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Propofol inhibits inflammation and lipid peroxidation following cerebral ischemia/reperfusion in rabbits☆

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Abstract

The present study established a rabbit model of global cerebral ischemia using the 'six-vessel' method, which was reperfused after 30 minutes of ischemia. Rabbits received intravenous injection of propofol at 5 mg/kg prior to ischemia and 20 mg/kg per hour after ischemia until samples were prepared. Results revealed that propofol inhibited serum interleukin-8, endothelin-1 and malondialdehyde increases and promoted plasma superoxide dismutase activity after cerebral ischemia/reperfusion. In addition, cerebral cortex edema was attenuated with little neuronal nuclear degeneration and pyknosis with propofol treatment. The cross-sectional area of neuronal nuclei was, however, increased following propofol treatment. These findings suggested that propofol could improve anti-oxidant activity and inhibit synthesis of inflammatory factors to exert a protective effect on cerebral ischemia/reperfusion injury.

Key Words: ischemia/reperfusion injury; propofol; endothelin-1; interleukin-8; malondialdehyde; superoxide dismutase; neuroprotection

Abbreviations: I/R, ischemia/reperfusion; IL-8, interleukin-8; ET-1, endothelin-1; MDA, malondialdehyde; SOD, superoxide dismutase

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INTRODUCTION

Acute cerebral ischemia/reperfusion (I/R) injury has a high incidence rate and poor prognosis[1-2], but currently has no effective treatment. Studies have demonstrated that propofol can attenuate neurological dysfunction and tissue pathology after I/R[3-5]. Propofol mainly protects the brain against I/R injury by inhibiting cell apoptosis and caspase-3 expression, and increasing Bcl-2, Bax, Toll-like receptor-4 and nuclear factor-κB mRNA expression[6-7]. However, a variety of factors can induce I/R injury[8-9], with the release of inflammatory mediators and leukocyte infiltration, playing important roles in this injury[10]. In the present study, we utilized a 'six vessel' brain I/R model to investigate the protective effect of propofol on cerebral ischemia in rabbits by assessing changes of serum interleukin-8 (IL-8), endothelin-1 (ET-1), malondialdehyde (MDA), and superoxide dismutase (SOD).

RESULTS

Quantitative analysis of experimental animals

A total of 24 rabbits were randomly assigned to three groups (n = 8 each): sham-surgery (no vessel occlusion), the I/R group (I/R model), and the propofol group (propofol + I/R model). All 24 rabbits were included in the final analysis.

Propofol suppressed serum IL-8 in rabbits with I/R injury

No statistical significance was seen in serum IL-8 15 minutes prior to ischemia (I_0) among groups or among different time points of the sham-surgery group ($P > 0.05$). Serum IL-8 was significantly increased after reperfusion for 30 minutes (R_1), 2 hours (R_2) and 4 hours (R_3) in the I/R group compared with the sham-surgery group. However, serum IL-8 remained unchanged during R_1–R_2 in the propofol group, but significantly increased at R_3 compared with the sham-surgery group ($P < 0.05$). Moreover, IL-8 levels were significantly decreased in the propofol group compared with the I/R group at R_1, R_2 and R_3 ($P < 0.05$; Figure 1).

Propofol suppressed serum plasma ET-1 in rabbits with I/R injury

Plasma ET-1 changes were similar to those seen with serum IL-8. After reperfusion, the concentration of ET-1 was significantly elevated in the I/R group ($P < 0.05$). ET-1 level gradually increased with prolonged reperfusion time in the propofol group, and was significantly higher than the sham-surgery group up to R_3 ($P < 0.05$).

However, the level of ET-1 in the propofol group was significantly reduced compared with the I/R group at different time points after reperfusion ($P < 0.05$; Figure 2).

