RESEARCH PAPER

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Human albumin aggravates cerebral edema by disrupting the blood-brain barrier in a rat model of ischemic stroke

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Cerebral edema and elevated intracranial pressure (ICP) are common complications observed following ischemic stroke. Osmotherapy has been used as a foundation to manage ICP induced by cerebral edema, and albumin is one of the most commonly used osmotic agents. The present study aimed to explore whether albumin lowered ICP by reducing cerebral edema when albumin elevated the colloid osmotic pressure (COP) of plasma. Sprague-Dawley rats that underwent middle cerebral artery occlusion were used to assess COP and ICP. Magnetic resonance imaging measurements were performed to evaluate the cerebral edema and infarct size. Evans blue was used to assess the blood-brain barrier (BBB) permeability. Western blotting was used to determine the expression levels of the tight junction proteins in cerebral vascular endothelial cells. The results showed that 25% albumin treatment (1.25 g/kg) by intravenous injection elevated the COP of plasma but did not reduce the ICP in rats that had undergone ischemic stroke. Additionally, albumin did not reduce the infarct size and instead aggravated the cerebral edema. Furthermore, the BBB permeability was increased by albumin. Concomitantly, albumin treatment significantly downregulated the expression of tight junction proteins (ZO-1, occludin and claudin-5) in cerebral vascular endothelial cells. Tight junction protein expression was significantly upregulated when the cells were treated with an MMP-9 inhibitor (GM6001). These results suggest that albumin aggravates cerebral edema in rats with ischemic stroke by increasing BBB permeability.

Key words: stroke, albumin, cerebral edema, intracranial pressure, blood-brain barrier

INTRODUCTION

Ischemic stroke is one of the major causes of death worldwide (Belayev et al., 2002; Beez and Steiger, 2019). Cerebral edema and elevated intracranial pressure (ICP) are common complications observed following ischemic stroke (Lee et al., 2009; Caltagirone et al., 2016), and without immediate and effective treatment, they may result in cerebral herniation, an immediate threat to life (Pallesen et al., 2018; Shah et al., 2019; Wei et al., 2018). Alleviation of ICP is achieved by two primary approaches: surgery and drug interventions. Surgical treatments to reduce ICP caused by ischemic stroke

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mainly involve decompressive craniotomy (Smith, 2019; Beez and Steiger, 2019). Medical treatments to reduce ICP include osmotherapy, diuretic therapy and hyperventilation (Freeman, 2015; Shah and Kimberly, 2016; Khan et al., 2017).

Osmotherapy is the primary strategy used to manage ICP induced by cerebral edema. Commonly used osmotic agents include hypertonic saline, mannitol and albumin (Walcott et al., 2012; Jeon et al., 2014; Decker et al., 2016). Although hypertonic saline and mannitol are common treatments for elevated ICP or cerebral edema in patients with acute ischemic stroke clinically, they inevitably induce adverse effects such as hypernatremia and renal dysfunction (Changa et al., 2019; Su et al., 2021). Albumin is a major component of plasma proteins and is an important circulating carrier that transports drugs, hormones, metabolites and fatty acids through the plasma (Horvathy et al., 2017; Erstad, 2018; Rabbani and Ahn, 2019). It has been reported that albumin is neuroprotective in animal models of ischemic stroke (Wang et al., 2013; Tuttolomondo, 2015). Owing to its beneficial properties, albumin has been used in clinical settings as an adjuvant therapy for ischemic stroke. However, albumin did not improve the outcomes of ischemic stroke patients in a randomized, double-blind, placebo-controlled trial. Furthermore, albumin increased the rates of intracerebral hemorrhage (Hill, 2015), which means albumin may aggravate cerebral vascular endothelial cell damage or increase the permeability of the blood-brain barrier (BBB) after ischemic stroke. The above clinical research results are contradictory to those of early animal experiments (Belayev et al., 2001; Huang and Xiao, 2021). Indeed, the effects of albumin treatment on BBB permeability, cerebral edema and ICP following ischemic stroke remain to be fully clarified.

Increased BBB permeability following ischemic stroke can lead to edema, a significant cause of elevated ICP (Michinaga and Koyama, 2015). BBB permeability is regulated by the integrity of tight junction proteins (TJPs). Occludin, claudins and zonula occludens are the major TJPs (Daneman and Prat, 2015). After ischemic stroke, damage to tight junctions disrupts the BBB integrity and aggravates cerebral edema.

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent endopeptidase expressed in pericytes, neurons and astrocytes in brain tissue and it has the ability to cleave gelatin (Vafadari et al., 2016). MMP-9 degrades the basement membrane and extracellular matrix of surrounding cells within the extracellular space. It makes a significant contribution to BBB damage (Qin et al., 2019). It has been reported that MMP-9 causes TJP degradation, resulting in BBB disruption, and these alterations can be inhibited by broad-spectrum MMP inhibitors (GM6001) (Rempe et al., 2016; Zhang et al., 2018). However, whether albumin regulates the BBB permeability by indirectly affecting the expression of MMP-9 and TJPs remains to be explained.

The purpose of the present study was to determine if albumin would lower ICP by reducing cerebral edema when albumin elevated the COP of plasma. Furthermore, we explored whether albumin affects BBB permeability and the expression of tight junction proteins.

METHODS

Animals

The rats were fed standard chow and water and housed under standard experimental conditions (temperature, 20-25°C; humidity, 50-70%) with a 12 h light/dark cycle. All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals guidelines and were approved by the Research Ethics Committee of Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China (grant no. GDREC2012106A(R1)).

Under the condition that the sample statistics were satisfied, as few animals were used in the experiments as possible. The estimation of sample size was based on the equation

$$n = \varphi^2(\sum S_i^2/g)/[\sum(\overline{X}_i - \overline{X})^2/(g-1)],$$

where n is the sample size required for each group and g stands for the number of groups. $\overline{X}_i$ and S_i are the means and standard deviations of each group, respectively, from our preliminary experiment. $\overline{X}$ can be calculated as $\overline{X} = \sum\overline{X}_i/g$.

A total of 165 rats were randomly divided into 6 groups: the sham-operated group (sham group), sham-operated+albumin group (Alb group), middle cerebral artery occlusion (MCAO) + normal saline group (MCAO+NS group), MCAO + albumin group (MCAO+Alb group) and MCAO + albumin + GM6001 group (MCAO+Alb+GM6001 group). Rats in the MCAO+NS group, MCAO+Alb group and MCAO+Alb+GM6001 group were subjected to MCAO. Rats in the sham group and Alb group were subjected to all of the same procedures but without occlusion.

The tail vein was cannulated for 25% albumin or normal saline infusion. After suture removal following the 2-h sham operation or MCAO, the rats in the MCAO+NS group, MCAO+Alb group and Alb group

were given normal saline (5 ml/kg) or 25% albumin (1.25 g/kg) by intravenous injection at a constant rate over 3 min (Huh et al., 1998; Yao et al., 2010; Wang et al., 2013). The volume of saline or 25% albumin administered was 0.5 ml/100 g. The rats in the MCAO+Alb+GM6001 group were injected with GM6001 (an MMP-9 inhibitor; MedChemExpress; cat. no. HY-15768) intraperitoneally at 30 mg/kg of body weight every 12 h for 3 doses (Winding et al., 2002; Klohs et al., 2009), starting 12 h before MCAO.

