


Neuroprotective effects of four different fluids on cerebral ischaemia/reperfusion injury in rats through stabilization of the blood–brain barrier

Reai Shan^{1,2,3} | Hongyan Zhou⁴ | Xinfang Liu² | Guangjun Su^{2,5} |
Guangsen Liu^{1,2,5} | Xiaoli Zhang^{1,2,5} | Cong Sun^{1,2,5} | Zining Yu^{2,5} |
Lifang Zhan^{1,2} | Zhihua Huang^{2,3,6} 

¹Department of Anesthesiology, First Affiliated Hospital of Gannan Medical University, Ganzhou, China

²Key Laboratory of Prevention and treatment of cardiovascular and cerebrovascular diseases of Ministry of Education, Gannan Medical University, Ganzhou, China

³Institute of Pain Medicine, Gannan Medical University, Ganzhou, China

⁴Department of Anesthesiology, Chongqing Hospital of Traditional Chinese Medicine, Chongqing, China

⁵Graduate School, Gannan Medical University, Ganzhou, China

⁶Department of Physiology, Gannan Medical University, Ganzhou, China

Correspondence

Zhihua Huang, Department of Physiology, Gannan Medical University, Ganzhou, China.
Email: zh.huang@gmu.edu.cn

Abstract

Protecting the blood–brain barrier (BBB) is a potential strategy to treat cerebral ischaemic injury. We previously reported that hypertonic sodium chloride hydroxyethyl starch 40 (HSH) treatment alleviates brain injury induced by transient middle cerebral artery occlusion (tMCAO). However, other fluids, including 20% mannitol (MN), 3% hypertonic sodium chloride (HTS) and hydroxyethyl starch 130/0.4 solution (HES), have the same effect as HSH in cerebral ischaemia/reperfusion injury (CI/RI) remains unclear. The present study evaluated the protective effects of these four fluids on the BBB in tMCAO rats. Sprague–Dawley (SD) rats were randomly assigned to six groups. A CI/RI rat model was established by tMCAO for 120 min followed by 24 h of reperfusion. The sham and tMCAO groups were treated with normal saline (NS), whereas the other four groups were treated with the four fluids. After 24 h of reperfusion, neurological function, brain oedema, brain infarction volume, permeability of the BBB, cortical neuron loss and protein and mRNA expression were assessed. The four fluids (especially HSH) alleviated neurological deficits and decreased the infarction volume, brain oedema, BBB permeability and cortical neuron loss induced by tMCAO. The expression levels of

Abbreviations: AHF, acute heart failure; ANOVA, analysis of variance; AQP4, aquaporin 4; BBB, blood–brain barrier; CI/RI, cerebral ischaemia/reperfusion injury; CNS, central nervous system; CPR, cardiopulmonary resuscitation; EB, Evans Blue; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclear antigen; HES, hydroxyethyl starch; HHS, hypertonic sodium chloride hydroxyethyl starch; HTS, hypertonic saline; IL-1 β , interleukin-1 β ; IL-6, interleukin-6; ICAM-1, intercellular adhesion molecule 1; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinase; MMPs, matrix metalloproteinases; MN, mannitol; NF- κ B, nuclear factor kappa B; NO, nitric oxide; NS, normal saline; PBS, phosphate-buffered saline; PDGFR- β , platelet-derived growth factor receptor β ; RGS5, regulator of G-protein signalling 5; RNS, reactive nitrogen species; ROS, reactive oxygen species; TLRs, Toll-like receptors; tMCAO, transient middle cerebral artery occlusion; TNF- α , tumour necrosis factor α ; TTC, 2,3,5-triphenyltetrazolium chloride.

Reai Shan and Hongyan Zhou contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *European Journal of Neuroscience* published by Federation of European Neuroscience Societies and John Wiley & Sons Ltd.

Funding information

Innovation Team Foundation of Gannan Medical University, Grant/Award Number: TD201705; Natural Science Foundation of Jiangxi Province, China, Grant/Award Number: 20142BAB205020; Science and Technology Foundation of the Bureau of Education of Jiangxi Province, China, Grant/Award Number: GJJ12548

Edited by: Ying-Shing Chan

GFAP, IL-1 β , TNF- α , MMP-9, MMP-3, AQP4, PDGFR- β and RGS5 were decreased, whereas the expression levels of laminin and claudin-5 were increased. These data suggested that small-volume reperfusion using HSH, HES, MN and HTS ameliorated CI/RI, probably by attenuating BBB disruption and postischaemic inflammation, with HSH exerting the strongest neuroprotective effect.

KEYWORDS

blood–brain barrier, cerebral ischaemia/reperfusion, fluid infusion, neuroprotection

1 | INTRODUCTION

Cerebral ischaemia/reperfusion injury (CI/RI), which is a major cause of death and long-lasting disability worldwide, is a pathological condition characterized by cerebral ischaemia and brain cell injury, and after reperfusion, the injury is further aggravated (Al-Mufti et al., 2018). It has been widely demonstrated that excitotoxicity, ionic imbalance, adhesion molecule expression upregulation, reactive oxygen species (ROS) and reactive nitrogen species (RNS) formation, inflammation and apoptosis are the main mechanisms involved in the pathophysiological process of CI/RI (Chen et al., 2017). All of these events contribute to blood–brain barrier (BBB) breakdown, which is considered a critical step in cerebral ischaemia pathogenesis. CI/RI occurs not only in a variety of cerebrovascular diseases but also during other acute events, such as cardiopulmonary resuscitation (CPR), acute heart failure (AHF), haemorrhagic shock, hypotension, metabolic disorders and cardiac arrest, particularly in elderly or critically ill patients. In addition, once neurons are injured or dead, it can be challenging to regenerate them. Therefore, strategies to alleviate or prevent CI/R-induced brain damage are urgently needed.

Clinically, reducing intracranial pressure and controlling cerebral oedema are the primary strategies for treating cerebrovascular disease, and the most effective treatment is osmotic therapy (Cook et al., 2020), which is the main treatment used to mitigate the development of cerebral oedema, decrease intracranial pressure and prevent the formation of cerebral hernias. In vivo experiments have shown that when the brain water content decreases by 1.7%, the brain tissue volume decreases by approximately 90 ml (Carney et al., 2017). Currently, the osmotic agents used for osmotic therapy include mannitol (MN), glycerol fructose and albumin hypertonic saline (HTS) (Deng et al., 2016).

Although many studies on the effect of MN and HTS liquid crystal infiltration on ischaemic brain oedema have been performed, there are relatively few reports about colloids. Many studies have shown that plasma colloid administration causes the osmotic pressure to decrease by 1 mmHg and the intracranial pressure to increase by 0.37 mmHg (Farasatinasab et al., 2018; Park et al., 2018). Our previous studies revealed that hypertonic sodium chloride hydroxyethyl starch 40 (HSH) has neuroprotective effects against CI/RI in vivo and the mechanism may be related to its antioxidative effects, such as regulation of nitric oxide (NO) levels, inhibition of inducible nitric oxide synthase (iNOS) activity and improvement of cerebral energy metabolism (Li et al., 2014). In the present study, we compared the neuroprotective effects of four fluids, that is, MN, 3% hypertonic HTS, hydroxyethyl starch 130/0.4 (HES) and hypertonic sodium chloride hydroxyethyl starch (HSH), against CI/RI and examined the potential mechanism by which they maintain the integrity of the BBB.

2 | MATERIALS AND METHODS

2.1 | Animals

Male Sprague–Dawley rats (6–8 weeks old, 250–300 g) were purchased from the Experimental Animal Center of Gannan Medical University (institutional licence no. SCXK (Gan) 2014-0001) and were housed with free access to food and water at a constant temperature of $22 \pm 2^\circ\text{C}$ and a humidity of $55 \pm 5\%$ on a 12-h light/dark cycle. All experimental protocols were approved by the Ethics Committee of Animal Experiments of Gannan Medical College. All procedures were approved by the Institutional Animal Care and Use Committees of Gannan Medical University and followed the guidelines

of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 | Materials

The experimental solutions included 20% MN (Jiangxi Kelun Pharmaceutical Co., Ltd., Jiangxi, China), 3% HTS (prepared from 10% HTS) and HES (Fresenius Kabi Pharmaceutical Co., Ltd., Bad Homburg, Germany) and hypertonic saline–hydroxyethyl starch (4.2% HTS + 7.6% HHS), which was purchased from Fuji Pharmaceutical Co., Ltd. (Shanghai, China). The following primary antibodies were obtained from Abcam (United States): anti-beta actin (ab8226), anti-RGS5 (ab172132), anti-PDGFR- β (ab32570), anti-MMP9 (ab38898), anti-GFAP (ab68428), anti-laminin (ab11575) and anti-NeuN (ab104224). An anti-claudin-5 antibody (sc-28670) was obtained from Santa Cruz (United States). An Alexa Fluor 488-conjugated mouse secondary antibody (#A11029), enhanced chemiluminescence reagent (ECL) western blotting detection reagents (#32106), a Reverse Transcription Master Mix Kit (#4374966) and SYBR Select Master Mix (#4472908) were purchased from Thermo Fisher Scientific (United States). 2,3,5-Triphenyl tetrazolium chloride (TTC, #T8877-25G) was purchased from Sigma (United States).

2.3 | Experimental protocol

The rats were randomly assigned to the following six groups (eight rats per group): the sham group, transient middle cerebral artery occlusion and reperfusion (tMCAO) group (tMCAO+4 ml/kg normal saline), MN group (tMCAO+4 ml/kg 20% MN), HTS group (tMCAO +4 ml/kg 3% HTS), HES group (tMCAO+4 ml/kg HES) and HSH group (tMCAO+4 ml/kg HSH). Focal cerebral ischaemia was induced by tMCAO using a nylon filament as described in previous studies (Hao et al., 2020; Li et al., 2017; Longa et al., 1989; Shu et al., 2020). Briefly, each rat was anaesthetized with 1% pentobarbital sodium (50 mg/kg, i.p.), and a midline incision was made on the neck from Vertebrae 3 to 10, followed by blunt dissection of the subcutaneous tissue and muscle. The common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were isolated. A 5-cm nylon filament (ϕ 0.26–0.28 mm, Xinongkeji, China) was inserted into the ICA via the ECA stump, and the suture was gently advanced to block the middle cerebral artery (MCA) blood flow for 120 min. Afterwards, the nylon filament was carefully removed to allow blood flow to return to the ischaemic artery. Finally, the incision was

closed with stitches. Sham-operated rats were subjected to the same surgical procedures without occlusion of the MCA. In the sham and tMCAO groups, intravenous infusion of 0.9% NS with a constant speed pump was conducted for 30 min after ischaemic insult for 120 min. The other four groups were intravenously infused with the corresponding liquid for 30 min.