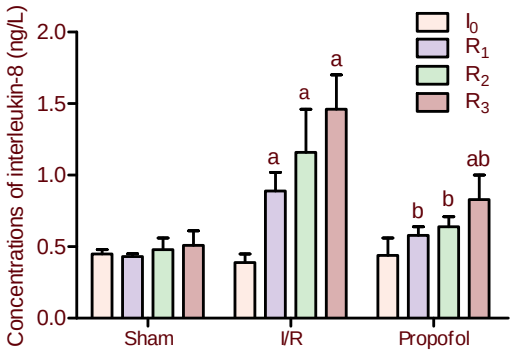

Figure 1 Serum interleukin-8 changes among the three different groups.

Data expressed as mean ± SD of eight rabbits in each group. [a]$P < 0.05$, *vs*. sham-surgery (sham) group; [b]$P < 0.05$, *vs*. I/R group (two-way analysis of variance, *q* test).

I_0, R_1, R_2 and R_3 corresponded to 15 minutes before ischemia, and 30 minutes, 2, and 4 hours after reperfusion, respectively. I/R: Ischemia/reperfusion.

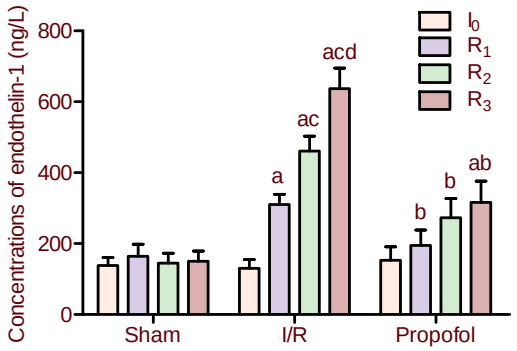

Figure 2 Plasma endothelin-1 concentration in three different groups.

Data expressed as mean ± SD of eight rabbits in each group. [a]$P < 0.05$, *vs*. sham-surgery (sham) group; [b]$P < 0.05$, *vs*. I/R group, [c]$P < 0.05$, *vs*. R_1 in I/R group; [d]$P < 0.05$, *vs*. R_2 in I/R group (two-way analysis of variance, *q* test).

I_0, R_1, R_2 and R_3 corresponded to 15 minutes before ischemia, and 30 minutes, 2, and 4 hours after reperfusion, respectively. I/R: Ischemia/reperfusion.

Effect of propofol on plasma MDA and SOD levels in rabbits with I/R injury

The MDA concentration of the propofol group remained unchanged after reperfusion, but was lower than that in the I/R group at corresponding time points ($P < 0.05$). However, MDA levels were elevated after reperfusion in the I/R group, and were significantly higher than those in the sham-surgery group at R_1, R_2 and R_3 ($P < 0.05$; Figure 3). The concentrations of SOD in the propofol group were lower than those in the sham-surgery group at R_2 and R_3, but higher than those in the I/R group at R_1, R_2 and R_3 ($P < 0.05$; Figure 4).

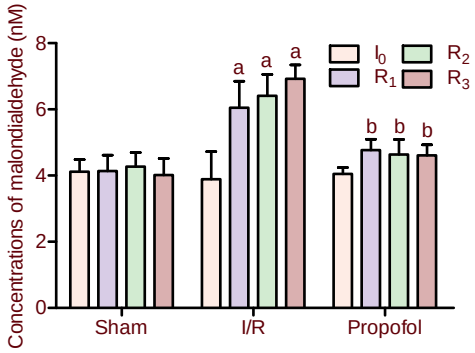

Figure 3 Effect of propofol on malondialdehyde concentration in plasma.

Data expressed as mean ± SD of eight rabbits in each group. [a]$P < 0.05$, *vs*. sham-surgery (sham) group; [b]$P < 0.05$, *vs*. I/R group (two-way analysis of variance, *q* test).

I_0, R_1, R_2 and R_3 corresponded to 15 minutes before ischemia, and 30 minutes, 2, and 4 hours after reperfusion, respectively. I/R: Ischemia/reperfusion.

