Does the intrathecal propofol have a neuroprotective effect on spinal cord ischemia?

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Abstract

The neuroprotective effects of propofol have been confirmed. However, it remains unclear whether intrathecal administration of propofol exhibits neuroprotective effects on spinal cord ischemia. At 1 hour prior to spinal cord ischemia, propofol (100 and 300 µg) was intrathecally administered in rats with spinal cord ischemia. Propofol pre-treatment greatly improved rat pathological changes and neurological function deficits at 24 hours after spinal cord ischemia. These results suggest that intrathecal administration of propofol exhibits neuroprotective effects on spinal cord structural and functional damage caused by ischemia.

Key Words: nerve regeneration; propofol; pre-treatment; spinal cord; ischemia; neuroprotection; paraplegia; neural regeneration


Introduction

Paraplegia, an occasional but serious complication seen after surgical repair of thoracoabdominal aortic aneurysms, has been confirmed to be attributed to ischemia of the spinal cord caused by interruption of blood flow during aortic cross-clamping (Hsieh et al., 2005). The reported incidence is between 4% and 33% (Ilhan et al., 1999, 2004). A variety of factors including microcirculatory disturbance, inflammatory factors, cellular necrosis and apoptosis, or biochemical auto-destruction (calcium ion overloading, free radicals, stimulatory amino-acids, etc.) have been proposed to explain the occurrence of paraplegia, the exact mechanisms remain largely unknown (Sahin et al., 2014). The pathophysiological processes responsible for the development of ischemic/ hypoxic injury of the spinal cord are obscure (Kale et al., 2011; Sahin et al., 2014). Lumbar drains, intercostal artery re-implantation, left heart bypass and hypothermic circulatory arrest can be protective against the development of paraparesia or paraplegia following aortic surgery (Tetik et al., 2000; Umehara et al., 2010; Saito et al., 2011; Smith et al., 2011), but their complex and invasive nature is inevitably associated with additional complications, limiting their widespread prophylactic utility (Saito et al., 2011). Pharmacological agent(s) with potent protective effects are currently unavailable, but some compounds have been shown to be useful in reducing the inflammatory and metabolic injury resulting from the destructive effect of ischemia/reperfusion on the spinal cord (Smith et al., 2011). Therefore, neurological deficits arising from ischemia/reperfusion injury may potentially be corrected using pharmacological treatments (Saafi et al., 2011). However, in spite of multimodal efforts aiming at reducing the incidence of spinal cord ischemia, a significant elimination of the risk has not been possible until now. Human studies on the reduction of spinal cord ischemia risk after thoracoabdominal aortic aneurysm repair focused mainly on additional invasive procedures, rather than using a preventive drug treatment (Tetik et al., 2000; Gravereaux et al., 2001; Chiesa et al., 2005). However, ischemia risk has not been eliminated so far using these methods. In addition, such methods (e.g., drainage of cerebrospinal fluid, intravenous steroid treatment, additional measures in high-risk patients, thoracic endograft repair) have their own risks (Gravereaux et al., 2001).

Propofol (2,6-diisopropylphenol) is a widely used short-acting intravenous anesthetic agent. In vitro studies suggest that effects of propofol are associated with the inhibition of N-methyl-D-aspartate (NMDA) receptor (Xu, 2004). Several reports have also suggested an inhibitor effect of propofol on gamma-aminobutyric acid (GABA) receptors (Nadeson et al., 1997; Nishiyama et al., 2004; Wang et al., 2004; Geo et al., 2005; Vasileiou et al., 2009). This agent also has analgesic properties (Xu et al., 2004; Ji et al., 2013). When administered at non-sedative doses, it exerts anxiolytic effects and has strong antioxidant properties. There is a study suggesting a neuro-protective effect of propofol (Vasileiou et al., 2009).

