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Berberine reduces the hypoxic-ischemic insult in rat pup brain

F **Benaissa**[1], H **Mohseni-Rad**[2], P **Rahimi-Moghaddam**[2,3], M **Mahmoudian**[2,3]

[1]Department of Neurology, [2]Razi Institute for Drug Research, [3]Department of Pharmacology,
Iran University of Medical Science, P.O. BOX 14155-6183, Tehran, Iran

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Berberine, an isoquinoline alkaloid extracted from medicinal herbs, has been used as antipyretic, antidiarrheal, bactericide and anti-inflammatory agent. In this study, berberine effects on neuronal damage have been examined. The right carotid artery of seven-day-old rat pups was ligated (ischemic insult), then berberine solution (0.2, 0.5, 1 or 2 mg/kg) was injected intra-peritoneally, and 30 minutes later pups were passed through hypoxic condition with breathing in air containing 10% oxygen and 90% nitrogen(hypoxic insult). The day after that the brains of pups were enucleated for pathologic assessment. Pathologic review of the samples obtained from rats treated with different doses of berberine in comparison with samples from pups treated by normal saline showed that there was a significant reduction of brain injury and edema in the rats treated with berberine. Our study also demonstrates that berberine reduces brain ischemic-hypoxic injury dose-dependently. Therefore, beberine may be considered as useful anti-stroke agent.

Keywords: berberine, hypoxic-ischemic insult, brain edema, rat pups, neuronal damage, brain injury, anti-stroke agent

Berberine is an isoquinoline alkaloid extracted from medicinal herbs such as *Hydrastis Canadensis*, *Rhizoma coptidis* and *Cortex phellodendri*. It is used as antipyretic, antidiarrheal and it alleviates stomachache as mentioned in Chinese old medicine (25). Nowadays, bactericidal, anti-cholera toxin and anti-inflammation effects of berberine is proved. Berberine inhibits cyclooxygenase-2 (COX-2) transcription, reduces local interleukin-8 (IL-8) concentration and lipoxygenase activity (6, 7, 23). Also, berberine

Corresponding author: Massoud Mahmoudian
Department of Pharmacology, Razi Institute for Drug Research
Iran University of Medical Sciences, Tehran, Iran
E-mail: masmah99@iums.ac.ir

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has been effective in lowering blood glucose and low density lipoprotein cholesterol (17). The chemical structure of berberine is shown in Fig. 1.

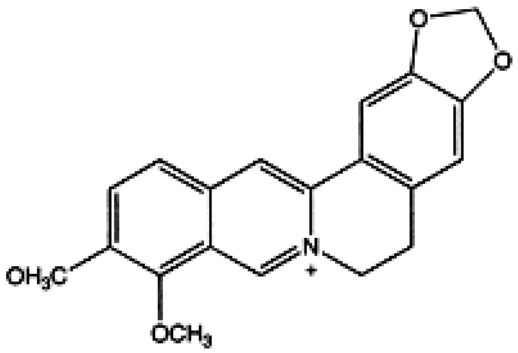

Fig. 1. Berberine structure formula

Cerebrovascular accident (CVA) is one of the main causes of death in the world. Cellular damage mechanism in relation to CVA is ischemic or hypoxic (8). Following to ischemic-hypoxic insult, neuronal cells enter to degenerative process. Immediately after ischemic-hypoxic injury the brain may seem normal, grossly and microscopically. Little by little, cortex and white matter border decreases. Then, in 12–24 hours microscopic changes reveal edema and shrinkage of neurons, eosinophilic cytoplasm, nucleus pyknosis and vacuolation of parenchyma. Perivascular and pericellular area widens which means vasogenic edema (3).

Berberine anti-inflammatory effect is explained in many articles. Kuo and his colleagues showed that berberine dose dependently decreased production rate of COX-2 and activator protein-1 in oral cancer cells (6). It has been shown that barberry extract reduces neuronal damage in gerbil hippocampus after transient forebrain ischemia (20). In this work, we investigated the effect of berberine, one of ingredients of barberry extract, on brain injury after hypoxic-ischemic insult.

