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ORIGINAL ARTICLE

Magnesium sulfate protects blood–brain barrier integrity and reduces brain edema after acute ischemic stroke in rats

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Abstract

Brain edema is a fatal complication of acute ischemic stroke and associated with worse outcomes in patients. This study was designed to evaluate the effects of magnesium sulfate on vasogenic brain edema formation and blood–brain barrier (BBB) disruption caused by ischemia-reperfusion (IR) in a rat model of ischemic stroke. A total of 72 male Sprague-Dawley rats were categorized into the following three primary groups: sham, control ischemic, magnesium-sulfate-treated (300 mg/kg loading dose, followed by an additional 100 mg/kg) ischemic ($n = 24$ in each group). Transient focal cerebral ischemia was induced by 60-min-long occlusion of the left middle cerebral artery, followed by 24-h-long reperfusion. Sensorimotor deficits, infarct volume, and brain edema were evaluated at the end of the reperfusion period. The BBB permeability was assessed by Evans Blue extravasation technique. Lipid peroxidation levels were assessed by measuring the malondialdehyde content in the brain tissue homogenate, and the activities of the antioxidant enzymes superoxide dismutase, catalase, and glutathione peroxidase were detected according to the technical manual of the assay kits. Induction of cerebral ischemia in the control group produced considerable BBB damage in conjunction with severe brain edema formation. Treatment with magnesium sulfate significantly attenuated brain edema and protected BBB integrity in the ischemic lesioned hemisphere. In addition, magnesium sulfate reduced lipid peroxidation and increased antioxidant protection of brain tissue by upregulating the activities of antioxidant enzymes. Treatment with magnesium sulfate protected BBB integrity against IR-induced damage and reduced vasogenic edema formation partly via antioxidant mechanisms in a rat model of acute ischemic stroke.

Keywords Stroke · Brain edema · Magnesium sulfate · Superoxide dismutase · Catalase · Malondialdehyde

Introduction

Brain edema is a fatal complication of ischemic stroke that causes herniation and death in several patients with severe brain injuries (Brouns et al. 2011). Ischemic brain edema is a combination of cytotoxic and vasogenic types. Cytotoxic edema initially develops due to disturbances in ionizing mechanisms in the cell membrane and is reversible, whereas vasogenic edema develops within the first few hours after stroke and continues for several days (Ayata and Ropper 2002). After ischemia-reperfusion (IR)-induced brain injuries, endothelial tight junctions are disrupted by oxidative stress and inflammatory cytokines are produced by activated astrocytes (Michinaga and Koyama 2015). Cerebrovascular damage could participate in hemorrhagic transformation, thereby limiting the safety and therapeutic time window of recombinant tissue plasminogen activator (r-tPA) that has been accepted for the treatment of patients with acute ischemic stroke (Wardlaw et al. 2012). Therefore, protecting the BBB against stroke-induced destruction and preventing the development of vasogenic brain edema can protect the brain from further damage and improve the patient's outcome (Michinaga and Koyama 2017).

The blood–brain barrier (BBB) is composed of endothelial cells of brain microvessels. This structure represents a dynamic interface that controls the trafficking of molecules between circulation and brain tissue. Under ischemic stroke conditions and subsequent reperfusion time, several pathological

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mechanisms such as inflammation, oxidative stress, enzymatic activity, and angiogenesis decrease BBB integrity and contribute to the development of vasogenic brain edema (Sandoval and Witt 2008). Oxidative stress is an important pathological contributor in vasogenic edema caused by uncontrolled production of reactive oxygen species in the brain tissue. Superoxide and hydroxyl radicals can damage cellular macromolecules and disrupt endothelial cell tight junctions, resulting in increased BBB permeability (Schreibelt et al. 2007). Previous studies have suggested that antioxidants protect BBB against ischemic damage by the inhibition of oxidative stress (Fagan et al. 2004; Margaill et al. 2005).

Magnesium (Mg^{2+}) is well known for its multiple critical cellular functions. It has been reported that Mg^{2+}concentration decreases in the brain tissue following ischemic brain injury, and reduction of Mg^{2+} level can be considered as an injury factor in the brain due to serious biological and metabolic dysfunctions (Kaya and Ahishali 2011). Previous studies have suggested that treatment with Mg^{2+}can reduce brain injury and improve motor and cognitive deficits (Westermaier et al. 2003; Zhou et al. 2003). Furthermore, magnesium sulfate treatment produced improvement against spinal cord (Gok et al. 2007) and traumatic brain injuries (Esen et al. 2003). Based on a review of the literature, we found no reports about the effects of magnesium sulfate on BBB integrity and brain edema formation after ischemic stroke. Therefore, this study was designed to evaluate the effects of treatment with magnesium sulfate on vasogenic brain edema formation, BBB integrity, and related mechanisms in a rat model of ischemic stroke.