Intrathecal drug administration is mainly used for the purpose of analgesia and anesthesia as an alternative route
usually when sufficient efficacy cannot be achieved with high oral dose or parenteral administration with acceptable side effects (Smith et al., 2008). A number of laboratory and clinical studies are currently focusing on drug administration into cerebrospinal fluid thus bypassing blood-brain barrier. Cerebrospinal administration has many theoretical advantages when compared to intravascular route. Intrathecal administration bypasses cerebrospinal fluid barrier thus providing high drug levels in cerebrospinal fluid rapidly. Since the drug is directly present in cerebrospinal fluid, lower doses can be used, resulting in potentially reduced systemic toxicity. In addition, drugs have longer half-lives in the cerebrospinal fluid due to very low level of protein binding and enzymatic activity when compared to plasma (Misra et al., 2003).

Considering the absence of literature data on the protective effect of propofol on spinal ischemia/reperfusion injury, we aimed to examine its potential protective role in ischemic spinal cord injury when administered as an intrathecal pre-treatment.

Materials and Methods

Ethics statement
Approval was obtained for protocols used in this study from the Animal Care Committee of Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey (Permission No. 2013/03-6). All efforts were made to minimize animal discomfort and reduce the number of animals used.

Animals
Fifty-four adult male Wistar rats, weighing 400–425 g, were selected and provided by Animal Research Center, Ataturk University, Erzurum, Turkey. Our study protocol was based on a previous animal study method reported by Sahin et al. (2014) and Hsieh et al. (2005). All rats were maintained under the same physiological and biological properties (22 ± 2°C and 12-hour dark/light cycle) and fed with standard rat food and water ad libitum.

Intrathecal catheterization
Three days before spinal cord ischemia, intrathecal catheterization for drug delivery was performed. Rats were placed in a plastic container and anesthetized with 1.5–2% isoflurane. After shaving the head and posterior neck, the head was fixed anteriorly to allow maintenance of isoflurane anesthesia with face masks (Anesthesia Workstation AWS, Hallowell EMC, Pittsfield, MA, USA). Heating pads were placed under the rats to maintain a normal body temperature. The tail artery was cannulated with a 22G catheter for intra-arterial heparin infusion. The left carotid artery proximal artery pressure (PAP) was measured after cannulation with a 20G catheter. For spinal cord ischemia, the left femoral artery was exposed, and a 2F Fogarty catheter (Edwards Lifesciences, Irvine, CA, USA) was advanced through the thoracic aorta. The tip of the catheter was localized at the junction of the left subclavian artery, which corresponds to a catheter length of 10.8–11.4 cm as reported in other studies (Yaksh et al., 1976; Taira et al., 1996). Immediately after the placement of the arterial catheter, 200 U (0.2 mL) of heparin (Nevarpin, Mustafa Nevzat Ilac, Istanbul, Turkey) was injected through the tail artery. The balloon was filled with 0.05 mL of physiological saline for 11 minutes to induce spinal cord ischemia. The diastolic arterial pressure (DAP) was measured at the tail artery to assess the effectiveness of occlusion. PAP was measured from the left carotid artery and maintained around 40 mmHg. DAP, PAP and body temperature were monitored before and during ischemia/reperfusion using the Philips Intellivalue MP30 (Philips, USA). After ischemia was obtained, the balloon was deflated. After completing all procedures, catheters were removed and wounds were closed. Protamine sulphate (Protamin HCl, Onko&Kocsel, Istanbul, Turkey) 4 mg was injected subcutaneously to counteract the anti-coagulant effect of heparin.

Drug administration
In the first part of the study, rats were randomized into four experimental groups (n = 6) to assess the effect of intrathecal propofol on neurological signs and histopathological changes: control group, propofol 100 and 300 µg groups, and the sham group.

One hour before the induction of ischemia, 10 µL of physiological saline was injected in the control group, while rats in the propofol 100 µg group received propofol 100 µg, rats in the propofol 300 µg group received propofol 300 µg in 10 µL of physiological saline solution (Nishiyama et al., 2004). A 50 µL of Hamilton syringe was used for drug administration. In the sham group, a Fogarty catheter was placed but the balloon was not inflated and PAP was decreased to 40 mmHg for 11 minutes. No medication was given to the sham group.

