Mitochondrial Calcium Uniporter Opener Spermine Attenuates the Cerebral Protection of Diazoxide through Apoptosis in Rats

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Background: It is reported that ischemic penumbra is a dynamic process, in which irreversible necrosis in the center of ischemia propagates to the neighboring tissue over time. Recent research has indicated that mitochondrial adenosine triphosphate (ATP)-sensitive potassium channels (mitoK_{ATP}) opener diazoxide plays an important role in cerebral protection; however, the role of mitochondrial calcium uniporter (MCU) in the effect of diazoxide on penumbra and infarct core remains unclear. *Methods:* Adult male Wistar rats were randomly divided into 5 groups: the Sham group, the ischemia-reperfusion (I/R) group, the diazoxide and ischemia-reperfusion (Dzx + I/R) group, the diazoxide and spermine and ischemia-reperfusion (Dzx + Sper + I/R) group, and the spermine and ischemia-reperfusion (Sper + I/R) group. The animals were exposed to 24-hour reperfusion after 2-hour ischemia. Diazoxide and spermine were administrated at 30 minutes or 10 minutes before the beginning of ischemia or reperfusion, respectively. After 24-hour reperfusion, animals were given neurologic performance tests and when overdosed with general anesthesia their brains were excised for infarct volume, apoptosis, and immunohistochemical. *Results:* Rats in the Dzx + I/R group displayed improved neurologic deficits, decreased infarct volume in cortex but not in subcortex, and apoptosis (evidenced by decreased terminal deoxynucleotidyl transferase-mediated dUTP nick end lebeling–positive percentage and the immunohistochemistry of cytochrome c) in cortex caused by ischemia/reperfusion. Rats in the Dzx + Sper + I/R group displayed worse neurologic deficits, larger infarct volume in cortex but not in subcortex, and more apoptosis both in penumbra and infarct core of cortex than those in the Dzx + I/R group. *Conclusions:* Results in our study suggested that diazoxide improved neurologic deficits, decreased infarct volume in cortex but not in subcortex, and apoptosis in cortex against ischemia/reperfusion injury is mediated by spermine. **Key Words:** Diazoxide—spermine—mitochondria—apoptosis.

Introduction

Responsible for about 5.4 million deaths annually worldwide, stroke is the major cause of disability.[1] It is reported that approximately 90% of stroke are known ischemic.[2] The restoration of perfusion and concomitant reoxygenation of the ischemic cerebral region is the most effective therapy for acute ischemia.[3] If reperfusion is not started in a short time, irreversible morphologic damage develops. However, reperfusion is frequently associated with an exacerbation of tissue injury.[4] Mitochondrial calcium

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overload has been closely related with mitochondrial damage in both necrotic and apoptotic forms of cell death.[5]

Activation of mitochondrial adenosine triphosphate (ATP)-sensitive potassium channel (mitoK$_{ATP}$) has been proposed to play an essential role in protecting brain from ischemia/reperfusion injury. Recent research indicates that mitoK$_{ATP}$ plays an initial or end-effective role in preconditioning-induced cerebral protection. MitoK$_{ATP}$ opener diazoxide-induced protective effects have been demonstrated in brain.[6] It is shown that mitoK$_{ATP}$-induced protection is involved in dissipating the mitochondrial membrane, thereby decreases calcium influx during ischemia.[7] Mitochondrial calcium uniporter (MCU), located in the inner mitochondrial membrane, is widely accepted to be responsible for the uptake of calcium by mitochondria, and it is activated by calcium concentrations greater than 200 nm in cytosolic microdomains or by physiological levels of polymines such as spermine.[8,9] Thus, it is likely that mitoK$_{ATP}$-induced cerebral protection can be attenuated by increasing the activity of MCU.

Our present study aimed to discuss the effect of MCU opener on diazoxide-induced protection in the ischemic cerebral cortex and subcortex in rats.

