia. The 144 rats subjected to cerebral ischemia were equally and randomly divided into model group, *Huoxue Rongluo* Tablet group and buflomedil pyridoxal phosphate group. The rats in the *Huoxue Rongluo* Tablet and buflomedil

pyridoxal phosphate groups were respectively administered *Huoxue Rongluo* Tablet solution and buflomedil pyridoxal phosphate solution after surgery for the ischemia model. The rats in the sham surgery and model groups were intragastrically administered distilled water. Rats that did not successfully model ischemia and those that died before anesthesia were supplemented with new rats. A total of 12 rats from each group were obtained at 24 hours, and 3, 5 and 7 days after surgery. Of these, 6 rats were used for immunohistochemistry, and the remaining 6 rats were used for 2,3,5-triphenyltetrazolium chloride (TTC) staining.

The effects of *Huoxue Rongluo* Tablet on general condition of rats with cerebral infarction

In the sham surgery group, the rats had quick reflexes, good appetite, bright eyes, shiny hair, strong muscles, and increasing body weight. The general condition of rats with cerebral infarction were poor-depressed, lying down, closed eyes, slow reflexes, poor appetite, rusty hair, thin muscles, and decreasing body weight. The general conditions in the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups were similar. They had a similar mental status, unsatisfactory appetite, average reflexes, and slightly reduced body weight.

Huoxue Rongluo Tablet improved neurological function in rats with cerebral infarction

The rats in the sham surgery group did not exhibit neurological impairment. In the model group, neurological scores were significantly decreased ($P < 0.05$), and they gradually increased 3 days later. Neurological scores were significantly higher in the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups than in the model group 3 days after cerebral infarction ($P < 0.05$), and they gradually increased over time. No significant difference in neurological score was detected between the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups ($P > 0.05$; Table 1).

Huoxue Rongluo Tablet reduced infarct volume in rats with cerebral infarction

With TTC staining, no infarction was visible in the sham surgery group. In the model group, cerebral infarction was detected, and infarct volume did not noticeably change over time. Infarct volume was significantly smaller in the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups compared with the model group ($P < 0.05$). Moreover, infarct volume decreased over time. The effects of *Huoxue Rongluo* Tablet on infarct volume were more significant compared with buflomedil pyridoxal phosphate ($P < 0.01$ or $P < 0.05$; Figure 1, Table 2).

Table 1 Effects of *Huoxue Rongluo* Tablet on neurological function in rats with cerebral infarction

Group	Time after cerebral infarction			
	24 hours	3 days	5 days	7 days
Sham surgery	5	5	5	5
Model	1.50±0.84 ^a	1.33±0.52 ^a	1.67±0.51 ^a	2.17±0.75 ^a
<i>Huoxue Rongluo</i> Tablet	1.67±0.82	2.50±0.55 ^b	3.00±0.89 ^b	3.83±0.98 ^b
Buflomedil pyridoxal phosphate	1.30±0.52	2.20±0.41 ^b	2.70±0.82 ^b	3.30±0.80 ^b

Neurological function was evaluated using the Zausinger's method: 0–5 points. High scores represent good neurological function. The data are expressed as mean ± SD. Each group contained six rats at each time point. The difference among multiple groups was compared using Kruskal-Wallis test. ^a $P < 0.05$, vs. sham surgery group; ^b $P < 0.05$, vs. model group.

