Saudi Journal of Biological Sciences (2017) **24**, 246–250

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

Effect of propofol on mitochondrial ATP content and ATPase activity in hippocampus of rats with cerebral ischemia-reperfusion injury

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Received 19 June 2016; revised 31 August 2016; accepted 2 September 2016
Available online 10 September 2016

KEYWORDS

Ischemia-reperfusion injury;
Propofol;
ATP;
Mitochondria;
Hippocampus

Abstract *Objective:* Study on the influence of the cerebral Ischemia-reperfusion Injury (IRI) on mitochondrial adenosine triphosphate (ATP) content and ATPase activity in hippocampus of rats, as well as the protective effect of propofol on IRI in rats.

Methods: A total of 40 male SD rats were randomly divided into 5 groups: sham operation group (Group A), ischemia reperfusion control group (Group B) and ischemic reperfusion with propofol pretreatment group (C group). Group C was further divided into three sub groups according to the different doses of propofol: Group C1 (50 mg/kg), Group C2 (100 mg/kg) and Group C3 (150 mg/kg). The rats from Groups B and C were applied for the IRI model preparation by blockage of the blood flow in arteria carotis communis. For the Groups A, arteria carotis communis were separated without blockage of the blood flow. Before preparation of IRI model for rats in Group C, different doses of propofol were intraperitoneally injected into the rats. For rats in Groups A and B, only saline solution with same volume was intraperitoneally injected at the same time. The ultrastructures of mitochondria in hippocampus of rats were observed under transmission electron microscope, and the mitochondrial degeneration rate was counted. The contents of ATP were determined by HPLC and the ATPase activity was characterized by ATPase activity assay kit.

Results: (1) Mitochondria in the hippocampus from Groups B and C showed different degrees of ultrastructural damage and more significant mitochondrial degeneration than those from Group A. The degree of damage and the rate of degeneration were in the order of B > C1 > C2 > C3 and the difference was statistically significant ($P < 0.01$). (2) The contents of ATP and the ATPase activity in hippocampus from Groups B and C were significantly lower than those of Group A, while these

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2016.09.007

indices from Group C were significantly higher than those in the B group, and the sequence was C3 > C2 > C1, indicating that the ATP content and ATPase activity were significantly correlated with the dose of propofol, and the difference was statistically significant ($P < 0.05$).

Conclusion: In summary, the contents of ATP and ATPase activity in hippocampus of rats can be decreased by cerebral IRI. The structure and function of the impaired mitochondria in IRI rats could be significantly improved by propofol, and the improvement effect is related to the dose of propofol.

1. Introduction

Ischemia-reperfusion injury (IRI) is a phenomenon in which the ischemic tissue and organs cannot recover their function and structure after blood reperfusion, and may even aggravate the injury. The occurrence of IRI is common in clinic, which is usually investigated in the cases of microcirculation dredging during shock, cardiopulmonary resuscitation after cardiac arrest, Organ transplantation after revascularization (such as artery bypass grafting), etc. IRI cannot only affect the structure of the ischemic tissues and organs, but also damage the function and metabolism of organisms in a more serious degree than that induced by ischemia alone. Therefore, alleviating or avoiding the occurrence and development of IRI has become a hot topic in the basic and clinical research. The occurrence mechanism of IRI is related to the free radicals, calcium overload and leukocyte. The previous studies (Wang and Han, 2013; Tu et al., 2013; Gharanei et al., 2013; Cui et al., 2013; Sun et al., 2013) show that both the IRI pretreatment and some drug pretreatment can increase the tolerance of the tissue to IRI, reducing the effect of the IRI. Propofol is a kind of common clinically applied non-barbiturate intravenous anesthetic. It is found that propofol has the functions of antioxidation, scavenging free radicals, as well as blocking the calcium channel. The protective effect of propofol on IRI in the hippocampus has been reported in Ref. (Guo et al., 2004). Mitochondria is the main site for energy metabolism of animals and plants, which can not only maintain the normal physiological function of the cell, but also to participate in various cellular activities, such as the Ca^{2+} regulation and the conduction of the signal transduction pathway (Hernandez-Resendiz et al., 2013). Meanwhile, it is also a key target for IRI (Ten and Starkov, 2012). IRI is caused by the mitochondrial damage through the action of free radicals and calcium overload, resulting in the synthesis function reduction of adenosine triphosphate (ATP), and further reducing the activity of ATPase. Therefore, it is of great significance to prevent the decreasing of mitochondrial ATP content and ATPase activity during IRI for the prevention of IRI occurrence and development as well as the cell function recovery. In this work, the protective effect and mechanism of propofol on IRI in the hippocampus were studied by investigating the influence of the propofol on the mitochondrial ATP content and ATPase activity.

