Effect of breviscapine on brain edema and neutrophil infiltration induced by focal cerebral ischemia and reperfusion in rats

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Abstract: **AIM** To investigate whether breviscapine attenuates brain edema and neutrophil infiltration and inhibiton ICAM-1 expression in the cerebral ischemia-reperfusion rats. **METHODS** Focal cerebral ischemia-reperfusion model in rats were made by transient occlusion of the middle cerebral artery for 2 h followed by 24 h reperfusion. Breviscapine 50 and 75 mg·kg^{-1} were administered ip at 1 h and repeated at 22 h after reperfusion. Twenty-four hours later, brain edema, neutrophil infiltration and the expression of intercellular adhesion molecule-1 (ICAM-1) were measured with dry-wet weight, myeloperoxidase (MPO) activity, and immunohistochemistry, respectively. **RESULTS** After ischemia reperfusion, brain water content was obviously increased, MPO activity and the expression of ICAM-1 in the ischemic hemisphere cortex of MCA area and caudate putamen were markedly increased. Treatment with breviscapine 50 and 75 mg·kg^{-1} reduced brain edema, inhibited MPO activity and the expression of ICAM-1. **CONCLUSION** Breviscapine attenuated brain edema and neutrophil infiltration after cerebral ischemia reperfusion.

Key words: breviscapine; cerebral ischemia; brain edema; neutrophils; intercellular adhesion molecule-1

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Intercellular adhesion molecule-1 (ICAM-1) is constitutively expressed at low levels in brain microvascular endothelium cells and can be markedly upregulated after focal cerebral ischemia-reperfusion (I-R). ICAM-1 mediates neutrophil adhesion and infiltration into lesion tissue after cerebral ischemia[1-3]. Neutrophils recruitment and infiltration induced by the response to cerebral I-R in ischemia area of brain contribute to the secondary neuronal injury through release of cytotoxicants and oxygen free radicals from activated neutrophils[4]. Breviscapine was extracted from a Chinese herb *Erigeron breviscapus* (Vant.) Hand.-Mazz. The active ingredient is 4′-OH-scutellarin-7-*o*-glucuronide[5]. Breviscapine exerted a remarkable activity against platelets and thrombus formation[6,7]. It has been used in the treatment of ischemic cerebral vascular diseases and demonstrated to exert protection against ischemic brain damage through eliminating free radicals and apoptosis. However, most of these studies show effect in animals only when administered either before or very shortly after the onset of ischemia, its therapeutic mechanism is not fully elucidated. Breviscapine has never been proved to have efficacy of anti-inflammatory through inhibiting expression of cellular adhesion molecule and infiltration of neutrophil following cerebral ischemia. Therefore, the present study was undertaken to investigate whether breviscapine attenuates neutrophil infiltration response to cerebral I-R and inhibits expression of ICAM-1.

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1 MATERIALS AND METHODS

1.1 Drug and animals

Breviscapine (purity > 95%, made in Yuxi

Pharmaceutical Co. Ltd., Yunnan Province, China) was dissolved in normal saline (NS) and adjusted pH to 6.5 with NaOH. Sprague-Dawley rats (♂, 200 – 235 g, Grade Ⅱ, certificate No. 19-053) were supplied by the Experimental Animal Center, Tongji Medical College, Huazhong University of Science and Technology.

1.2 Focal cerebral ischemia-reperfusion model

Rats were randomly divided into 4 groups: sham-operated group, I-R model group, breviscapine 50 and 75 mg·kg^{-1} groups, respectively. All animals were anesthetized with an intraperitoneal injection of 10% chloral hydrate (350 mg·kg^{-1}). Transient middle cerebral artery (MCA) occlusion was induced as described by Longa, *et al*[8]. Briefly, the right carotid rejion was exposed through a ventral midline cervical incision. Right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were isolated. The pterygopalatine branch of ICA was ligated to prevent incorrect insertion of the occluder filament. A 5 cm length of fish nylon thread(φ 0.23 mm), whose tip had been rounded by heating near a flame, was introduced from the ECA into ICA until a mild resistance was felt (18 to 19 mm). Thereby the origin of the MCA was occluded. Two hours after MCA occlusion, the thread was removed to allow reperfusion of the ischemic area *via* the right CCA. The sham-operated animals were subjected to the same surgical procedures but not occluded the MCA. The rectal temperature was maintained at 37 – 38℃ with a heating lamp and heating pad during the operation. The room temperature was controlled in the range of 25 – 27℃ throughout the experimental procedure.