Materials and Methods

Hypoxic-ischemic insult was performed as described by Mahmoudian et al. (10). Seven-day-old rat pups of either sex, weighing 10–15 g were used for the experiments. They were kept in cages in which their mothers fed them under optimal conditions (4 pups for each mother). All experiments were approved by the Institute of Animal Care Committee at Iran University of Medical Sciences. The pups were categorized in 6 groups, seven in each. Five groups were operated for ligation of the right carotid artery. One group did not undergo any surgery or hypoxic-ischemic insult and served as morphologic standard for normal brain tissue. Before operation, the pups were anesthetized with ether, and then the right common carotid artery was explored with an oblique 5-millimeter incision in front of the neck, and it was ligated with 6-0 surgical

silk thread. The incision was sutured with the same thread. After 5 minutes, 0.1 ml of the desired solution was injected intraperitoneally. The operated rats were randomly divided to five groups. Group 1 (control) received an injection of normal saline, while groups 2–5 received 0.2, 0.5, 1.0 and 2 mg/kg berberine in normal saline, respectively. All rats were returned to their dams for a 2.5-hour rest and then they were placed in jars with mixture of a 10% oxygen and 90% nitrogen circulation and 37±2 °C. After 2.5 hours, the jars were opened to room air and the surviving pups were returned to their dams for 24 hours. Animals were killed and the whole brain was removed and put in formalin for three days for neuropathologic assessment.

To assess the extent of edema and ischemia, the brains were embedded in paraffin and 10-mm slices were prepared by a fine microtome from the brain cortex, specially hippocampal cortex and basal ganglia. Slides were stained by H&E and examined under light microscope by a neuropathologist in a double-blind trial. Ischemic-hypoxic insult induced neuronal damage and edema in cortex and basal ganglia was assessed according to Rice et al. (15) as follows:

For edema, grade 0 means no visible swelling, grade 1 means slight swelling, grade 2 means moderate swelling and grade 3 means marked swelling.

For neuronal damage (shrinkage of neurons, eosinophilic cytoplasm, nucleus pyknosis and vacuolation of parenchyma), grade 0 means no damage, grade 1 means a few neurons damaged, grade 2 means a moderate number of neurons damaged, grade 3 means the majority of neurons damaged and grade 3+ means infarction (2, 15).

For statistical analysis, SPSS package was used to carry out one way analyses of variance (ANOVA) on the grade of edema and ischemia.

Results

The hypoxic-ischemic insult induced edema and ischemia in neonatal rats brains. This could be reduced by 0.2–2 mg/kg of berberine (Figs 2 and 3). There is a significant difference between berberine-treated groups and normal-saline-treated group. Also, groups that receive berberine in higher concentration (1 and 2 mg/kg) showed more reduction of ischemic insult in comparison with groups received berberine in lower concentrations (0.2 and 0.5 mg/kg). This means that effect of berberine in ameliorating ischemic-hypoxic injury is dose-dependent. There was no difference in the effect of berberine in cortex and basal ganglia (Fig. 4).

