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Research Report

Protective effects of propofol against whole cerebral ischemia/reperfusion injury in rats through the inhibition of the apoptosis-inducing factor pathway

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ABSTRACT

Cerebral ischemia/reperfusion (I/R) injury could cause neural apoptosis that involved the signaling cascades. Cytochrome *c* release from the mitochondria and the followed activation of caspase 9 and caspase 3 are the important steps. Now, a new mitochondrial protein, apoptosis-inducing factor (AIF), has been shown to have relationship with the caspase-independent apoptotic pathway. In this study, we investigated the protective effects of propofol through inhibiting AIF-mediated apoptosis induced by whole cerebral I/R injury in rats. 120 Wistar rats that obtained the permission of the animal care committee of Harbin Medical University were randomly divided into three groups: sham group (S group), cerebral ischemia/reperfusion injury group (I/R group), and propofol treatment group (P group). Propofol (1.0 mg/kg/min) was administered intravenously for 1 h before the induction of ischemia in P group. The apoptotic rate in three groups was detected by flow cytometry after 24 h of reperfusion. The mitochondrial membrane potential (MMP) changes were detected via microplate reader. The expressions of B-cell leukemia-2 (Bcl-2), Bcl-2 associated X protein (Bax) and AIF were evaluated using Western blot after 6 h, 24 h and 48 h of reperfusion. The results of our study showed that apoptotic level was lower in P group compared with I/R group and propofol could protect MMP. The ratio of Bcl-2/Bax was significantly higher in P group compared with I/R group. The translocation of AIF from mitochondrial to nucleus was lower in P group than that in I/R group. Our findings suggested that the protective effects of propofol on cerebral I/R injury might be associated with inhibiting translocation of AIF from mitochondrial to the nucleus in hippocampal neurons.

1. Introduction

Apoptosis has been tested play a critical role in lots of neurologic diseases. Apoptosis included activation of caspase-dependent and caspase-independent signaling pathways. While caspase-dependent pathway via release of cytochrome c from mitochondria, and then followed by apoptosome formation and activation of caspase3, caspase6, have been studied in detail. By now, little is known about the contribution of caspase-independent signaling after global cerebral ischemia (Harukuni and Bhardwaj, 2006).

Apoptosis inducing factor (AIF) is expressed in central nervous system and appears to play an important role in neuronal apoptosis induced by glutamate toxicity or oxidative stress (Yu et al., 2002; Zhang et al., 2002). AIF is a mitochondrial flavor protein which could mediate caspase-independent apoptotic cell death through its nuclear degrading activities (Daugas et al., 2000a, 2000b; Dumont et al., 2000; Ferri and Kroemer, 2000; Susin et al., 1999; Vieira et al., 2000). AIF translocated from mitochondria to nuclei where it induces caspase-independent DNA fragmentation. AIF is synthesized as a cytoplasmic ~67-kDa precursor which gives rise to a mature ~62-kDa protein that located in the mitochondrial inter-membrane space. When pathological permeabilization of the mitochondria, AIF is then processed to a ~57-kDa protein and followed released and translocated to the nucleus. The translocation to nucleus of AIF is associated with chromatin condensation and caspase-independent large-scale DNA fragmentation (Cheung

Abbreviations: I/R, cerebral ischemia/reperfusion; AIF, apoptosis-inducing factor; MMP, mitochondrial membrane potential; Bcl-2, B-cell leukemia-2; Bax, Bcl-2-associated X protein; MAP, mean arterial blood pressure; PaO2, arterial oxygen pressure; PaCO2, arterial carbon dioxide pressure; FCM, flow cytometry; CypA, cyclophilin A

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et al., 2005).

Propofol is an intravenous anesthetic that is commonly used in induce and maintain anesthesia. Many experimental evidences suggested that propofol had neuroprotective effect against cerebral ischemia injury (Ergun et al., 2002; Ito et al., 1999; Yano et al., 2000; Zheng et al., 2008). Although many studies have evaluated the mechanisms of propofol, it is still not totally clear. The brain protective mechanisms of propofol are complex. Our prior study had shown that propofol could inhibit the caspade-dependent apoptosis pathway. It is unknown, however, whether the brain protective effect of propofol is due to the caspase-independent pathway. Accordingly, the authors speculated that AIF signaling pathway is a candidate for contributing to the caspase-independent apoptotic process after cerebral ischemic injury. To test this hypothesis, we studied if apoptosis inducing factor (AIF), a major representative of caspase-independent apoptotic signaling is released from mitochondria and translated to nucleus following whole cerebral I/R injury and whether propofol could inhibit the process.

2. Results

2.1. Physiological parameters

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 kept within the normal range. The MAP was kept within predetermined limits (40 ± 5 mm Hg) during the ischemic period in the I/R group and propofol group of rats.