After anesthesia with pentobarbital sodium (30 mg/kg intraperitoneal injection), normal saline perfusion and exsanguination were used for euthanasia when all of the experimental operations were completed.

Rat model of cerebral ischemia

Prior to the surgical procedure, all rats were fasted with access to water overnight. Cerebral ischemia was induced by MCAO as described previously (Salehi et al., 2020; Ding et al., 2021). To minimize suffering and distress, the rats were anesthetized with pentobarbital sodium (30 mg/kg intraperitoneal injection) followed by a midline incision. The right common carotid artery, internal carotid artery and external carotid artery were carefully exposed. A head-end spherical nylon suture was inserted from the external carotid artery into the middle cerebral artery until resistance was felt. The suture remained in place for 2 h, after which it was withdrawn to allow reperfusion. The rats were examined after suture removal. Those did not walk spontaneously and had a depressed level of consciousness, were excluded from the study and euthanized with normal saline perfusion and exsanguination.

Measurement of COP and ICP

COP and ICP were measured after 2 h of MCAO. The right common carotid artery was cannulated to collect arterial blood samples for COP measurement. Two hundred microliter blood samples were collected at 0, 2, 6, 12, 18 and 24 h after surgery. A medical membrane osmometer (Onkometer BMT-923; BMT Messtechnik GMBH) was used to measure the COP. To evaluate the ICP, a midline incision over the vertex was performed following anesthesia with pentobarbital sodium (30 mg/kg intraperitoneal injection), and then a hole caudal to the coronal suture was drilled, 4 mm from the midline. The dura was punctured, and a microsensor for ICP was inserted intraparenchymally (Ding et al., 2021). An ICP monitor (Integra CAMO2; Integra LifeSciences, Ltd.) was used to measure the ICP for 24 h.

Magnetic resonance imaging (MRI)

After anesthesia with pentobarbital sodium (30 mg/kg intraperitoneal injection), MRI measurements were performed 24 h post-MCAO using a 7.0 T scanner (Bruker BioSpin) with a 30 mm diameter rat head coil. T2-weighted MRI was performed with the following parameters: slice thickness (THK) = 1 mm; interslice gap = 0.5 mm, field of view (FOV) = 3.0×3.0 cm^2; matrix = 256×256; repetition time (TR) = 1,000 ms; echo time (TE) = 50 ms. The infarct volume and infarct volume ratio were calculated on T2WI maps using ImageJ software. The infarct volume ratio = infarct volume/whole brain volume. The DTI parameters were THK = 1 mm; interslice gap = 0.5 mm; FOV = 3.0×3.0 cm^2; matrix = 128×128; TR = 3,000 ms; TE = 32 ms; Δ = 20 ms; δ = 4 ms; in-plane image resolution = 250×250 μm^2; NEX = 4, 30 gradient directions; b values = 0, 1,000 s/mm^2. To compensate for the effects of brain swelling, apparent diffusion coefficient (ADC) maps were produced. Relative ADC (rADC) was calculated using the following equation: rADC = (ADC of the infarcted hemisphere/ADC of the contralateral hemisphere) ×100.

Assessment of BBB permeability

Evans blue (EB; 2% solution 4 ml/kg) was injected 1 h prior to sacrifice through the caudal vein. At 24 h after reperfusion, the rats were perfused transcardially with normal saline to remove the intravascular dye, and 4% paraformaldehyde was used to perfuse the brain. The brains were harvested, homogenized, and incubated in formamide (1 ml/100 mg) at 60°C for 24 h. The supernatant was separated after centrifugation at 12,000 × g for 20 min. EB standard solution (100, 50, 25, 12.5, 3.125, 1.5625, 0.78125, 0 μg/ml) was prepared. The optical density (OD) values were measured at 620 nm using a spectrophotometer. The EB content was calculated according to the concentration-OD standard curve.

Western blotting analysis

We collected approximately 100 mg of peri-infarct tissue from rats that underwent MCAO, while the middle cerebral artery blood-supplying area tissue was collected from sham rats. Peri-infarct tissue is defined as the ischemic penumbra, the brain tissue at a level between the thresholds of functional impairment and morphological integrity, presenting with edema and other pathological changes not present in normal brain tissue (Ermine et al., 2021). Total proteins from

the peri-infarcted cerebral cortex (n=5 per group) were extracted using a Total Protein Extraction kit (BestBio; cat. no. BB-3101-100T). The protein concentration was determined using a BCA Protein assay kit (Gibco; Thermo Fisher Scientific, Inc.; cat. no. 23227). Equal quantities of protein from each sample were loaded on a 10% SDS-gel, resolved using SDS-PAGE and transferred to a PVDF membrane. The membranes were blocked using 5% nonfat milk for 1 h at room temperature. Subsequently, the membranes were incubated with one of the following primary antibodies overnight at 4°C: MMP-9 (1:1,000; Abcam; cat. no. Ab76003), ZO-1 (1:1,000; Thermo Fisher Scientific, Inc.; cat. no. SL258826), occludin (1:1,000; Abcam; cat. no. Ab216327) and claudin-5 (1:1,000; Abbkine Scientific Co., Ltd.; cat. no. Abp50990). The membranes were washed the following day and incubated with an HRP-conjugated goat anti-rabbit antibody (1:2,000; Cell Signaling Technology, Inc.; cat. no. 7074S) for 2 h at 4°C. The immunoblots were visualized using a chemiluminescence kit (Bioworld Technology, Inc.; cat. no. AC36131) and detected using an imaging densitometer (ImageQuant LAS 500, GE Healthcare Bio-Sciences). The gray values were quantified using FluorChem 8900 version 4.0.1 (Alpha Innotech Corporation). β-actin was used as the loading control. The relative density was calculated by dividing the gray value of the respective β-actin by that of the target protein.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0 (SPSS, Inc.). All values are expressed as the mean ± standard deviation. Differences between 2 groups were compared using Student's t-test. One-way analysis of variance (ANOVA) was used to analyze the data of 3 or more group univariate-factor measurements. Multiple comparisons were analyzed using Tukey's test if the data were distributed normally; otherwise, they were analyzed using Dunnett's T3 method. Repeated measurement data were analyzed using a repeated-measures ANOVA with LSD *post hoc* analysis. P<0.05 was considered to indicate a statistically significant difference.

RESULTS

Mortality and exclusion rates

A total of 165 rats were used in this experiment. The mortality rate was 19.16%, and 15 (8.98%) rats were ruled out by their inability to walk spontaneously and a depressed level of consciousness.