2.4 | Neurobehavioural evaluation

All rats underwent assessment of neurobiological function according to the Longa and Garcia methods at 24 h after tMCAO (Garcia et al., 1995; Longa et al., 1989). Longa scores were assigned according to a 5-point scale as follows: *no neurological deficit* = 0, *failure to fully extend the left forepaw* = 1, *circling to the left when walking* = 2, *falling to the left when walking* = 3 and *failure to walk spontaneously with a decreased level of consciousness* = 4. All rats were assessed according to the Garcia method at 24 h after cerebral ischaemia reperfusion (I/R) to evaluate their neurobiological function. The evaluation includes tests of the following six parameters scored on a scale from 3 to 18: spontaneous activity, symmetry in movement of the four limbs, forepaw outstretching, climbing, body proprioception and response to vibrissae touch. Each test was repeated three times, and the average score is presented as the evaluation score. The evaluation was conducted in a blinded manner.

2.5 | Assessment of brain oedema

Brain oedema was analysed using the wet/dry method. After neurological assessment, the rats were anaesthetized with 1% pentobarbital sodium (50 mg/kg, i.p.) and sacrificed. Their brains were collected and weighed immediately (wet weight). After 72 h of drying at 70°C, the brains were weighed again (dry weight). The degree of brain oedema was calculated as follows: [(wet weight – dry weight)/wet weight] \times 100%.

2.6 | Evaluation of cerebral infarction

After neurological assessment, the rats were anaesthetized with 1% pentobarbital sodium (50 mg/kg, i.p.) and sacrificed. The brains were placed in a dish containing phosphate-buffered saline (PBS), and the meninges were isolated and cut into five coronal sections with a thickness of 2 mm. The brain sections were immersed in 0.5% TTC solution for 15 min at 37°C and then fixed with

4% paraformaldehyde solution. The brain tissues were analysed according to colour as follows: White indicated the infarct area and red indicated the non-infarcted area. ImageJ software was used to calculate the cerebral infarct area. The infarct areas in each section were summed and multiplied by the section thickness to calculate the infarction volume.

2.7 | Measurement of BBB permeability

The rats were injected with 2% Evans Blue (EB; E2129, Sigma) solution (3 ml/kg) via the femoral vein 30 min before death and then anaesthetized with 1% pentobarbital sodium. The heart was perfused with 0.9% normal saline (200–300 ml) to flush the EB dye and then reperfused with 200 ml 4% PFA. The whole brain was taken and placed on filter paper. After the whole brain was blotted dry, it was placed on black paper and photographed with a small animal imaging system (IVIS Lumina Series III, PerkinElmer, USA). The emission wavelength was 540 nm, the absorption wavelength was 620 nm, and the fluorescence intensity was calculated.

2.8 | Immunofluorescence staining

The rats were anaesthetized intraperitoneally as described previously and transcardially perfused with heparinized saline, followed by transcardial perfusion with 4% paraformaldehyde (Sigma, China). After perfusion, the rats were decapitated, and the brain tissue was removed from the skull, embedded in OCT medium (Leica, Germany) and stored at -80°C . Coronal sections (8 μm) were obtained from the frozen brain tissue for immunohistochemical staining. The sections were covered with 5% BSA (Sigma) for 30 min at 37°C . The sections were then incubated with primary antibodies against NeuN (1:500) overnight at 4°C . The sections were washed with PBS three times and then incubated with biotinylated anti-goat IgG (1:1000, A-11029, Invitrogen) for 2 h at 37°C in the dark. DAPI (P36971, Invitrogen) was used to stain the nuclei. Finally, fluorescence was observed by using a fluorescence microscope (Olympus). Six sections were selected from each group, and three photos were taken of each section. Using ImageJ software (National Institutes of Health, Bethesda, MD, USA), the number of NeuN-positive and DAPI-positive cells in the entire photo was counted, and the NeuN-positive cell rate was calculated and is expressed as the percentage of DAPI-positive cells.

2.9 | Western blot analysis

Ischaemic penumbra tissues were collected after 24 h of perfusion for western blotting. The brain tissue samples were mechanically homogenized in protein lysis buffer (Beyotime, China). The lysates were centrifuged at $12,000\times g$ for 10 min at 4°C . The protein concentration was determined using a BCA Protein Assay Kit (Beyotime, China). The samples (50 μg per lane) were separated by 10% SDS-PAGE and electrotransferred onto a nitrocellulose membrane. The non-specific proteins on the membrane were blocked with 5% skimmed milk for 1 h at room temperature and incubated overnight at 4°C with primary antibodies against GFAP (1:1000), MMP-9 (1:300), RGS5 (1:300), laminin (1:300) and PDGFR- β (1:300). After washing three times for 5 min, the membrane was incubated with secondary antibodies (1:5000) for 1 h at 37°C . The bands were visualized by incubation with the chemiluminescence reagents provided in the ECL kit for 1 min. Finally, the protein bands were imaged, and the grey value of each band was analysed using a chemiluminescence imaging system (Bio-Rad, Hercules, CA, USA). The grey value of the target band relative to that of the β -actin band was calculated, and then, the value was normalized to that of the sham group.

2.10 | Quantitative polymerase chain reaction analysis

Total RNA was isolated from the penumbra of the cerebral cortex using TRIzol reagent according to the manufacturer's instructions. Four micrograms of total RNA was used to synthesize first strand cDNA using the Reverse Transcription Master Mix Kit and SYBR[®] Select Master Mix. Then, polymerase chain reaction (PCR) was performed using the following reaction system: 2 μl cDNA, 10 μl $1\times$ SYBR Select Master Mix, 2 μl 10 μM primer and ddH₂O to a total volume of 20 μl . The parameters for PCR were as follows: pre-denaturation at 95°C for 20 min followed by 40 cycles (denaturation at 95°C for 10 s, annealing at 61°C for 20 s and extension at 72°C for 25 s). Relative mRNA levels were calculated by the $2^{-\Delta\Delta\text{Ct}}$ method and were normalized to β -actin mRNA levels. The primers for PCR were purchased from Sangon Biotech, Co., Ltd., Shanghai, China. The sequences were as follows: GFAP, sense: 5'-GCAAGAAACAGAAGAGTGGTATCG-3', antisense: 5'-CTCTCCAAGGACTCGTTCGTG-3'; IL-1 β , sense: 5'-CACCTCAATGGACAGAACATAAGC-3', antisense: 5'-TCTTCTTTGGGTATTGTTTGGGA-3'; TNF- α , sense: 5'-CCACCACGCTTCTGTCTACTG-3', antisense:

5'-GGGCTACGGGCTTGTCACTC-3'; laminin, sense: 5'-TGGCTTCACAGGCTGCTCA-3', antisense: 5'-GGTCGCTCACCTTCATTTTCG-3'; MMP-9, sense: 5'-GGGGCTAGGCTCAGAGGTAAC-3', antisense: 5'-TCACCCGGTTGTGGAAACTC-3'; MMP-3, sense: 5'-GCAGGTTATCCTAAAAGCATTAC-3', antisense: 5'-TGAAGATCCGCTGAAGAAGTAAAG-3'; AQP4, sense: 5'-TGAATCCAGCTCGATCCTTTG-3', antisense: 5'-CAATGTCGATCATGCACCA-3'; RGS5, sense: 5'-GCCAATCCCTGGACAACTTC-3', antisense: 5'-ACAGGCAACCCAGAACTCAAG-3'; PDGFR- β , sense: 5'-CCTGCAAGTGTGAGACAGTAGTGA-3', antisense: 5'-CATGGGTGTGCTTAAACTTTTCG-3'; and β -actin, sense: 5'-TGTGACGTTGACATCCGTAAAGAC-3', antisense: 5'-GGACTCATCGTACTCTGCTTG-3'.

2.11 | Statistical analysis

All data are presented as the mean \pm SEM. Statistical analysis was performed using GraphPad Prism 5.0 software. Differences between groups were compared by one-way ANOVA followed by the Newman-Keuls post hoc test. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ were considered to indicate statistical significance.

3 | RESULTS

3.1 | Small-volume liquid reperfusion ameliorated brain injury and improved neurological function in rats subjected to ischaemic brain injury

Transient MCAO was used to induce severe cerebral ischaemia injury and neurobehavioural dysfunction (Figure 1). The Longa score (Figure 1a) and infarct size (Figure 1c,d) were significantly increased, and the Garcia JH scale score (Figure 1b) was increased in the tMCAO (NS) group compared with the sham group ($P < 0.05$); however, the Longa score and infarct size were significantly decreased, and the Garcia JH scale score was increased in the groups treated with the four fluids compared with the tMCAO (NS) group ($P < 0.05$). Compared with that in the MN group and HES group, the infarct size in the HSH group was significantly smaller ($P < 0.001$); additionally, the infarct size in the HTS group was smaller than that in the MN group and HES group, but the difference did not reach significance. These results showed that all four fluids induced neuroprotection against CI/RI induced by tMCAO in rats and that HSH might have been the most effective.

3.2 | Small-volume liquid reperfusion reduces brain oedema and BBB permeability

When the BBB is disrupted, EB-labelled albumin extravasates into brain tissues from the vasculature, and EB extravasation can be measured to quantify BBB permeability. EB signals (Figure 2a), fluorescence intensity (Figure 2b) and brain water content (Figure 2c) were increased in the tMCAO (NS) group compared with the sham group ($P < 0.05$). Compared with those in the tMCAO (NS) group, these indicators in the groups treated with the four fluids were significantly lower ($P < 0.05$). The differences in BBB permeability among the groups treated with the four fluids were not statistically significant, but HSH was the most effective in reducing brain oedema ($P < 0.001$ vs. the MN and HES groups).

3.3 | Small-volume liquid reperfusion increased the NeuN expression of protein in brain tissue

NeuN is expressed in the nuclei of neurons and is often used to evaluate the number of neurons. Figure 3 shows that the fluorescence intensity (Figure 3a,b) and the number of NeuN-positive cells (Figure 3a,c) in rat brain tissue were decreased in the tMCAO group compared with the sham group ($P < 0.05$). Compared with those in the tMCAO (NS) group, these indicators in the groups treated with the four fluids were significantly increased ($P < 0.05$).