1.3 Drug administration

Breviscapine was given ip twice at 1 and 22 h after reperfusion. Sham-operated and I-R model groups received NS 10 mL·kg^{-1} according to the same protocol.

1.4 Brain edema

After 24 h reperfusion, rats were decapitated. Brain water content was measured with dry-wet weight. The degree of brain edema was represented by water content, which was calculated by following equation: water content = (wet weight – dry weight)/wet weight × 100%.

1.5 Tissue myeloperoxidase (MPO) content

After 24 h reperfusion, neutrophil infiltration was measured with the MPO activity assay[9,10]. Brain samples of the ischemic hemisphere were taken from the cortex of the MCA area and the caudate putamen on ice, and immediately frozen in liquid nitrogen and store at – 80℃ for later biochemical analysis. The procedures used to quantify MPO activity were according to the description of the MPO kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

1.6 Immunohistochemical study

After 24 h reperfusion, rats were anesthetized with 10% chloral hydrate (350 mg·kg^{-1}, ip) and transcardially perfused with 100 mL of NS, followed by 250 mL of 4% paraformaldehyde in 0.1 mol·L^{-1} phosphate buffer solution (PBS, pH 7.4, 4℃). Brain was then postfixed 1 h in the same fixative at 4℃, cryoprotected in 30% sucrose in 0.1 mol·L^{-1} PBS (pH 7.4, 4℃) until sinking. Coronal brain sections(30 μm) were cut at 1.2 – 3.3 mm bregma level on a cryostat(Leica CM1850, Germany). Tissue was reacted with a monoclonal mouse anti-rat ICAM-1 antibody (1∶50 from Santa Cruz, USA). Antibody-binding was detected using an Histostain^{TM}-SP kit (Beijing Zhongshan Biotechnology, Co. Ltd. Beijing, China), according to the manufacturers instructions and with diaminobenzidine tetrahydrochloride (Fluka) as the chromogen.

The values of mean absorbance of peroxidase stained microvessels in the parietal cortex and center part of caudate putamen were counted in 4 nonoverlapping fields under the light microscope (Olympus, Japan) at 400 × magnification.

HPIAS-1000 image analysis system was used to quantify the levels of ICAM-1 expression and the values of mean absorbance was used to show the level.

1.7 Statistical analysis

The data were presented as $\bar{x} \pm s$, and analyzed using ANOVA followed by Student-Newman-

Keul′s test.

2 RESULTS

2.1 Effect of breviscapine on brain edema

The water content of the right forebrain hemisphere of the model group increased markedly as compared with the sham-operated group. Treatment with breviscapine 75 mg·kg^{-1} alleviated the increased brain water content (Tab 1).

Tab 1. Effect of breviscapine on brain edema induced by cerebral ischemia-reperfusion (I-R) in rats

Group	Brain edema/%
Sham-operated	78.6 ± 0.3
I-R	80.9 ± 1.4**
I-R + breviscapine 50	79.5 ± 0.8*
I-R + breviscapine 75	79.0 ± 0.7$^{\#}$

I-R: The model of focal cerebral I-R was induced by 2 h of the middle cerebral artery occlusion followed by 24 h of reperfusion. Breviscapine 50 or 75 mg·kg^{-1} was given ip twice at 1 and 22 h after reperfusion. Sham-operated and I-R model groups received NS 10 mL·kg^{-1} according to the same protocol. $\bar{x} \pm s$, $n = 6$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with sham-operated group; $^{\#}P < 0.05$, compared with I-R group.

