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The Effect of Diazoxide on Ultrastructural Changes Following Ischemia-Reperfusion Injury of Rat Brain

Mehdi Mehdizadeh[2]*, Majid Katebi [1], Mansoure Soleimani [2], Mohammad Hassan Farahani Pad [3], S.Ali Hoseini Tehrani [3], Mohammad Taghi Joghataei [2]

1. Department of Anatomy, School of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.
2. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
3. School of Medicine, Azad University, Tehran, Iran.

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ABSTRACT

Background & Objective: Even today there is no effective drug therapy to prevent neuronal loss after brain stroke. In the present study we studied the effect of mitochondrial KATP channel regulators on neuronal ultrastructure after ischemia reperfusion in the rat.

Materials & Methods: Rats temporarily subjected to four vessels occlusion for 15 minutes followed by 24 hours reperfusion with or without K-ATP channel regulators.

Results: Neuronal ultrastructure significantly improved in K-ATP channel opener (diazoxide) treated ischemia-reperfusion group compared with control group.

Conclusion: Our results showed that dizoxide treatment after ischemia reperfusion leads to better preservation of cortical neurons in rat.

Introduction

There is increasing evidence that the functional recovery after cerebral lesions and ischemia may be influenced by pharmacotherapy (Goldstein LB, 1993). There is escalating evidence that mitochondria play a key role in both necrotic and apoptotic neuronal cell death after acute cerebral ischemia (Mattson, Clumsee & Yu ZF, 2000; Nicholls, & Budd, 2000). Mitochondrial dysfunction is one factor that plays a critical role in mediating both apoptotic and necrotic neuronal cell death and is involved in the pathophysiology of cerebral ischemia (Saraste, & Pulkki, 2000). There is also increasing evidence about the diverse functions of mitochondrial KATP channels in the regulation of mitochondrial matrix volume, ATP production, and Ca2+ homeostasis in mitochondria, essential factors determining the outcome of ischemic stress on cellular function and survival (Holmuhamedov, Jovanovic, Dzeja, Jovanovic, & Terzic,

* ***Corresponding Author:***
Mehdi Mehdizadeh, Ph.D of Anatomical Sciences, Fellowship of Transgenic Animals, Department of Anatomical Sciences, Cellular & Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran,
PO Box:15875-1454Tel/Fax:+98(21)88058689
maranaoo2004@yahoo.com

1998; Szewczyk, Czyz, Wojcik, Wojczak, & Nalecz, 1996). Mitochondrial KATP channels show selective sensitivity to opening by diazoxide (Domoki, Perciaccante, Veltkamp, Bari, & Busija, 1999). The mechanism by which activation of mitochondrial KATP channels protect ischemia remains to be clarified. We tried to investigate the effects of selective mitochondrial KATP channel opener diazoxide (Garlid, Paucek, Yarov-Yarovoy, Murray, Darbenzio, D'Alonzo, Lodge, Smith, & Grover, 1997) on neuronal ultrastructre after ischemia-reperfusion.

Materials & Methods

Animal Treatment

Male Wistar rats weighing 180 to 200 g were used in this study. Rats were housed in an air-conditioned room with a 12-hour light/dark cycle. One week before surgery, the rats were randomly divided into 8 groups. The first group subjected to the anesthetic and surgical procedures of 4VO without interruption of the cerebral blood flow (Sham, n=6) ; The second group was subjected to 4VO received saline intraperitoneally (Vehicle, n=6); the third group was subjected to 4VO received glibenclamide, 1 mg/kg (Experimental 1, n=6), and fourth group was subjected to 4VO received glibenclamide, 5 mg/kg (Experimental 2, n=6), and fifth groups was subjected to 4VO received glibenclamide, 25 mg/kg (Experimental 3, n=6), and the sixth group was subjected to 4VO received diazoxide, 2 mg/kg (Experimental 4, n=6), and seventh group was subjected to 4VO received diazoxide, 6 mg/kg (Experimental 5, n=6), and eighth group was subjected to 4VO received diazoxide, 18 mg/kg (Experimental 6, n=6).

