



Research Article

Propofol Reduces Reactive Oxygen Radical Dependent Lipid Peroxidation after Experimental Spinal Cord Injury in Rats

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Abstract

Primary spinal cord injury (SCI) is frequently inevitable; however secondary injury may be ameliorated by treatment that reduces reactive oxygen radical (ROR) dependent lipid peroxidation (LP). In this study, we investigated the effect of propofol on ROR dependent LP subsequent to SCI in anesthetized rats. Thirty four rats were divided into four groups: In Group L (n=7), only laminectomy was performed. In Group T (n=7), after performing laminectomy, SCI was inflicted. In Group P1 (n=10), after bolus propofol injection, the procedure was started and SCI was inflicted following laminectomy. In Group P2 (n=10), propofol bolus plus infusion was maintained throughout laminectomy and SCI procedure. SCI resulted in significant increase in production of ROR and level of LP respectively. These increases were significantly prevented by propofol in both Groups. There were also significant difference between Group P1 and both Groups L and P2 when both production of ROR and the level of LP were evaluated. In conclusion, these results clearly show propofol significantly reduces reactive oxygen radical dependent lipid peroxidation after experimental spinal cord injury in rats.

Keywords: Chemiluminescence reactivity, lipid peroxidation, propofol, reactive oxygen radicals, spinal cord injury

Propofol, Sıçanlarda Deneysel Omurilik Travması Sonrasında Reaktif Oksijen Radikallerinin Neden Olduğu Lipid Peroksidasyonu Azaltmaktadır

Özet

Primer omurilik hasarı (OH) sıklıkla önlemez iken; sekonder hasar reaktif oksijen radikalleri (ROR)'nin neden olduğu lipid peroksidasyon (LP)'un azalmasını sağlayan tedavilerle hafifletilebilir. Bu çalışmada, propofol anestezisinin sıçanlarda OH'nda meydana gelen ROR'ne bağlı LP üzerine olan etkisini araştırmayı hedefledik. Otuzdört sıçan rastgele olarak 4 gruba ayrıldılar: Grup L (n=7)'de sadece laminektomi ve Grup T (n=7)'de laminektomiyi takiben OH oluşturulurken; Grup P1 (n=10)'de bolus propofölü yapıldıktan sonra OH ve Grup P2 (n=10)'de ise OH oluşumu sırasında propofol hem bolus hem de infüzyon olarak uygulandı. Omurilik hasarı sonrasında ROR ve LP seviyelerinde belirgin artış olduğu saptandı. Bu artışların her iki propofol grubunda da belirgin olarak azaldığı görüldü. Hem Grup P2, hem de Grup L'deki ROR ve LP değerlerinin Grup P1' den belirgin düşük olduğu görüldü. Bu sonuçlar propofölün sıçanlarda deneysel OH sonrasında ortaya çıkan ROR'a bağlı LP'ü önemli ölçüde azaldığını göstermiştir.

Anahtar Kelimeler: Kimyasal ışık reaktivliği, lipid peroksidasyon, propofol, reaktif oksijen radikalleri, omurilik hasarı

INTRODUCTION

Major spinal surgery, tumor excision, traction, instrumentation, and surgical trauma may cause spinal cord injury (SCI) and surgery may result in either a new neurological deficit or deterioration of previous neurological deficit.⁽¹⁾ Primary SCI is frequently inevitable; however secondary injury may be ameliorated by treatment that reduces reactive oxygen radical (ROR) dependent lipid peroxidation (LP).^(3,6,12,13,17,21)

Therefore, neuroanesthesiologists should concern about the usage of anesthetic agent having anti-oxidant property throughout the neurosurgery in order to prevent/decrease effect of secondary insult.

In literature, propofol has been shown to decrease the neuronal metabolism and oxygen consumption more than it decreases the cerebral blood flow both in cerebrum and spinal cord.⁽⁵⁾ Therefore, anesthesia induction and/or maintenance with propofol infusion might be a preferable method for neuroanesthesia.

In this study, we investigated the effect of propofol on ROR dependent LP subsequent to SCI in ketamine anesthetized rats.

METHODS

The experimental protocol was approved by the Animal Care and Use Committee. Thirty-four male Sprague-Dawley rats weighing approximately 250-300 g were housed 2 animals per cage at the Institute of Neurological Science. They were fed a standard rodent chow diet and water ad libitum, and were kept at a constant temperature (22°C) on 12-hour cycles of light and dark.