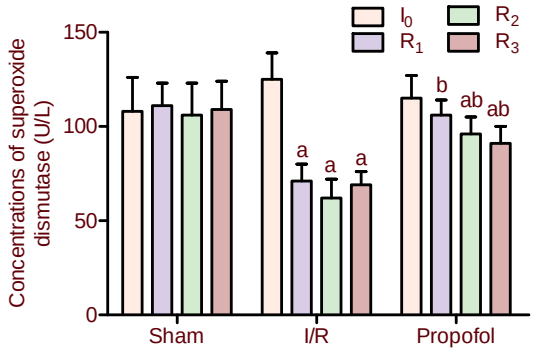

Figure 4 Effect of propofol on superoxide dismutase concentration in plasma.

Data expressed as mean ± SD of eight rabbits in each group. [a]$P < 0.05$, *vs*. sham-surgery (sham) group; [b]$P < 0.05$, *vs*. I/R group (two-way analysis of variance, *q* test).

I_0, R_1, R_2 and R_3 corresponded to 15 minutes before ischemia, and 30 minutes, 2, and 4 hours after reperfusion, respectively. I/R: Ischemia/reperfusion.

Propofol attenuated cerebral edema in rabbits with I/R injury

Water content of the cerebral cortex was significantly lower in the propofol group (80.02 ± 1.80%) compared to the I/R group (83.96 ± 2.59%; $P < 0.05$), but significantly higher compared with the sham-surgery group (77.03 ± 1.68%; $P < 0.05$).

Propofol ameliorated pathological changes of the cerebral cortex in rabbits with I/R injury

Mild cerebral cortex edema, as well as neuronal nucleus degeneration and pyknosis, were observed in the

propofol group, but no leukocyte infiltration or vascular cuffing was found. By contrast, evident cerebral cortex edema, blood stagnation and even hemorrhage were seen in the I/R group. Neuronal nucleus degeneration and pyknosis were also evident with remarkable leukocyte infiltration and vascular cuff formation. Normal neuron structure and no edema were found in samples from the sham-surgery group (Figure 5).

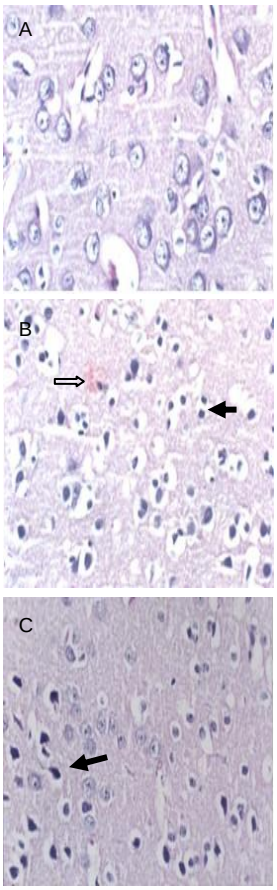

Figure 5 Pathological changes in the cerebral cortex of the experimental groups (hematoxylin-eosin staining, × 400).

Sham-surgery group (A) was normal, while evident cerebral cortex edema, blood stagnation (white arrow), pyknosis (black arrow) and even hemorrhage were seen in the ischemia/reperfusion group (B).

Cortex edema, neuronal nucleus degeneration (black arrow) and pyknosis were also observed in the propofol group (C).

Propofol increased the cross-sectional area of neuronal nuclei and the average absorbance of Nissl bodies in rabbits with I/R injury

The cross-sectional area of neuronal nuclei was lower in the propofol group compared with the sham-surgery group, but higher compared with the I/R group. The average absorbance of Nissl bodies in the sham-surgery group was the highest, followed by the propofol group, and then the I/R group ($P < 0.05$; Figure 6).

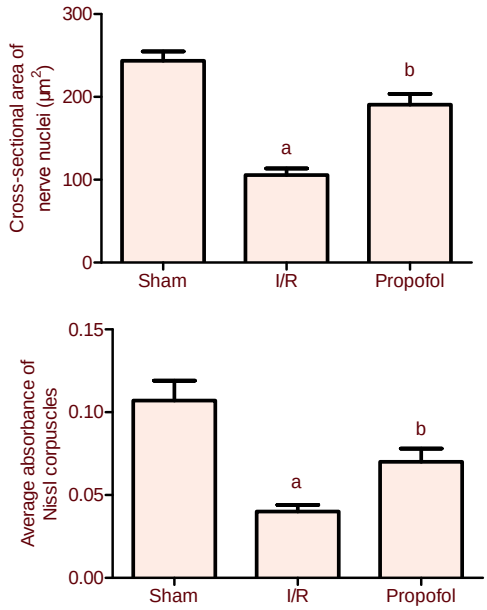

Figure 6 Cross sectional area of neuronal nuclei and average absorbance in three experimental groups.