Methods

Animal preparation

Male Sprague-Dawley rats (weighing 320–370 g) were obtained from the central animal house facility of Ardabil University of Medical Sciences (Ardabil, Iran). All study protocols were approved by the institutional animal ethics committee of Ardabil University of Medical Sciences, which follows the NIH guidelines for care and use of animals (ethical number: IR.ARUMS.REC.1397.014). Anesthesia was induced by an intraperitoneal (IP) injection of 10% solution of chloral hydrate (Fluka, Germany, 400 mg/kg). A local anesthetic, lidocaine hydrochloride 2% solution (SAMEN Co, Iran,0.2 ml), was locally spread into the surgical area and repeated at the end of surgery for analgesia. Transient focal cerebral ischemia was induced by 60-min left middle cerebral artery occlusion (MCAO) using intraluminal filament method, followed by a 24-h reperfusion period (Panahpour et al. 2014). MCAO was accepted by an 80% decrease in regional cerebral blood flow recorded by laser Doppler flowmeter pencil probe (MNP100, AD instrument, Australia). Body temperature was maintained at 37 ± 0.5 °C using a heating feedback control system during surgery.

Experimental protocol

Three primary groups of animals were randomly selected and examined in this study. *Group1* (sham; $n = 24$) animals received the surgery at the neck region without being exposed to MCAO and received the vehicle (0.9% NaCl solution, IP injection) 60 min after the beginning of surgery and 20 min later. *Group 2* (control ischemic; n = 24) animals underwent brain ischemia by 60-min MCAO, followed by 24-h reperfusion and received the vehicle at the end of MCAO and 20 min later. *Group 3* (magnesium-sulfate-treated ischemic rats; n = 24) rats underwent ischemia and reperfusion in a similar manner as that of the control group and received IP injection of magnesium sulfate ($MgSO_4.7H_2O$, Merck, Germany) of 300 mg/kg loading dose at the end of MCAO, followed by an additional 100 mg/kg 20 min later. Magnesium sulfate was dissolved in 0.9% NaCl solution (300 mg/5 ml = 0.24 mmol/ml, 487.7 mOsm/l) (Kaya et al. 2004).

Three subgroups of animals ($n = 8$) were examined in each primary group. The first subgroup of animals was used for the assessment of sensorimotor deficits, infarct size, and edema formation. The BBB permeability was evaluated in the second set of animals. The third subgroupof animals was used for the measurement of lipid peroxidation and the activities of antioxidant enzymes. Systolic blood pressure (SBP) was recorded in randomly selected animals from each group using a noninvasive blood pressure system for rodents (AD Instruments, Australia) before and after the injection of vehicle or magnesium sulfate.

Assessment of neurological sensorimotor deficits

Neurological sensorimotor deficits were evaluated 24 h after the surgery by a blinded observer using a six-point scale test previously described by Longa et al. (Longa et al. 1989) with some modifications (Table 1). In addition, quantitative measurement of strength was used to monitor changes in motor function caused due to ischemic stroke. Contralateral (right) forelimb motor paralysis was assessed using the grip strength test in animals before surgery and again 24 h after MCAO. Three consecutive trials were performed for each rat, and grip strength performance was expressed as the ratio to baseline (pre-stroke) value (Atif et al. 2013).

The hot plate test was used for quantitative sensory testing of animals 24 h after surgery. The pain response was evaluated by recording the latency time for licking a hind paw by the animal when placed on a 50 °*C* plate. The rat was removed from the plate immediately upon licking the hind paw or when no response was observed for 50s (Gunn et al. 2011).

Table 1 Neurological deficit score test for assessment of the neurological disabilities in studied animals 24 h after MCAO

Neurological Dysfunction	Score
Normal motor function without any apparent deficit	1
Flexion of contralateral forelimb when suspended vertically by the tail and, or decreased the griping capacity of animal when pulled by tail	2
Spontaneous movement in all direction at the rest and circling to contralateral side when pulled with the tail	3
Spontaneous circling to contralateral side	4
loss of righting reflex and decreased resistance to lateral push	5
No spontaneous motor activity	6

Measurement of infarct size and brain edema formation

Six coronal brain slices (2-mm-thick) were prepared using a brain matrix and stained with a 2% solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Molekula, UK) at 37 °C for 15 min. Digital images of the stained sections were prepared, and the infarction areas were quantified by the image analyzer software (NIH Image Analyzer).

The water content of the brain tissue was measured as the indicator of edema formation using the wet/dry weight method (Tian et al. 2008). Stained sections of the brain were precisely divided into the left and right hemispheres and used for the detection of water content. The difference between the water content of the left (lesioned) hemisphere and that of the right hemisphere of the brain was calculated as the edema formation index.