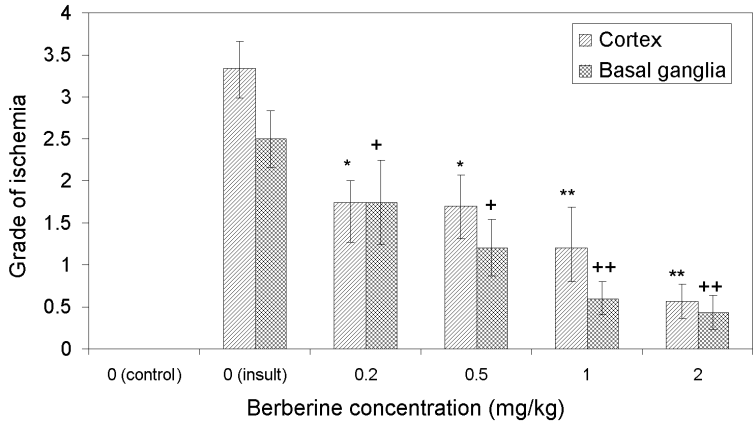

Fig. 2. Effect of berberine treatment on neuronal damage
The injury was evaluated by histological method and graded as described in the text. The control group did not have any surgery or hypoxic insult. The insult group received an injection of normal saline after ischemic condition. Each region was compared with the similar region (cortex: brick column, basal ganglia: dotted column) of the control group. *significant difference ($p<0.05$) with the insult group in cortex. **significant difference ($p<0.001$) with the insult group in cortex. +significant difference ($p<0.05$) with the insult group in basal ganglia. ++significant difference ($p<0.001$) with the insult group in basal ganglia

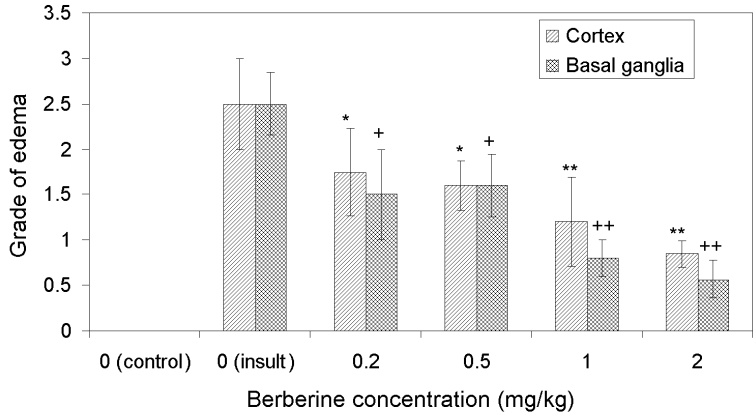

Fig. 3. Effect of berberine treatment on edema
The injury was evaluated by histological method and graded as described in the text. The control group did not have any surgery or hypoxic insult. The insult group received an injection of normal saline after ischemic condition. Each region was compared with the similar region (cortex: brick column, basal ganglia: dotted column) of the control group. *significant difference ($p<0.05$) with the insult group in cortex. **significant difference ($p<0.001$) with the insult group in cortex. +significant difference ($p<0.05$) with the insult group in basal ganglia. ++significant difference ($p<0.001$) with the insult group in basal ganglia