2. Experimental

2.1. Experimental materials

2.1.1. Animals

A total of 40 male SD rats (10–12 months old, weighting 250–350 g) in SPF grade were provided by XXX. The animals were free fed with food animal laboratory, at room temperature (23 ± 2 °C), humidity 50–75%, natural light, adaptive feeding for 2 weeks.

2.1.2. Apparatus and reagents

(1) Apparatus: High performance liquid chromatograph (HPLC); Transmission Electron Microscope (TEM) (Jiangsu Yixing Co., Ltd., China); High Speed Freezing Centrifuge (Wuhan Scientific Instrument Factory, CAS); Ultrasonic Cell Disruption System (Beijing Keer Instrument Co., Ltd., China).
(2) Reagents: Propofol (Chinese MedicineH20051842: Guangdong Gabo Pharmaceutical Co. Ltd., China); Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase assay Kit (Suchow Keming Co., Ltd., China); Coomassie blue reagent (Suchow Keming Co., Ltd., China).

2.2. Methods

2.2.1. Animal grouping

The SD rats were randomly divided into 5 groups with 8 rats in each group: sham operation group (Group A), ischemia reperfusion control group (Group B) and ischemic reperfusion with propofol pretreatment group (C group). Group C was further divided into three sub groups according to the different dose of propofol: Group C1 (50 mg/kg), Group C2 (100 mg/kg) and Group C3 (150 mg/kg).

2.2.2. Preparation and Treatment method for IRI model of rats

IRI model of rats was prepared using rats from Groups B and C. The specific operation methods were as follows: After intraperitoneal injection of 45 mg/kg pentobarbital sodium for anesthesia, bilateral arteria carotis communis of the rats were carefully separated, and cerebral blood flow was then blocked using noninvasive arterial clamp for 10 min to bring about forebrain ischemia, followed by releasing the noninvasive arterial clamp for Reperfusion. For the Groups A, arteria carotis communis were separated without blockage of the blood flow. About 30 min before preparation of IRI model for rats in Group C, 5 ml saline solution with propofol was intraperitoneal injected into the rats. For rats in Groups A and B, 5 ml saline solution without propofol was intraperitoneally injected at the same time.

All the experiments were performed in the mobile air bath with temperature monitor for the membrane. In order to avoid the effects of brain temperature on the results of the experiment, the temperature of the membrane was kept in 37.0 ± 0.5 °C by light bulb heating or ice cooling.

2.2.3. Specimen extraction

The brain was taken out after the guillotining, and then the hippocampal tissue was rapidly separated on the ice plate, put into liquid nitrogen for cryopreservation in −75 °C refrigerator.

2.3. Observation index

2.3.1. Pathological examination

A total of 1 mg tissues in the same parts of the hippocampus were taken out and fixed in 4% poly formaldehyde solution at 4 °C for a week, and then in 1% osmic acid at 4 °C for 1–2 h, After acetone dehydration step by step, the samples were soaked for 2 h in percolate (Acetone embedding solution 1:1), and then embedded in epoxy resin 618 for 2 times with 3 h each time. After drying and polymerization the Ultrathin section was prepared, followed by double staining with uranium acetate-lead citrate. The mitochondrial ultrastructure was observed under TEM, and randomly selected 6 non-overlapping fields of view to carry out the percentage of denatured mitochondria.

2.3.2. ATP content detection

The ATP content was detected by High performance liquid chromatography (HPLC). A total of 50 mg hippocampus was weighted and added into 100 mL of 3.6% cold perchlorate. The mixture was made into a homogenate under the ice bath and then centrifuged in 8000 r/min for 15 min at 4 °C. The supernatant was obtained and the PH was adjusted to neutral using 6 mol/L K_2CO_3 solution, and then centrifuged in 3000 r/min for 15 min at 4 °C. A total of 20 mL supernatant was applied for sampling, using ODS-C18 chromatographic column. The mobile phase was 0.1 mol/L phosphate buffer (PH = 6.0), and the flow rate was 1 mL/min. A 254 nm adjustable UV-detector was applied for detection.

2.3.3. ATPase activity

The Na^+-K^+-ATPase activity was detected by a Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity assay kit. The activity was indexed by the content of the produced inorganic *P* from degenerated tissue protein (/mg) by enzyme (/h). Specific processes include: a certain amount of hippocampal tissue was taken out and made into 10% homogenate with cold saline. After 1200 r/min it was centrifuged for 10 min at 4 °C, the supernatant was taken out for further centrifugation in 4000 r/min for 30 min at 4 °C. The deposition contained mitochondria. The obtained mitochondria were dehydrated with acetone and dried 2 times. The mitochondria and Tris-HCl were mixed in the proportion of 10 mg: 1 ml, followed by breaking the mitochondria in the ultrasonic apparatus for 20 min. The tissue proteins were determined by coomassie blue reagent strictly according to the instructions of enzyme activity assay kit.