Assessment of BBB permeability

The BBB permeability was evaluated using the Evans Blue (EB) extravasation technique (Kaya et al. 2003). Briefly, a catheter filled with heparinized serum was inserted into the external branch of the left jugular vein. After 30 min of surgery, 2% EB solution in saline (1 ml/kg, IV) was infused. After 24 h, the chest was opened under deep anesthesia and the entire body was perfused with approximately 250 ml warm saline solution (37 °C) to washout the EB dye from the circulation.

After decapitation of the animal, each hemisphere tissue was accurately weighed and homogenized in 2.5 ml phosphate-buffered saline. Then, 2.5 ml trichloroacetic acid (60%) was added to the homogenized solution and centrifuged at 3500 rpm (1640 g) for 30 min. EB absorbance was measured at 610 nm by a spectrophotometer (UV 1800, Shimadzu, Japan); then, the concentrations were calculated against a standard curve and the results were expressed as μg/g of brain tissue.

Assessment of lipid peroxidation and antioxidant enzyme activities

The ischemic lesioned hemisphere was separated, weighed, and homogenized in phosphate-buffered saline (pH 7.4) containing 0.1 mM EDTA with a weight-to-volume ratio of 1:6. The homogenate was centrifuged at 10,000 *g* for 15 min at 4 °C, and the resulting supernatant was used for biochemical studies. Lipid peroxidation was estimated by determining the amount of malondialdehyde (MDA) levels in the brain homogenateaccording to the technical manual of the assay kits (Cayman Chemical Company, USA), and the results were expressed as nmol/g of brain wet tissue. The activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), in the brain tissue homogenate were measured according to the method provided by the assay kit (Cayman Chemical Company, USA). Results were expressed as the percentage value of average amount in the sham-treated group.

Statistical analyzes

Data were represented as mean ± SEM. Infarct size data were analyzed by Student's *t* test. Other results were evaluated by one-way analysis of variance, followed by Dunnett's or Tukey's post hoc tests. Statistical significance was accepted at $P < 0.05$.

Results

SBP recording

The results of the noninvasive recordings of SBP indicated that the blood pressure values of animals in sham, control ischemic, and magnesium-sulfate-treated groups were in the normal physiological range, and there were no significant differences among them before and after the IP injections of vehicle or magnesium sulfate (Table 2).

Behavioral assessments of sensorimotor deficits

Ischemia significantly increased the neurological deficit score (NDS) of ischemic rats in the control group (3.5 ± 0.42) compared to that in sham animals ($P < 0.05$), and

Table 2 Systolic blood pressure (SBP) was measured in studied groups before and after vehicle or magnesium sulfate injection

Group	SBP (mm Hg) Preinjection	SBP (mm Hg) Postinjection
Sham	115 ± 4.2	113 ± 2.3
Control	112 ± 2.7	109 ± 2.7
MgSO4	108 ± 3.1	100 ± 1.5

Values are mean ± SEM. ($n = 6$). SBP: systolic blood pressure, MgSO4: magnesium sulfate

treatment with magnesium sulfate significantly improved the neurological function and reduced the NDS (1.88 ± 0.23, $P < 0.05$) (Fig. 1b).

Motor function of the contralateral forelimb was examined by the grip strength test in the study animals. When control ischemic rats had severe motor deficits and decreased grip strength compared to those in the sham-operated animals ($P < 0.001$), treatment with magnesium sulfate resulted in significantly improved motor performance and increased grip strength and muscle force ($P < 0.001$, Fig. 1c).

The hot plate test was also used to evaluate sensory function in the studied animals. Ischemia significantly enhanced the foot licking time of control animals compared with the sham group ($P < 0.001$). Treatment with magnesium sulfate significantly improved the sensory function in the hot plate test compared to that in the control group ($P < 0.05$, Fig. 1d). These findings together demonstrated that treatment with magnesium sulfate could significantly recover the animals from stroke-induced motor and sensory disabilities.

Assessment of cerebral infarct volumes

Sham-operated rats had no brain infarction volume. However, 60-min ischemia and 24-h reperfusion caused 46.9% of cerebral infarction in the left hemisphere in the ischemic control group. Treatment with magnesium sulfate significantly