Neurological investigation
After spinal ischemia, rats were transferred to their cages for recovery and their neurological functions were assessed in the first 24-hour postoperative period. For the assessment of motor functions, hind limb placing/stepping reflex was recorded. Ambulation in the hind limbs was graded as follows (Hsieh et al., 2005): 0, normal (symmetrical and coordinated hind limb movements); 1, the first toe is immobile while walking, but ataxia is present; 2, knuckle walking; 3, absence of knuckling, but some mobility exists in the hind limbs; 4,
no movement in the hind limbs. The placing/stepping reflex was assessed by dragging the dorsum of the hindpaw along the edge of a surface. Normally, this requires a coordinated pulling-up and placing response, and was graded as follows (Hsieh et al, 2005): 0, Normal; 1, weak; 2, no stepping.

A motor deficit index (MDI) score was calculated for each rat as the sum of both scores with a maximum of 6 (score of 4 for ambulation and 2 for the lacing/stepping reflex). MDI was calculated by an observer blinded to the treatments used.

**Tissue samples and histopathological assessment**

After observation of the motor behavior, rats were anesthetized by intraperitoneal ketamine injection (10 mg/kg), which was followed by the transcardiac perfusion of 100 mL of heparinized physiological saline. Immediately after this, 150 mL of 4% paraformaldehyde with phosphate buffer was given. Then, the lumbar expansion of the spinal cord at L3 or L4 was removed and kept in the same fixative at 4°C overnight. The samples were embedded in paraffin and 5 µm thick transverse cross sections were prepared and stained with hematoxylin and eosin. All slides were evaluated using a light microscope (Olympus, Tokyo, Japan). The samples were assessed by a pathology specialist blinded to treatment groups. For all rats, acute grey matter injury was calculated on the basis of the proportion of death or abnormal cells in the ventral horn as follows: 0, No neuronal injury or death; 1, mild injury (< 10%); 2, moderately severe injury (10–50%); and 3, severe injury (> 50%). For each rat, the score corresponds to the findings in the right and left hemicords in three consecutive sections.

**Survival evaluation**

In the second part of the study, the survival rate during the 28 day follow-up period was assessed in the remaining 30 rats, which were randomized into three groups with 10 rats in each group: physiological saline, propofol 100 µg, and propofol 300 µg groups. Interventions to these groups were the same as the first part of the study.

**Statistical analysis**

Statistical analysis was performed using SPSS software (version 17.0; SPSS, Chicago, IL, USA). Differences in motor and histopathological scores (MDI and histopathologic score expressed as a median (Q1–Q3)) were evaluated using the non-parametric Mann-Whitney U test (Monte Carlo significance test). Fisher’s exact probability test was used for the comparisons in survival analyses. P values less than 0.05 were considered statistically significant.

**Results**

**Neurological function**

There was no significant difference in body weight among the groups. The rats were weighted at the beginning of the study. There were significant differences in MDI and histopathology scores between the groups at 24 hours after spinal cord ischemia (Tables 1, 2). After the 11th minute of the aortic occlusion by proximal controlled hypotension (40 mmHg), normal motor functions were observed in the sham group, while acute flaccid paraplegia developed in the control group. At 24 hours after reperfusion, acute flaccid paraplegia and spastic paraplegia occurred in the control group. At 24 hours after spinal cord ischemia, one of the six rats in the propofol 100 µg group showed a slight decrease in the mobility of the hind limbs (MDI 2 and 3) with no significant paraplegia in the remaining five rats (MDI 1). In the propofol 300 µg group, no rats had severe paraplegia (MDI 0 and 1).