2.4. Statistical analysis

All statistical analyses were performed using SPSS software. Data were expressed as mean ± SEM ($\bar{x} \pm s$). The statistical significance was determined by using the Student *t* test for differences between two groups and one-way ANOVA for differences among multiple groups. A *P* value of less than 0.05 was considered statistically significant.

3. Results

3.1. Ultrastructural changes of mitochondria in the hippocampus of IRI rats

Under electron microscope investigation, mitochondria in the hippocampus from Groups B and C showed different degrees of ultrastructural damage, and the main characterizations involve a high degree of mitochondria swelling, many vacuoles in the matrix, the reduction or breakage of the ridge, condensation of the nuclear chromatin, high degree of the endoplasmic reticulum swelling, destruction of endometrial integrity, and the increasing of electron density. Among these groups, the damage of Group B was the most serious, followed by C3, C2 and C1 group. The mitochondrial degeneration rate in B group was significantly higher than that in other groups ($P < 0.01$), while the mitochondrial structure of A group was very clear without obvious swelling or degeneration. The damage of mitochondria in Groups C1–C3 was significantly mitigated than that in Group B, and the degeneration rate was significantly lower than that in B group. Their degeneration rates were in order of C1 > C2 > C3, and the difference was statistically significant (< 0.01 *P*)in Table 1.

3.2. The content variationof ATP and adenylate pool

Table 2 shows that the contents of ATP, ADP, AMP and total adenine in hippocampus from Groups B and C were lower than that from Group A, while the contents in all the three subgroups C1–C3 were significantly higher than that in Group B. The content sequence was C3 > C2 > C1, which indicates that the contents of ATP and adenylate pool in C1–C3 group were related to the dose of propofol, and the difference was statistically significant ($P < 0.05$).

3.3. Comparison of ATP activity in hippocampus of rats from each group

As shown in Table 3, the activities of ATPase (Ca^{2+}-Mg^{2+}-ATPase, Na^+-K^+-ATPase and total ATP ase) in the hip-

Table 1 The percentage of mitochondrial degeneration in the hippocampus of the rats from each group (%).

Groups	Percentage
Group A	2.80 ± 0.02
Group B	85.34 ± 5.09[a]
Group C1	67.01 ± 5.48[a,b]
Group C2	60.03 ± 3.14[a,b,c]
Group C3	51.34 ± 7.14[a,b,c,d]

Note: a means comparing with Group A, $P < 0.05$; b means comparing with Group B, $P < 0.05$; c means comparing with Group C1, $P < 0.05$; d means comparing with Group C2, $P < 0.05$.

Table 2 Effect of propofol on the content of ATP in the hippocampus of IRI rats (μmol/g, $\bar{x} \pm s$).

Groups	ATP	ADP	AMP	Total adenine
Group A	1.21 ± 0.09	1.46 ± 0.12	1.24 ± 0.19	3.94 ± 0.41
Group B	0.43 ± 0.07[a]	0.74 ± 0.06[a]	0.69 ± 0.11[a]	1.79 ± 0.22[a]
Group C1	0.71 ± 0.08[a,b]	0.80 ± 0.08[a]	0.81 ± 0.15[a]	2.54 ± 0.30[a,b]
Group C2	0.85 ± 0.07[a,b,c]	0.85 ± 0.08[a]	0.89 ± 0.16[a]	2.81 ± 0.29[a,b,c]
Group C3	1.01 ± 0.09[a,b,c,d]	0.88 ± 0.10[a]	0.93 ± 0.21[a]	3.16 ± 0.38[abcd]

Note: a means comparing with Group A, $P < 0.05$; b means comparing with Group B, $P < 0.05$; c means comparing with Group C1, $P < 0.05$; d means comparing with Group C2, $P < 0.05$.

Table 3 Effect of propofol on the activity of ATP in the hippocampus of IRI rats (μmolPi $mgpro^{-1}$ h^{-1}, $\bar{x} \pm s$).

Groups	Na^+-K^+-ATPase	Ca^{2+}-Mg^{2+}-ATPase	Total ATPase
Group A	10.68 ± 1.23	6.82 ± 0.53	21.35 ± 1.93
Group B	6.74 ± 0.51[a]	3.67 ± 0.21[a]	14.32 ± 1.61[a]
Group C1	7.13 ± 0.58[a,b]	4.16 ± 0.35[a,b]	16.31 ± 1.54[a,b]
Group C2	8.04 ± 0.69[a,b,c]	4.93 ± 0.51[a,b,c]	17.24 ± 1.69[a,b,c]
Group C3	9.19 ± 0.64[a,b,c,d]	5.82 ± 0.46[a,b,c,d]	19.20 ± 1.63[a,b,c,d]

Note: a means comparing with Group A, $P < 0.05$; b means comparing with Group B, $P < 0.05$; c means comparing with Group C1, $P < 0.05$; d means comparing with Group C2, $P < 0.05$.

pocampus from Groups B and C were significantly lower than those from Group A, while the activities in all the three subgroups C1–C3 were significantly higher than those in Group B. The content sequence was C3 > C2 > C1, which indicates that the activity of ATP in Groups C1–C3 were related to the dose of propofol, and the difference was statistically significant ($P < 0.05$).