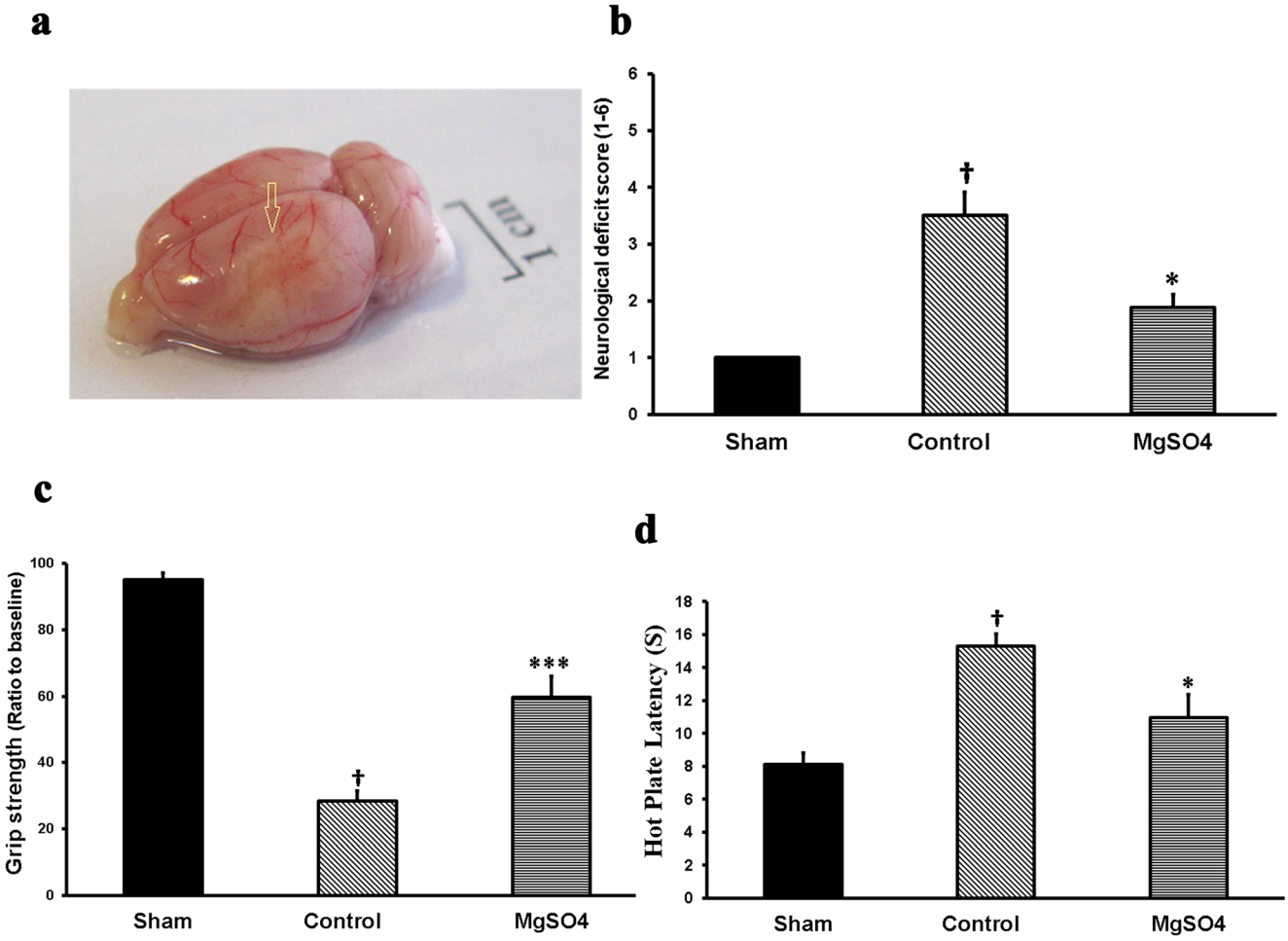

Fig. 1 Effects of magnesium sulfate on sensorimotordisabilities. a Photograph shows infarction region caused by MCAO in left side of the brain. **b** Neurological deficit score, **c** Grip strength test results on paretic (right) forelimb and **d** and Hot plate latency evaluation 24 h after MCAO in the studied animals. Values are mean ± SEM ($n = 8$). † $P < 0.001$ compared with sham group, ***$P < 0.001$ and *$P < 0.05$ compared with control group

reduced the infarction rate compared to that in the control group (32.4%, $P < 0.05$).

The total infarct volume in the control group was 396 ± 28 mm^3 and treatment with magnesium sulfate significantly decreased it in comparison with the control group (245 ± 26 mm^3, $P < 0.01$). Furthermore, magnesium sulfate administration significantly reduced the infarct volumes in the cortex and striatum of the brain compared to those in the control ischemic group ($P < 0.01$, Fig. 2a–c).

Detection of edema formation and BBB permeability

IR caused severe edema formation in control ischemic rats (3.86 ± 0.28%). Treatment with magnesium sulfate significantly reduced brain edema formation compared to that incontrol animals (1.87 ± 0.34%, $P < 0.001$, Fig. 3b).

Ischemia and subsequent reperfusion period weakened the BBB and significantly increased the EB concentration in the ischemic hemisphere in the control group compared to those in sham-operated animals ($P < 0.001$). Magnesium sulfate administration resulted in protection of BBB integrity and significantly decreased the EB concentration compared to those in the ischemic control group ($P < 0.05$, Fig. 3a and c).

