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Cerebralcare Granule® attenuates blood–brain barrier disruption after middle cerebral artery occlusion in rats

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ABSTRACT

Disruption of blood–brain barrier (BBB) and subsequent edema are major contributors to the pathogenesis of ischemic stroke, for which the current clinical therapy remains unsatisfied. Cerebralcare Granule® (CG) is a compound Chinese medicine widely used in China for treatment of cerebrovascular diseases. CG has been demonstrated efficacy in attenuating the cerebral microcirculatory disturbance and hippocampal neuron injury following global cerebral ischemia. However, the effects of CG on BBB disruption following cerebral ischemia have not been investigated. In this study, we examined the therapeutic effect of CG on the BBB disruption in a focal cerebral ischemia/reperfusion (I/R) rat model. Male Sprague–Dawley rats (250 to 300 g) were subjected to 1 h middle cerebral artery occlusion (MCAO). CG (0.4 g/kg or 0.8 g/kg) was administrated orally 3 h after reperfusion for the first time and then once daily up to 6 days. The results showed that Evans blue extravasation, brain water content, albumin leakage, infarction volume and neurological deficits increased in MCAO model rats, and were attenuated significantly by CG treatment. T2-weighted MRI and electron microscopy further confirmed the brain edema reduction in CG-treated rats. Treatment with CG improved cerebral blood flow (CBF). Western blot analysis and confocal microscopy showed that the tight junction proteins claudin-5, JAM-1, occludin and zonula occluden-1 between endothelial cells were significantly degradated, but the protein expression of caveolin-1, the principal marker of caveolae in endothelial cells, increased after ischemia, all of which were alleviated by CG treatment. In conclusion, the post-treatment with CG significantly reduced BBB permeability and brain edema, which were correlated with preventing the degradation of the tight junction proteins and inhibiting the expression of caveolin-1 in the endothelial cells. These findings provide a novel approach to the treatment of ischemic stroke.

Introduction

Ischemic stroke and post-ischemia/reperfusion (I/R) induced by thrombolytic therapy result in the blood–brain barrier (BBB) disruption, leading to the development of brain edema and hemorrhagic conversion (Hacke et al., 1999; Jung et al., 2010). Despite the advances in the overall management of acute stroke, the propensity of ischemic brain tissue to develop edema remains the major cause of death within the first few days of stroke in patients with serious herniation (Davalos et al., 1999; Moulin et al., 1985). Thus, drugs protected the BBB may be a promising management strategy for treatment of ischemic stroke.

The endothelial cells of the BBB are the first-line of the defense between the blood and the brain (Abbott et al., 2010). The permeability of the endothelial barrier is regulated by two different routes, one is the paracellular pathway, which is through interendothelial junctions, and the other is transcellular pathway which is via caveolae-mediated vesicular transport. Tight junction (TJ) between adjacent endothelial cells plays critical role in the BBB disruption during ischemic stroke (Jiao et al., 2011). The TJ consists of three types of integral transmembrane proteins: claudins, occludin, and junction adhesion molecules (JAMs), and several cytoplasm accessory proteins including zonula occludens (ZOs) (Abbott et al., 2010; Wolburg et al., 2009), among which claudin-5, occludin, JAM-1 and ZO-1 play a pivotal role in the ischemic BBB injury (Coisne and Engelhardt, 2011; Sandoval and Witt, 2008; Simard et al., 2007; Zehendner et al., 2009).

Caveolae, 50–100 nm plasmalemmal vesicles in the cytoplasm, are important for regulation of a range of endothelial cell functions, such

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as production of nitrogen oxide (Bernatchez et al., 2005), signaling along MAP kinase cascade (Engelman et al., 1998), as well as the transcytosis of albumin across endothelial cells (Ghitescu et al., 1986; Schubert et al., 2001). Caveolin proteins represent the principal structural proteins of the caveolae. As the principal marker of caveolae (Rothberg et al., 1992), cavelin-1 is required for the caveolae-mediated transcytosis of albumin (Minshall et al., 2003). Under pathological condition the breakdown of BBB causes the increase in albumin leakage via both paracellular and transcellular pathway.

Cerebralcare Granule (CG; Tasly Pharmaceutical Co Ltd, Tianjin, China) is a newly developed compound Chinese medicine composed of eleven herbs (Xu et al., 2009). The CG was approved in 1996 by the Chinese State Food and Drug Administration (1002004736603642) and had been widely used in China for treatment of headache and dizziness associated with cerebrovascular diseases. Our previous studies have demonstrated that CG can inhibit the production of superoxide in cerebral venular endothelium, albumin leakage across venules, alleviate the overall microcirculatory disturbances and hippocampal neuron damage in global I/R of Mongolian gerbil (Sun et al., 2010; Xu et al., 2009). However, the effect of CG on BBB injury elicited by focal cerebral I/R has not been well explored. Therefore, this study was designed to investigate the role of CG in BBB disruption after I/R, with particularly addressing the involvement of tight junction and caveolae.

Materials and methods

Animals

Male Sprague–Dawley rats weighing 250 to 300 g were purchased from the Animal Center of Peking University Health Science Center (Beijing, certificate no. SCXK 2006-0008). The animals were housed in cages at 22 °C ± 2 °C and humidity of 40% ± 5% under a 12-hour light/dark cycle, and received standard diet and water ad libitum. The rats were fasted for 12 h before experiment but allowed free access to water. The experimental procedures were carried out in accordance with the European commission guidelines (2010/63/EU). All animals were handled according to the guidelines of the Peking University Animal Research Committee. The protocols were approved by the Committee on the Ethics of Animal Experiments of the Health Science Center of Peking University (LA2011-38).