Electron Microscopy

Rat brain tissue was perfused with normal saline solution followed by phosphate-buffered 2% glutaraldehyde and 4% paraformaldehyde. The brain was carefully removed and immersed in the same fixative. Each tissue sample was then post-fixed in 1% potassium-ferrocyanide–reduced osmium tetroxide, dehydrated in graded acetones, and embedded in TAAB resin. The sections were stained with 0.25% lead citrate and 5% uranyl acetate in 50% methanol. A single blinded investigator using an objective grading system analyzed electron micrographs of neurons.

Results

Electron Microscopy

In rats treated with 25 mg/kg glibenclamid (G3 group), perinuclear space was widened and severe cytoplasmic edema was seen. The integrity of the nuclear membrane of neurons was completely degraded and the nuclear contents leaked out into the cytoplasm. (Figure 1-A). Mitochondria in varying stages of destruction with irregular morphology with poor structural integrity and matrix lucency contained hardly distinguishable cristae.

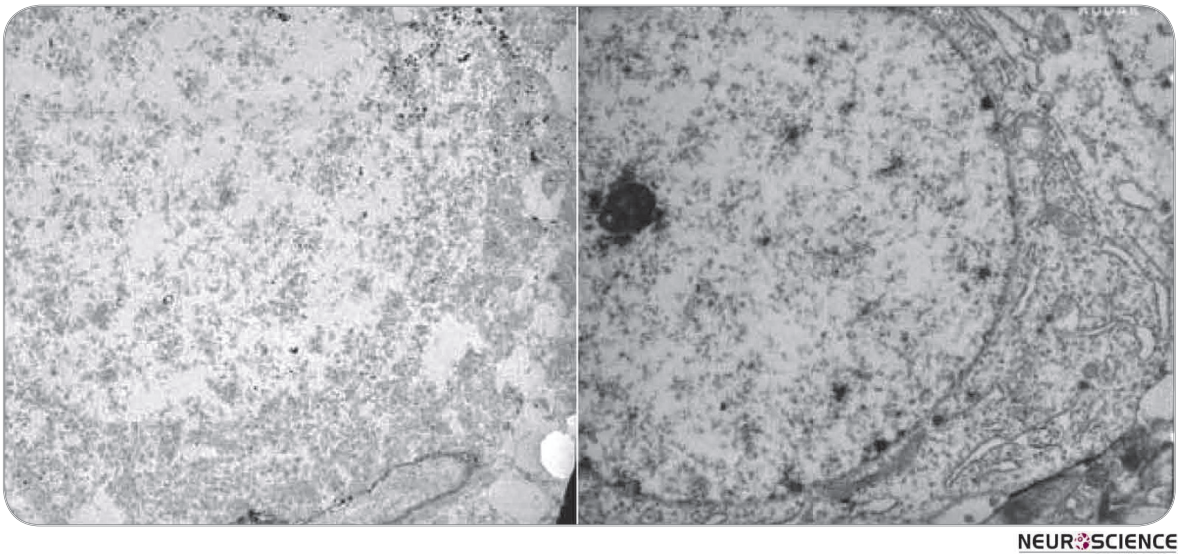

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Fig. 1-A: Serve cytoplasmic edema with a paucity of orangelles in cortical neurons of rats after 24 hours of reperfusion treated with 25 mg/kg glibenclamid (G3 group) B: Cortical neurons of rats after 24 hours of reperfusion treated rats treated with 18 mg/kg KATP channel opener diazoxide, (D3 group). (magnification X5000).

Mitochondria contain electron-dense deposits and show sign of autophagy with no discernible cristae (Figure 2-A). In addition, organelles showed more vacuolization compared to ischemic vehicle group.

In rats treated with 18 mg/kg KATP channel opener diazoxide, the general structure of neurons well preserved (Figure 1-B) and mitochondrial morphology greatly improved after ischemia/reperfusion compared to glibenclamide-treated rats (Figure 2-B).