3.4 | Small-volume liquid reperfusion increased laminin and claudin-5 expression levels and decreased matrix metalloproteinase expression levels

MMP-3 and MMP-9 are two matrix metalloproteinases (MMPs) that can digest vascular basement membrane components. Laminin is a special non-collagen glycoprotein of the basement membrane that comprises the basement membrane structure of the BBB. Claudin-5 is an important molecule in gap junctions. Therefore, the protein and mRNA expression levels of these molecules reflect the structure of the BBB. In this study, the expression levels of laminin and claudin-5 were significantly lower in the tMCAO group than in the sham group (Figure 4a,d,f); in contrast, the expression levels of MMP-3 (Figure 4c) and MMP-9 (Figure 4b,e) were markedly higher, showing that the BBB was

FIGURE 1 The effect of the four different liquids on brain infarction and neurological scores. Transient MCAO rats were treated with HSH, HES, MN, HTS or equivalent NS 30 min before reperfusion. 24 h following reperfusion, the brain infarcted volume, neurological function scores and brain water content were evaluated. (a,b) The results of neurological function scores. (c) The representative images of TTC staining for infarcted brain. (d) The analysis results of brain infarcted volume. One-way ANOVA followed by Newman-Keuls test, *** $P < 0.001$ vs. the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the tMCAO group; * $P < 0.05$, *** $P < 0.001$ vs. the HSH group, $n = 9$

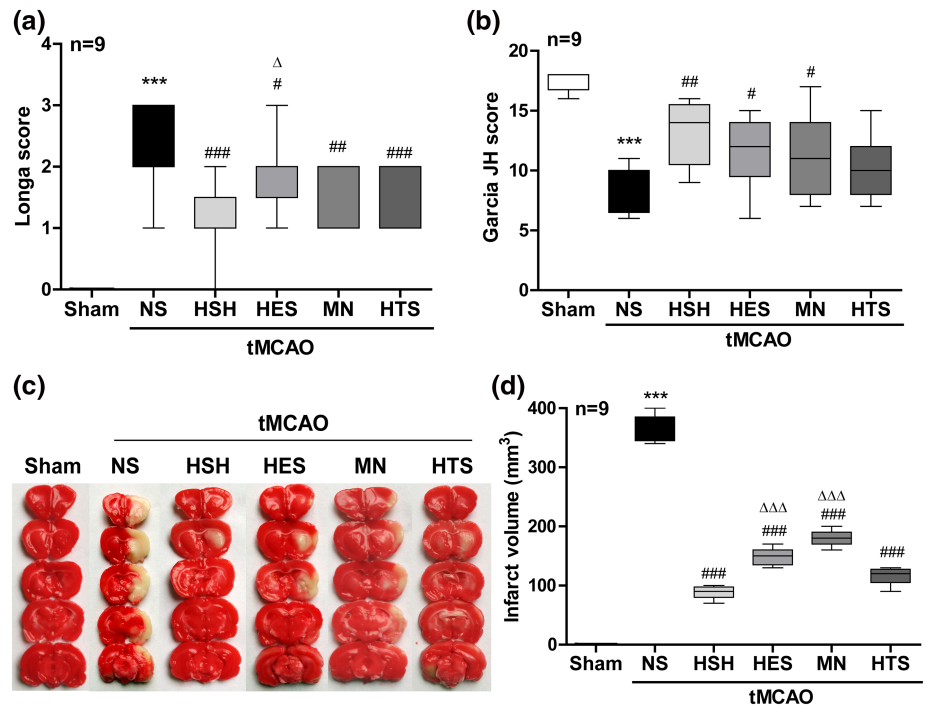
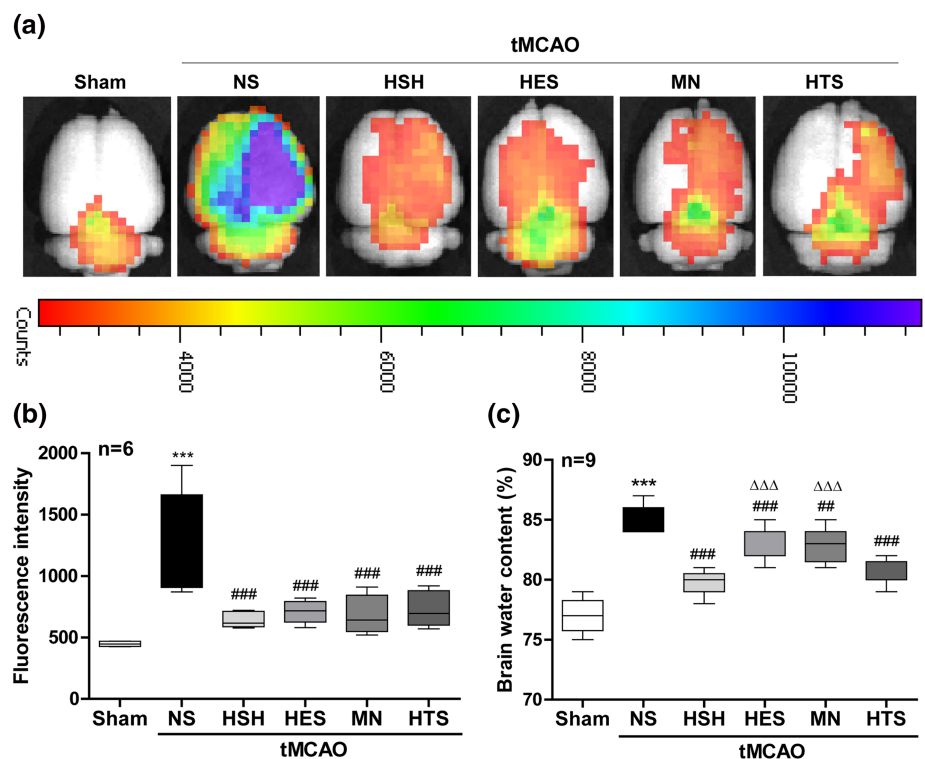


FIGURE 2 Four different liquids treatment decreased BBB permeability. Transient MCAO rats were treated with HSH, HES, MN, HTS or equivalent NS 30 min before reperfusion. 24 h following reperfusion, EB test and brain water content were evaluated. (a) The fluorescence photo of brain after staining EB. (b) The fluorescence intensity of EB. (c) The brain water content. One-way ANOVA followed by Newman-Keuls test, *** $P < 0.001$ vs. the sham group; *** $P < 0.001$ vs. the tMCAO group; *** $P < 0.001$ vs. the HSH group, $n = 6-9$



severely damaged after tMCAO. However, compared with those in the tMCAO group, the expression levels of laminin and claudin-5 in the groups treated with the four fluids were higher (Figure 4a,d,f), whereas the MMP-3 (Figure 4c) and MMP-9 (Figure 4b,e) expression levels were lower, indicating that reperfusion with HSH, HES and HTS maintained BBB

integrity by increasing laminin and claudin-5 expression levels and decreasing MMP expression levels. HSH was the most effective in increasing laminin expression ($P < 0.05$ vs. the MN and HES groups) and reducing MMP-3 ($P < 0.01$ vs. the HTS group) and MMP-9 ($P < 0.001$ vs. the MN, HES and HTS groups) expression.

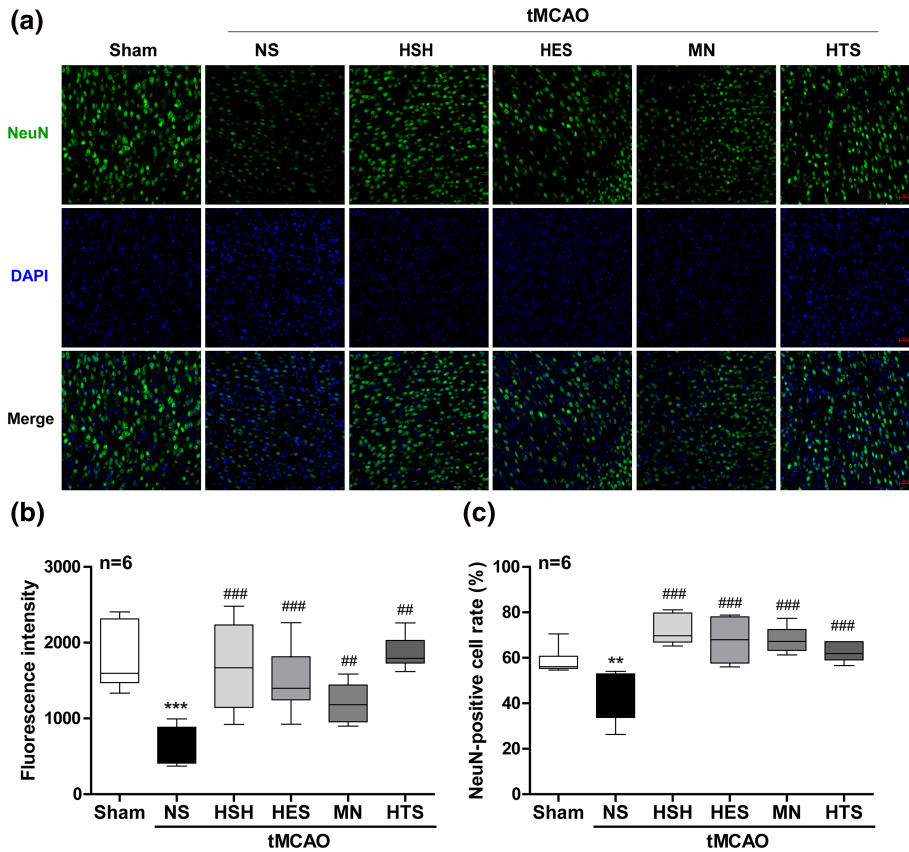


FIGURE 3 Four different liquids treatment reduced neuronal loss. Transient MCAO rats were treated with HSH, HES, MN, HTS or equivalent NS 30 min before reperfusion. 24 h following reperfusion, immunofluorescence staining was used to test NeuN expression. (a) The fluorescence photo of cortex after staining NeuN (scale bar = 50 μ m). (b) The fluorescence intensity of NeuN. (c) The NeuN-positive cell rate. One-way ANOVA followed by Newman–Keuls test, ** $P < 0.01$, *** $P < 0.001$ vs. the sham group; ** $P < 0.01$, *** $P < 0.001$ vs the tMCAO group, $n = 6$