Effects of albumin on COP and ICP following MCAO

The COP levels of the MCAO+Alb group (0 h, 12.15±0.58 mmHg; 2 h, 19.20±0.47 mmHg; 6 h, 18.00±0.87 mmHg; 12 h, 17.69±1.43 mmHg; 18 h, 16.70±0.65 mmHg; 24 h, 14.79±0.42 mmHg) were significantly higher than the MCAO+NS group (0 h, 11.96±0.23 mmHg; 2 h, 11.99±0.33 mmHg; 6 h, 12.04±0.47 mmHg; 12 h, 12.06±0.42 mmHg; 18 h, 12.08±0.26 mmHg; 24 h, 11.91±0.15 mmHg; MCAO+Alb group *vs.* MCAO+NS group: F_1=1241.21, P=0.00) and sham group (0 h, 12.28±0.51 mmHg; 2 h, 12.31±0.52 mmHg; 6 h, 12.00±0.39 mmHg; 12 h, 12.24±0.82 mmHg; 18 h, 12.30±0.47 mmHg; 24 h, 11.80±0.68 mmHg; MCAO+Alb group *vs.* sham group: $F_{1,95}$=563.49, P<0.001). There was no significant difference between the MCAO+NS group and sham group ($F_{1,95}$=1.15, P<0.01) (Fig. 1A). The exclusion rate and mortality in the MCAO+NS group were 9.09% and 27.27%, respectively, and 10.00% and 20.00% in the MCAO+Alb group, respectively.

The ICP levels of the MCAO+Alb group (0 h, 7.56±0.58 mmHg; 2 h, 12.06±0.95 mmHg; 6 h, 14.31±1.01 mmHg; 12 h, 16.95±0.90 mmHg; 18 h, 19.68±1.71 mmHg; 24 h, 22.48±0.73 mmHg) and MCAO+NS group (0 h, 7.16±0.68 mmHg; 2 h, 11.50±1.43 mmHg; 6 h, 13.59±1.24 mmHg; 12 h, 16.24±0.92 mmHg; 18 h, 18.59±0.92 mmHg; 24 h, 22.76±0.72 mmHg) were significantly increased compared with the sham group (0 h, 7.60±0.79 mmHg; 2 h, 7.95±0.92 mmHg; 6 h, 7.69±0.77 mmHg; 12 h, 7.35±0.56 mmHg; 18 h, 7.51±0.75 mmHg; 24 h, 8.15±1.09 mmHg) (MCAO+Alb group *vs.* sham group: $F_{1,95}$=1227.86, P<0.01; MCAO+NS group *vs.* sham group: $F_{1,95}$=710.62, P<0.01). However, the ICP levels in the MCAO+Alb group did not differ significantly from those in the MCAO+NS group ($F_{1,95}$=2.80, P>0.05) (Fig. 1B). The exclusion rate and mortality in the MCAO+NS group were 8.33% and 33.33%, respectively, while they were 9.09% and 27.27% in the MCAO+Alb group, respectively.

Albumin does not reduce the infarct size following MCAO based on T2WI imaging

T2WI images 24 h after MCAO or sham surgery from NS-treated and albumin-treated rats are shown in Fig. 2A. Quantification of infarct size from T2WI images showed no significant differences between the MCAO+NS group and MCAO+Alb group in the volume ratio of the lesion (35.12±5.07% *vs.* 35.91±6.11%; t_{10}=-0.245, P=0.717; Fig. 2B). The exclusion rate and mortality in the MCAO+NS group were 11.11% and 33.33%, respectively, while they were 12.50% and 25.00% in the MCAO+Alb group, respectively.

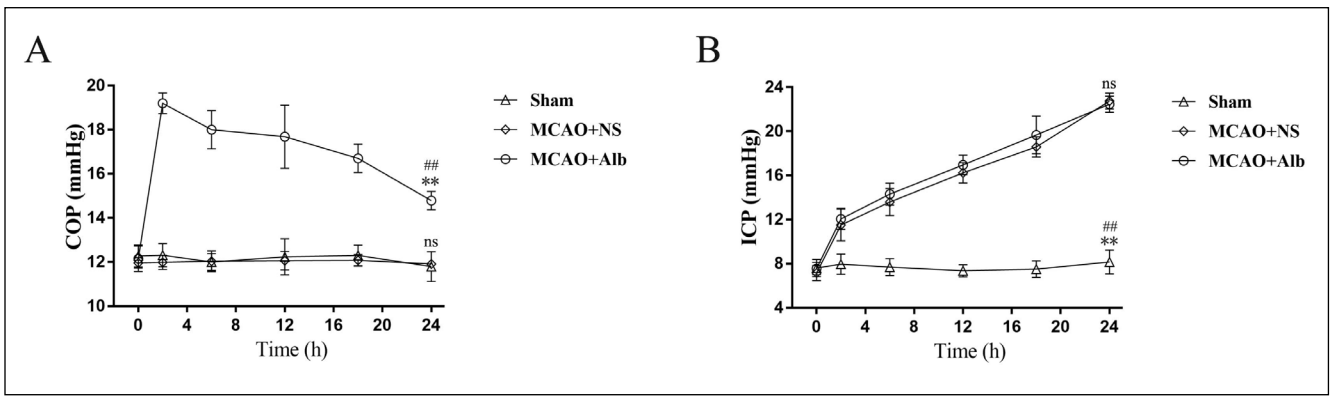

Fig. 1. Effects of albumin on the COP and ICP following MCAO. (A) The COP levels of the MCAO+Alb group were significantly higher compared with the MCAO+NS group and sham group. There were no significant differences between the MCAO+NS group and sham group. $^{ns}P>0.05$, $^{**}P<0.01$ for the MCAO+Alb group *vs.* the sham group, $^{\#\#}P<0.01$ for the MCAO+Alb group *vs.* the MCAO+NS group. (B) The ICP levels of the MCAO+Alb group and MCAO+NS group were significantly increased compared with the sham group. However, the ICP levels in the MCAO+Alb group did not differ significantly compared with the MCAO+NS group. $^{ns}P>0.05$, $^{**}P<0.01$ for the MCAO+Alb group *vs.* the sham group, $^{\#\#}P<0.01$ for the MCAO+NS group *vs.* the MCAO+sham group. n=8. (COP), colloid osmotic pressure; (ICP), intracranial pressure; (MCAO), middle cerebral artery occlusion; (NS), normal saline; (Alb), albumin.