Assessment of antioxidant enzyme activities and lipid peroxidation

In the control group, 60-min ischemia and 24-h reperfusion led to considerably enhanced MDA concentrations and

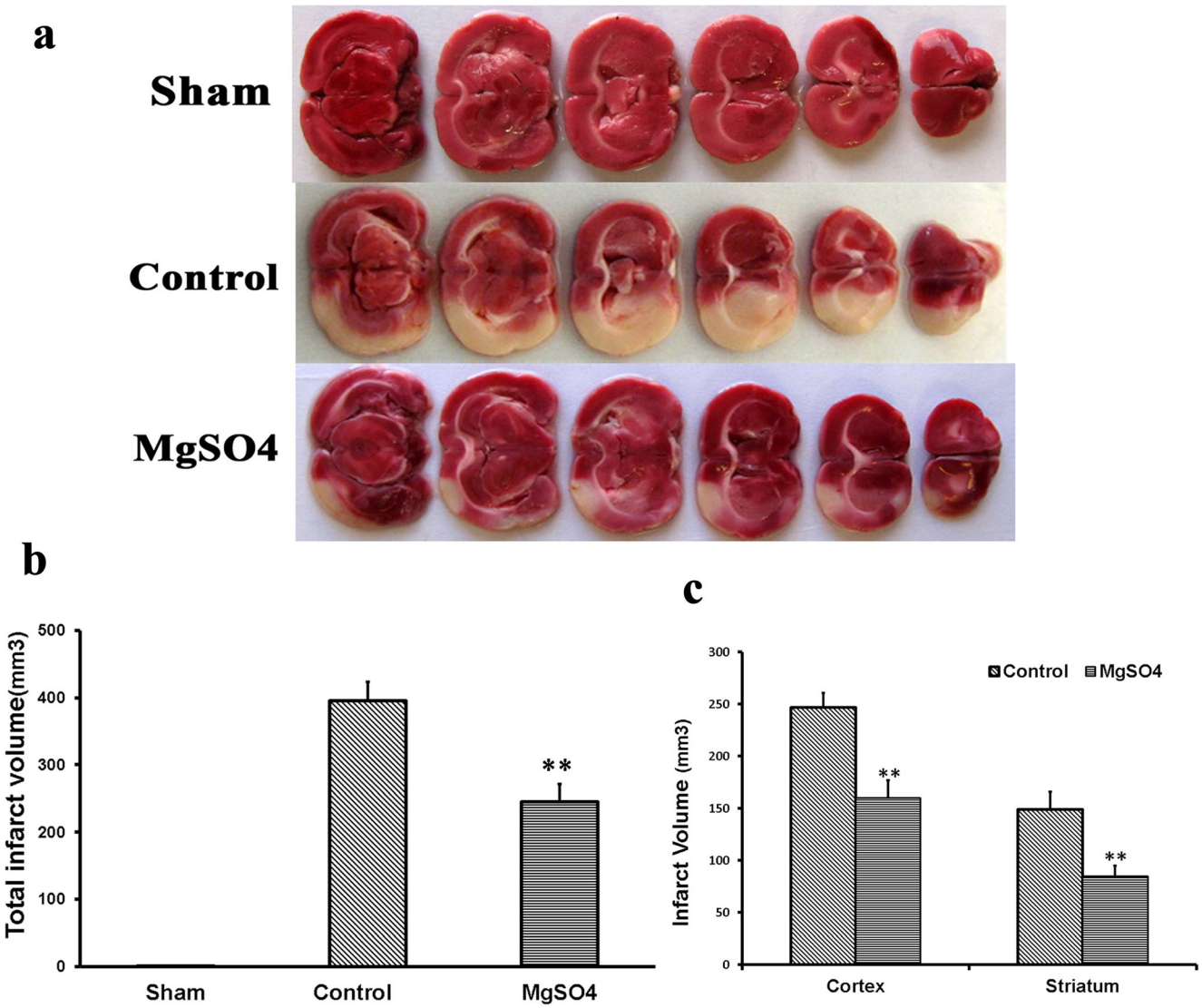

Fig. 2 Effects of magnesium sulfate on cerebral infarction volume. a Photograph of brain slices stained with TTC in the studied groups. Infarct region was showed with white color. **b** Total infarct volume and **c** infarct volumes in cortex and striatum of studied groups ($n = 8$). Values are mean ± SEM. $^{**}P < 0.01$ compared to control group