2.2. Apoptosis assay assessed by flow cytometry (FCM)

Neuronal apoptosis in the hippocampal from different groups was detected by FCM at 24 h after reperfusion. The distribution of apoptotic neurons was observed in each group. Few apoptotic neurons were found in the sham group. However, many apoptotic neurons were induced in the I/R group. Compared with the I/R group, the level of apoptosis was significantly decreased in propofol group. The percentage of apoptotic neurons was also calculated in the three groups. The percentage of apoptotic neurons in the I/R group ($50.9 \pm 3.2\%$) was higher ($n=5$, $P<0.05$ vs. sham), compared with the sham group ($6.4 \pm 0.8\%$); the percentage of apoptotic neurons in the propofol treatment group ($24.7 \pm 1.2\%$) was lower compared with the I/R group, ($n=5$, $P<0.05$ vs. I/R, see detail in Fig. 1).

Table 1
Physiological parameters of groups, a controlled parameter ($n=40$).

Parameters	Group	Time		
		Baseline	Ischemia	Recovery
MAP (mm Hg)	S	100 ± 5	102 ± 6	104 ± 9
	I/R	103 ± 4	$38 \pm 2^*$	107 ± 6
	P	102 ± 5	$40 \pm 3^*$	106 ± 5
PaO_2 (mm Hg)	S	130 ± 11	133 ± 8	132 ± 9
	I/R	134 ± 9	131 ± 6	135 ± 5
	P	136 ± 7	136 ± 8	137 ± 6
$PaCO_2$ (mm Hg)	S	38 ± 3	39 ± 2	39 ± 2
	I/R	39 ± 3	38 ± 3	39 ± 2
	P	39 ± 2	38 ± 3	40 ± 3
pH	S	7.35 ± 0.04	7.38 ± 0.04	7.39 ± 0.05
	I/R	7.37 ± 0.03	7.37 ± 0.04	7.38 ± 0.05
	P	7.37 ± 0.03	7.39 ± 0.03	7.38 ± 0.04

2.3. Mitochondrial membrane potential analysis

Mitochondrial membrane potential (MMP) changes were detected at 6 h, 24 h, and 48 h after reperfusion and were determined via microplate reader. A lower light absorption photometric value of mitochondria reflected a lesser opening of the mitochondrial permeability transition pore, and the functionality of the mitochondria was taken as an approximation of normal neuron. The light absorption photometric values were 0.28 ± 0.08, 2.41 ± 0.4, and 1.2 ± 0.27 at sham group, I/R group and propofol group at 6 h after reperfusion, respectively; 0.28 ± 0.08, 2.56 ± 0.26, and 1.48 ± 0.19 at sham group, I/R group and propofol group at 24 h after reperfusion, respectively; 0.28 ± 0.08, 2.64 ± 0.16, and 1.68 ± 0.21 at sham group, I/R group and propofol group at 48 h after reperfusion, respectively.

The light absorption in I/R group and propofol group are higher than that in the sham group ($P<0.05$ at each time point, respectively). The light absorption in propofol group was lower than that in the I/R group (as shown in Fig. 2, $P<0.05$ at each time point, respectively).

2.4. Western blot analysis of Bcl-2 and Bax

The expression of Bcl-2 and Bax was evaluated by Western blot analysis. The results showed that the ratio of Bcl-2/Bax in P group (2.2 ± 0.63) was significantly higher than that in I/R group (1.1 ± 0.31) at 24 h after reperfusion ($P<0.05$ vs I/R, Fig. 3).

2.5. Translocation of AIF

The expressions of AIF protein in different part of neuron were examined using Western blot in sham group, I/R group and propofol group at 6 h, 24 h and 48 h after reperfusion. The results showed that the total AIF protein level was not significantly changed after ischemia, and clear translocation of AIF from mitochondria to the nucleus was observed in 6 h, 24 h and 48 h after reperfusion. The AIF relative levels of mitochondrial fraction in I/R group and propofol group were lower than that in sham group ($n=5$, $P<0.05$ vs sham). The AIF relative level of mitochondrial fraction in I/R group was lower than that in propofol group ($n=5$, $P<0.05$ vs I/R). The AIF relative levels of nuclear fraction in I/R group and propofol group were higher than that in sham group ($n=5$, $P<0.05$ vs sham). The AIF relative level of nuclear fraction in I/R group was higher than that in propofol group ($n=5$, $P<0.05$ vs I/R, see detail in Fig. 4).

3. Discussion

Our study showed that propofol (1.0 mg/kg/min) significantly reduced the neural apoptosis level, ameliorated the opening of the mitochondrial permeability transition pore, increased the expression of apoptosis related gene Bcl-2, decreased the expression of Bax and decreased the translocation of AIF from mitochondria to the nucleus after cerebral ischemia/reperfusion injury.

In this study, we choose the clinical dose 1.0 mg/kg/min propofol as the test dose, and according to our past study, this dose could provide better cerebral protective effect and less circulation depress. The current study performed that AIF is a caspase-independent signaling pathways and important mediator of neuronal apoptosis and propofol treatment could decrease the translocation of AIF from mitochondria to nucleus after whole cerebral I/R injury. To our knowledge, this is the first report to implicate AIF in neuronal apoptosis about propofol treatment in whole cerebral I/R