Data were expressed as mean ± SD of eight rabbits in each group. [a]$P < 0.05$, *vs*. sham-surgery (sham) group; [b]$P < 0.05$, *vs*. I/R group (one-way analysis of variance). I/R: Ischemia/reperfusion.

DISCUSSION

In the present study, IL-8 increased only at R_3 in the propofol group, and rose sharply after reperfusion in the I/R group, demonstrating that propofol affected the synthesis and release of IL-8. Furthermore, the level of serum MDA in the propofol group was lower, but the level of SOD was higher, than that in the I/R group. IL-8 continuously accumulates due to its tolerance to degradation by peptidase, and therefore promotes the release of oxygen radicals. Increasing free radicals can exacerbate the inflammatory reaction and promote the release of cytokines, such as IL-8, which creates a vicious cycle and further escalates the biochemical cascade[11-12].

Light microscopy revealed no obvious leukocytic infiltration, accumulation or perivascular cuffing in the propofol group, but large leukocytic infiltrations and perivascular cuffs were observed in the I/R group. Attenuating leukocyte stimulation was positively correlated with a decline in the release of IL-8 in the propofol group. Based on the relationships of IL-8 with leukocyte stimulation and MDA, as well as the pathology seen in sections from the three groups, inhibiting synthesis and release of IL-8 might be one of the mechanisms by which propofol attenuates cerebral I/R injury.

Previous studies have shown that detecting ET-1 concentration invariably reflects the injury extent of endotheliocytes and cerebral I/R[13-14]. In the present

study, the concentration of ET-1 in the propofol group during the reperfusion period was significantly lower than the I/R group, and the cross-sectional area of the nucleus was higher than the I/R group. Additionally, karyopyknosis, completion of nuclear membranes and nucleolus clarity were observed in pathological sections. The concentration of ET-1 in the I/R group increased slowly with increasing reperfusion time, which was the result of the increasing synthesis and release of ET-1. The increase of neuron autocrine activity may have been caused by stimulation of endothelial anoxia and ischemia[15]. Large amounts of neuronal necrosis and damage were observed in the I/R group compared with the other two groups, which demonstrated that propofol suppressed the synthesis and release of ET-1 after reperfusion and attenuated brain I/R injury[16]. These observations are in accordance with results published by Bodelsson *in vitro*[17].

These findings illustrate that propofol could suppress the generation of oxygen free radicals and improve the activity of SOD, due to the chemical structure of propofol. Considering the alteration of ET-1, SOD and MDA levels, we concluded that ET-1 could promote the generation of oxygen free radicals and activation of leukocytes and endotheliocytes after cerebral I/R injury. Also increased oxygen radicals could further stimulate the synthesis and release of ET-1, leading to cerebral I/R injury through various pathways[18].

In summary, propofol increased the activity of anti-oxidants and inhibited the release of inflammatory cytokines, thus exerting neuroprotective effects.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

The experiment was performed at the Laboratory of Anesthesiology, Renmin Hospital of Wuhan University, China from March to October 2010.

Materials

A total of 24 specific pathogen-free New Zealand white rabbits, aged 6–8 months, weighing 2.0–2.5 kg, of either gender, were provided by the Institute of Laboratory Animal Science, Wuhan University, China (license No. SCXK (E) 2003-0004). All rabbits were housed under constant temperature (20 ± 2°C) in 12-hour light/dark cycles. They were allowed free access to water and food. The animal protocol complied with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China in 2006[19].