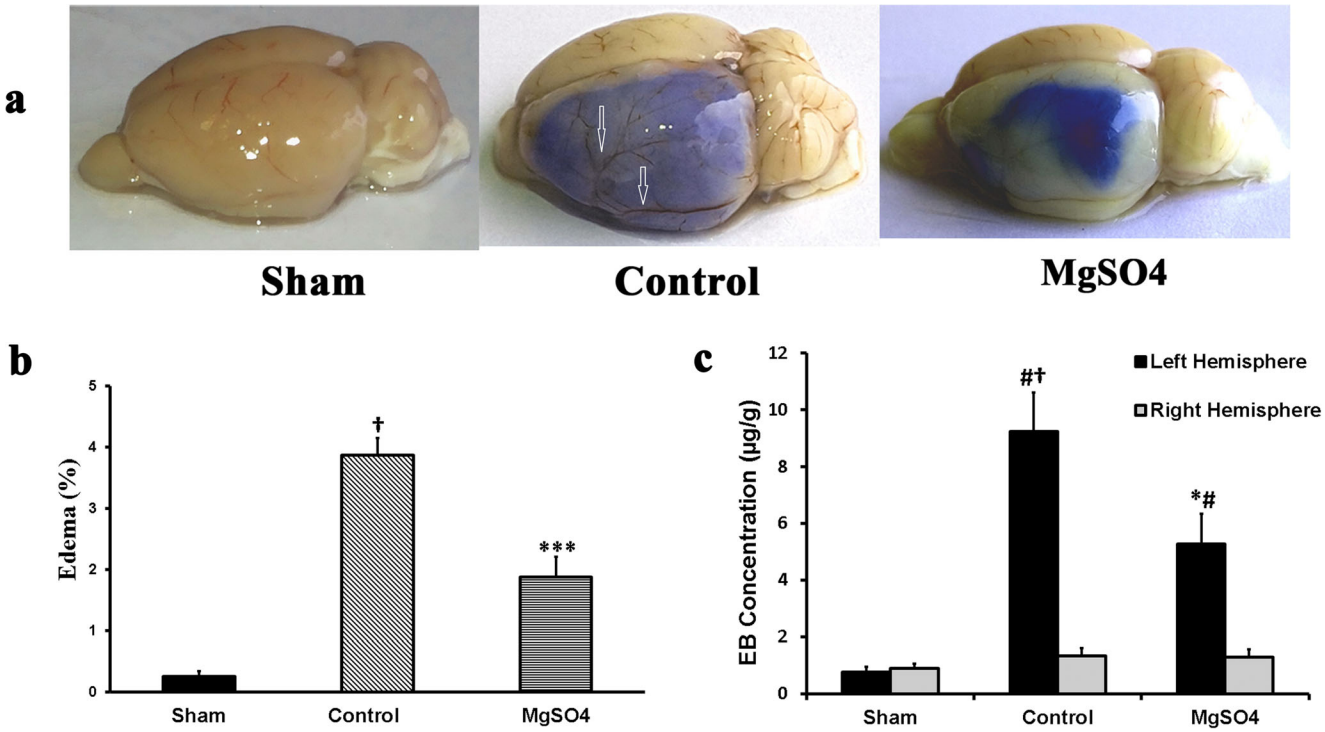

Fig. 3 Effects of magnesium sulfate on brain edema formation and BBB disruption. a Photograph of brains 24 h after MCAO and EB injection. The intensity of blue color is related to the extent of BBB damage. White arrows point to vessels located in the surface of the brain and damaged following IR injury. **b** brain edema formation and **c** EB concentration in the brain tissues in the studied groups (n = 8). Values are mean ± SEM, †$P < 0.001$ compared with sham group; ***$P < 0.001$ and *$P < 0.05$ compared with control group;# $P < 0.001$ compared with right side of the same group

decreased antioxidant enzyme activities in the left side of the brain tissues compared to those in the sham group. Treatment with magnesium sulfate significantly reduced the MDA concentration ($P < 0.05$) and elevated SOD and CAT activities ($P < 0.01$and $P < 0.05$, respectively) compared to those in the control ischemic group (Fig.4a–c). However, treatment with magnesium sulfate showed no significant improvement in GPX activity (Fig. 3d).

Discussion

This study has demonstrated that administration of magnesium sulfate attenuated BBB permeability and reduced vasogenic brain edema formation induced by a transient ischemic stroke model. These protective effects were associated with an increase in the antioxidant capacity of the brain tissue.

The BBB is composed of brain capillary endothelial cells connected through tight junctions, astrocyte end-feet, and pericytes. This dynamic structure selectively controls transport between circulation and brain tissue and provides a unique microenvironment for cerebral neuronal function. Disruption of the BBB with subsequent vasogenic brain edema formation is a common complication of acute ischemic stroke and associated with worse outcome (Brouns et al. 2011). Protection of the BBB integrity against IR-induced damage could be considered as a therapeutic strategy for ischemic brain injury and vasogenic edema (Michinaga and Koyama 2017). During acute ischemic stroke, IR-induced BBB damage occurs in two phases. There is a reversible initial opening (0–24 h) that is followed by a later irreversible damage (24–72 h) (Sifat et al. 2017; Yang and Rosenberg 2011). Moreover, thrombolytic therapy with r-tPA after acute ischemic stroke by disruption of tight junction proteins could contribute to BBB damage and promote vasogenic edema (Sifat et al. 2017).