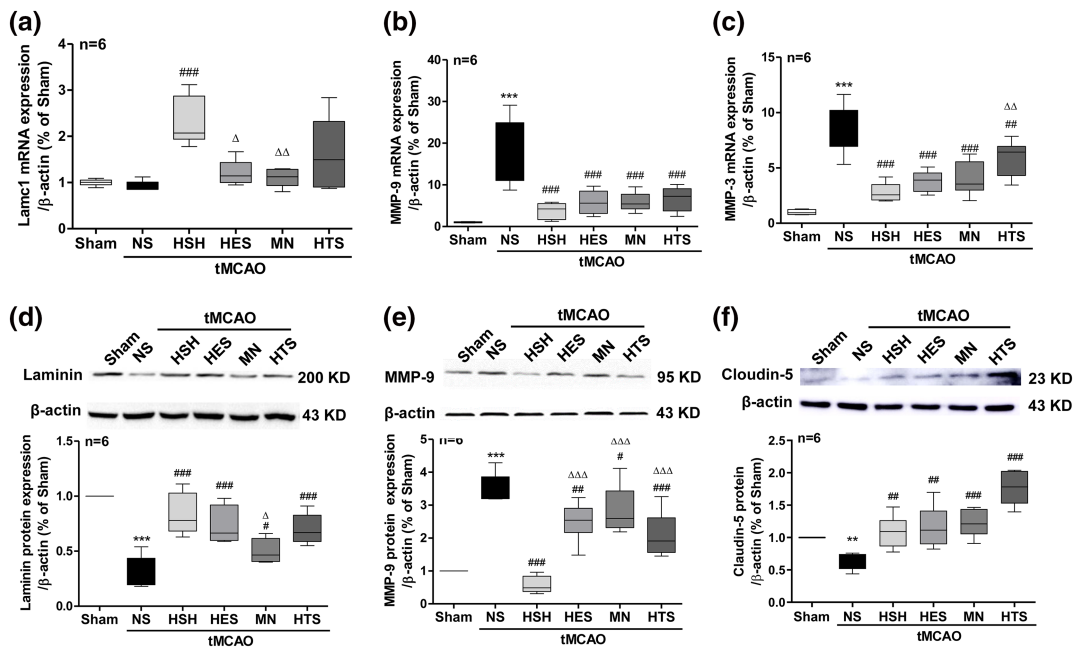


FIGURE 4 The effect of the four different liquids on the expression of BBB relevant factors. The laminin, MMP-3, and MMP-9 mRNA or/and protein expression in ischaemic penumbra regions were measured 24 h after four different liquids treatment. (a–c) qPCR results of laminin, MMP-3 and MMP-9 mRNA expression. (d–f) Western blot results of laminin, MMP-9 and claudin-5 protein expression. One-way ANOVA followed by Newman–Keuls test, *** $P < 0.001$ vs. the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the tMCAO group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the HSH group, $n = 6$

3.5 | Small-volume liquid reperfusion decreased the expression levels of RGS5 and PDGFR- β

RGS5 and PDGFR- β are the characteristic proteins of pericytes. By evaluating RGS5 and PDGFR- β protein expression, we can indirectly determine the condition of pericytes to determine the degree of BBB damage. The mRNA and protein expression of RGS5 and PDGFR- β was upregulated after CI/R, and the expression levels of these two molecules were lower in the groups treated with the four fluids than in the tMCAO group ($P < 0.05$), suggesting that the four fluids stabilized the BBB and might have alleviated CI/RI (Figure 5a–d). HSH was the most effective in reducing PDGFR- β ($P < 0.001$ vs. the MN group) and RGS5 ($P < 0.01$ vs. the HES group) protein expression.

3.6 | Small-volume liquid reperfusion inhibited the activation of astrocytes

Astrocytes are an important component of the BBB. During CI/RI, astrocytes are activated, which is manifested by increased expression of GFAP, followed by increased AQP4 and pro-inflammatory cytokine levels, accelerating the damage and permeability of the BBB. As shown in

Figure 6a–c, the mRNA and protein expression of GFAP in the sham group was increased. Activation of this astrocyte marker was significantly inhibited in the four liquid-treated groups. Moreover, the mRNA expression levels of AQP4, IL-1 β and TNF- α showed the same change in expression as GFAP (Figure 6d–f).

4 | DISCUSSION

CI/RI is a major cause of death and long-lasting disability worldwide. It has been widely demonstrated that excitotoxicity, ionic imbalance, adhesion molecule expression upregulation, ROS and RNS formation, inflammation and apoptosis induced by CI/R contribute to BBB breakdown, which is considered a critical step in cerebral ischaemia pathogenesis (Guell & Bix, 2014; Kassner & Merali, 2015). An increase in BBB permeability results in endogenous and exogenous injuries, which are the primary causes of brain oedema and high cranial pressure (Gu et al., 2011; Shang et al., 2010). Therefore, the current key strategy for treating cerebral oedema involves maintaining the normal structure of the BBB.

The BBB is a physical and functional barrier between the central nervous system (CNS) and blood that maintains CNS homeostasis. The BBB is the primary component of the neurological vascular cell structure, and it is

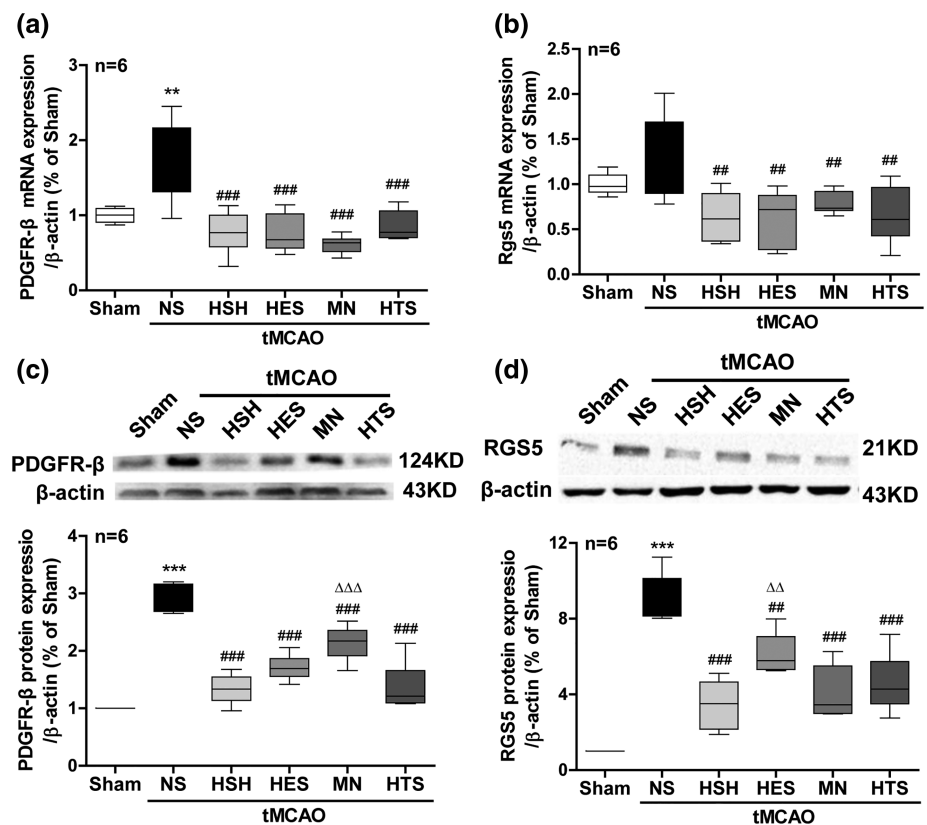


FIGURE 5 The effect of the four different liquids on the expression of the characteristic proteins of pericytes. The PDGFR- β and RGS5 mRNA and proteins expression in ischaemic penumbra regions were measured 24 h after four different liquids treatment. (a,b) qPCR results of PDGFR- β and RGS5 mRNA expression. (c,d) Western blot results of PDGFR- β and RGS5 protein expression. One-way ANOVA followed by Newman-Keuls test, ** $P < 0.01$, *** $P < 0.001$ vs. the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the tMCAO group; ** $P < 0.01$, *** $P < 0.001$ vs. the HSH group, $n = 6$

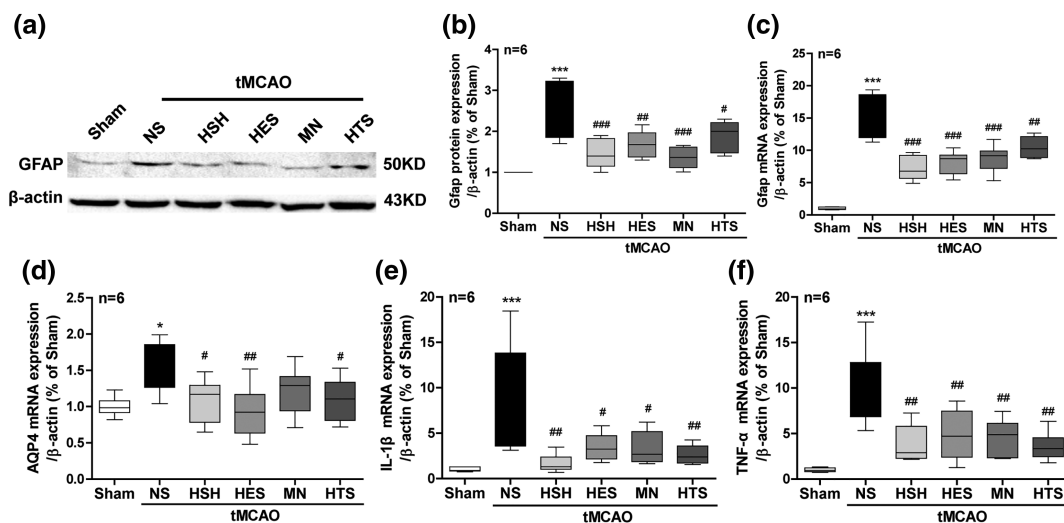


FIGURE 6 Effects of the four different liquids on activity of astrocytes and the expression of inflammatory factors. The GFAP, AQP4, IL-1 β and TNF- α mRNA and/or protein expression in ischaemic penumbra regions were measured 24 h after four different liquids treatment. (a,b) Western blot result of GFAP protein expression. (c-f) qPCR results of Gfap, AQP4, IL-1 β and TNF- α mRNA expression. One-way ANOVA followed by Newman-Keuls test, * $P < 0.05$, *** $P < 0.001$ vs. the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. the tMCAO group, $n = 6$

mainly composed of cellular structures (endothelial cells, pericytes, astrocytes and immunocytes) and molecular structures (tight junctions, basement membranes and leukocyte adhesion molecules) (Obermeier et al., 2016), which form a highly selective permeability barrier that separates the circulating blood from the extracellular fluid in the brain and maintains the microenvironment in the brain (Obermeier et al., 2016). In the physiological state, the BBB strictly regulates the transport of electrolytes, water-soluble substances and small molecules and only permits lipid-soluble substances to be transported into brain tissue (Abbott et al., 2010). The components of the BBB are activated in response to acute ischaemic stroke and then secrete inflammatory factors that directly or indirectly disrupt its integrity (Shan et al., 2019), which results in increased permeability of the BBB and leads to the formation of cerebral oedema (Borlongan et al., 2012; Feng et al., 2010; Gu et al., 2011; Obermeier et al., 2013). As we found in our study, EB signals were increased in the tMCAO (NS) group, which suggests that the permeability of the BBB was increased after CI/RI.