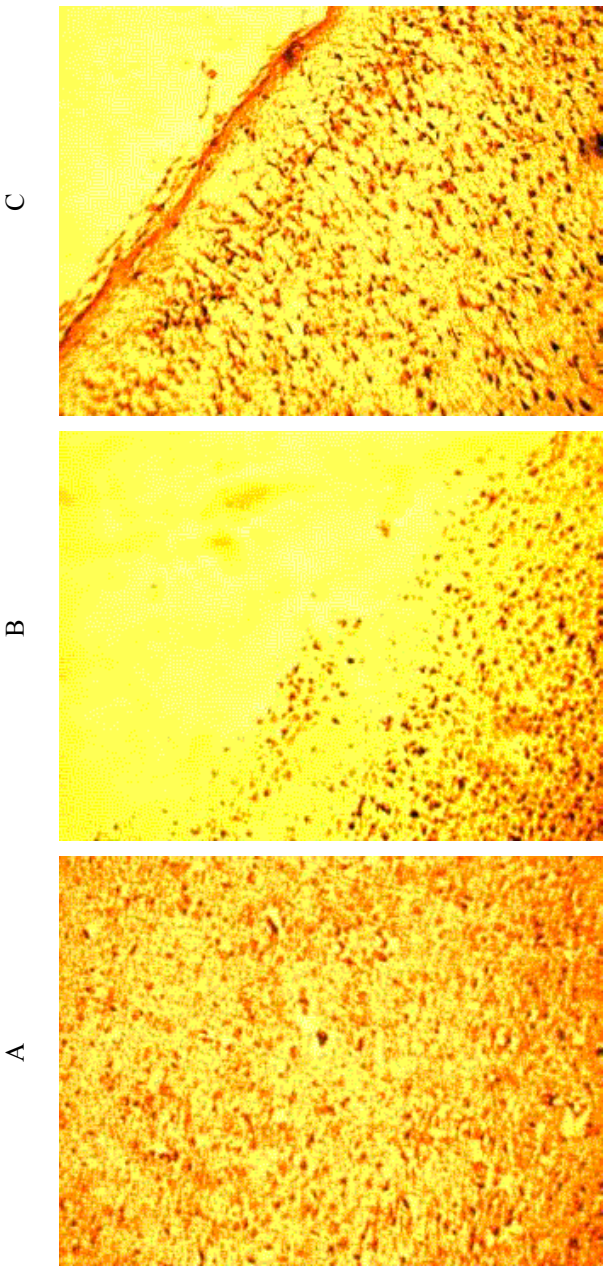

Fig. 4. Representative histogram of normal rat brain (A), of rat brain after ischemic-hypoxic insult (B), and of rat brain treated with 2 mg/kg berberine after ischemic-hypoxic insult (C). The sections were taken from hippocampal cortex, stained by H&E

Discussion

Stroke or cerebrovascular accident (CVA) is the third cause of death in USA. Stroke is divided to hemorrhagic and infarction types. Cellular damage mechanism in relation to CVA is ischemic or hypoxic. Following to ischemic-hypoxic insult, neuronal cells enter to degenerative process. Because anaerobic ATP production pathway (glycolysis) along with hypoxia persists, ischemia destroys cells sooner than hypoxia. Lack of ATP inactivates sodium pumps and glycolysis increases lactic acid concentration, so cells go through inflammation and acidosis. Astrocytes take up glucose of the peri-ischemic region to store glycolysis substrate for neurons (4, 11).

The hypoxic-ischemic model used in the present study was developed by Rice et al. (15). The model has the advantages of inducing highly-reproducible brain damage in spontaneously breathing animals, with low mortality rate, and in the absence of convulsions or cardiopulmonary complications for at least the first 50 hours of survival. The model is also inexpensive and relatively simple to prepare, so that large numbers of predictably brain-damaged animals can be produced for study of drugs.

Berberine administration to 35 rat pups showed that berberine had an effective neuroprotection against ischemic-hypoxic insult. When comparing groups which received various doses of berberine with the group which received normal saline, groups treated with berberine expressed less injury. Vascular supply of the basal ganglia in the rat-brain is variable (16). Ischemic cell necrosis within the basal ganglia may also be different. Therefore, case to case variability of the cortical and subcortical cell damage is not avoidable. However, we observed a significant reduction in injury in all berberine-treated animals. Therefore, we can conclude that berberine reduced ischemic-hypoxic neuronal damage dose-dependently.

Berberine anti-inflammatory effect is explained in many articles. Kuo and colleagues showed that berberine dose-dependently decreases production rate of COX-2 and AP-1 in oral cancer cells (6). Oral berberine reduces histological lesions, morphological damage, and myeloperoxidase activity in trinitrobenzene sulfonic acid-induced colitis in rats. Also, berberine can inhibit the increased production of IL-8 in trinitrobenzene sulfonic acid-induced colitis in rats (23). Another study in 2007 demonstrated that berberine, the protoberberine alkaloid widely distributed in the plant kingdom, was capable of suppressing inflammatory-agents-induced cytokine production in lung cells. These proinflammatory agents are interleukin-1-beta (IL-1-beta) and tumor necrosis factor-alpha (TNF-alpha). Moreover, the suppression of berberine on the cytokine production resulted from the inhibition of inhibitory kappaB-alpha phosphorylation and degradation (7). Ischemia makes cells release substances like matrix metalloproteinase which cause secretion of mediators like IL-1-beta and results in parenchymal insult (1). Because berberine has anti-inflammatory effect (6), it can help to decrease ischemic damage. Some studies have demonstrated the protective effect of berberine in cardiac ischemia (19, 22, 24).