Cerebralcare granule

CG was produced by Tasly Pharmaceutical Co. Ltd (Tianjin, China). The batch number of the CG used in this experiment was 100200 4736603642. No steroid is included in the content of CG. The processing of the product followed a strict quality control, and the ingredients were subjected to standardization. The drugs were manufactured as granules after dynamic cycle extraction and concentrated by evaporating and spray drying. The CG was packed with aluminum foil composite, 4 g per bag. The CG was dissolved in saline to a concentration of 80 mg/mL or 160 mg/mL before use (Xu et al., 2009).

Middle cerebral artery occlusion model and experimental groups

Male Sprague–Dawley rats were subjected to MCAO and reperfusion, as reported previously (Longa et al., 1989). Briefly, after anesthetized with pentobarbital sodium (0.1 g/kg body weight) intraperitoneally, neck vessels were exposed through a midline incision, and branches of the right external carotid artery were isolated and cauterized. A 4-0-monofilament nylon suture was prepared by rounding the tip to approximately 0.35 mm in diameter with a cautery. The right middle cerebral artery was occluded with the suture by inserting it through the right external carotid artery and gently advancing into the internal carotid artery up to a point approximately 18 mm distal to the bifurcation of the carotid artery. Reperfusion was achieved by slowly pulling the suture back after 60 min occlusion. The incision on the neck was closed and the animals were allowed to recover. Rectal temperature was maintained at 37 ± 0.5 °C throughout the procedure from the start of the surgery until the recovery of the animals from anesthesia with a thermostat-controlled heating pad.

A total of 165 rats were included and randomly divided into 5 groups: (1) sham group (n = 33), (2) I/R 3H group (n = 33), (3) I/R 6D group (n = 33), (4) I/R6D + CG0.4 group (n = 33), and (5) I/R 6D + CG0.8 group (n = 33) (see Table 1 for further details). In the sham group, rats were operated in the same way, but without occlusion of middle cerebral artery. In CG post-treated groups, CG was administrated by gavage (5 mL/kg) 3 h after the beginning of reperfusion for the first time at a dose of either 0.4 g/kg (I/R6D + CG 0.4 group) or 0.8 g/kg (I/R6D + CG 0.8 group), and received the same dose of CG every 24 h for 6 days. The animals in sham group and I/R 6D groups were given equivalent volume of saline in the same manner. Determination of the doses of CG used in the present study was based on our previous works (Sun et al., 2010; Xu et al., 2009). All variables in this study were examined at 3 h (I/R 3H group), or on day 6 (remaining groups), after reperfusion, unless otherwise mentioned.

MRI examination

Rat brain edema was examined in a 3.0-Tesla (T) MRI animal scanner (Magnetom Trio with TIM system, Siemens, Erlangen, Germany). The rats in each group (n = 5) were submitted to examine by MRI for two times, the one at 3 h after reperfusion, and the other on day 6. The animal's head was positioned in a custom-made "birdcage coil" (inner diameter of 30 mm) for signal excitation and detection. MRI parameters were set at TE = 92 ms, TR = 3620 ms, FOV = 8 × 8 cm^2, M = 256 × 256, NA = 2, thickness = 2 mm, and gap = 0 mm. After the optimal adjustment of contrast, hemisphere intensity was examined by Image-Pro Plus 5.0 software (Media Cybernetic, Bethesda, MD, USA) using the operation "mean density value." The intensity percentage of the ipsilateral hemisphere against the contralateral hemisphere was calculated, and statistical analysis was performed (Zhang et al., 2011).

Albumin leakage

The animal's head was secured in a stereotactic frame. With a hand-held drill, a 4 × 6 mm^2 cranial window was performed through an incision 1 mm behind the coronal suture, and 1 mm on the right side of the sagittal suture. This location corresponds to the margin of the MCA territory. The dura was removed and the pia mater was superfused contiguously with 37 °C warm physiological saline.

Table 1
The number of animals for different experimental groups and various parameters.

	Sham	I/R3H	I/R6D	I/R6D + CG0.4	I/RD + CG0.8	Total
Evans blue extravasation	5	5	5	5	5	25
Albumin leakage	6	6	6	6	6	30
Cerebral blood flow, TTC staining and neurological score	6	6	6	6	6	30
Confocal	3	3	3	3	3	15
MRI and brain water content	5	5	5	5	5	25
Western blot assay	5	5	5	5	5	25
Ultrastructure examination	3	3	3	3	3	15
Total	33	33	33	33	33	165

The same animals were used for detection of CBF, TTC staining and neurological score. The same animals were used for detection of MRI and brain water content. Sham: Sham group; I/R 3H: I/R 3 h group; I/R 6D: I/R 6 days group; I/R 6D + CG 0.4: I/R 6 days plus post-treatment with CG 0.4 g/kg group; I/R 6D + CG 0.8: I/R 6 days plus post-treatment with CG 0.8 g/kg group. MRI: Magnetic Resonance Imaging; TTC: triphenyl tetrazolium chloride.