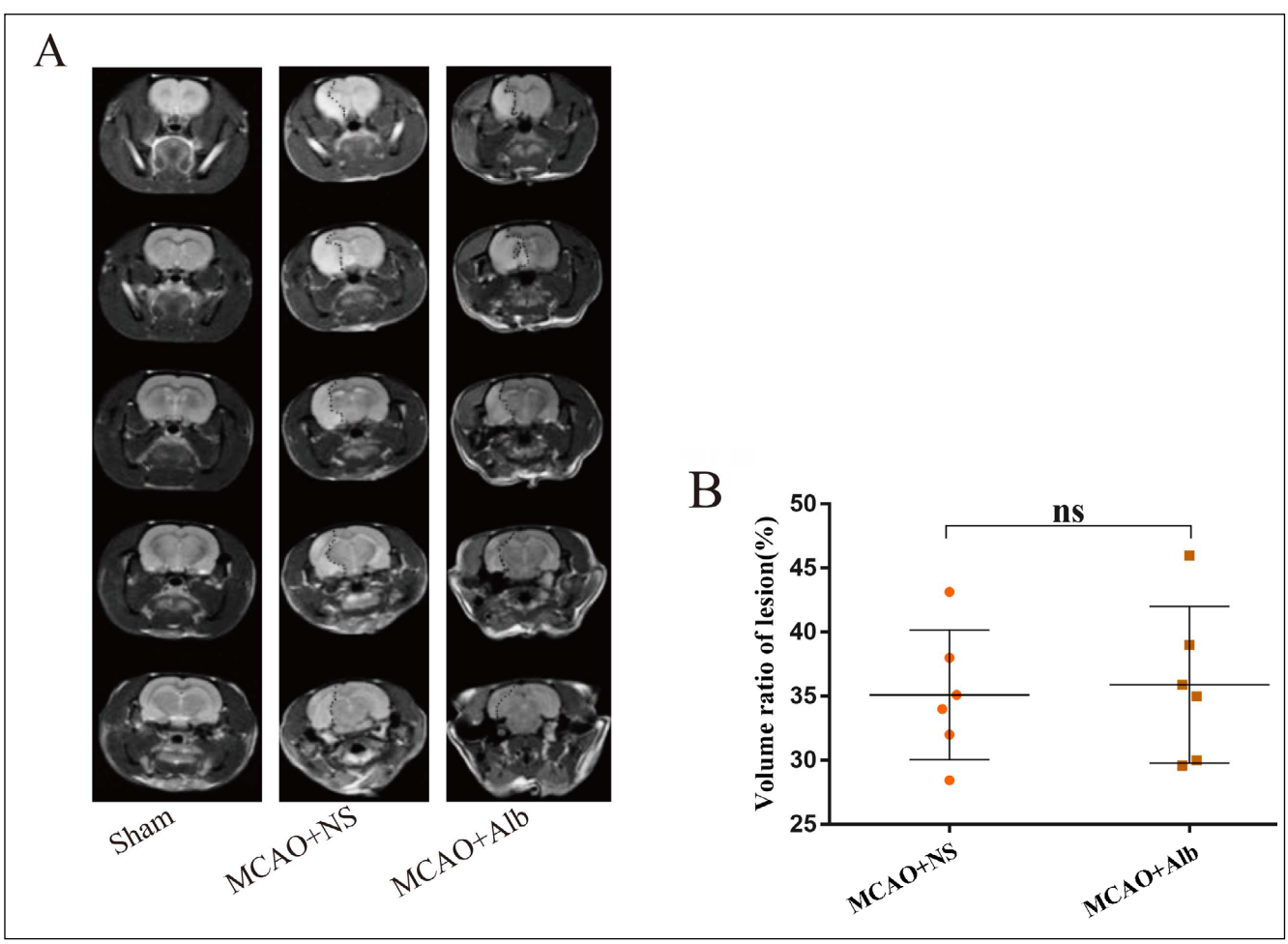

Fig. 2. Albumin does not reduce the infarct size following MCAO. (A) T2WI images 24 h after MCAO or sham surgery from the NS- and albumin-treated rats. (B) Quantification of the infarct size from the T2WI images. There was no significant difference between the MCAO+NS group and MCAO+Alb group for the volume ratio of the lesion. n=6. The data are expressed as the mean ± standard deviation. $^{ns}P>0.05$. (MCAO), middle cerebral artery occlusion; (NS), normal saline; (Alb), albumin.

Albumin aggravates cerebral edema following MCAO based on ADC images

ADC images 24 h after MCAO or sham surgery from NS-treated and albumin-treated rats are shown in Fig. 3A. There were significant differences in the rADC levels among the MCAO+Alb group, the MCAO+NS group and the sham group, as shown by one-way ANOVA ($F_{2,95}$=154.95, P<0.01). The rADC levels in the MCAO+NS group were significantly lower compared with the sham group (0.70±0.05 *vs.* 1.14±0.07; P=0.00). The rADC levels in the MCAO+Alb group were significantly lower than those in the sham group (0.56±0.06 *vs.* 1.14±0.07; P=0.00) and MCAO+NS group (0.56±0.06 *vs.* 0.70±0.05; P=0.00; Fig. 3B). The exclusion rate and mortality in the MCAO+NS group were 12.50% and 25.00%, respectively, while they were 28.57% and 14.29% in the MCAO+Alb group, respectively.

Albumin increases BBB permeability to EB after MCAO

Among the MCAO+Alb group, MCAO+NS group, Alb group and sham group, the concentration of EB in the brain tissue differed significantly according to one-way ANOVA ($F_{3,95}$=215.97, P<0.01). There was no significant difference between the Alb group and sham group in the concentration of EB in the brain tissue (1.10±0.28 μg/g *vs.* 1.00±0.30 μg/g; P=0.63). The concentration in the MCAO+NS group was significantly higher than that in the sham group (3.80±0.37 μg/g *vs.* 1.00±0.30 μg/g; P=0.00). The concentration of EB in the brain tissue of the MCAO+Alb group was significantly higher than that of the MCAO+NS group (5.12±0.38 μg/g *vs.* 3.80±0.37 μg/g; P=0.00) (Fig. 4). The exclusion rate and mortality in the MCAO+NS group were 11.11% and 33.33%, respectively, while they were 12.50% and 25.00% in the MCAO+Alb group, respectively.

