Therapeutic Effect Analysis of Sinomenine on Rat Cerebral Ischemia–Reperfusion Injury

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Objective: The objective of this study is to investigate the therapeutic effect of sinomenine (SIN) on rat cerebral ischemia–reperfusion (IR) injury and the molecular mechanism. *Methods:* One hundred thirty-five rats were equally randomized into sham-operated group, middle cerebral artery occlusion (MCAO) group, and SIN group, and reversible rat MCAO model was made according to the Longa method for the MCAO and SIN groups. Then, 15 rats from each group were decapitated at 6, 12, and 24 hours after reperfusion to obtain brain tissue samples. Rats in the SIN group were injected with sinomenine by tail vein (90 mg/kg) 1 hour before ischemia; rats in the MCAO and sham-operated groups were administrated with the same volume of saline. Neurological severity score (NSS), infarction volume, ischemic brain water content, and blood–brain barrier (BBB) permeability were determined at corresponding time points. Acid-sensing ion channel (ASIC) 1a mRNA level was determined by quantitative real-time polymerase chain reaction; ischemic brain contents of lactic acid (LD), lactic dehydrogenase (LDH), ATPase, and inflammatory factors were determined by spectrophotometric method. *Results:* At 12 hours after reperfusion and since then, NSS in the SIN group decreased obviously; infarction volume, brain water content, and BBB permeability in the SIN group were lower than those in the MCAO group ($P < .05$). IR injury resulted in the upregulation of the contents of ASIC1a mRNA, LD, LDH, and inflammatory factors and the downregulation of the contents of ATPase, while SIN could reverse the upregulation/downregulation effect induced by IR injury ($P < .05$). *Conclusion:* Through its anti-inflammation effect, which alleviates acidosis, improves energy metabolism, and inhibits ASIC1a level, SIN protects ischemic rat brain against cerebral IR injury. **Key Words:** Cerebral ischemia–reperfusion—acidosis—ATPase—ASIC1a—inflammatory factors.

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Received December 25, 2015; revision received February 12, 2016; accepted February 16, 2016.

This work was supported by a grant from Shandong Provincial Natural Science Foundation, China (No. ZR2014HL037).

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1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2016.02.023

Introduction

Ischemic cerebrovascular disease is the focal dysfunction resulting from focal cerebral insufficiency of blood supply, oxygen deficit, or oxygen interruption, and this disease commonly occurs in transient ischemic attacks, cerebral embolism, and cerebral thrombosis. Ischemia–reperfusion (IR) injury is a pathological phenomenon that aggravates the degree of tissue damage after a certain time of ischemia and recovering blood supply from ischemia subsequently. The mechanisms of IR injury are quite complicated, involving energy metabolic disorders,[1] acidosis,[2] blood–brain barrier (BBB) destruction, high expression of proinflammatory factors and molecular adhesion, calcium dyshomeostasis, production of free radicals,[3] activation of apoptosis gene, and inflammation.[4] Tissue acidosis accompanies with ischemia, and most of the in vivo studies indicate that acidosis aggravates ischemic brain injury, and the accumulation of lactic acid (LD) as a by-product of glycolysis, together with the production of protons by ATP hydrolysis, cause a drop in the pH level of the ischemic brain.[5]

Acid-sensing ion channels (ASICs) belong to the amiloride-sensitive epithelial Na^+ channel/degenerin (ENaC/DEG) superfamily. Six subtypes of ASICs have been found: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. ASICs may contribute to poor stroke prognosis due to a localized drop in brain pH level, resulting in excessive calcium overload, independent of glutamate activation. Among the 6 ASIC subtypes, ASIC1a is the primary acid sensor in mammalian brain and plays a major role in neuronal injury following cerebral ischemia. Xiong et al[6] proved that ASIC1a was related to IR injury by using the acid-induced cortical nerve injury model in rat neuronal cells in vitro and the rat reversible middle cerebral artery occlusion (MCAO) model. McCarthy et al[7] verified that psalmotoxin-1, the ASIC1a-selective blocker, could markedly reduce cortical and striatal infarct volumes measured 72 hours post stroke in conscious, spontaneously hypertensive rats, which correlated with improvements in neurological score, motor function, and preservation of neuronal architecture.

Sinomenine (SIN) is an alkaloid extracted from the traditional Chinese medicine *Caulis sinomenii*, with pharmacological effects of anti-inflammation,[8] immunosuppression,[9] analgesia,[10] decompression,[11] and antiarrhythmia.[12] For example, Zhao et al[13] found that SIN inhibited nuclear factor-κB transcriptional activity to suppress IR-induced inflammatory response in the kidney while attenuating mitogen-activated protein kinase signaling to prevent tubular cells apoptosis after IR insult. In addition, SIN is also used for the treatment of carcinoma.

Wu et al[14] found that the extracellular application of SIN inhibited the currents mediated by ASIC1a and L-type voltage-gated calcium channels in rat cultured neurons, and pointed out that the inhibitory effects of SIN on ASIC1a were involved in this neuroprotection. Based on the multiple effects of SIN, through the administration of SIN on the rat MCAO model, we explored the outcomes and how ASIC1a, inflammatory factors, and LD were involved in IR injury.

Materials and Methods

Grouping and Administration

Male Sprague-Dawley rats, aged 7-9 weeks and weighing 250-290 g, were kept in diurnal lighting conditions (12-hour darkness/light). The rats were allowed to acclimate for 1 week with general feed and free drinking water. One hundred thirty-five rats were randomly divided into 3 groups: sham-operated group, MCAO group, and SIN group (n = 45). Fifteen rats from each group were sacrificed 6, 12, and 24 hours after reperfusion to obtain brain tissue samples. In the SIN group, rats were injected with sinomenine by tail vein (90 mg/kg) 1 hour before ischemia; in the MCAO and sham-operated groups, the rats were administrated with the same volume of saline.

Experiments were performed according to the international guidelines for animal research and approved by the Ethical Committee of Tai'an City Central Hospital, China.

Establishment of Rat MCAO Model

The model was established according to the Longa method.[15] Procedures were as follows: the rats were anesthetized by intraperitoneal injection with 10% chloral hydrate (.35 mL/100 g body weight) then fixed in supine position on the operating table. The right common carotid artery (CCA), external carotid artery (ECA), internal carotid artery (ICA), pterygopalatine artery, and vagus were exposed after slitting the neck midline and separating the tissues layer by layer. Then, the ECA trunk was ligated and the pterygopalatine artery was also ligated at its origin. After that, vessels were occluded temporarily at the proximal end of the CCA and at the start of the ICA. A small hole was made on the CCA near the bifurcation of the CCA and ICA by ophthalmic scissors and the .25-mm nylon line was inserted into the hole. Then the artery clamp was loosened on the ICA, and the nylon line was inserted into the ICA after going through the CCA. The nylon line was gently advanced into the internal carotid artery until it encountered small resistance, which implied that right middle cerebral artery occlusion was achieved. At that time, the artery clamp at the proximal end of the CCA was removed. Then, the line was ligated to fix it. After 2 hours of ischemia, the middle cerebral artery reperfusion was achieved through gently pulling the line. For the sham-operated group, the CCA, ECA, ICA, and vagus were just isolated without inserting the nylon line.

Before ischemia, during ischemia, and after reperfusion, the body temperature of the animals was monitored. In