The main management in ischemic (infarction) type of stroke is thrombolysis to re-canalize occluded vessels. But this approach has critical side effects such as hemorrhage especially in older patients. According to a study in 1995, berberine inhibits platelet aggregation and adhesion induced by ADP, arachidonic acid and collagen in rats with 24 hours of reversible middle cerebral artery occlusion. This study showed that the thromboxane B2 (TXB2) levels after drug treatment were lower than those in ischemia control rats. Therefore, the decline of platelet aggregation and decrease of TXB2 content may be one of the important factors involved in the anti-cerebral ischemia effect of berberine (18).

Decreasing of accessible oxygen is hypoxia and decreasing of perfusion means ischemia, although differentiating between these two terms is difficult. Brain cortex includes perikaryon, dendrites, initial segments of axons and glial cells arranging in 6 layers. Although neurons in birds' brain can regenerate, mammals' neurons usually cannot divide and degeneration is irreversible. Among neurons some like hippocampi pyramidal cells and globus pallidus neurons are more damageable in relation to ischemic-hypoxic insult. This sensitivity is because of increase in glutamic acid and its various metabolites in different cells in ischemia. Through ischemic process, other components like free radicals increase, then plasma membrane ion is disturbed leading to expression of apoptosis genes (3, 8). Berberry extract reduces N-methyl-D-aspartate (NMDA) receptor immunoreactivity after ischemia and in this way it reduces neuronal damage (20). As berberine has a central sympatholytic effect through alpha adrenoceptor blockade (5, 9), its hypotensive effect in stroke patients can have disastrous results, since the blood supply to ischemic but as yet uninfarcted brain tissue may be further compromised (13). This would be a disadvantage in berberine use for stroke patients.

Many herbs like barberry are overfilled with berberine and have been applied for so many years in traditional medicine particularly in China. It seems that berberine has a good potential for widespread medical treatment notably in stroke as our research shows.

We should note that all steps of the study such as adjustment of temperature and oxygen concentration were done manually and technical errors are possible. On the other hand, observation of the samples for assessing the grade of edema and neuronal damage was qualitative, so confounding variables are likely.

In conclusion, berberine can be used as a neuroprotective agent in ischemia. It is relatively non-toxic to humans (14, 21) and its genotoxicity study has shown no significant mutagenic effect (12). The most probable neuroprotective mechanism of berberine might be due to its anti-inflammatory and anticoagulatory effects.

REFERENCES

1. Amantea D, Russo R, Gliozzi M, Fratto V, Berliocchi L, Bagetta G, Bernardi G, Corasaniti MT: Early upregulation of matrix metalloproteinases following reperfusion triggers neuroinflammatory mediators in brain ischemia in rat. Int. Rev. Neurobiol. 82, 149–169 (2007)