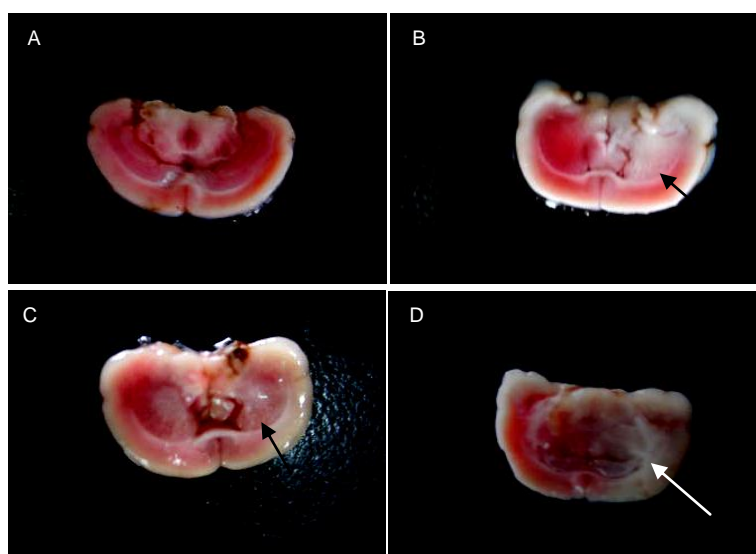


Figure 1 Infarct volume in rats at 7 days after cerebral infarction (2,3,5-triphenyltetrazolium chloride staining).

(A) Sham surgery group: No cerebral infarction. (B) Model group: Apparent cerebral infarction. (C, D) *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups: Infarct volumes are noticeably smaller. Red: Normal brain tissue; white: infarcted tissue. Infarct region is the basal ganglia (arrows).

Table 2 Effects of *Huoxue Rongluo* Tablet on infarct volume (%) in rats with cerebral infarction

Group	Time after cerebra infarction			
	24 hours	3 days	5 days	7 days
Sham surgery	0	0	0	0
Model	54.50±1.93 ^a	54.60±1.02 ^a	54.75±1.46 ^a	54.57±1.22 ^a
<i>Huoxue Rongluo</i> Tablet	54.18±1.30 ^{be}	51.08±0.88 ^{bd}	49.26±0.78 ^{be}	47.22±0.83 ^{be}
Buflomedil pyridoxal phosphate	54.05±0.87 ^c	52.77±1.03 ^c	50.46±0.86 ^c	48.60±1.17 ^c

Infarct volume (%) = infarct area/brain area on the detected layer × 100%. The data are expressed as mean ± SD. Each group contained six rats at each time point. Intergroup comparison was done using one-way analysis of variance. ^a $P < 0.05$, vs. sham surgery group; ^b $P < 0.01$, ^c $P < 0.05$, vs. model group; ^d $P < 0.01$, ^e $P < 0.05$, vs. buflomedil pyridoxal phosphate group.

***Huoxue Rongluo* Tablet diminished matrix metalloproteinase-9 expression in rats with cerebral infarction**

Immunohistochemistry demonstrated matrix metalloproteinase-9 expression in the brain tissues of rats in the sham surgery group. In the model group, matrix metalloproteinase-9 expression increased ($P < 0.05$), peaked

at 3 days, and then gradually decreased. Matrix metalloproteinase-9 expression was significantly lower in the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups than in the model group ($P < 0.01$ or $P < 0.05$). Moreover, the therapeutic efficacy of *Huoxue Rongluo* Tablet was better than that of buflomedil pyridoxal phosphate ($P < 0.05$; Figure 2, Table 3).