In each case, the rat was anesthetized with an i.p. injection of ketamine 90 mg/kg and then placed on a board in prone position. A rectal probe of the monitor (Kontron minimon 7138 plus) was insert and recorded with 5 min intervals. The dorsal hair was closely shaved with an electrical razor, and the surgical field was disinfected

with povidone-iodine and draped with sterile towels. A dorsal midline incision between 5th-10th thoracic vertebrae was performed. After paravertebral dissection of the muscle, laminectomy was performed on the 9th thoracic vertebra. The rats were randomized by using sealed envelopes and allocated to one of four treatment groups:

Group L (laminectomy-only; n=7): Only laminectomy was performed.

Group T (trauma, n=7): After performing laminectomy, spinal cord injury was inflicted (see details for trauma method below).

Group P1 (propofol bolus; n=10): Bolus IV 25mg/kg propofol was injected via 26 G catheter into the tail vein. Then, the procedure was started. After performing laminectomy, spinal cord injury was inflicted (see details for trauma method below).

Group P2 (propofol bolus plus infusion; n=10): Propofol 25 mg/kg bolus was injected and then throughout the laminectomy and spinal cord injury procedure (see details for trauma method below), 25 mg kg/hr infusion was maintained via 26 G catheter in the tail vein.

The dorsal wound margins were apposed with a non-absorbable interrupted suture. After 1-hr, all rats were sacrificed through decapitation. Between 8th and 10th thoracic vertebrae, 1 cm spinal cord specimens were obtained and studied. The samples were evaluated by a blinded biochemist.

Allen's Spinal Cord Trauma Method

Spinal cord trauma was performed as described by Allen⁽²⁾ over the intact dura mater with weight-dropped. The apparatus was a 10 cm guide tube positioned perpendicular to the center of the spinal cord with an inner stainless steel rod (weighing 5 g). The animals were subjected to an impact of 50 g/cm² to the dorsal surface of the spinal cord.

Measurement of Reactive Oxygen Radicals

Reactive oxygen radicals were determined by tissue supernatant electrophoresis through chemiluminescence with luminol and lucigenin. In this procedure, tissue samples were transferred to the tube containing 2-mL hanks (0.44mM potassium phosphate; 5.37mM potassium chloride; 0.34mM sodium phosphate; 136.89mM sodium chloride; 136.89mM D-glucose) and HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulfonic acid]) buffer solution. Then, 0.2Mm luminol and lucigenin were added. Chemiluminescence's reactivity measurements were performed at 15-sec intervals for 10 min. While Luminol is selective for hydrogen peroxide, hydroxyl radicals, hypochloride and peroxyxynitrate; Lucigenin is selective for superoxide anion radicals.⁽³⁾ Time-dependent data obtained during chemiluminescence's reactivity analysis were expressed in a graphic. The value of ROR was obtained from the area under graphic curve (AUC), and it was expressed as AUC rlu mg⁻¹ tissue.⁽¹⁹⁾

Measurement of Lipid Peroxide Level

Autocatalytic process at the cellular level was studied through lipid peroxidation. This level was analyzed with thiobarbituric acid reactive species (TBA-RS) in injured rat spinal cord. Tissue samples were frozen at -20°C, and then homogenized in trichloroacetic acid (TCA) (1 mg tissue in 1 mL 10% TCA). Supernatant was mixed with equal volume of 67% TBA and washed in 100°C tap water for 15 min. This mixture was dried at the room temperature and then spectrometric analysis was done. Lipid peroxide level was expressed as malondialdehyde nmolmg⁻¹ tissue.

Statistical Analysis

All data were evaluated in blinded fashion and expressed as mean \pm SD. Group data were statistically compared using one-way analysis of variance (ANOVA). If a statistical difference was identified using ANOVA, Tukey-Kramer test was performed as a post-hoc. Probability values < 0.05 were considered to indicate significant difference.

RESULTS

Rats' body temperature was within normal range in all groups (Group L, $37.4 \pm 0.1^\circ\text{C}$; Group T, $37.5 \pm 0.2^\circ\text{C}$; Group P1, $37.4 \pm 0.2^\circ\text{C}$; Group P2, $37.5 \pm 0.1^\circ\text{C}$). None of the rats experienced respiratory depression throughout the operation.