Rapid administration of r-tPA remains the only accepted therapeutic intervention for patients with acute ischemic stroke (Powers et al. 2015). The clinical application of r-tPA is restricted to a small patient population (~3%) due to its limited therapeutic window (4.5 h) and significant adverse effects (Alexandrov and Grotta 2002). Intracranial hemorrhage (ICH) is an important adverse event that has been reported in patients with stroke treated with r-tPA (Group 1997). Disruption of the BBB limits the administration of r-tPA by increasing the incidence of hemorrhagic transformation, brain edema formation, and neuroinflammation (Cheng et al. 2014; Khanna et al. 2014). In addition, activation of matrix metalloproteinase-9 (MMP-9) may be caused by the possible entrance of r-tPA into the brain parenchyma through impaired BBB (Tsuji et al. 2005).

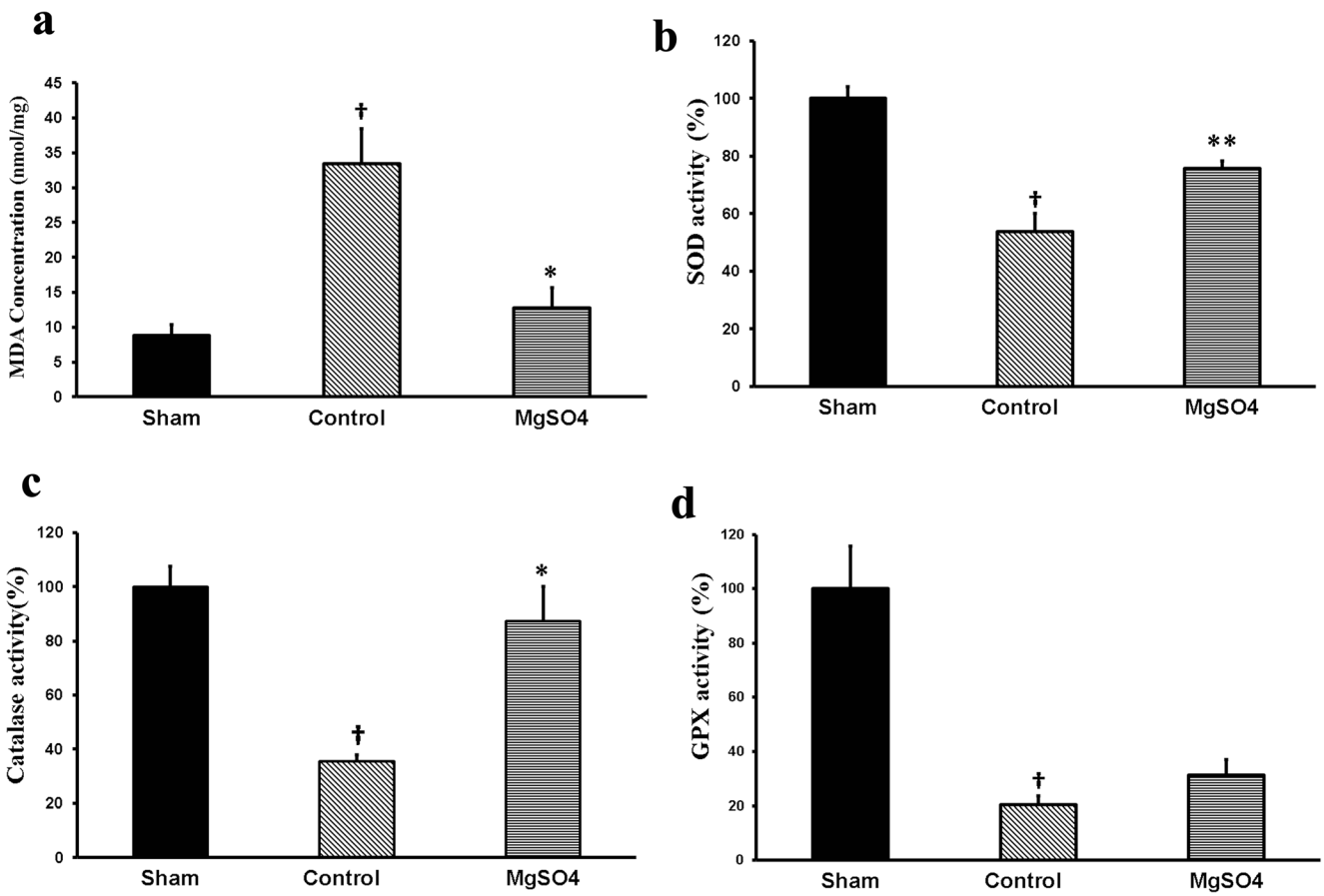

Fig. 4 Effects of magnesium sulfate on lipidperoxidation and brain tissue antioxidant enzyme activities. a MDA content, **b** SOD, **c** CAT and **d** GPX activities in brain tissue homogenate of studied animals 24 h after MCAO (n = 8). Values are mean ± SEM. †$P < 0.05$ compared with sham group; **$P < 0.01$ and *$P < 0.05$ compared with control group

Furthermore, r-tPA-induced activation of MMP-9 could be facilitated by oxidative stress during transient acute ischemic stroke that is followed by reperfusion (Harada et al. 2012).