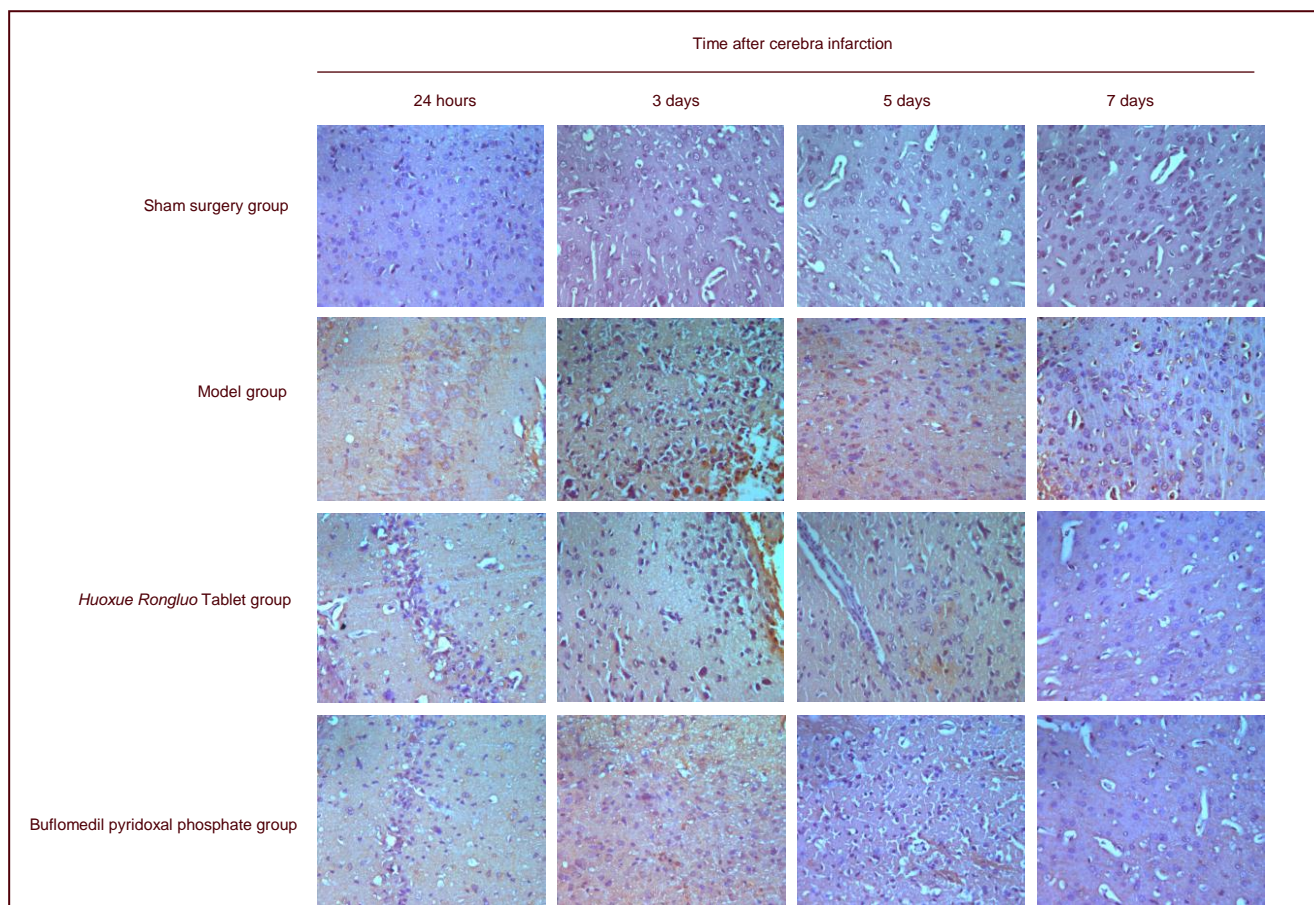


Figure 2 Matrix metalloproteinase-9 (MMP-9) expression in rats with cerebral infarction (immunohistochemical staining, $\times 400$).

MMP-9 expression was observed mainly in the cytoplasm as brown staining. In the sham surgery group, no obvious MMP-9 expression was detected at the various time points. In the model group, MMP-9 protein expression increased and peaked at 3 days. In the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups, MMP-9 protein expression increased, but it was less than in the model group.

Table 3 Effects of *Huoxue Rongluo* Tablet on matrix metalloproteinase-9 expression (mean absorbance) in rats with cerebral infarction

Group	Time after cerebra infarction			
	24 hours	3 days	5 days	7 days
Sham surgery	0.23 \pm 0.05	0.24 \pm 0.07	0.20 \pm 0.05	0.19 \pm 0.03
Model	0.46 \pm 0.06 ^a	0.57 \pm 0.07 ^a	0.44 \pm 0.10 ^a	0.41 \pm 0.06 ^a
<i>Huoxue Rongluo</i> Tablet	0.36 \pm 0.06 ^{ab}	0.44 \pm 0.08 ^{ab}	0.33 \pm 0.05 ^{ab}	0.27 \pm 0.04 ^{abd}
Buflomedil pyridoxal phosphate	0.38 \pm 0.04 ^{ac}	0.47 \pm 0.09 ^{ac}	0.36 \pm 0.06 ^{ac}	0.33 \pm 0.05 ^{ac}