2.2 Effect of breviscapine on myeloperoxidase activity

To estimate the infiltration of neutrophils into ischemic areas, brain MPO activity, as a marker of infiltrated neutrophils, was measured in rats. The MPO activity in the cortex and caudate putamen of model group increased markedly as compared with sham-operated group. Administration of breviscapine 75 mg·kg^{-1} significantly inhibited the increase in MPO activity in the cortex of MCA area and caudate putamen (Tab 2).

2.3 Effect of breviscapine on intercellular adhesion molecule-1 expression

ICAM-1 expression was low on endothelial surface of microvessels in the sham-operated group. In the model group, ICAM-1 was clearly expressed on the microvessels in the ischemic cortex and caudate putamen. In the rats treated with breviscapine 50 or 75 mg·kg^{-1}, the expression of ICAM-1 was markedly decreased compared with

Tab 2. Effect of breviscapine on myeloperoxidase (MPO) activity after cerebral ischemia-reperfusion in rats

Group	MPO activity/μmol·min^{-1}·g^{-1} wet weight	
	Cortex	Caudate putamen
Sham-operated	0.36 ± 0.13	0.32 ± 0.07
I-R	0.71 ± 0.10**	0.66 ± 0.11**
I-R + breviscapine 50	0.53 ± 0.15	0.54 ± 0.11*
I-R + breviscapine 75	0.51 ± 0.09$^{\#}$	0.40 ± 0.10$^{\#\#}$

See Tab 1 for I-R and breviscapine treatments. $\bar{x} \pm s$, $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with sham-operated group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with I-R group.

Tab 3. Effect of breviscapine on intercellular adhesion molecule-1 (ICAM-1) expression after cerebral ischemia-reperfusion in rats

Group	Absorbance of ICAM-1 positive stain	
	Cortex	Caudate putamen
Sham-operated	0.21 ± 0.02	0.15 ± 0.04
I-R	0.37 ± 0.08**	0.32 ± 0.06**
I-R + breviscapine 50	0.27 ± 0.08$^{*\#}$	0.24 ± 0.02$^{**\#}$
I-R + breviscapine 75	0.22 ± 0.05$^{\#\#}$	0.19 ± 0.02$^{*\#\#}$

See Tab 1 for I-R and breviscapine treatments. $\bar{x} \pm s$, $n = 12$ (4 fields each rat, 3 rats in one group). $^{*}P < 0.05$, $^{**}P < 0.01$, compared with sham-operated group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, compared with I-R group.

I-R model group(Tab 3, Fig 1).

3 DISCUSSION

Neutrophils are known to mediate injury in acute ischemic stroke especially during reperfusion. Migration of neutrophils into regions of ischemic injury involves binding to ICAM-1 of endothelial cells through its CD11/CD18 leukointegrin. Following focal cerebral ischemia, the interactions between CD11/CD18 and ICAM-1 contribute to neutrophil infiltration into the tissue of ischemic brain[2,3]. With ischemically compromised tissue, neutrophils contribute to secondary injury by releasing active oxygen species, generating lipid mediators, activating thrombosis, disrupting to blood brain barrier, increasing cere-