2. Cadavid HMD, Rushing EJ: Human cerebral infarct: a proposed histopathologic classification based on 137 cases. Acta Neuropathol. 108, 524–530 (2004)
3. Graham SH, Chen J: Programmed cell death in cerebral ischemia. J. Cereb. Blood Flow Metab. 21, 99–109 (2001)
4. Kajihara H, Tsutsumi E, Kinoshita A, Nakano J, Takagi K, Takeo S: Activated astrocytes with glycogen accumulation in ischemic penumbra during the early stage of brain infarction: immunohistochemical and electron microscopic studies. Brain Res. 909, 92–101 (2001)
5. Ko ST, Lim DY: Influence of berberine on the blood pressure of rabbits. Arch. Pharm. Res. 3, 23–30 (1980)
6. Kuo C-L, Chia C-W, Liu T-Y: The anti-inflammatory potential of berberine in vitro and in vivo. Cancer Letters 203, 127–137 (2004)
7. Lee CH, Chen JC, Hsiang CY, Wu SL, Wu HC, Ho TY: Berberine suppresses inflammatory agents-induced interleukin-1beta and tumor necrosis factor-alpha productions via the inhibition of kappa-B degradation in human lung cells. Pharmacol. Res. 56, 193–201 (2007)
8. Lipton P: Ischemic cell death in brain neurons. Physiol. Rev. 79, 1431–1568 (1999)
9. Liu JC, Chan P, Chen YJ, Tomlinson B, Hong SH, Cheng JT: The antihypertensive effect of the berberine derivative 6-protoberberine in spontaneously hypertensive rats. Pharmacol. 59, 283–289 (1999)
10. Mahmoudian M, Siadatpour Z, Ziai SA, Mehrpour M, Benaissa F, Nobakht M: Reduction of the prenatal hypoxic-ischemic brain edema with Noscapine. Acta Physiol. Hung. 90, 313–318 (2003)
11. Melani A, Turchi D, Vannucchi MG, Cipriani S, Gianfriddo M, Pedata F: ATP extracellular concentrations are increased in the rat striatum during in vivo ischemia. Neurochem. Int. 47, 442–448 (2005)
12. Pasqual MS, Lauer CP, Moyna P, Henriques JAP: Genotoxicity of the isoquinoline alkaloid berberine in prokaryotic and eukaryotic organisms. Mut. Res. 286, 243–252 (1993)
13. Powers WJ: Acute hypertension after stroke: the scientific basis for treatment decisions. Neurol. 43, 461–467 (1993)
14. Rabbani GH, Butler T, Knight J, Sanyal SC, Alam K: Randomized controlled trial of berberine sulfate therapy for diarrhea due to enterotoxigenic Escherichia coli and Vibrio cholera. J. Infect. Dis. 155, 979–984 (1987)
15. Rice JE,III BS, Robert C, Vannucci MD, Lames B, Brierley, MD: The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann. Neurol. 9, 131–141 (1981)
16. Rieke GK, Bowers DE, Penn P: Vascular supply pattern to rat caudatoputamen and globus pallidus: scanning electromicroscopic study of vascular endocasts of stroke-prone vessels. Stroke 12, 840–847 (1981)
17. Sun FL: Chinese herbs for diabetes mellitus. Chinese Prescription Drug 7, 86–89 (2002)
18. Wu JF, Liu TP: Effects of berberine on platelet aggregation and plasma levels of TXB2 and 6-keto-PGF1 alpha in rats with reversible middle cerebral artery occlusion. Yao Xue Xue Bao. 30, 98–102 (1995)
19. Xuan B, Li DX, Wang W: Protective effects of tetrahydroprotoberberines on experimental myocardial infarction in rats. Zhongguo Yao Li Xue Bao. 13, 167–171 (1992)
20. Yoo KY, Hwang IK, Lim BO, Kang TC, Kim DW, Kim SM, Lee HY, Kim JD, Won MH: Berberry extract reduces neuronal damage and N-Methyl-D-aspartate receptor 1 immunoreactivity in the gerbil hippocampus after transient forebrain ischemia. Biol. Pharm. Bull. 29, 623–628 (2006)
21. Zeng X-H, Zeng X-J, Li Y-Y: Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am. J. Cardiol. 92, 173–176 (2003)
22. Zheng L, Zhou Z, Tao D, Lan T: Protective effect of berberine on cardiac myocyte injured by ischemia-reperfusion. Sichuan Da Xue Xue Bao Yi Xue Ban. 34, 452–454 (2003)
23. Zhou H, Mineshita S. The effect of berberine chloride on experimental colitis in rats in vivo and in vitro. J. Pharmacol. Exper. Therapeut. 294, 822–829 (2000)
24. Zhou J, Xuan B, Li DX: Effects of tetrahydroberberine on ischemic and reperfused myocardium in rats. Zhongguo Yao Li Xue Bao. 14, 130–133 (1993)
25. Zhu F, Qian C: Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease. BMC Neurosci. 7, 78 (2006)