Methods

Establishment of the cerebral I/R model

Rabbits were initially anesthetized using 1% pentobarbital sodium [30 mg/kg, intravenously (i.v.)] with fentanyl (10 μg/kg, i.v.). An endotracheal tube was placed through a tracheostomy, and the rabbits were mechanically ventilated for the duration of the experiment. The $P_{ET}CO_2$ was maintained between 35 and 45 mm Hg (1 mm Hg = 0.133 kPa). After general heparinization, unilateral femoral arterial and venous lines were inserted by surgical cutdown. A multiple channel physiological recorder was used to monitor mean arterial blood pressure, central venous pressure, heart rate, $P_{ET}CO_2$, electrocardiogram and temperature. Tympanic temperature was monitored and maintained at 38 ± 1.0°C. A tube was retrogradely inserted into the glomus jugulare through the internal jugular vein and used as a bleeding tube. The rabbit model of complete cerebral ischemia was established using the 'six-vessel' method[20]. The bilateral internal and external carotid arteries were separated, along with the vertebral artery, and all of bilateral internal, external carotid and vertebral arteries were blocked for 30 minutes. Reperfusion was initiated by removing the occlusion and continued for 4 hours. Rabbits were treated with Lactated Ringer's Solution (Baxter, Shanghai, China) during the observing time (10 mL/kg per hour, i.v.)[20].

Propofol treatment

The propofol group received bolus propofol infusion (5 mg/kg, Diprivan; AstraZeneca, S.P.A., Italy) 10 minutes prior to vessel occlusion, followed by continuous propofol (20 mL/kg per hour, i.v.) throughout the procedure. Normal saline at the same dose was used in the sham-surgery and I/R groups.

Detection of serum IL-8 and plasma ET-1, MDA and SOD

Blood from the internal jugular vein was harvested at I_0, R_1, R_2, and R_3 in the I/R and propofol groups, and at corresponding time points in the sham-surgery group[20]. Plasma concentrations of ET-1, as well as the serum level of IL-8, were measured using radioimmunoassay with kits being provided by the East Asia Immunization and Technique Institute (Beijing, China). Plasma concentrations of MDA and SOD were assayed using colorimetry and kits were obtained from Nanjing Institute of Biomedical Engineering (Nanjing, Jiangsu Province, China).

Detection of water content in brain tissue

Animals were sacrificed after 4 hours of reperfusion. Unilateral new cortex, part of the temporal lobe, was then separated through the hemisphere[21] and the wet weight of each sample was determined on a precision scale (Jingke Co., Shanghai, China). Dry weight was determined by drying tissue in an oven at 100°C until the weight did not decrease any further. The water content of each hemisphere was calculated as follows[22]:

Water content (%) = (wet weight − dry weight)/wet weight × 100%.

Hematoxylin-eosin and Nissl staining for pathological changes

Tissue was cut randomly into five pieces and paraffin sections were created (4 μm thick). Pathology was observed under a light microscope (Olympus, Tokyo, Japan) after hematoxylin-eosin staining and Nissl

staining. Photographs were analyzed using a MPIAS-500 color pathological image analysis system (Qingping Co., Wuhan, Hubei, China). Neuronal nuclear cross-sectional area was measured with a 0.199 μm pixel length and 1.109×10^4 μm^2 measuring range, and the average absorbance of the Nissl bodies was analyzed with a 0.389 μm pixel length and 4.22×10^4 μm^2 measuring range. The level of neuronal injury was assessed using the neuronal nuclear cross-sectional area and the average absorbance of the Nissl bodies[23].

Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA). Data were expressed as mean ± SD. The homogeneity of variance was tested using the Levene test. Variance was used for analysis if it was homogenous, with the *q* test was used to compare differences within and between groups. If variance was non-homogenous, a rank test was used. *P* values of less than 0.05 were considered statistically significant.

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Author contributions: Xiaodong Wei designed this study, wrote the manuscript and processed the experimental data. Bangchang Cheng guided the experiment and revised the manuscript. Xing Wan and Jiabao Hou translated and corrected this manuscript. Bo Zhao and Ming Liu also designed the study and analyzed the data. Bo Zhao contributed to the statistical analysis.

Conflicts of interest: None declared.

Ethical approval: The experimental procedures and protocols were approved by the Animal Ethics Committee of Wuhan University, China.

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