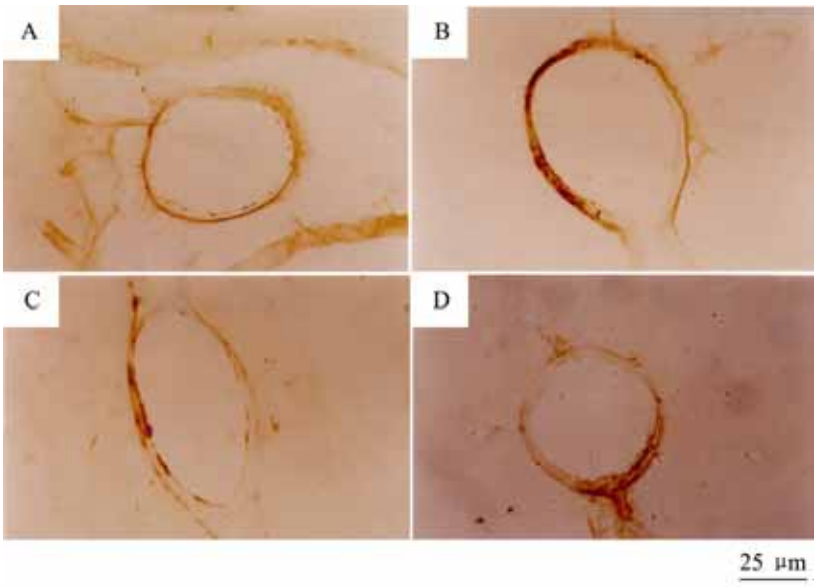

Fig 1. Immunohistochemical study of intercellular adhesion molecule-1 expression on microvessels (in parietal cortex of the middle cerebral artery area) after focal cerebral ischemia-reperfusion in rats. See Tab 1 for I-R and breviscapine treatments. A: sham-operated group; B: I-R group; C, D: I-R + breviscapine 50 and 75 mg·kg $^{-1}$, respectively.

bral edema, and plugging the cerebral microvasculature[4,11,12].

MPO is an enzyme localized in neutrophils. MPO activity reflected neutrophils infiltration. By measuring its activity, the degree of neutrophils accumulation and infiltration after stroke can be quantified[9,10].

Our present study demonstrated that transient focal cerebral ischema (2 h) and reperfusion (24 h) in the rat provided sufficient stimulation to enhance the expression of ICAM-1 on the endothelial cells of microvessels and to induce infiltration of neutrophils into injuried tissue and brain edema formation. While, treatment with breviscapine at 1 and 22 h after reperfusion significantly inhibited the post-ischemic increase in expression of ICAM-1 and MPO activity in the cortex of MCA area and caudate putamen, reduced the interaction between ICAM-1 on endothelial cells and neutrophils, and attenuated the invasion of neutrophils in the development of post-ischemic brain inflammation and brain edema.

In conclusion, cerebral I-R enhances the expression of ICAM-1 on microvessels endothelial cells and induces infiltration of neutrophils into ischemic brain. Breviscapine can inhibit the expression of ICAM-1, infiltration of neutrophils and brain edema, which contribute to its preventive effects on the ischemic brain inflammation.

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灯盏花素对大鼠局灶性脑缺血再灌注后脑水肿和中性粒细胞浸润的影响

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摘要: 目的　研究灯盏花素对大鼠局灶性脑缺血再灌注后脑水肿和中性粒细胞浸润的影响,探讨其抑制脑缺血再灌注损伤后炎症反应的作用机制。**方法**　大鼠大脑中动脉短暂阻塞制成局灶性脑缺血 2 h,再灌注 24 h 模型。再灌注后 1 h 分别 ip 灯盏花素 50 或 75 mg·kg^{-1},再灌注后 22 h 重复给药 1 次。再灌注 24 h 后干湿重法测定脑水肿,分光光度法测定缺血区大脑皮质和尾壳核髓过氧化物酶(MPO)活性,免疫组织化学染色测定大脑皮质和尾壳核细胞间粘附分子-1(ICAM-1)的表达。**结果**　缺血再灌注后,脑组织含水量明显增加,缺血区大脑皮质及尾壳核 MPO 活性和 ICAM-1 表示显著增加,灯盏花素 50 和 75 mg·kg^{-1}治疗用药能减轻脑水肿,降低 MPO 活性和抑制 ICAM-1 表达。**结论**　灯盏花素可减轻大鼠局灶性脑缺血再灌注后脑水肿和中性粒细胞浸润。

关键词: 灯盏花素; 脑缺血; 脑水肿; 中性粒细胞; 细胞间黏附分子-1

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