The BBB is connected by tight junction proteins on the endothelial cell surface. There are three types of transmembrane tight junction proteins: claudins, occludin and junctional adhesion molecules. Their dysfunction could contribute to failure of the BBB (Gloor et al., 2001), trigger brain oedema and cause cerebral injuries (Ayata & Ropper, 2002). At the BBB, claudin-5 is the most highly expressed tight junction protein, and its dysfunction has been implicated in stroke and brain injury (Greene et al., 2019). In this study, we found that

in the tMCAO (NS) group, the expression of claudin-5 was significantly downregulated, indicating destruction of tight junctions. Decreased expression of claudin-5 was promoted by the four fluids, suggesting the potential mechanism underlying the effects of the four fluids in maintaining the structure and function of the BBB.

Astrocytes play an important role in maintaining the integrity of the BBB under physiological conditions. However, under pathological conditions, such as traumatic and ischaemic injury, astrocytes secrete inflammatory factors, such as MMPs, chemokines and cytokines; these factors directly or indirectly aggravate brain damage and BBB disruption (Wiese et al., 2012). MMPs mainly contribute to degrading the BBB extracellular matrix structure (Zhao et al., 2020). Numerous studies have shown that MMP-3 and MMP-9 are two critical factors in BBB damage. In normal tissues, MMP-3 and MMP-9 are expressed at low levels, but the levels of these proteins are significantly increased after CI/RI (Fan et al., 2018; Ljubicavljovic et al., 2015).

A recent report showed that suppressing BBB disruption and inflammation alleviates CI/RI (He et al., 2017; Ji et al., 2015; Zhang, Park, et al., 2017). Laminin is mainly found in the basement membrane structure, which is a distinct non-collagen glycoprotein of the basement membrane. Laminin has a major role in assembly of the basement membrane, forming a network structure that allows cells to attach to the basement membrane (Jyoti et al., 2016). In this study, we analysed the expression of MMP-3, MMP-9 and laminin to determine the degree of BBB damage. Our results showed that the expression

of MMP-3 and MMP-9 was increased, whereas that of laminin was decreased after CI/RI, which is consistent with previous studies (Morancho et al., 2010; Jickling et al., 2014; Yang & Rosenberg, 2015; Zhang, Fan, et al., 2017). The four treatment groups had lower MMP expression and higher laminin expression than the tMCAO group, which indicates that these treatments stabilized the BBB.

Astrocytes are a major source of inflammatory factors in the damage zone after acute ischaemic stroke (Chen et al., 2018; Gelderblom et al., 2012; Shan et al., 2019; Wiese et al., 2012), which contribute greatly to peripheral immune cell recruitment, microglial activation, brain damage aggravation and BBB disruption (Gelderblom et al., 2012; Shan et al., 2019). Astrocyte activation is characterized by increased expression of GFAP, followed by increased expression of pro-inflammatory cytokines, such as IL-6, IL-1 β and TNF- α , which directly affect the BBB. In addition, the activation of astrocytes increases AQP4 expression, inducing astrocyte swelling and promoting BBB injury (Chu et al., 2017; Jennifer et al., 2011). In this study, the four fluids decreased the expression of GFAP, AQP4, IL-1 β and TNF- α , indicating that they can inhibit the activation of astrocytes and then alleviate inflammation and BBB damage.

Changes in the expression of RGS5 and PDGFR- β , as the characteristic proteins of pericytes, cellular components of the BBB, can indirectly reflect the condition of these cells and reveal the degree of damage to the BBB after CI/R injury (Özen et al., 2018; Yang et al., 2019). PDGF and the PDGF receptor (PDGFR) are widely expressed in the CNS and function as mitotic agents and growth factors by acting on a series of cells, promoting mitosis and chemotaxis of glial cells and mesenchymal cells. They are also involved in angiogenesis after vascular injury, which plays an important role in neuroprotective and vascular remodelling after cerebral apoplexy (Funa & Sasahara, 2014). The PDGF-B/PDGFR- β signalling system also has a function in maintaining the integrity of the BBB. When brain tissue ischaemia occurs, the expression of PDGF and PDGFR is upregulated to promote peripheral cell proliferation and migration (Arimura et al., 2012). RGS5 belongs to the family of G-protein signalling regulators, which negatively regulate G-protein signal transduction and play an important role in maintaining the morphology and migration of peripheral cells (Roth et al., 2019). In addition, RGS5 inhibits downstream tyrosine kinases and activates the MAPK signalling pathway, which is closely related to hypoxia and angiogenesis (Wang et al., 2019). RGS5 is an important oxygen receptor, and its protein level increases in response to hypoxia, which suggests that hypoxia inhibits the degradation of RGS5, resulting

in many RGS5 aggregates (Zhou et al., 2008). In our results, the mRNA and protein expression of RGS5 and PDGFR- β was increased in the tMCAO group compared with the sham group. This could be attributed to CI/RI-induced stimulation of peripheral cell proliferation, secretion, recruitment and migration, which all indicate damage to the BBB. The four treatment groups had lower expression of RGS5 and PDGFR- β than the tMCAO group, which indicates that these treatments stabilized pericytes and protected the BBB.

NeuN is a neuron-specific marker that is mainly expressed in the cytoplasm and nuclei of neurons in the nervous system (Jia et al., 2010). In some studies on cerebral ischaemic injury, neuronal necrosis was assessed by evaluating the expression of NeuN (Unal-Cevik et al., 2004). Liu et al. (2009) found that CI/RI can downregulate the expression of NeuN in the damaged area, and this can be observed during the early stages of ischaemia. This shows that we can judge changes in the number of neurons by assessing the expression of NeuN. In this study, we studied the expression of NeuN in rats with CI/RI through an immunofluorescence assay to analyse the changes in the number of neurons. Compared with that in the sham group, the expression of NeuN in the injured areas was significantly reduced in the tMCAO (NS) group, which indicates that CI/RI can damage neurons in the brain and may promote neuronal apoptosis. The expression of NeuN was significantly increased after reperfusion with the four fluids, especially HSH. These results indicate that these four fluids can reduce the apoptosis of neurons and exert a neuroprotective effect.

MN is a classical osmotic dehydrating agent, and its short-term application can effectively reduce intracranial pressure (Wise & Chater, 1962). Both clinical and animal experiments have shown that MN is more effective than other solutions in reducing intracranial pressure and relieving cerebral oedema. Its mechanism involves reducing the water content of brain tissue through permeability dehydration. In addition, MN can reduce the swelling of astrocytic end feet, preventing the microvascular compression and stasis they cause and consequently reducing the infarct size (Ito et al., 2014). Neurological scores, brain water content and infarct size were decreased in the MN group compared with the tMCAO group, which indicates that MN has a protective effect against CI/RI. Although the protein expression level of laminin was higher in the MN group than in the tMCAO group, there was no significant difference. The expression levels of MMP-3, MMP-9, RGS5, PDGFR- β , GFAP, AQP4, IL-1 β and TNF- α were decreased in MN-treated rats, which may be associated with its ability to alleviate brain oedema and improve the function of pericytes and astrocytes. Although MN has been demonstrated to clearly

reduce cranial pressure, an increasing number of studies have suggested that long-term treatment with or large doses of MN can cause BBB injury, intracranial pressure rebound, hypotension, electrolyte disorder, etc. (Witherspoon & Ashby, 2017). There is some evidence showing that repeated administration of MN may even aggravate brain oedema (Cho et al., 2007).

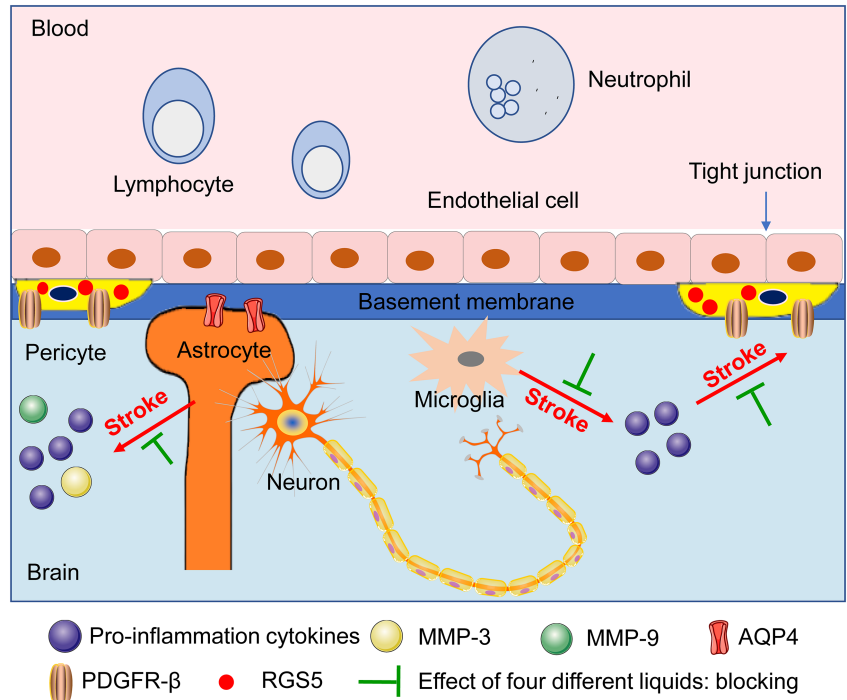
Because of the above-mentioned limitations of MN, HTS solutions have been investigated as alternatives for the treatment of cerebral oedema (Lewandowski-Belfer et al., 2014; Rallis et al., 2017). HTS is a sodium chloride solution with a concentration greater than 0.9%. Currently, it is commonly used at concentrations of 3%, 5%, 7.5%, 10% and 23.4%. In this study, we used 3% HTS with an osmotic pressure equal to that of 20% MN. HTS has many theoretical advantages over MN, such as the ability to increase plasma volume and augment cerebral blood flow, whereas MN reduces the intravascular volume and cerebral perfusion pressure (Fink, 2012). In addition, HTS has a larger osmotic reflection coefficient than MN; therefore, it is more effective in reducing intracranial pressure and alleviating cerebral oedema. As we showed in this study, neurological function scores, brain water content and infarct size were reduced in the HTS group compared with the MN group, which is consistent with previous studies. HTS can also improve cerebral perfusion pressure and relieve tissue ischaemia and hypoxia to avoid the aggravation of oedema and cell dysfunction. In this study, we also demonstrated that HTS protected the BBB. This finding was supported by our results showing that the expression level of laminin was reduced, whereas the expression levels of MMP-3, MMP-9, PDGFR- β , RGS5, GFAP, AQP4, IL-1 β and TNF- α were increased in the HTS group compared with the tMCAO group, which may be associated with oxygen free radical scavenging, a reduced inflammatory response and inhibition of MMP activation (Huang et al., 2014; Theobaldo et al., 2012).