The findings of this study showed that ischemia and subsequent reperfusion increased the BBB permeability as demonstrated by the EB extravasation technique and resulted in severe brain edema formation. A major finding of this study is that early treatment with magnesium sulfate had protective effects on BBB integrity and significantly reduced ischemia-induced brain edema formation. These results are consistent with previous studies reporting that treatment with magnesium could produce protective effects against BBB breakdown in head trauma(Heath and Vink 1998) and sepsis models of brain injury in rats (Esen et al. 2004). Furthermore, Kaya et al. demonstrated that treatment with magnesium sulfate significantly attenuated BBB disruption caused due to hyperosmotic mannitol (Kaya et al. 2004) and insulin-induced hypoglycemia (Kaya et al. 2001) in rats. In general, all these reports support the protective effects of magnesium sulfate on the structure of the BBB and cerebral edema formation.

In pathological conditions, including ischemic stroke, a decrease in magnesium levels may directly influence BBB integrity (Kaya and Ahishali 2011; Maier et al. 2004). Low magnesium concentration in microcirculation of the brain tissue can increase the production of cytokines and oxidative products and cause a progressive damage to BBB structures, leading to hemorrhage and cerebral edema (Maier et al. 2004). Therefore, systemic administration of magnesium may contribute both to improve BBB integrity and to attenuate brain vasogenic edema. An impaired BBB attenuates the potential of r-tPA, which is the only accepted therapeutic strategy in patients with acute ischemic stroke. Therefore, protecting BBB integrity could increase the therapeutic window of r-tPA and provide this important life-saving treatment for an increased number of patients with stroke (Sifat et al. 2017). The reversible MCAO model that was used in this study mimics the clinical recanalization of an embolized or thrombosed vessel by r-tPA (Liu and McCullough 2011). The findings of this study demonstrated that early administration of magnesium sulfate protects the brain against IR-induced cerebrovascular damage. Magnesium sulfate may be an ideal neuroprotective agent because it is safe with no significant adverse side effects, readily available, and easy to administer. Therefore, it can be used for earlier administration

in patients with stroke before brain imaging based on clinical symptoms in the prehospital setting and may be potentially beneficial for the prevention of BBB leakage and vasogenic edema formation (Saver and Starkman 2011). In addition, the combination of a BBB protective agent such as magnesium sulfate with r-tPA may also reduce the risk for ICH and increase the time frame of the therapeutic window for r-tPA, which requires further investigations.

The possible mechanisms involved in the protective effects of magnesium in different pathological conditions could include its antioxidant and anti-inflammatory properties (Chen et al. 2018; Kaya and Ahishali 2011). Moreover, it has been suggested that magnesium may provide protection against edema through downregulation of aquaporin-4 channels in the brain tissue following traumatic brain injury (Ghabriel et al. 2006). The beneficial effects of magnesium in decreasing cerebral edema may also be related to its role as a physiological calcium antagonist, blocking the NMDA glutamate receptor (Imer et al. 2009) and attenuating the oxidative stress (Chen et al. 2018).

Oxidative stress is an important pathological mechanism of BBB ischemic damage and vasogenic edema formation, especially during reperfusion period. Excessive production of reactive oxygen species during ischemic stroke overwhelms the endogenous antioxidant capacity of the brain tissue and damages cerebrovascular endothelial tight junctions (Schreibelt et al. 2007). In this study, administration of magnesium sulfate immediately after acute stroke reduced the elevated levels of lipid peroxidation and increased the activity of antioxidant enzymes (SOD and CAT). Similarly, Chen et al. (2018) reported that magnesium sulfate may produce protective effects against radiation-induced brain injury by increasing SOD activities and decreasing MDA levels (Chen et al. 2018). Furthermore, Üstün et al. (2001) reported that magnesium sulfate augments intrinsic antioxidant levels in the brain tissue by increasing the activities of SOD and GPX in an experimental model of head trauma (Üstün et al. 2001). Therefore, magnesium may partly provide protective effects on BBB integrity and reduce brain edema through antioxidant actions in the rat brain following ischemic stroke.

In conclusion, the results of this study have demonstrated that early treatment with magnesium sulfate protects BBB integrity against acute ischemic stroke and reduces vasogenic brain edema. The beneficial effects of magnesium on BBB functions and brain edema may be caused due to the upregulation of the antioxidant enzyme activities and the reduction of lipid peroxidation in the brain tissue. Treatment with magnesium sulfate may increase the therapeutic time window for thrombolysis or thrombectomy. However, this issue requires further investigations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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