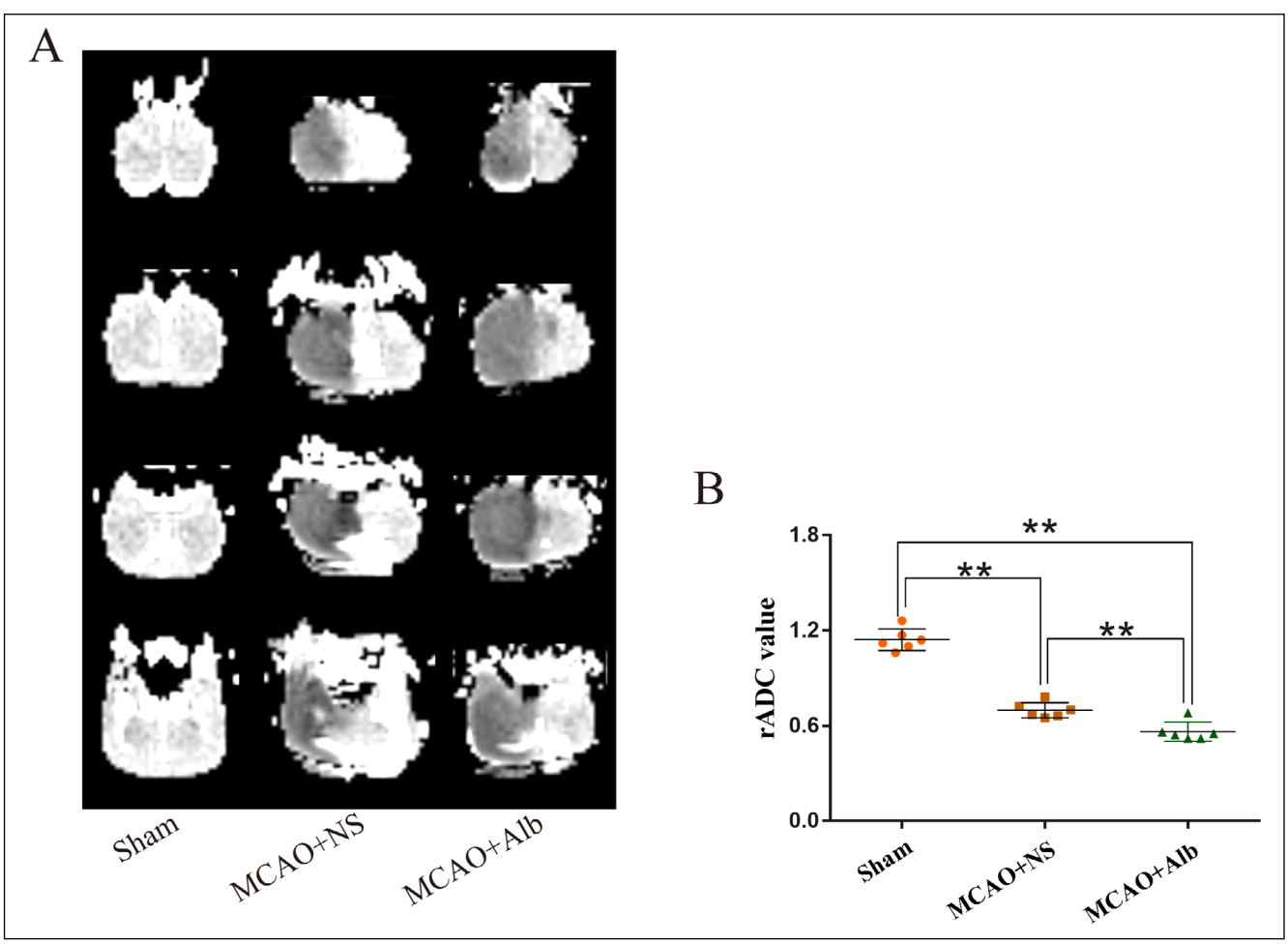

Fig. 3. Albumin aggravates brain edema following MCAO. (A) ADC images 24 h after MCAO or sham surgery from NS- and albumin-treated rats. (B) rADC levels in the MCAO+NS group were significantly lower compared with the sham group. The rADC levels in the MCAO+Alb group were significantly lower compared with the sham group and the MCAO+NS group. Data are expressed as the mean ± standard deviation. **P<0.01. n=6. (MCAO), middle cerebral artery occlusion; (NS), normal saline; (Alb), albumin; (ADC), apparent diffusion coefficient; (rADC), relative ADC.

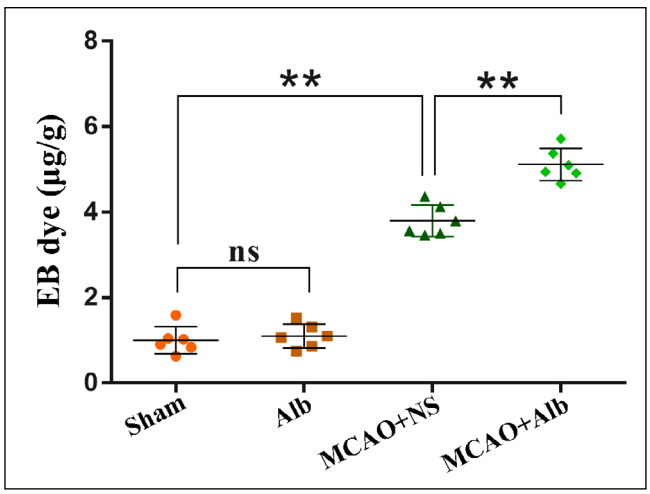

Fig. 4. Albumin increases blood-brain barrier permeability to EB following MCAO. There was no significant difference in the concentration of EB in the brain tissue between the Alb and sham groups. The concentration in the MCAO+NS group was significantly higher than that in the sham group. The concentration of EB in the brain tissue of the MCAO+Alb group was significantly higher compared with the sham group and MCAO+NS group. Data are expressed as the mean ± standard deviation. $^{ns}P>0.05$, $^{**}P<0.01$. n=6. (EB), Evans blue; (MCAO), middle cerebral artery occlusion; (NS), normal saline; (Alb), albumin.

Albumin decreases the expression of tight junction proteins through upregulation of MMP-9

The expression levels of MMP-9, ZO-1, occludin, and claudin-5 24 h after stroke were analyzed. One-way ANOVA showed that the expression of MMP-9 was significantly different among the Alb group and the sham group, MCAO+NS group, and MCAO+Alb group ($F_{3,95}$=86.51, P<0.01). The expression of MMP-9 did not differ significantly between the Alb group and sham group (0.13±0.04 *vs.* 0.12±0.05; P=0.98). The expression of MMP-9 in the MCAO+NS group was significantly higher compared with the sham group (0.42±0.11 *vs.* 0.12±0.05; P=0.00). The expression of MMP-9 in the MCAO+Alb group was significantly higher compared with the MCAO+NS group (0.92±0.13 *vs.* 0.42±0.11; P=0.00; Fig. 5A and B). Significant differences in TJP expression were observed among the Alb group, sham group, MCAO+NS group, and MCAO+Alb group (ZO-1, F_4=126.02, P=0.00; occludin, $F_{4,95}$=145.26, P<0.01; claudin-5, $F_{4,95}$=142.80, P<0.01). Tight junction protein expression did not differ significantly between the Alb group and sham group (ZO-1, 0.97±0.09 *vs.* 0.98±0.08, P=0.90; occludin, 1.22±0.11 *vs.* 1.19±0.12, P=0.57; claudin-5, 0.87±0.08 *vs.* 0.86±0.07; P=0.74). Compared with the sham group, the expression of tight junction proteins was significantly decreased in the MCAO+NS group (ZO-1, 0.42±0.07 *vs.* 0.98±0.08, P=0.00; occludin, 0.40±0.08 *vs.* 1.19±0.12, P=0.00; claudin-5, 0.33±0.06 *vs.* 0.86±0.07, P=0.00). The expression of tight junction proteins was significantly decreased in the MCAO+Alb group compared with the MCAO+NS group (ZO-1, 0.12±0.05 *vs.* 0.42±0.07, P=0.00, occludin, 0.10±0.06 *vs.* 0.40±0.08, P=0.00; claudin-5, 0.08±0.03 *vs.* 0.33±0.06, P=0.00). Tight junction protein expression was significantly upregulated following GM6001 treatment (ZO-1, 0.43±0.08 *vs.* 0.12±0.05, P=0.00; occludin, 0.44±0.09 *vs.* 0.1±0.06, P=0.00; claudin-5; 0.41±0.07 *vs.* 0.08±0.03, P=0.00; Fig. 5C-F). The exclusion rate and mortality were 12.50% and 37.50% in the MCAO+NS group, respectively; 28.57% and 28.57% in the MCAO+Alb group, respectively; and 14.29% and 28.57% in the MCAO+Alb+GM6001 group, respectively.

DISCUSSION

In the present study, it was shown that 25% albumin treatment by intravenous injection elevated the COP of plasma but did not reduce the ICP. Additionally, albumin aggravated cerebral edema and increased BBB permeability in rats with ischemia.

Osmotherapy has been used as a basic treatment to manage ICP induced by cerebral edema. Exogenous human serum albumin has been reported to be neuroprotective by alleviating brain swelling, preventing postischemic thrombosis, providing antioxidant activity and hemodilution and increasing perfusion to ischemic tissue (Prajapati et al., 2011). However, whether albumin decreases ICP following ischemic stroke has not been assessed previously. The present study showed that 25% albumin treatment (1.25 g/kg) by intravenous injection elevated the COP of plasma but did not reduce the ICP. A possible explanation for this is that COP contributes to only a small proportion of the total serum osmolality. It has been reported that the proportion of osmotic pressure of electrolytes is ~95%, whereas serum proteins contribute <0.5% of the total serum osmolality (Rasouli, 2016). This, therefore, may explain the effect of hypertonic saline, rather than albumin, on ICP reduction (Zeng et al., 2010; Huang et al., 2014; Maguigan et al., 2017).