Because of the rapid reestablishment of the osmotic balance between intra- and extracellular fluids, HTS has a short duration of action. However, colloidal solutions are different, such as HES, which can be retained in vessels for a longer period to effectively maintain the intravascular volume by elevating the colloid osmotic pressure. Hydroxyethyl starch 130/0.4 solution (HES), a synthetic colloid with a molecular weight of 130 kDa, has a good capacity for expansion and rarely induces anaphylactic reactions. Kaplan et al. (2000) proposed that HES reduces leukocyte adhesion and vascular injury under hypoxic conditions. In our study, we observed that HES had a neuroprotective effect by decreasing neurological scores, brain water content and infarct size. Previous studies indicated that the main mechanism of

hydroxyethyl neuroprotection includes haemodilution treatment, which reduces blood viscosity, leading to an increased blood flow and oxygen supply. Furthermore, haemodilution treatment has also been shown to be beneficial in scavenging harmful metabolites, such as oxygen free radicals, catecholamines and malondialdehyde, in brain tissues and reducing nerve cell damage. In addition to haemodilution treatment, reduced permeability of microvessels is also involved in the neuroprotective effect of hydroxyethyl. Using EB as an indicator of microvascular permeability, it has been shown that HES reduces the permeability of microvessels and brain injury after ischaemia and reperfusion. Subsequent research has confirmed that this effect of HES may be associated with decreased levels of inflammatory cytokines and ICAM-1, suppression of NF- κ B activation and downregulation of the expression of TLR2 and TLR4 in the brain (Feng et al., 2010). Previous studies showed that HES has a beneficial effect in decreasing BBB permeability (Feng et al., 2010). Our results showed that HES stabilized the BBB by decreasing the expression of MMP-3, MMP-9, PDGFR- β , RGS5, GFAP, AQP4, IL-1 β and TNF- α and increasing laminin expression, which may be associated with anti-inflammatory activity (Jung et al., 2018; Öztürk et al., 2015).

HHS is a mixture of two components: sodium chloride, which is mainly responsible for the osmotic gradient, and HES, which prolongs the expansion effect of the HTS and has anti-inflammatory effects. Therefore, HHS is more effective than the two components individually (Wang et al., 2015). Several studies have demonstrated that HHS improves the restoration of microperfusion by alleviating endothelial perivascular oedema. Similarly, there are an increasing number of reports showing that HHS improves neurological function and neuronal survival and reduces infarction size and mortality (Noppens et al., 2012). In this study, we used HSH injection, which is composed of 4.2% sodium chloride and 7.6% hydroxyethyl starch 40. Our results are consistent with the hypothesis that HSH is the most effective of the four fluids. It significantly decreased neurological scores, brain water content and infarct size and was clearly superior to the other three fluids. In addition, the HSH group showed the lowest expression levels of MMP-3, MMP-9, RGS5, PDGFR- β , GFAP, AQP4, IL-1 β and TNF- α and the highest expression of laminin among the four treatment groups, indicating that HSH alleviates brain injury due to its protective effect on the BBB and ability to suppress the inflammatory response. Our previous studies suggested that after cerebral ischaemia, MDA, NO and iNOS levels decreased dramatically, whereas the activities of SOD, GSH-Px, Na⁺/K⁺-ATPase and Ca²⁺/Mg²⁺-ATPase significantly increased in response to HSH

FIGURE 7 Schematic diagram of the mechanisms underlies the four different liquids against brain injury in tMCAO rats. The four different liquids treatment inhibits the activation of astrocyte and pericyte and increases the expression of tight junction proteins and then stabilizes BBB and protects neurons in tMCAO rats



infusion, which is consistent with other studies (Shan et al., 2011, 2012; Yu et al., 2012).

However, there are some limitations to this study. First, we did not use an EB dye tracer, which can measure BBB permeability directly. Second, we did not assess the long-term outcomes of the rats, including the survival rate and mortality rate. In future studies, we will extend the observation period and examine the effects on other organs.

In conclusion, as shown in Figure 7, our study showed that reperfusion with a small volume of 20% MN, 3% hypertonic sodium chloride (HTS), HES or HSH leads to cerebroprotective effects against brain ischaemia. The mechanisms are probably attenuation of BBB disruption and a reduction in postischaemic inflammation, and HSH achieved the best neuroprotection.

ACKNOWLEDGEMENTS

This study was supported by the Science and Technology Foundation of the Bureau of Education of Jiangxi Province, China (GJJ12548), the Natural Science Foundation of Jiangxi Province, China (20142BAB205020) and the Innovation Team Foundation of Gannan Medical University (TD201705).

CONFLICT OF INTERESTS

All authors declared that they had no conflict interests with each other.

AUTHOR CONTRIBUTIONS

All authors listed contributed immensely to this study. Reai Shan, Xinfang Liu and Guangjun Su performed the experiments. Hongyan Zhou, Xiaoli Zhang, Guangsen Liu, Cong Sun, Zining Yu and Lifang Zhan helped to perform the animal experiments. Zhihua Huang and Reai Shan analysed the data and designed the study. Hongyan Zhou drafted the paper.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/ejn.15385>.

DATA AVAILABILITY STATEMENT

Data can be requested from the first author.

ORCID

Zhihua Huang  <https://orcid.org/0000-0002-2610-1999>

REFERENCES

- Abbott, N. J., Patabendige, A. A., Dolman, D. E., Yusof, S. R., & Begley, D. J. (2010). Structure and function of the blood-brain barrier. *Neurobiology of Disease*, 37(1), 13–25. <https://doi.org/10.1016/j.nbd.2009.07.030>
- Al-Mufti, F., Amuluru, K., Roth, W., Nuoman, R., El-Ghanem, M., & Meyers, P. M. (2018). Cerebral ischemic reperfusion injury following recanalization of large vessel occlusions. *Neurosurgery*, 82, 781–789. <https://doi.org/10.1093/neuros/nyx341>