The data are expressed as mean \pm SD. Each group contained six rats at each time point. Intergroup comparison was done using one-way analysis of variance. ^a $P < 0.05$, vs. sham surgery group; ^b $P < 0.01$, ^c $P < 0.05$, vs. model group; ^d $P < 0.05$, vs. buflomedil pyridoxal phosphate group.

DISCUSSION

Matrix metalloproteinase-9, a matrix metalloproteinase, degrades extracellular matrix. It plays an important role in cerebral hematoma formation and blood-brain barrier damage, and it can disrupt the interaction between cells and matrix, and induce nerve cell death and neurolog-

ical dysfunction^[28]. Tall gastrodis tuber suppresses ex-udation in early inflammation, inhibits matrix metallo-proteinase-9 activation and expression, and protects brain tissues^[25]. Dahurian angelica root decreases se-rum proinflammatory cytokine content, elevates an-ti-inflammatory cytokine levels, and regulates nuclear factor- κ B p65 activity^[29]. An increase in inflammatory factors upregulates matrix metalloproteinase-9 protein

expression. Thus, expression of the metalloproteinase can be suppressed by inhibiting inflammatory factors. Extract of peach seed increases cerebral blood flow, decreases vascular resistance, improves hemodynamics, and prolongs coagulation time. D-catechin, a component of red peony root, alters hemodynamics, and reduces whole blood viscosity and erythrocyte electrophoretic time. Simultaneously, red peony root affects calcium metabolism, regulates thromboxane A₂/prostacyclin, diminishes blood plasma lipid peroxidation, and has beneficial effects on arterial wall lipids, calcium and phospholipid metabolism, and reduces aortic plaque area. Honeysuckle stem suppresses atherosclerosis. Papaya resists blood coagulation and scavenges oxygen free radicals^[25]. Together, these components downregulate matrix metalloproteinase-9 expression and protect brain tissues.

Results of this present study show that neurological function in rats with cerebral infarction improves gradually with *Huoxue Rongluo* Tablet therapy. *Huoxue Rongluo* Tablet reduced matrix metalloproteinase-9 expression to a significantly greater extent than buflomedil pyridoxal phosphate capsule.

MATERIALS AND METHODS

Design

A randomized controlled animal study.

Time and setting

Experiments were performed in the SPF Animal Experimental Center, Hunan University of Chinese Medicine, China in August 2012.

Materials

Experimental animals

A total of 192 clean healthy male Sprague-Dawley rats aged 6–7 weeks and weighing 250 ± 20 g were provided by the Experimental Animal Center, Hunan University of Chinese Medicine, China, license No. SCXK (Xiang) 2011-0003. All rats were housed in the SPF Animal Experimental Center, Hunan University of Chinese Medicine, China at 24–26°C and a humidity of 55–60%. Each cage contained five rats. They were allowed free access to food and water. Underpadding was replaced, and the cages were cleaned regularly. Procedures using experimental animals were in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China^[30].

Drugs

Huoxue Rongluo Tablet, made of tall gastrodis tuber, dahurian angelica root, honeysuckle stem, grassleaf sweetflag rhizome, common flowering quince fruit, figwort root, red peony root and peach seed at a proportion of 3:2:6:2:3:3:3:3, was provided by the Department of Pharmaceutics, the First Hospital of Hunan University of Chinese Medicine, China (lot No. Xiangyaozhizi Z20080472). This drug was verified by chief pharmacist Liu SG from the Department of Pharmaceutics, the First Hospital of Hunan University of Chinese Medicine, China. Specification: 100 tablets/bottle, 0.35 g/tablet, 0.14 g crude drug/tablet. Before administration, the tablet was dissolved in grade three purified water, and prepared into a liquid containing 2.8 mg/mL crude drug.