Analysis of Reactive Oxygen Radicals

Spinal cord injury resulted in a significant increase in (lucigenin- and luminol-) chemiluminescence reactivity respectively ($p < 0.001$ for both). These increases were significantly prevented by propofol in both Groups P1 and P2 ($p < 0.001$ for both) (Fig. 2). There was statistical significant difference between Group P1 and both Groups L ($p < 0.01$) and P2 ($p < 0.05$) when lucigenin-chemiluminescence was evaluated (Fig. 2).

Measurement of Lipid Peroxidation and Tissue Injury

In Group T, spinal cord injury resulted in a significant increase for level of TBA-RS ($p < 0.001$). However, it was significantly prevented by propofol bolus in Group P1 and bolus plus infusion in Group P2 ($p < 0.001$ for both) (Fig. 2). There were statistical significant difference between Group P1 and both Groups L ($p < 0.01$) and P2 ($p < 0.05$) when both the levels of MDA were evaluated respectively (Fig. 2).

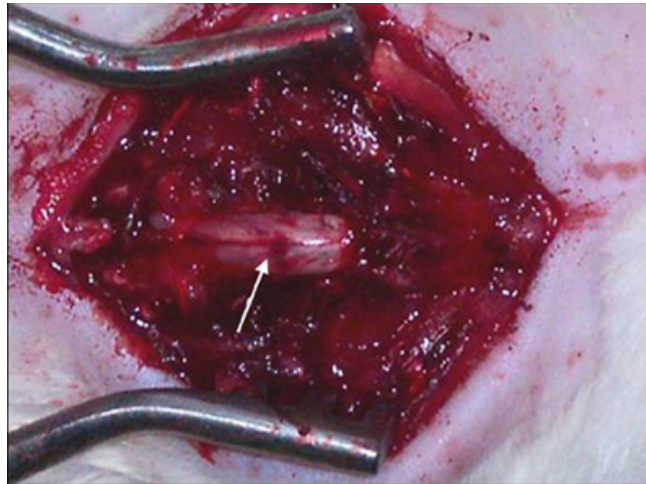


Fig 1: After performing laminectomy at the 9th thoracic vertebra, spinal cord injury was inflicted.

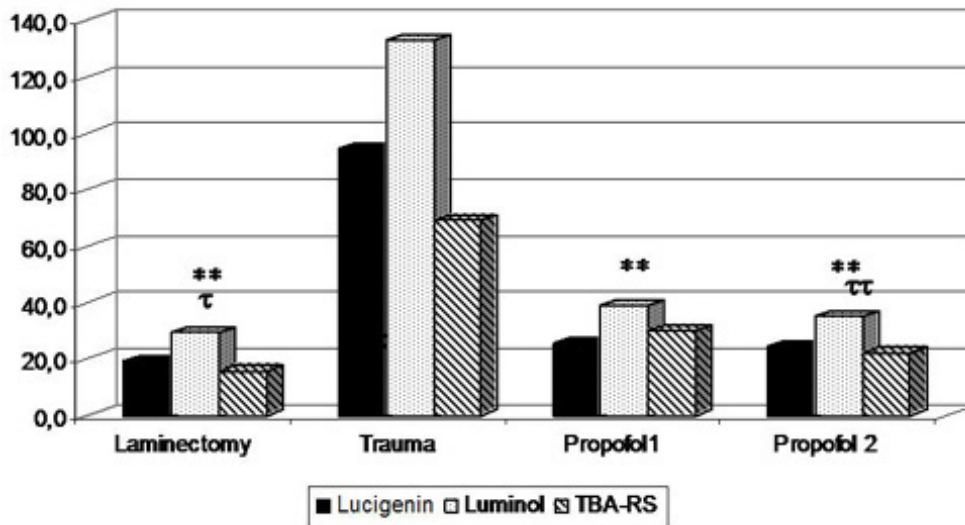


Fig 2: Lucigenin- and luminol- chemiluminescence reactivity and level of lipid peroxidation as thiobarbituric acid reactive species (TBA-RS). Lucigenin- and luminol- chemiluminescence reactivity were expressed as AUC $\mu\text{mol/g}$ tissue and TBA-RS was expressed as malondialdehyde nmol/mg tissue