The MRI ADC images showed that 25% albumin treatment aggravated cerebral edema. The BBB integrity is disrupted after ischemic stroke, resulting in increased BBB permeability, and albumin may enter the interstitial space of the brain through the disrupted BBB. This may explain why albumin treatment did not reduce the ICP in the present study. Given its aggravating effect on cerebral edema, 25% albumin treatment may potentially increase the ICP. This result is contradictory to previous studies. However, higher doses or more frequent dosing were used to increase

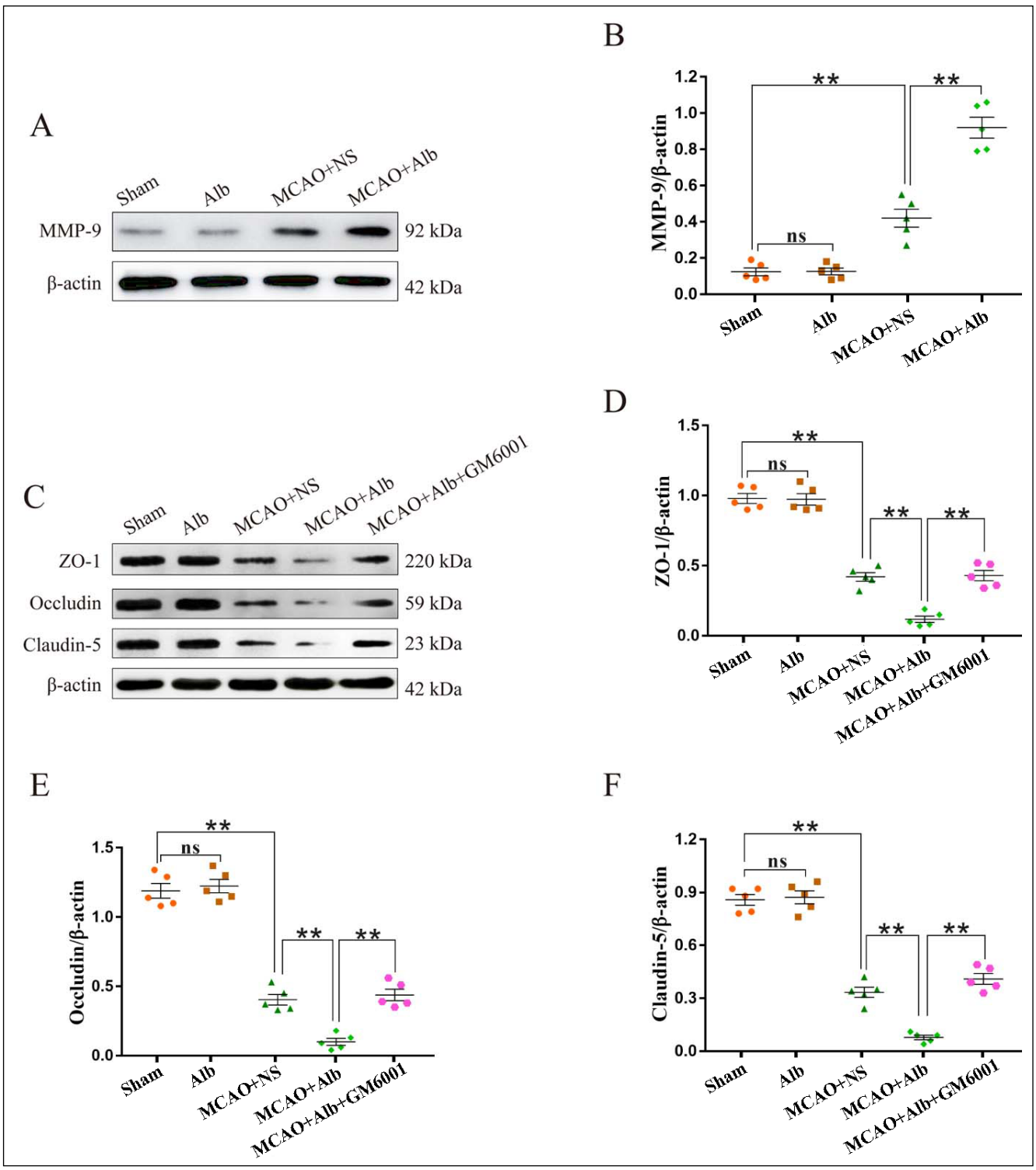

Fig. 5. Albumin decreases the expression of tight junction proteins 24 h after MCAO. (A, C) western blots of MMP-9 (92 kDa), ZO-1 (220 kDa), occludin (59 kDa), claudin-5 (23 kDa), and β-actin (42 kDa). (B) Expression of MMP-9 did not differ significantly between the Alb and sham groups. The expression of MMP-9 in the MCAO+NS group was significantly higher than that in the sham group. The expression of MMP-9 in the MCAO+Alb group was significantly higher compared with the MCAO+NS group. (D-F) The expression of tight junction proteins did not differ significantly between the Alb group and the sham group. In comparison with the sham group, the expression of tight junction proteins was significantly decreased in the MCAO+NS group. The expression of tight junction proteins was significantly decreased in the MCAO+Alb group compared with the MCAO+NS group. Tight junction protein expression was significantly upregulated following GM6001 treatment. $^{ns}P>0.05$, $^{**}P<0.01$. n=5. (MCAO), middle cerebral artery occlusion; (NS), normal saline; (Alb), albumin.

or maintain serum albumin levels in previous studies (Belayev et al., 1998; Yao et al., 2010). It has been reported that prolonged administration of high-dose albumin (2 g/kg per day for 3 days) to rats with ischemia can exert some beneficial effects (Matsui et al., 1993). The intervention time was longer and the dosage level of albumin was higher in previous studies than in the present study (1.25 g/kg once). Besides, albumin was reported to exert its protective effect associated with reversing stagnation, thrombosis, and corpuscular adherence within cortical venules in the reperfusion phase (Belayev et al., 2002). Therefore, we suppose that in the stroke with a larger infarction size the extent to which albumin can improve hemodynamics is reduced in the reperfusion phase, resulting in albumin not being beneficial for alleviating increased ICP. But whether the infarction size determines the albumin treatment effect still remains unexplained. Additionally, albumin treatment did not improve the functional or neurological outcomes of patients with acute ischemic stroke in a randomized, double-blind, placebo-controlled trial (Ginsberg et al., 2013). However, the situation in clinical studies is relatively more complicated, where the outcome of patients after acute ischemic stroke is related to the volumes of intravenous fluids, combinations of drugs, and the time window for recruitment (Bath, 2013).