Buflomedil pyridoxal phosphate capsule was provided by Kunming Jida Pharmaceutical Co., Ltd., Kunming, Yunnan Province, China, Approval No. GYZZ H20064618; specification: 0.2 g (89.2 mg pyridoxal phosphate and 110.8 mg buflomedil). Before administration, the tablet was dissolved in grade three purified water, prepared into a 2.1 mg/mL liquid, and stored in the refrigerator before use.

Methods

Establishment of a rat model of acute middle cerebral artery ischemia

The rats were raised in the laboratory for 1 week. They were fasted, but did not suffer from water deprivation before surgery. Using a modification of Longa *et al*'s method^[31], a rat model of acute middle cerebral artery ischemia was established. The rats were intraperitoneally anesthetized with 10% chloral hydrate (3 mL/kg, Changsha Lixin Biotechnology Co., Ltd., Changsha, Hunan Province, China). The head and limbs were fixed on a table in a supine position. After shaving and sterilizing, a median incision was made in the middle of the neck. Subcutaneous tissue and muscle were bluntly isolated along the inner margin of the sternocleidomastoid. The right common carotid artery, and the external and internal carotid arteries were isolated. Three threads were respectively placed at the proximal and distal parts of the common carotid artery, the crotch of the common carotid artery (external carotid artery branched) for further use. Blood vessels were isolated along the internal carotid artery in the direction of the head until the crotch of the internal carotid and pterygopalatine arteries. The pterygopalatine artery was ligated, and the external carotid artery and the proximal end of the common carotid artery were ligated. Subsequently, the internal carotid artery was clamped with a bulldog clamp. The thread for

ligating the common carotid artery at the proximal part was gently lifted, and a small cut slanting to the head was made using eye scissors. The thread was inserted into the skull from the common carotid artery bifurcation along the internal carotid artery until resistance was felt. The depth was about 18 mm (including the common carotid artery bifurcation). The thread at the distal end of the common carotid artery was tightly tied and placed under the skin to avoid removal by the rat. Penicillin powder was coated onto the skin to prevent infection. The skin at the neck was sutured and then sterilized. After the surgery, the rats were left alone under heat until consciousness was regained. At 6–8 hours after the surgery, the rats were fed. A successful model rat had an uneventful operation and no intraoperative massive hemorrhage. Neurological function was assessed with Zausinger's method^[32]. Rats with a score < 5 points were considered to have undergone successful modeling. Those with 1–4 points were included in this study. In the sham surgery group, blood vessels in the neck were isolated only.

Intragastric administration of drugs

The rats in the *Huoxue Rongluo* Tablet and buflomedil pyridoxal phosphate groups were intragastrically administered 1 mL/100 g *Huoxue Rongluo* Tablet (2.8 mg/mL) and 1 mL/100 g buflomedil pyridoxal phosphate (2.1 mg/mL), respectively. The drugs were first given 6 hours after surgery, followed by intragastric administration twice a day (interval 8 hours) until the rats were sacrificed. The rats in the sham surgery and model groups were intragastrically administered 2 mL distilled water.

Zausinger's method for evaluation of neurological function

Mental status, diet, activity and hair were observed at 24 hours, and 3, 5 and 7 days after surgery. Neurological function was assessed using Zausinger's method^[32]: 0, no spontaneous walking; 1, rotating to the opposite side of affected side during free walking; 2, getting hold of his tail, rotating to the opposite side of affected side; 3, a decrease in resistance to contralateral pressure on the affected side; 4, failure to extend fully the forepaw of the side opposite to the affected side, including flexion of the whole body towards the opposite side; 5, no neurologic impairment.

Sample collection

Six rats from each group were obtained at 24 hours, and 3, 5 and 7 days after surgery. They were sacrificed 2 hours after the final drug administration. After intraper-

itoneal anesthesia with 10% chloral hydrate (3 mL/kg), their carotid arteries were cut off, and then the brains were obtained by opening the cranial cavity. The brains were fixed in 4% paraformaldehyde for 24 hours.