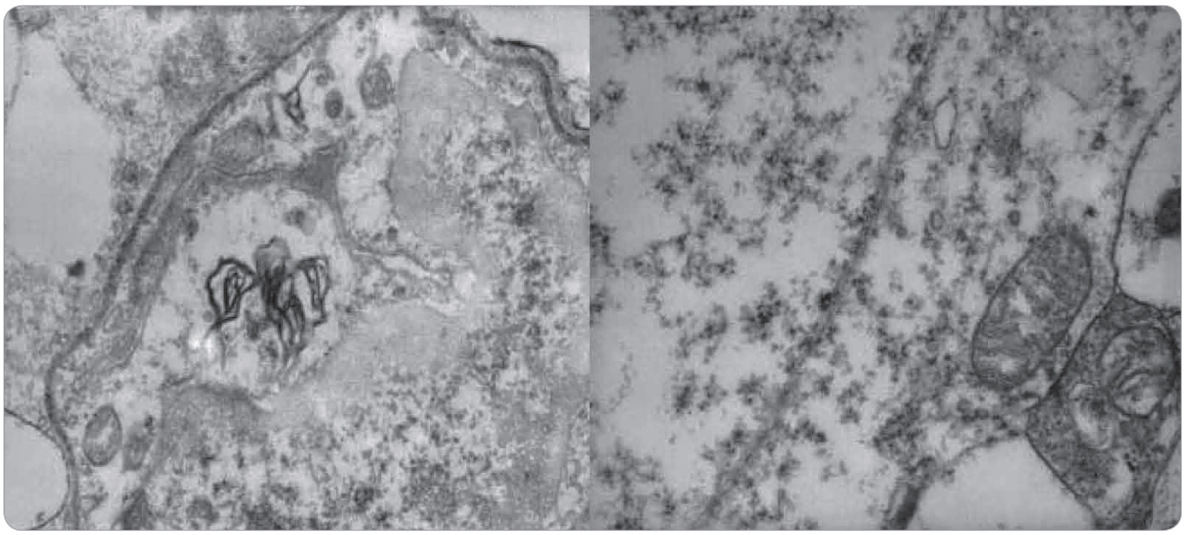

Fig. 2-A**:** Cortical neuron of rat after 24 hours of reperfusion treated with 25 mg/kg glibenclamid (G3 group) with variably disorganized cristae and a tubulo vesicular internal structure. B: Mitochondrial morphology improved in rats treated with 18 mg/kg KATP channel opener diazoxide, (D3 group). (magnification X5000).

Discussion

Mitochondrial ATP-sensitive potassium channels play a key role in modulating neuronal survival under ischemic conditions. Diazoxide is a mitochondrial membrane K-ATP channel opener that has been shown to attenuate the response to ischemia-reperfusion injury. It was reported that administration of diazoxide to newborn piglets enhances functional recovery after transient global cerebral ischemia (Domoki, Perciaccante, Veltkamp, Bari, & Busija, 1999). The mechanism by which activation of mitochondrial

KATP channels is translated into the observed protection is not known. However, we found that changes in structure were correlated to treated dose of KATP channel regulators in cells subject to ischemia and reperfusion in vivo.

We observed significant morphological differences by electron microscopy correlated to increasing dose of diazoxide. After 15 minutes of ischemia and 2 hour of reperfusion, there was severe cytoplasmic edema with a paucity of organelles and disintegration of the mitochondria. Review of the literature indicates similar findings (Solenski, DiPierro, Trimmer, Kwan, Gregory, & Helms, 2002).

We also found that mitochondrial architecture was preserved in animals treated with diazoxide. Similar changes in structure have been reported in spinal cord neurons subjected to ischemia and reperfusion in vitro (Roseborough, Gao, Chen, Trush, Zhou, Williams & Wei, 2006). The neuroprotective actions of diazoxide in vivo were largely abolished by glibenclamide, strongly suggesting that the effects of diazoxide were mediated by activation of Mito-KATP. In addition, a previous study indicated that opening of mitochondrial KATP channels is involved in the regulation of cell viability (Holmuhamedov, Jovanovic, Dzeja, Jovanovic & Terzic, 1998). In agreement with these results we demonstrated the direct effect of diazoxide on neuronal cell morphology.

Conclusion

The direct neuroprotective action of diazoxide suggests that this drug may be particularly effective in preventing neuronal damage after a stroke. However, for diazoxide to be beneficial in human stroke patients, it must be effective when given within a defined post-ischemic period, which remains to be established.

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