- Arimura, K., Ago, T., Kamouchi, M., Nakamura, K., Ishitsuka, K., Kuroda, J., Sugimori, H., Ooboshi, H., Sasaki, T., & Kitazono, T. (2012). PDGF receptor β signaling in pericytes following ischemic brain injury. *Current Neurovascular Research*, 9(1), 1–9. <https://doi.org/10.2174/156720212799297100>
- Ayata, C., & Ropper, A. H. (2002). Ischaemic brain oedema. *Journal of Clinical Neuroscience*, 9, 113–124. <https://doi.org/10.1054/jocn.2001.1031>
- Borlongan, C. V., Rodrigues, A. A. Jr., & Oliveira, M. C. (2012). Breaking the barrier in stroke: What should we know? A mini-review. *Current Pharmaceutical Design*, 18(25), 3615–3623. <https://doi.org/10.2174/138161212802002670>
- Carney, N., Totten, A. M., O'Reilly, C., Ullman, J. S., Hawryluk, G. W., Bell, M. J., ... Ghajar, J. (2017). Guidelines for the management of severe traumatic brain injury, fourth edition. *Neurosurgery*, 80(1), 6–15. <https://doi.org/10.1227/NEU.0000000000001432>
- Chen, C., Chu, S. F., Liu, D. D., Zhang, Z., Kong, L. L., Zhou, X., & Chen, N. H. (2018). Chemokines play complex roles in cerebral ischemia. *Neurochemistry International*, 112, 146–158. <https://doi.org/10.1016/j.neuint.2017.06.008>
- Chen, X., Zhang, X., Xue, L., Hao, C., Liao, W., & Wan, Q. (2017). Treatment with enriched environment reduces neuronal apoptosis in the periinfarct cortex after cerebral ischemia/reperfusion injury. *Cellular Physiology and Biochemistry*, 41(4), 1445–1456. <https://doi.org/10.1159/000468368>
- Cho, J., Kim, Y. H., Han, H. S., & Park, J. (2007). Accumulated mannitol and aggravated cerebral edema in a rat model of middle cerebral artery infarction. *Journal of Korean Neurosurgical Society*, 42(4), 337–341. <https://doi.org/10.3340/jkns.2007.42.4.337>
- Chu, H., Yang, X., Huang, C., Gao, Z., Tang, Y., & Dong, Q. (2017). Apelin-13 protects against ischemic blood-brain barrier damage through the effects of aquaporin-4. *Cerebrovascular Diseases*, 44(1-2), 10–25. <https://doi.org/10.1159/000460261>
- Cook, A. M., Morgan Jones, G., Hawryluk, G. W. J., Mailloux, P., McLaughlin, D., Papangelou, A., ... Shutter, L. (2020). Guidelines for the acute treatment of cerebral edema in neurocritical care patients. *Neurocritical Care*, 32(3), 647–666. <https://doi.org/10.1007/s12028-020-00959-7>
- Deng, Y. Y., Shen, F. C., Xie, D., Han, Q. P., Fang, M., Chen, C. B., & Zeng, H. K. (2016). Progress in drug treatment of cerebral edema. *Mini-Reviews in Medical Chemistry*, 16(11), 917–925. <https://doi.org/10.2174/1389557516666160304151233>
- Fan, F., Yang, J., Xu, Y., & Guan, S. (2018). MiR-539 targets MMP-9 to regulate the permeability of blood-brain barrier in ischemia/reperfusion injury of brain. *Neurochemistry Research*, 43(12), 2260–2267. <https://doi.org/10.1007/s11064-018-2646-0>
- Farasatinasab, M., Amouzegar, A., Safari, S., Ghanbari, B., Darkahian, M., Emami, S., Pakdaman, N., & Salili, M. (2018). Albumin utilization evaluation in a major teaching hospital in Iran: Recommendations for guideline development. *Journal of Research in Pharmacy Practice*, 7(3), 157–163. https://doi.org/10.4103/jrpp.JRPP_18_4
- Feng, X., Zhang, F., Dong, R., Wang, H., Liu, J., Liu, X., Li, W., Yao, J., Xu, J., & Yu, B. (2010). Effects of hydroxyethyl starch (130 kD) on brain inflammatory response and outcome during normotensive sepsis. *International Immunopharmacology*, 10(8), 859–864. <https://doi.org/10.1016/j.intimp.2010.04.020>
- Fink, M. E. (2012). Osmotherapy for intracranial hypertension: Mannitol versus hypertonic saline. *Continuum (Minneapolis)*, 18(3), 640–654. <https://doi.org/10.1212/01.CON.0000415432.84147.1e>
- Funa, K., & Sasahara, M. (2014). The roles of PDGF in development and during neurogenesis in the normal and diseased nervous system. *Journal of Neuroimmune Pharmacology*, 9(2), 168–181. <https://doi.org/10.1007/s11481-013-9479-z>
- Garcia, J. H., Wagner, S., Liu, K. F., & Hu, X. J. (1995). Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats: Statistical validation. *Stroke*, 26(4), 627–634. <https://doi.org/10.1161/01.str.26.4.627>
- Gelderblom, M., Weymar, A., Bernreuther, C., Velden, J., Arunachalam, P., Steinbach, K., Orthey, E., Arumugam, T. V., Leypoldt, F., Simova, O., Thom, V., Friese, M. A., Prinz, I., Hölscher, C., Glatzel, M., Korn, T., Gerloff, C., Tolosa, E., & Magnus, T. (2012). Neutralization of the IL-17 axis diminishes neutrophil invasion and protects from ischemic stroke. *Blood*, 120(18), 3793–3802. <https://doi.org/10.1182/blood-2012-02-412726>
- Gloor, S. M., Wachtel, M., Bolliger, M. F., Ishihara, H., Landmann, R., & Frei, K. (2001). Molecular and cellular permeability control at the blood-brain barrier. *Brain Research*, 36(2-3), 258–264. [https://doi.org/10.1016/s0165-0173\(01\)00102-3](https://doi.org/10.1016/s0165-0173(01)00102-3)
- Greene, C., Hanley, N., & Campbell, M. (2019). Claudin-5: Gatekeeper of neurological function. *Fluids Barriers CNS*, 16, 3(1), 3. <https://doi.org/10.1186/s12987-019-0123-z>
- Gu, Y., Dee, C. M., & Shen, J. (2011). Interaction of free radicals, matrix metalloproteinases and caveolin-1 impact blood-brain barrier permeability. *Frontiers in Biosciences (Schol Ed)*, 3, 1216–1231. <https://doi.org/10.2741/222>
- Guell, K., & Bix, G. J. (2014). Brain endothelial cell specific integrins and ischemic stroke. *Expert Review of Neurotherapeutics*, 14(11), 1287–1292. <https://doi.org/10.1586/14737175.2014.964210>
- Hao, R., Sun, B., Yang, L., Ma, C., & Li, S. (2020). RVG29-modified microRNA-loaded nanoparticles improve ischemic brain injury by nasal delivery. *Drug Delivery*, 27(1), 772–781. <https://doi.org/10.1080/10717544.2020.1760960>
- He, F., Duan, X., Dai, R., Li, Y., & Lin, Q. (2017). Protective effect of 4-methoxy benzyl alcohol on the blood-brain barrier after cerebral ischemia reperfusion injury. *Journal of Stroke and Cerebrovascular Diseases*, 26(6), 1258–1265. <https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.05.011>
- Huang, L. Q., Zhu, G. F., Deng, Y. Y., Jiang, W. Q., Fang, M., Chen, C. B., Cao, W., Wen, M. Y., Han, Y. L., & Zeng, H. K. (2014). Hypertonic saline alleviates cerebral edema by inhibiting microglia-derived TNF- α and IL-1 β -induced Na-K-Cl cotransporter up-regulation. *Journal of Neuroinflammation*, 11, 102–122. <https://doi.org/10.1186/1742-2094-11-102>
- Ito, U., Hakamata, Y., Watabe, K., & Oyanagi, K. (2014). Mannitol infusion immediately after reperfusion suppresses the development of focal cortical infarction after temporary cerebral ischemia in gerbils. *Neuropathology*, 34(4), 360–369. <https://doi.org/10.1111/neup.12113>
- Jennifer, M. R., Breanna, K. W., Phyllis, M. W., & Martha, E. O'. D. (2011). Effects of estradiol on ischemic factor-induced astrocyte swelling and AQP4 protein abundance. *American Journal*

- of *Physiology-Cell Physiology*, 301(1), C204–C212. <https://doi.org/10.1152/ajpcell.00399.2010>
- Ji, F. T., Liang, J. J., Miao, L. P., Wu, Q., & Cao, M. H. (2015). Profound post-conditioning protects the blood brain barrier by decreasing matrix metalloproteinase-9 and aquaporin-4 expression and improves the neurobehavioral outcome in a rat model of focal cerebral ischemia-reperfusion injury. *Molecular Medicine Reports*, 12(2), 2049–2055. <https://doi.org/10.3892/mmr.2015.3585>
- Jia, Q., Liu, L. P., & Wang, Y. J. (2010). Stroke in China. *Clinical and Experimental Pharmacology & Physiology*, 37(2), 259–264. <https://doi.org/10.1111/j.1440-1681.2009.05290.x>
- Jickling, G. C., Liu, D., Stamova, B., Ander, B. P., Zhan, X., Lu, A., & Sharp, F. R. (2014). Hemorrhagic transformation after ischemic stroke in animals and humans. *Journal of Cerebral Blood Flow and Metabolism*, 34(2), 185–199. <https://doi.org/10.1038/jcbfm.2013.203>
- Jung, D. M., Ahn, H. J., Yang, M., Kim, J. A., Kim, D. K., Lee, S. M., & Park, J. H. (2018). Hydroxyethyl starch is associated with early postoperative delirium in patients undergoing esophagectomy. *Journal of Thoracic and Cardiovascular Surgery*, 155(3), 1333–1343. <https://doi.org/10.1016/j.jtcvs.2017.10.077>
- Jyoti, G., Zhang, X. M., & Yao, Y. (2016). The role of pericytic laminin in blood brain barrier integrity maintenance. *Scientific Reports*, 6, 36450. <https://doi.org/10.1038/srep36450>
- Kaplan, S. S., Park, T. S., Gonzales, E. R., & Gidday, J. M. (2000). Hydroxyethyl starch reduces leukocyte adherence and vascular injury in the newborn pig cerebral circulation after asphyxia. *Stroke*, 31(9), 2218–2223. <https://doi.org/10.1161/01.str.31.9.2218>
- Kassner, A., & Merali, Z. (2015). Assessment of blood-brain barrier disruption in stroke. *Stroke*, 46(11), 3310–3315. <https://doi.org/10.1161/STROKEAHA.115.008861>
- Lewandowski-Belfer, J. J., Patel, A. V., Darracott, R. M., Jackson, D. A., Nordeen, J. D., & Freeman, W. D. (2014). Safety and efficacy of repeated doses of 14.6 or 23.4% hypertonic saline for refractory intracranial hypertension. *Neurocritical Care*, 20(3), 436–442. <https://doi.org/10.1007/s12028-013-9907-1>
- Li, L. D., Xue, J. H., Liu, R. Z., Li, X., Lai, L. J., Xie, J. L., Huang, Z. H., & Huang, C. (2017). Neuroprotective effects of genistein-3'-sodium sulfonate on focal cerebral ischemia in rats. *Neuroscience Letters*, 646, 43–48. <https://doi.org/10.1016/j.neulet.2017.02.046>
- Li, T., Li, J. H., Li, H. B., Xia, Z. Y., Shi, X. Y., Li, X. Y., & Liu, Y. T. (2014). Effects of hypertonic sodium chloride hydroxyethyl starch solution on cerebral vasospasm following subarachnoid hemorrhage and its mechanism. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*, 26(8), 589–593. <https://doi.org/10.3760/cma.j.issn.2095-4352.2014.08.014>
- Liu, F., Schafer, D. P., & McCullough, L. D. (2009). TTC, fluoro-jade B and NeuN staining confirm evolving phases of infarction induced by middle cerebral artery occlusion. *Journal of Neuroscience Methods*, 179(1), 1–8. <https://doi.org/10.1016/j.jneumeth.2008.12.028>
- Ljubisavljevic, S., Stojanovic, I., Basic, J., Vojinovic, S., Stojanov, D., Djordjevic, G., & Pavlovic, D. (2015). The role of matrix metalloproteinase 3 and 9 in the pathogenesis of acute neuroinflammation. *Implications for Disease Modifying Therapy. Journal of Molecular Neuroscience*, 56(4), 840–847. <https://doi.org/10.1007/s12031-015-0521-x>
- Longa, E. Z., Weinstein, P. R., Carlson, S., & Cummins, R. (1989). Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke*, 20(1), 84–91. <https://doi.org/10.1161/01.str.20.1.84>
- Moranco, A., Rosell, A., García-Bonilla, L., & Montaner, J. (2010). Metalloproteinase and stroke infarct size: Role for anti-inflammatory treatment? *Annals of the New York Academy of Sciences*, 1207, 123–133. <https://doi.org/10.1111/j.1749-6632.2010.05734.x>
- Noppens, R. R., Kelm, R. F., Lindemann, R., Engelhard, K., Werner, C., & Kempfski, O. (2012). Effects of a single-dose hypertonic saline hydroxyethyl starch on cerebral blood flow, long-term outcome, neurogenesis, and neuronal survival after cardiac arrest and cardiopulmonary resuscitation in rats. *Critical Care Medicine*, 40(7), 2149–2156. <https://doi.org/10.1097/CCM.0b013e31824e6750>
- Obermeier, B., Daneman, R., & Ransohoff, R. M. (2013). Development, maintenance and disruption of the blood-brain barrier. *Nature Medicine*, 19(12), 1584–1596. <https://doi.org/10.1038/nm.3407>
- Obermeier, B., Verma, A., & Ransohoff, R. M. (2016). The blood-brain barrier. *Handb Clinical Neurology*, 133, 39–59. <https://doi.org/10.1016/B978-0-444-63432-0.00003-7>
- Özen, I., Roth, M., Barbariga, M., Gaceb, A., Deierborg, T., Genové, G., & Paul, G. (2018). Loss of regulator of G-protein signaling 5 leads to neurovascular protection in stroke. *Stroke*, 49(9), 2182–2190. <https://doi.org/10.1161/STROKEAHA.118.020124>
- Öztürk, T., Onur, E., Cerrahoğlu, M., Çalgan, M., Nizamoglu, F., & Çivi, M. (2015). Immune and inflammatory role of hydroxyethyl starch 130/0.4 and fluid gelatin in patients undergoing coronary surgery. *Cytokine*, 74(1), 69–75. <https://doi.org/10.1016/j.cyto.2014.10.002>
- Park, J., Choi, E., Shin, S., Lim, S., Kim, D., Baek, S., Lee, K. P., Lee, J. J., Lee, B. H., Kim, B., Jeong, K., Baik, J. H., Kim, Y. K., & Kim, S. (2018). Nootropic nanocomplex with enhanced blood-brain barrier permeability for treatment of traumatic brain injury-associated neurodegeneration. *Journal of Controlled Release*, 284, 152–159. <https://doi.org/10.1016/j.jconrel.2018.06.021>
- Rallis, D., Poulos, P., Kazantzi, M., Chalkias, A., & Kalampalikis, P. (2017). Effectiveness of 7.5% hypertonic saline in children with severe traumatic brain injury. *Journal of Critical Care*, 38, 52–56. <https://doi.org/10.1016/j.jccr.2016.10.014>
- Roth, M., Gaceb, A., Enström, A., Padel, T., Genové, G., Özen, I., & Paul, G. (2019). Regulator of G-protein signaling 5 regulates the shift from perivascular to parenchymal pericytes in the chronic phase after stroke. *FASEB Journal*, 33(8), 8990–8998. <https://doi.org/10.1096/fj.201900153R>
- Shan, R. A., Bi, R. X., Zhan, L. F., & Huang, Z. H. (2012). The antioxidant effects of HH40 on rat cerebral ischemia/reperfusion injury. *Journal of Apoplexy and Nervous Diseases*, 29(8), 1087–1088. <https://doi.org/10.1096/fj.201900153R>
- Shan, R. A., Zhou, S. B., & Huang, Z. H. (2011). Effect of hypertonic sodium chloride hydroxyethyl starch 40 on NO and iNOS after rat cerebral ischemia reperfusion injury. *Journal of Guangdong Medical*, 32, 2510–2511.