It has been reported that BBB disruption contributes to the infiltration of neurotoxic compounds and leukocytes from the blood into the brain (Ryu et al., 2015), which could result in neuroinflammation and cerebral edema (Jiang et al., 2018). Furthermore, the disruption of tight junctions, including the expression of ZO-1, occludin and claudin-5, may have resulted in increased permeability of the BBB (Ryu et al., 2015). In the present study, it was shown that 25% albumin treatment upregulated MMP-9 expression. Albumin has been reported to activate astrocytes to release MMP-9, which could be the reason for the increase in MMP-9 expression caused by albumin treatment in our study (Ralay Ranaivo et al., 2012). Additionally, our study's results implied that upregulated MMP-9 significantly downregulated the expression of tight junction proteins and increased the permeability of the blood-brain barrier, which is similar to the findings of previous studies (Dhanda and Sandhir, 2018; Pan et al., 2021). Tight junction protein expression is significantly upregulated in response to MMP-9 inhibitors. These results suggest that the deleterious effects of albumin on cerebral edema are associated with the impairment of tight junctions of cerebral vascular endothelial cells and increased BBB permeability.

There are three limitations to the present study. First, the serum albumin levels were not comparable between rats and humans. In the present study, 25% human albumin treatment by intravenous injection elevated the COP of plasma; however, it aggravated cerebral edema in rats with cerebral infarcts from ischemic damage. This result was in contrast to previous studies, but a lower albumin dosage and administration frequency were used compared with the earlier studies (Belayev et al., 1998; Yao et al., 2010). In a previous report, albumin was shown to exert neuroprotection only with residual cortical cerebral blood flow between 25% and 50% during ischemia (Chen et al., 2009). Determining the change in the cerebral microcirculation following cerebral ischemia and the effect of albumin on the cerebral microcirculation may provide more support for the results of the present study. Second, the mechanism by which albumin downregulated tight junction protein expression and increased BBB permeability was not established. Therefore, if the specific pathways by which albumin can regulate tight junction proteins are identified, the results of the present study should be validated. Third, although albumin worsened the cerebral edema, increased ICP was not observed following albumin treatment. The effects of albumin on COP and brain edema are possibly associated with its dosage. However, different albumin concentrations were not administered in our study. The COP increases as the dose of albumin is increased. The dose of albumin administered should be considered and evaluated further to provide evidence to support the effect of albumin on ICP elevation.

CONCLUSIONS

The results of the present study demonstrated that the administration of albumin is not beneficial for controlling cerebral edema following cerebral infarction. These preliminary findings suggest that albumin treatment for reducing ICP or relieving cerebral edema after ischemic stroke may not be a suitable choice.

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REFERENCES

Bath PM (2013) Albumin for hyperacute stroke: another failed neuroprotectant. Lancet Neurol 12: 1036–1037.

Beez T, Steiger HJ (2019) Impact of randomized controlled trials on neurosurgical practice in decompressive craniectomy for ischemic stroke. Neurosurg Rev 42: 133–137.

Belayev L, Zhao W, Pattany PM, Weaver RG, Huh PW, Lin B, Busto R, Ginsberg MD (1998) Diffusion-weighted magnetic resonance imaging confirms marked neuroprotective efficacy of albumin therapy in focal cerebral ischemia. Stroke 29: 2587–2599.

Belayev L, Liu Y, Zhao W, Busto R, Ginsberg MD (2001) Human albumin therapy of acute ischemic stroke: marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. Stroke 32: 553–560.

Belayev L, Pinard E, Nallet H, Seylaz J, Liu Y, Riyamongkol P, Zhao W, Busto R, Ginsberg MD (2002) Albumin therapy of transient focal cerebral ischemia: In vivo analysis of dynamic microvascular responses. Stroke 33: 1077–1084.

Caltagirone C, Cisari C, Schievano C, Di Paola R, Cordaro M, Bruschetta G, Esposito E, Cuzzocrea S (2016) Co-ultramicronized palmitoylethanolamide/luteolin in the treatment of cerebral ischemia: from rodent to man. Transl Stroke Res 7: 54–69.

Changa AR, Czeisler BM, Lord AS (2019) Management of elevated intracranial pressure: a review. Curr Neurol Neurosci Rep 19: 99.

Chen J, Ji X, Ding Y, Luo Y, Cheng H, Ling F (2009) Role of residual flow on the neuroprotective efficacy of human albumin in the rat with transient cerebral ischemia. Neurol Res 31: 396–401.

Daneman R, Prat A (2015) The blood-brain barrier. Cold Spring Harb Perspect Biol 7: a020412.

Decker D, Collier L, Lau T, Olivera R, Roma G, Leonardo C, Seifert H, Rowe D, Pennypacker KR (2016) The effects of clinically relevant hypertonic saline and conivaptan administration on ischemic stroke. Acta Neurochir Suppl 121: 243–250.

Dhanda S, Sandhir R (2018) Blood-brain barrier permeability is exacerbated in experimental model of hepatic encephalopathy via MMP-9 activation and downregulation of tight junction proteins. Mol Neurobiol 55: 3642–3659.

Ding H, Li Y, Wen M, Liu X, Han Y, Zeng H (2021) Elevated intracranial pressure induces IL-1β and IL-18 overproduction via activation of the NLRP3 inflammasome in microglia of ischemic adult rats. Int J Mol Med 47: 183–194.

Ermine CM, Bivard A, Parsons MW, Baron JC (2021) The ischemic penumbra: From concept to reality. Int J Stroke 16: 497–509.

Erstad BL (2018) Albumin disposition in critically Ill patients. J Clin Pharm Ther 43: 746–751.

Freeman WD (2015) Management of intracranial pressure. Continuum 21: 1299–1323.

Ginsberg MD, Palesch YY, Hill MD, Martin RH, Moy CS, Barsan WG, Waldman BD, Tamariz D, Ryckborst KJ (2013) High-dose albumin treatment for acute ischaemic stroke (ALIAS) Part 2: A randomised, double-blind, phase 3, placebo-controlled trial. Lancet Neurol 12: 1049–1058.

Hill MD, Martin RH, Palesch YY, Moy CS, Tamariz D, Ryckborst KJ, Jones EB, Weisman D, Pettigrew C, Ginsberg MD (2015) Albumin administration in acute ischemic stroke: Safety analysis of the ALIAS part 2 multicenter trial. PloS One 10: e131390.

Horvathy DB, Simon M, Schwarz CM, Masteling M, Vacz G, Hornyak I, Lacza Z (2017) Serum albumin as a local therapeutic agent in cell therapy and tissue engineering. Biofactors 43: 315–330.

Huang LQ, Zhu GF, Deng YY, Jiang WQ, Fang M, Chen CB, Cao W, Wen MY, Han YL, Zeng HK (2014) Hypertonic saline alleviates cerebral edema by inhibiting microglia-derived TNF-alpha and IL-1beta-induced Na-K-Cl cotransporter up-regulation. J Neuroinflammation 11: 102.

Huang Y, Xiao Z (2021) Albumin therapy for acute ischemic stroke: a meta-analysis. Neurol Sci 42: 2713–2719.

Huh PW, Belayev L, Zhao W, Busto R, Saul I, Ginsberg MD (1998) The effect of high-dose albumin therapy on local cerebral perfusion after transient focal cerebral ischemia in rats. Brain Res 804: 105–113.

Jeon SB, Koh Y, Choi HA, Lee K (2014) Critical care for patients with massive ischemic stroke. J Stroke 16: 146–160.

