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Research Report

Propofol prevents neuronal mtDNA deletion and cerebral damage due to ischemia/reperfusion injury in rats

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ABSTRACT

Propofol is a commonly used intravenous anesthetic that has been demonstrated to be neuroprotective against cerebral ischemia–reperfusion (*I/R*) injury. It remains unclear whether this protective effect has any relationship with the prevention of neuronal mitochondrial deoxyribonucleic acid (mtDNA) deletion. In this study, 81 Wistar rats were randomly divided into three groups ($n=27$ each): sham (S group), ischemia/reperfusion (*I/R* group), or propofol (P group). Cerebral ischemia was induced by clamping the bilateral common carotid arteries for 10 min. A polymerase chain reaction (PCR) was conducted to determine mtDNA deletion. The mitochondrial membrane potential (MMP) changes were detected via microplate reader. The neuronal ultrastructure was visualized via electron microscope. MMP significantly decreased after *I/R* ($P<0.05$ compared with the S group). Severe damage to the ultrastructure of neuronal mitochondria was observed in cerebral *I/R* injury. When propofol (1.0 mg/kg/min) was administered intravenously for 1 h prior to the induction of *I/R*, the neuronal structure and MMP were well preserved, and mtDNA deletion was reduced after ischemia/reperfusion injury compared with the *I/R* group ($P<0.05$). These data suggested that propofol prevented mtDNA deletion and preserved a normal structure and MMP, which are important for normal mitochondrial function and increase neuronal resistance to *I/R* injury.

Abbreviations: Bax, Bcl-2-associated X protein; Bcl-2, B-cell leukemia-2; EEG, electroencephalogram; *I/R*, ischemia–reperfusion; MAP, mean arterial blood pressure; MMP, mitochondrial membrane potential; MPTP, mitochondrial permeability transition pore; mtDNA, mitochondrial deoxyribonucleic acid; $PaCO_2$, arterial carbon dioxide pressure; PaO_2, arterial oxygen pressure; PCR, polymerase chain reaction

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1. Introduction

The brain is very susceptible to ischemia and hypoxia. Reperfusion after ischemia may not improve the function of cells but may instead induce further damage. This has attracted the attention of anesthesiologists and has promoted the search for anesthetics that decrease the brain's vulnerability to ischemia/reperfusion injury.

Propofol is an intravenous anesthetic that has a neuroprotective effect against I/R injury in animal models (Yano et al., 2000; Ergun et al., 2002; Zheng et al., 2008). Our previous study showed that propofol had a protective effect against I/R injury by regulating the apoptosis regulatory genes B-cell leukemia-2 (Bcl-2) and Bcl-2-associated X protein (Bax) (Song et al., 2011; Xi et al., 2011). Many studies showed that mitochondria play an important role in cell apoptosis. The neurological impairment after I/R injury is related to mitochondrial function deficits (Chen et al., 2001). Cerebral ischemia causes mtDNA deletion (Zeng et al., 1999). The mtDNA segment is the coding area of many important genes that make up the oxidative phosphorylation and electronic transfer process. Many studies have shown that hippocampal neuronal cells are sensitive to ischemia, and mtDNA was damaged in this case (Abe et al., 1996). Some studies have shown that propofol may inhibit mitochondrial swelling and promote the recovery of mitochondrial respiratory enzyme activity (Adembri et al., 2006). However, it remains unclear whether propofol can reduce mtDNA deletion to protect the brain after cerebral I/R injury, and few studies have reported the effects of propofol on mitochondria following cerebral I/R injury (Chen et al., 2013; Li et al., 2014; Lee et al., 2000). Therefore, we hypothesized that propofol can reduce mtDNA deletion and protect mitochondrial structure and function after whole cerebral I/R injury in rats. To test our hypothesis, we established a cerebral I/R injury animal model by clamping the bilateral common carotid arteries. We observed mitochondrial microstructure damage and detected MMP change. We also detected mtDNA deletion via PCR.

2. Results

2.1. Physiological parameters

Wistar rats ($n=81$) were randomly divided into three groups ($n=27$): sham (S group), ischemia/reperfusion (I/R group), or propofol (P group). The P group rats received propofol (1.0 mg/kg/min) intravenously for 1 h prior to the induction of ischemia (see Section 4). The physiological variables of each group of rats were not statistically significant in terms of weight, mean arterial blood pressure (MAP), or arterial blood gas tension analysis, as shown in Table 1. The levels of arterial oxygen pressure (PaO_2) and arterial carbon dioxide pressure ($PaCO_2$), as well as the blood pH were maintained within the normal range. There were no between-group differences in baseline or recovery MAP. The MAP was maintained within predetermined limits (40±5 mmHg) during the ischemic period in the I/R group and P group.

Table 1 – Physiological parameters of groups, a controlled parameter ($n=9$).

Parameters	Group	Time		
		Baseline	Ischemia	Recovery
MAP (mmHg)				
	S	101±2	103±3	106±4
	I/R	106±2	39±1*	108±3
	P	105±2	41±1*	108±3
PaO_2 (mmHg)	S	132±6	138±4	135±4
	I/R	137±4	138±4	139±3
	P	139±3	140±4	141±3
$PaCO_2$ (mmHg)	S	38±1	39±1	40±1
	I/R	38±1	39±1	39±1
	P	39±1	38±1	40±1
pH	S	7.36±0.02	7.39±0.02	7.39±0.02
	I/R	7.38±0.01	7.38±0.02	7.39±0.03
	P	7.38±0.02	7.41±0.01	7.39±0.02

* $P<0.05$, compared with S group

2.2. Effect of propofol on mtDNA deletion

The PCR amplification products (using S1 and A1 primers) of each sample had bright DNA bands at 770 bp, demonstrating that mtDNA was successfully extracted from all of the samples (Fig. 1A). mtDNA deletion was demonstrated to have occurred if the PCR products using primers S2 and A2 had bright DNA bands at 459 bp (Fig. 1B).

No deletion product was found in the S group at 6 h, 24 h or 48 h after the procedure with mtDNA deletion ratios (calculated by dividing the number of DNA bands by the total bands) of 0%, 16% and 0%, respectively. Obvious deletion-amplified fragments were present in 2 of 6 (33%), 6 of 6 (100%), and 5 of 6 (83%) in the I/R group at 6 h, 24 h, and 48 h, respectively, after ischemia/reperfusion ($P<0.05$ compared with the S group). The P group had higher mtDNA deletion ratios (16%, 67%, and 33% at 6 h, 24 h, and 48 h, respectively) than the S group but lower ratios than the I/R group (Fig. 2, Table 2).

Both I/R and P groups had mtDNA deletion, indicating a significant difference compared with the S group. mtDNA deletion in the P group was significantly reduced at each time point compared with that in the I/R group ($P<0.05$, Table 3).

2.3. Transmission electron microscopic examination findings

Neuronal mitochondrial damage was evaluated using transmission electron microscopic examination of brain sections at the level of the hippocampus. Tissue samples from the S group showed normal nucleus and mitochondria (Fig. 3G). I/R injury caused significant mitochondrial damage in the brain tissue (Fig. 3A–C). Nearly all of the mitochondria showed pathological ultrastructure changes, and most of the mitochondria were swollen. Large vacuoles and intercellular edema were observed in the cytoplasm. No additional ultrastructural pathology was detected in the nuclei, cell membranes or other organelles of