4. Discussion

As a common and important problem in clinic, Prevention and reduction of patients' IRI is essential for the function recovery of the organ and tissue. Therefore, research on IRI has been drawing more and more attention in recent years. The etiology and pathogenesis of IRI are very complex, involving energy metabolism disorder, intracellular calcium homeostasis, free radical generation, intracellular acidosis, increasing of excited amino acid release, apoptosis gene activation, etc. These factors are reciprocal causation, cascade response to the interaction, and eventually leading to the apoptosis or necrosis of the cell (Ten and Starkov, 2012). Among these factors, energy metabolism disorder is considered to be the initial factor for IRI. As the main site for aerobic respiration, mitochondria provide about 95% of the energy required for cell life activities. Hence, it is known as the "power plant" for intracellular energy supply. Mitochondrial energy depletion will generate a variety of transport barriers of intracellular ion pumps, which depended on ATP energy (Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase), leading to the Na^+ and Ca^{2+} overload. The increasing of intracellular osmotic pressure caused by Na^+ increasing is an important reason for the Cerebral edema and cell necrosis in early stage of IRI. Meanwhile, the Ca^{2+} overload becomes even worse through the Na^+-Ca^{2+} exchange, and the Ca^{2+} could be deposited in mitochondria in the form of calcium phosphate, which is harmful to the mitochondrial function, resulting in ATP synthesis disorders. Moreover, Ca^{2+} could also lead to the generation of intracellular free radicals and the damage of cytomembrane structure. The increasing of cell membrane permeability could further lead to and external environment imbalance internal of the cells, accelerating cell apoptosis. It was found that the generation of mitochondrial ROS can be reduced during IRI by inhibiting Ca^{2+} overload (Jeong et al., 2013; Bround et al., 2013), thus improving mitochondrial dysfunction. Therefore, the variations of ATP content and ATPase activity in mitochondria can be applied as an index to reflect the severity of IRI, which is also an important target to mitigate or prevent the occurrence and development of IRI.

The results show that the structural degenerations of hippocampal mitochondria from the rats in groups B and C are different to those in group A. Comparing with group A, the ATP content and ATPase activity were significantly decreased in groups A and B, which is consistent with the previous study (Lu and Zheng, 2013). This indicates that the mitochondria is an important target of IRI. The damage of mitochondria may be related to the oxidative stress reaction. The content of reactive oxygen species (ROS) could be enhanced by IRI, which oxide the mercaptanan the mitochondrial permeability transition pores (MPTP), inducing MPTP channel opening. The continued increase in oxygen free radicals leads to the sustained opening of the channel (Penna et al., 2013). On the other hand, the ROS also induces the dissociation of mitochondrial phospholipid and cytochrome C, leading to mitochondrial structural damage and even apoptosis. Moreover, mitochondrial DNA is vulnerable to oxygen free radicals, because it is lack of protective effect from histone and DNA binding proteins, and direct exposure to high oxygen concentration in the process of oxidative phosphorylation reaction. The mitochondrial structure damage will decrease its ability to synthesize ATP and the ATPaseactivity.

It was found that the ATP content and ATP activity of the rats in C1–C3 groups were significantly higher than those in B group, and C3 > C2 > C1, indicating that mitochondria in

IRI rats could be improved by propofol, and the improvement effect is related to the dose of propofol. The reason may be related to the antioxidant, scavenging free radicals, and calcium blockade of the propofol. Propofol can reduce the generation of intracellular ROS and inhibit the overload of Ca^{2+}, which can control the oxidative stress injury. Yin et al. Yin et al. (2014) found that propofol can inhibit the opening of MPTP, reduce the concentration of Ca^{2+}, intervene the expression of apoptosis protein Bcl-2 and Bax by inhibiting the AMPK signaling pathway, and thus have a positive effect on IRI. It is found that (Cao, 2013) the propofol can inhibit the learning and memory function of normal rat's brain. Hence, the propofol dosage should be very careful in clinical use, and the optimal dosage needs to be further studied.

5. Conclusions

In summary, the contents of ATP and ATPase activity in hippocampus of rats can be decreased by cerebral IRI. The structure and function of the impaired mitochondria in IRI rats could be improved by propofol, and the improvement effect is related to the dose of propofol.

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