- Shan, Y. L., Tan, S., Lin, Y. Y., Liao, S. Y., Zhang, B. J., Chen, X. D., Wang, J., Deng, Z., Zeng, Q., Zhang, L., Wang, Y., Hu, X., Qiu, W., Peng, L., & Lu, Z. Q. (2019). The glucagon-like peptide-1 receptor agonist reduces inflammation and blood-brain barrier breakdown in an astrocyte-dependent manner in experimental stroke. *Journal of Neuroinflammation*, *16*, 242. <https://doi.org/10.1186/s12974-019-1638-6>
- Shang, E., Fang, F., Lv, P., Bai, L., Qi, Y., Yan, F., & Mao, L. (2010). Blood brain barrier permeability and therapeutic time window of ginkgolide B in ischemia-reperfusion injury. *European Journal of Pharmaceutical Sciences*, *39*, 8–14. <https://doi.org/10.1016/j.ejps.2009.10.002>
- Shu, L., Shen, X., Zhao, Y., Zhao, R., He, X., Yin, J., Su, J., Li, Q., & Liu, J. (2020). Mechanisms of transformation of nicotinamide mononucleotides to cerebral infarction hemorrhage based on MCAO model. *Saudi Journal of Biological Sciences*, *27*, 899–904. <https://doi.org/10.1016/j.sjbs>
- Theobaldo, M. C., Barbeiro, H. V., Barbeiro, D. F., Petroni, R., & Soriano, F. G. (2012). Hypertonic saline solution reduces the inflammatory response in endotoxemic rats. *Clinics (São Paulo, Brazil)*, *67*, 1463–1468. [https://doi.org/10.6061/clinics/2012\(12\)18](https://doi.org/10.6061/clinics/2012(12)18)
- Unal-Cevik, I., Kiling, M., Gürsoy-Ozdemir, Y., Gurer, G., & Dalkara, T. (2004). Loss of NeuN immunoreactivity after cerebral ischemia does not indicate neuronal cell loss: A cautionary note. *Brain Research*, *1015*, 169–174. <https://doi.org/10.1016/j.brainres.2004.04.032>
- Wang, D., Xu, Y., Feng, L., Yin, P., Song, S. S., Wu, F., Yan, P., & Liang, Z. (2019). RGS5 decreases the proliferation of human ovarian carcinoma-derived primary endothelial cells through the MAPK/ERK signaling pathway in hypoxia. *Oncology Reports*, *41*, 165–177. <https://doi.org/10.3892/or.2018.6811>
- Wang, Y. L., Chen, J. H., Zhu, Q. F., Yu, G. F., Luo, C. F., Luo, G. J., Li, S. R., & Hei, Z. Q. (2015). In vivo evaluation of the ameliorating effects of small-volume resuscitation with four different fluids on endotoxemia-induced kidney injury. *Mediators of Inflammation*, *2015*, 726243. <https://doi.org/10.1155/2015/726243>
- Wiese, S., Karus, M., & Faissner, A. (2012). Astrocytes as a source for extracellular matrix molecules and cytokines. *Frontiers in Pharmacology*, *3*, 120. <https://doi.org/10.3389/fphar.2012.00120>
- Wise, B. L., & Chater, N. (1962). The value of hypertonic mannitol solution in decreasing brain mass and lowering cerebrospinal-fluid pressure. *Journal of Neurosurgery*, *19*, 1038–1043. <https://doi.org/10.3171/jns.1962.19.12.1038>
- Witherspoon, B., & Ashby, N. E. (2017). The use of mannitol and hypertonic saline therapies in patients with elevated intracranial pressure: A review of the evidence. *Nursing Clinics of North America*, *52*, 249–260. <https://doi.org/10.1016/j.cnur.2017.01.002>
- Yang, E., Cai, Y., Yao, X., Liu, J., Wang, Q. X., Jin, W. L., Wu, Q., Fan, W., Qiu, L., Kang, C., & Wu, J. L. (2019). Tissue plasminogen activator disrupts the blood-brain barrier through increasing the inflammatory response mediated by pericytes after cerebral ischemia. *Aging (Albany NY)*, *11*, 10167–10182. <https://doi.org/10.18632/aging.102431>
- Yang, Y., & Rosenberg, G. A. (2015). Matrix metalloproteinases as therapeutic targets for stroke. *Brain Research*, *1623*, 30–38. <https://doi.org/10.1016/j.brainres.2015.04.024>
- Yu, G., Chi, X., Hei, Z., Shen, N., Chen, J., Zhang, W., & Li, S. (2012). Small volume resuscitation with 7.5% hypertonic saline, hydroxyethyl starch 130/0.4 solution and hypertonic sodium chloride hydroxyethyl starch 40 injection reduced lung injury in endotoxin shock rats: Comparison with saline. *Pulmonary Pharmacology & Therapeutics*, *25*(1), 27–32. <https://doi.org/10.1016/j.pupt.2011.10.003>
- Zhang, H., Park, J. H., Maharjan, S., Park, J. A., Choi, K. S., Park, H., Jeong, Y., Ahn, J. H., Kim, I. H., Lee, J. C., Cho, J. H., Lee, I. K., Lee, C. H., Hwang, I. K., Kim, Y. M., Suh, Y. G., Won, M. H., & Kwon, Y. G. (2017). Sac-1004, a vascular leakage blocker, reduces cerebral ischemia-reperfusion injury by suppressing blood-brain barrier disruption and inflammation. *Journal of Neuroinflammation*, *14*(1), 122. <https://doi.org/10.1186/s12974-017-0897-3>
- Zhang, Y., Fan, F., Zeng, G., Zhou, L., Zhang, Y., Zhang, J., Jiao, H., Zhang, T., Su, D., Yang, C., Wang, X., Xiao, K., Li, H., & Zhong, Z. (2017). Temporal analysis of blood-brain barrier disruption and cerebrospinal fluid matrix metalloproteinases in rhesus monkeys subjected to transient ischemic stroke. *Journal of Cerebral Blood Flow and Metabolism*, *37*(8), 2963–2974. <https://doi.org/10.1177/0271678X16680221>
- Zhao, B., Yin, Q., Fei, Y., Zhu, J., Qiu, Y., Fang, W., & Li, Y. (2020). Research progress of mechanisms for tight junction damage on blood-brain barrier inflammation. *Archives of Physiology and Biochemistry*, *1*, 1–12. <https://doi.org/10.1080/13813455.2020.1784952>
- Zhou, D., Wang, J., Zapala, M. A., Xue, J., Schork, N. J., & Haddad, G. G. (2008). Gene expression in mouse brain following chronic hypoxia: Role of sarcospan in glial cell death. *Physiological Genomics*, *32*, 370–379. <https://doi.org/10.1152/physiolgenomics.00147.2007>

How to cite this article: Shan, R., Zhou, H., Liu, X., Su, G., Liu, G., Zhang, X., Sun, C., Yu, Z., Zhan, L., & Huang, Z. (2021). Neuroprotective effects of four different fluids on cerebral ischaemia/reperfusion injury in rats through stabilization of the blood–brain barrier. *European Journal of Neuroscience*, *54*(4), 5586–5600. <https://doi.org/10.1111/ejn.15385>