Jiang X, Andjelkovic AV, Zhu L, Yang T, Bennett MVL, Chen J, Keep RF, Shi Y (2018) Blood-brain barrier dysfunction and recovery after ischemic stroke. Prog Neurobiol 163–164: 144–171.

Khan NA, McAlister FA, Pilote L, Palepu A, Quan H, Hill MD, Fang J, Kapral MK (2017) Secondary prevention treatment after acute stroke in older South Asian, Chinese and other Canadians: A retrospective data analysis. CMAJ Open 5: E702-E709.

Klohs J, Baeva N, Steinbrink J, Bourayou R, Boettcher C, Royl G, Megow D, Dirnagl U, Priller J, Wunder A (2009) In vivo near-infrared fluorescence imaging of matrix metalloproteinase activity after cerebral ischemia. J Cereb Blood Flow Metab 29: 1284–1292.

Lee SW, Kim WJ, Jun HO, Choi YK, Kim KW (2009) Angiopoietin-1 reduces vascular endothelial growth factor-induced brain endothelial permeability via upregulation of ZO-2. Int J Mol Med 23: 279–284.

Maguigan KL, Dennis BM, Hamblin SE, Guillamondegui OD (2017) Method of hypertonic saline administration: Effects on osmolality in traumatic brain injury patients. J Clin Neurosci 39: 147–150.

Matsui T, Sinyama H, Asano T (1993) Beneficial effect of prolonged administration of albumin on ischemic cerebral edema and infarction after occlusion of middle cerebral artery in rats. Neurosurgery 33: 293–300.

Michinaga S, Koyama Y (2015) Pathogenesis of brain edema and investigation into anti-edema drugs. Int J Mol Sci 16: 9949–9975.

Pallesen LP, Barlinn K, Puetz V (2018) Role of decompressive craniectomy in ischemic stroke. Front Neurol 9: 1119.

Pan P, Li G, Shen M, Yu Z, Ge W, Lao Z, Fan Y, Chen K, Ding Z, Wang W, Wan P, Shereen MA, Luo Z, Chen X, Zhang Q, Lin L, Wu J (2021) DENV NS1 and MMP-9 cooperate to induce vascular leakage by altering endothelial cell adhesion and tight junction. PLoS Pathog 17: e1008603.

Prajapati KD, Sharma SS, Roy N (2011) Current perspectives on potential role of albumin in neuroprotection. Rev Neurosci 22: 355–363.

Qin W, Li J, Zhu R, Gao S, Fan J, Xia M, Zhao RC, Zhang J (2019) Melatonin protects blood-brain barrier integrity and permeability by inhibiting matrix metalloproteinase-9 via the NOTCH3/NF-κB pathway. Aging 11: 11391–11415.

Rabbani G, Ahn SN (2019) Structure, enzymatic activities, glycation and therapeutic potential of human serum albumin: A natural cargo. Int J Biol Macromol 123: 979–990.

Ralay Ranaivo H, Hodge JN, Choi N, Wainwright MS (2012) Albumin induces upregulation of matrix metalloproteinase-9 in astrocytes via MAPK and reactive oxygen species-dependent pathways. J Neuroinflammation 9: 68.

Rasouli M (2016) Basic concepts and practical equations on osmolality: Biochemical approach. Clin Biochem 49: 936–941.

Rempe RG, Hartz AMS, Bauer B (2016) Matrix metalloproteinases in the brain and blood-brain barrier: Versatile breakers and makers. J Cereb Blood Flow Metab 36: 1481–1507.

Ryu JK, Petersen MA, Murray SG, Baeten KM, Meyer-Franke A, Chan JP, Vagena E, Bedard C, Machado MR, Rios Coronado PE, Prod'homme T, Charo IF, Lassmann H, Degen JL, Zamvil SS, Akassoglou K (2015) Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. Nat Commun 6: 8164.

Salehi MS, Pandamooz S, Safari A, Jurek B, Tamadon A, Namavar MR, Dianatpour M, Dargahi L, Azarpira N, Fattahi S, Shid Moosavi SM, Keshavarz S, Khodabandeh Z, Zare S, Nazari S, Heidari M, Izadi S, Poursadeghfard M, Borhani-Haghighi A (2020) Epidermal neural crest stem cell transplantation as a promising therapeutic strategy for ischemic stroke. CNS Neurosci Ther. 26: 670–681.

Shah A, Almenawer S, Hawryluk G (2019) Timing of decompressive craniectomy for ischemic stroke and traumatic brain injury: A review. Front Neurol 10: 11.

Shah S, Kimberly WT (2016) Today's approach to treating brain swelling in the neuro intensive care unit. Semin Neurol 36: 502–507.

Smith WS (2019) Endovascular stroke therapy. Neurotherapeutics 16: 360–368.

Su Y, Liu Y, Chen Z, Cui L (2021) Comparison of equiosmolar doses of 10% hypertonic saline and 20% mannitol for controlling intracranial hypertention in patients with large hemispheric infarction. Clin Neurol Neurosurg 200: 106359.

Tuttolomondo A, Pecoraro R, Arnao V, Maugeri R, Iacopino DG, Pinto A (2015) Developing drug strategies for the neuroprotective treatment of acute ischemic stroke. Expert Rev Neurother 15: 1271–1284.

Vafadari B, Salamian A, Kaczmarek L (2016) MMP-9 in translation: from molecule to brain physiology, pathology, and therapy. J Neurochem Suppl 2: 91–114.

Walcott BP, Kahle KT, Simard JM (2012) Novel treatment targets for cerebral edema. Neurotherapeutics 9: 65–72.

Wang M, Wang Y, He J, Wei S, Zhang N, Liu F, Liu X, Kang Y, Yao X (2013) Albumin induces neuroprotection against ischemic stroke by altering Toll-like receptor 4 and regulatory T cells in mice. CNS Neurol Disord Drug Targets 12: 220–227.

Winding B, NicAmhlaoibh R, Misander H, Høegh-Andersen P, Andersen TL, Holst-Hansen C, Heegaard AM, Foged NT, Brünner N, Delaissé JM (2002) Synthetic matrix metalloproteinase inhibitors inhibit growth of established breast cancer osteolytic lesions and prolong survival in mice. Clin Cancer Res 8: 1932.

Wei CC, Zhang ST, Tan G, Zhang SH, Liu M (2018) Impact of anemia on in-hospital complications after ischemic stroke. Eur J Neurol 25: 768–774.

Yao X, Miao W, Li M, Wang M, Ma J, Wang Y, Miao L, Feng H (2010) Protective effect of albumin on VEGF and brain edema in acute ischemia in rats. Neurosci Lett 472: 179–183.

Zeng HK, Wang QS, Deng YY, Fang M, Chen CB, Fu YH, Jiang WQ, Jiang X (2010) Hypertonic saline ameliorates cerebral edema through downregulation of aquaporin-4 expression in the astrocytes. Neuroscience 166: 878–885.

Zhang S, An Q, Wang T, Gao S, Zhou G (2018) Autophagy- and MMP-2/9-mediated reduction and redistribution of ZO-1 contribute to hyperglycemia-increased blood-brain barrier permeability during early reperfusion in stroke. Neuroscience 377: 126–137.