** $: p < 0.001$ when compared with the values in Group T

t $: p < 0.05$ when compared with the values in Group P1

tt $: p < 0.01$ when compared with the values in Group P1

DISCUSSION

Traumatic events at the spinal cord result in neuronal tissue injury by direct (primary) and indirect (secondary-auto destructive) mechanisms.^(6,17,21) Primary injury caused by neuronal and vascular damage occurs at the time of trauma and results in loss of neuronal tissue. It is a biological process related to the biomechanical factors. Secondary injury following primary injury is a chain of physiopathological events and causes additional neuronal damage. However, although direct injury cannot be prevented, chain of pathological events can be broken.⁽¹⁰⁾ In previous studies, antioxidant agents increased the neuronal healing process by either preventing or restricting secondary injury.^(5,15)

Main factor responsible from this injury is oxygen-dependent lipid peroxidation. In previous studies, it was shown that lipid peroxidation triggered by free oxygen radicals, takes place following spinal cord injury.⁽¹⁵⁾ Lipid peroxidation was at its maximum at one-hour after injury.^(4,14) In the light of this information; our specimens were analyzed at one hour after injury.

Malondialdehyde is the most important aldehyde formed as a secondary product of lipid peroxidation. The analysis of its level with TBA-RS reveals lipid peroxidation status of the tissue.^(7,8)

Whether propofol is a neuroprotective agent remains controversial. The studies asserting its neuroprotective effect have not clearly described the mechanism by which it shows its protection. Furthermore, there are conflicting results on the role of its plasma concentration in clinical application.

In literature, it is claimed that neuroprotective effect of propofol is also related to its inhibitory effect on GABA receptors and its lowering effect on intracellular Ca ion level.⁽²²⁾ Increase of intracellular Ca ion enhances the xanthine

oxidase, resulting in the production of free radicals.

In this study, it was shown that during experimental SCI, propofol injection -as a bolus or infusion plus- could significantly decrease free oxygen radical formation and lipid peroxidation independent from total dosage in vivo. In vitro rat studies have shown that propofol has an antioxidant effect on liver microsomes, mitochondria, and cerebral synaptosomes.^(9,11,25) Antioxidant effect of propofol was believed to be related to the structural similarity to alpha-tocopherol.^(18,24)

Shibuta et al.⁽²⁰⁾ suggested that neuroprotective effect of propofol is limited and propofol does not offer advantages over thiopental against N-methyl-D-aspartate/nitrous oxide-induced cytotoxicity. Similarly, Feiner et al.⁽¹⁰⁾ claimed that mild hypothermia, but not propofol, protects CA1 and CA3 neurons in hippocampal slice cultures subjected to oxygen and glucose deprivation. Their findings showed that propofol was not neuroprotective at concentrations that reduce glutamate and NMDA receptor responses in cortical and hippocampal neurons.

Unlike the findings of the aforementioned studies, propofol showed a neuroprotective effect in an in vitro model of oxygen-glucose deprivation, which was apparently mediated by a GLUT1-independent restoration of the glutamate uptake impaired during the injury.^(10,23) Under the in vitro conditions of the experiment by Lee et al.⁽¹⁶⁾ propofol was an excellent and a very potent antioxidant against kainic acid-induced lipid peroxidation. They thought that the antioxidant properties of propofol at clinically relevant anesthetic concentrations might have a neuroprotective effect.

As a conclusion, our results revealed that in a dose dependent manner, propofol significantly reduces reactive oxygen

radical dependent lipid peroxidation after experimental spinal cord injury in rats.