Materials and Methods

Animals

All experimental protocols and animal handling procedures were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals, and the experimental protocols were approved by the Institutional Animal Care and Use Committee of Qingdao University Medical College. Adult male Wistar rats (220-280 g) were purchased from Shandong Jining Lukang Co., Ltd, Jining, Shandong, China. The rats were maintained at 24 ± 1.0°C with a 12:12-hour light-to-dark cycle and given food and water ad libitum.

Transient Focal Middle Cerebral Artery Occlusion

Transient focal brain ischemia was produced by the filament model as initially described, with some modifications.[10] Briefly, rats had free access to water before the surgery. The animals were anesthetized with intraperitoneal (IP) injection of 10% chloral hydrate (10 mg/kg) and allowed to breathe spontaneously. During surgery, rectal temperature was maintained from 36.5°C to 37.0°C by a heating pad. Briefly, the common carotid artery (CCA), the external carotid artery, and the internal carotid artery (ICA) were dissected from the surrounding connective tissues via a ventral neck midline incision. The external carotid artery and the proximal end of CCA were ligated, and the proximal end of ICA was clamped, then a small cut was made on the distal end of the CCA, and a suture loop was put around the distal end of the cut. A 26mm diameter carbon fishing line with a pretreated rounded tip was introduced via the CCA cut into the ICA, and then, the suture loop was slightly fastened till no bleeding occurred. After removal of the clip, the filament was advanced further into the ICA until a resistance was felt at approximately 16 to 20 mm from the carotid bifurcation. The filament was fastened by tightening the loop around the distal CCA stump, and the neck incision was closed. After 2-hour middle cerebral artery occlusion

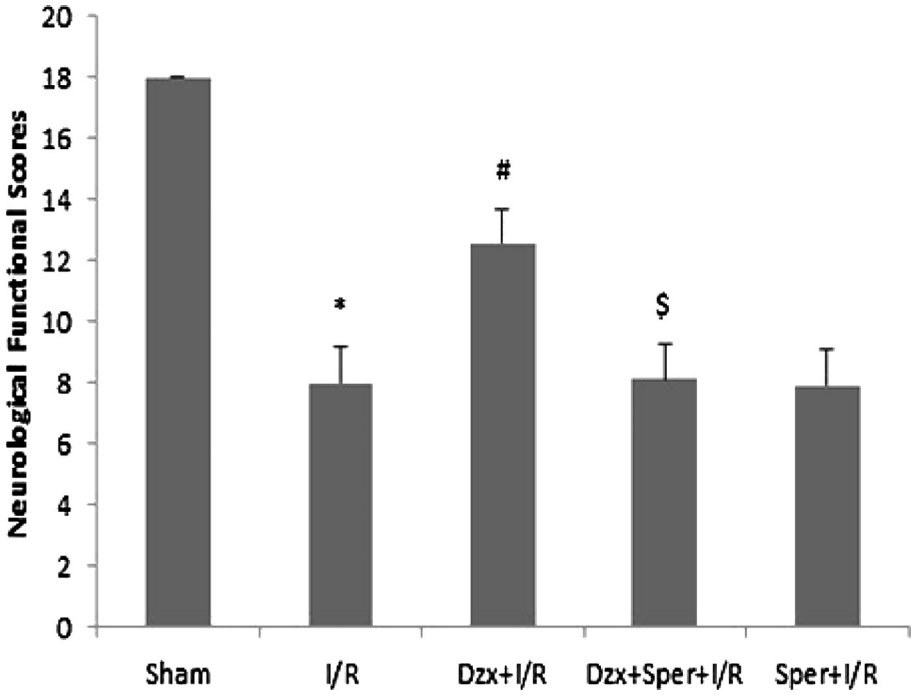

Figure 1. *The neurologic score for all 5 groups. * P < .05, compared with the Sham group; # P < .05, compared with the I/R group; $ P < .05, compared with the Dzx + I/R group. Abbreviations: Dzx, diazoxide; Dzx + I/R, diazoxide and ischemia-reperfusion; Dzx + Sper + I/R, diazoxide and spermine and ischemia-reperfusion; I/R, ischemia-reperfusion; Sper + I/R, spermine and ischemia-reperfusion.*