TTC staining for infarct volume

After removal of the olfactory bulb, cerebellum and brain stem, brain tissues were sliced into sections. The sections were placed in 1% TTC (Shanghai Branch, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), agitated, and incubated at 37°C for 15 minutes. Normal brain tissues were stained red, but infarct tissues were white. Subsequently, the sections were photographed and analyzed using Motic Images Advanced 3.0 software (Motic China Group Co., Ltd., Xiamen, Fujian Province, China). Infarct volume (%) was calculated as infarct area/brain area of the detected layer × 100%.

Immunohistochemistry for matrix metalloproteinase-9 expression in rat brain tissues

Brain tissues were embedded in paraffin, sliced into 5- μ m-thick sections, dewaxed with xylene, rehydrated in gradient alcohol, incubated in 3% H₂O₂ at room temperature for 5–10 minutes to deactivate endogenous enzyme activity, and washed three times in distilled water. The sections were immersed in 0.01 mol/L citric acid buffer (pH 6.0) and boiled in a microwave oven. After an interval of 5–10 minutes, the procedure was repeated (twice), and the sections were cooled at room temperature. After two washes in PBS (pH 7.2–7.6), the sections were blocked in normal goat serum at room temperature for 20 minutes. The unnecessary liquid was discarded, and no wash was given. The sections were incubated in rabbit anti-rat matrix metalloproteinase-9 polyclonal antibody (1:100; Boster, Wuhan, Hubei Province, China) at 37°C for 1 hour, washed with PBS for 2 minutes (three times), and then incubated in biotinylated goat anti-rabbit IgG (Boster) at room temperature for 20 minutes. They were then washed with PBS (pH 7.2–7.6) for 2 minutes (three times) and then incubated with streptavidin-biotin complex (Boster) at room temperature for 15 minutes, and then washed with PBS for 3 minutes (three times). The sections were visualized with 3,3'-diaminobenzidine, washed with distilled water, terminated by washing in running water, counterstained with hematoxylin, dehydrated, permeabilized, and mounted. The sections were observed and analyzed using MIAS medical image analysis software (Beihang Software Development Company, Beijing, China), and average absorbance was calculated.

Statistical analysis

The data were analyzed using SPSS 16.0 software (SPSS,

Chicago, IL, USA), and expressed as mean \pm SD. Infarct volume and matrix metalloproteinase-9 protein expression obeyed normal distribution. Intergroup comparisons and intragroup comparisons were performed using one-way analysis of variance. Neurological deficit score did not follow a normal distribution. The differences among multiple groups were compared utilizing Kruskal-Wallis test. A value of $P < 0.05$ was considered statistically significant.

Research background: Increased matrix metalloproteinase-9 expression is a pathophysiological feature in cerebral infarction. Matrix metalloproteinase-9 plays an important role in the occurrence, development and hemorrhagic transformation of cerebral infarction.

Research frontiers: *Huoxue Rongluo* Tablet can effectively treat cerebral infarction and vascular dementia. Modern pharmacological studies have shown that *Huoxue Rongluo* Tablet alters blood flow and suppresses blood coagulation.

Clinical significance: This study shows that *Huoxue Rongluo* Tablet can reduce infarct volume and effectively treat cerebral infarction by regulating matrix metalloproteinase-9 protein expression.

Academic terminology: An enzyme inhibitor is a molecule which binds to enzymes and decreases their activity. Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, many drugs are enzyme inhibitors.

Peer review: This study investigated the effects of *Huoxue Rongluo* Tablet on matrix metalloproteinase-9 protein expression in a rat model of cerebral infarction, used buflomedil pyridoxal phosphate as a positive control, and showed that *Huoxue Rongluo* Tablet is effective in the treatment of cerebral infarction.

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(Reviewed by Patel B, Raye W, Yang DG, Chen X)

(Edited by Yu J, Qiu Y, Li CH, Song LP, Liu WJ, Zhao M)