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REFERENCES

1. Albert TJ, Kim DH. Timing of surgical stabilization after cervical and thoracic trauma. *J Neurosurg Spine* 2005;3:182-90.
2. Allen AR. Remarks on the histopathological changes in the spinal cord due to impact. An experimental study. *J Nerv Ment Dis* 1914;4:141-7.
3. Anderson DK, Demediuk P, Saunders RD, et al. Spinal cord injury and protection. *Ann Emerg Med* 1985;14:316-21.
4. Barut S, Canbolat A, Bilge T, et al. Lipid peroxidation in experimental spinal cord injury: time-level relationship. *Neurosurg Rev* 1993;16:53-9.
5. Cenic A, Craen RA, Lee TY, Gelb AW. Cerebral blood volume and blood flow responses to hyperventilation in brain tumors during isoflurane or propofol anesthesia. *Anesth Analg* 2002;94:661-6.
6. Cho Y, Ueda T, Mori A, et al. Protective use of N-methyl-D-aspartate receptor antagonists as a spinoplegia against excitatory amino acid neurotoxicity. *J Vasc Surg* 2005;42:765-71.
7. Dahle LK, Hill EG, Holman RT. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. *Arch. Biochem. Biophys* 1962;98:253-61.
8. Davies GR, Simmonds NJ, Stevens TR, et al. *Helicobacter pylori* stimulates antral mucosal reactive oxygen metabolite production in vivo. *Gut* 1994;35:179-85.
9. Faden AI, Jacobs TP, Holiday JW. Opiate antagonist improves neurologic recovery after spinal cord injury. *Science* 1981;211:493-4.
10. Feiner JR, Bickler PE, Estrada S, et al. Mild hypothermia, but not propofol, is neuroprotective in organotypic hippocampal cultures. *Anesth Analg* 2005;100:215-25.
11. Grasshoff C, Gillessen T. The effect of propofol on increased superoxide concentration in cultured rat cerebrocortical neurons after stimulation of N-Methyl-D-Aspartate receptors. *Anesth Analg* 2002;95:920-2.
12. Ginsberg MD, Sternau LL, Globus MY, Dietrich WD, Busto R. Therapeutic modulation of brain temperature-relevance to ischemic brain injury. *Cerebrovasc Brain Metab Rev* 1992;4:189-225.
13. Hall ED. Lipid antioxidants in acute central nervous system injury. *Ann Emerg Med* 1993;22:1022-7.
14. Hsu CY, Halushka PV, Hogan EL, et al. Alteration of thromboxane and prostacyclin levels in experimental spinal cord injury. *Neurology* 1985;35:1003-9.
15. Kaptanoglu E, Sen S, Beskonakli E, et al. Antioxidant actions and early ultrastructural findings of thiopental and propofol in experimental spinal cord injury. *J Neurosurg Anesth* 2002;14:114-22.
16. Lee H, Jang YH, Lee SR. Protective effect of propofol against kainic acid-induced lipid peroxidation in mouse brain homogenates: comparison with trolox and melatonin. *J Neurosurg Anesthesiol* 2005;17:144-8.
17. McIlvoy LH. The effect of hypothermia and hyperthermia on acute brain injury. *AACN Clin Issues* 200;6:488-500.
18. Murphy PG, Myers DS, Davies MJ, Webster NR, Jones JG. The antioxidant potential of propofol (2,6-diisopropylphenol). *Br Anaesth* 1992;68:613-8
19. Ozben T. Chemiluminescence measurements for the detection of free radicals species. In: Yalcin AS, Haklar G, Kucukkaya B, Yuksel M, Dalaman G, eds. *Free Radicals, Oxidative Stress and Antioxidants*. New York: Plenum Press, 1999:385-90.
20. Shibuta S, Sriranganathan V, Inoue T, et al. The effects of propofol on NMDA- or nitric oxide-mediated neurotoxicity in vitro. *Neuroreport* 2001;12:295-8.
21. Solaroglu I, Kaptanoglu E, Okutan O, et al. Magnesium sulfate treatment decreases caspase-3 activity after experimental spinal cord injury in rats. *Surg Neurol* 2005;642:17-21.
22. Southorn PA, Powis G. Free radicals in medicine. Chemical nature of biologic reactions. *Mayo Clin Proc* 1988;63:381-8.
23. Velly LJ, Guillet BA, Masmajan FM, et al. Neuroprotective Effects of Propofol in a Model of Ischemic Cortical Cell Cultures: Role of Glutamate and Its Transporters. *Anesthesiology* 2003;99:368-75.
24. Wilson JX, Gelb AW. Free radicals, antioxidant and neurologic injury: Possible relationship to cerebral protection by anesthetics. *J Neurosurg Anesth* 2002;4:66-79.
25. Yamaguchi S, Midorikawa Y, Okuda Y, Kitajima T. Propofol prevents delayed neuronal death following transient forebrain ischemia in gerbils. *Can J Anaesth* 1999;46:593-8.