**Histopathological change in spinal cord tissue**

At 24 hours, in histopathological evaluation, two rats in the control group had severe neuronal injury of the lumbar spinal cord, while the remaining four rats had moderately severe neuronal injury (Figure 1A). In the sham group, two
rats had mild neuronal injury, whereas four rats had no neuronal injury (Figure 1B). In the propofol 100 µg group, four rats had moderate neuronal injury, and two rats had no injury (Figure 1C). In the propofol 300 µg group, one rat had mild neuronal injury, and the remaining five rats had no injury (Figure 1D).

Survival evaluation
The therapeutic effects of propofol 100 and 300 µg on rat survival were examined. In the control group (n = 10), only three rats survived beyond 28 days after spinal cord injury. Six rats from propofol 100 µg group and eight rats from propofol 300 µg group survived beyond 28 days, respectively, without loss of good-level motor functions after spinal cord ischemia. These findings corresponded to higher survival rates (i.e., 60% and 80%) in propofol 100 µg and propofol 300 µg groups than in the control group (30%) during this time period (Figure 2). Fisher’s exact probability test results showed there was no significant difference in survival rate between control and propofol 100 µg groups but it existed between control and propofol 300 µg groups.

Discussion
Our results show that pre-treatment with propofol (100 µg or 300 µg, intrathecal) was associated with a significant decrease in hind limb motor dysfunction due to ischemic spinal cord injury 24 hours after ischemia/reperfusion in rats. In addition, pre-treatment with propofol 100 or 300 µg was able to prevent histopathological changes 24 hours after ischemia in the spinal cord.

The underlying mechanisms for the neuroprotective effect of propofol are not well understood. However, in a rabbit model of experimental spinal cord ischemia, Zeng et al. (2009a) demonstrated a neuroprotective effect of propofol infusion at room temperature or at 4°C throughout ischemia. Both approaches were associated with lower malonylaldehyde concentrations in the spinal cord as compared to control and sham groups, while they were associated with higher superoxide dismutase concentrations. Thus, they proposed that propofol may exhibit neuroprotective effects on spinal cord injury through regulating malonylaldehyde and superoxide dismutase concentrations. In the study by Zeng et al. (2009a), the infusion was performed via the left femoral artery during cross-clamping. In a rabbit study by Lin et al. (2008), intra-aortic and intravenous propofol infusions were performed during infrarenal occlusion. In rabbits receiving intra-aortic infusion, propofol concentration at the spinal level of L₄₋₆ was higher than at the level of T₆₋₈. Intravenous administration did not result in significant increases in both treatment and control groups and at both L₄₋₆ and T₆₋₈ segments, and no significant difference in propofol concentration was observed between treatment and control groups.
The incidence of paraplegia was lower in the intra-aortic infusion group than in the control and intravenous infusion groups, while there was no significant difference between the control and intravenous infusion groups.

These authors concluded that intra-aortic propofol infusion resulted in better neurological outcomes than intravenous propofol infusion. Similarly, in a rabbit study by Ke et al. (2005) where spinal cord ischemia was induced by aortic cross-clamping, intravenous infusion of propofol was found to prevent cell apoptosis in the spinal cord and this effect was attributed to changes in Bax and Bcl-2 protein levels, which were achieved by its regulation of malonialdehyde and superoxide dismutase concentrations. In the study by Zeng et al. (2009b), propofol infused through a catheter positioned at the distal part of the aortic clamp inhibited the accumulation of excitatory amino acids in the ischemic spinal cord and thereby provide neuroprotective effects.

Although propofol is a widely utilized general anesthetic agent, its mechanism of action has not been well elucidated (Wang et al., 2004; Ji et al., 2013). Several reports have also suggested a certain degree of anti-inflammatory effect of propofol (Ke et al., 2005; Line et al., 2008; Zeng et al., 2009a, b).

To conclude, our results suggest that pre-treatment with intrathecal propofol at 100 µg and 300 µg can prevent against spinal cord ischemia. This approach also benefits for the prevention of spinal cord ischemia-related complications.

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References

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