



## Neuroprotective effect of ketamine on acute spinal cord injury in rats

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**ABSTRACT.** The aim of this study was to investigate the neuroprotective effects of ketamine during acute spinal cord injury in rats. Sprague Dawley (SD) rats (N = 70) were randomly divided into three groups: sham-operated (N = 10), control (N = 30), and treatment (N = 30) groups. The moderate spinal cord injury model was established. After injury, the sham-operated group received no drug, the treatment group received intraperitoneal ketamine injections, and the control group received intraperitoneal normal saline injections. Serum levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and spinal cord malondialdehyde (MDA) were assessed, and nerve cell apoptosis was evaluated in each group at varying time points. After spinal cord injury, TNF- $\alpha$ , IL-6, and MDA levels, and the number of TUNEL-positive cells among 2500 cells significantly increased ( $P < 0.05$ ). Further, compared with the control group, the treatment group showed significantly lower TNF- $\alpha$ , IL-6, and MDA levels, and fewer TUNEL-positive cells among 2500 cells at each time point ( $P < 0.05$ ). Our data indicate that ketamine exerts a neuroprotective effect on injured spinal cord.

**Key words:** Ketamine; Rats; Acute spinal cord injury; Tumor necrosis factor- $\alpha$ ; Interleukin-6; Malondialdehyde

## INTRODUCTION

Acute spinal cord injury (ASCI), a complete or incomplete dysfunction of spinal cord movement, sensation, and sphincter function caused by external forces, is a common and devastating disease. Recent basic science and clinical research has advanced the treatment of spinal cord injury (Esposito et al., 2011; Ha et al., 2011). Different methods have been used to treat patients with spinal cord injury and have achieved some effect (Lemcke et al., 2010; Jamous et al., 2010). However, treatments remain unsatisfactory, partially because the mechanisms of ASCI remain unclear, thereby preventing the development of improved therapeutics. Over the last decade, detailed studies have revealed that secondary spinal cord injuries play a critical role in the occurrence, development, and prognosis of ASCI (Margaryan et al., 2010). Some data indicate that neuronal apoptosis mediates spinal cord injury (Jiao et al., 2010), since the inhibition of apoptosis significantly reduces secondary spinal cord injury. Tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6 are important cytokines that have a variety of biological effects and are implicated in some diseases (Carmen et al., 2009; Carvajal et al., 2009; Kato et al., 2009). Previous research indicated that, after spinal cord injury, TNF- $\alpha$  and IL-6 levels increased significantly in rat serum (van Neerven et al., 2010). Thus, understanding how to inhibit apoptosis and reduce inflammatory cytokines is of interest for ASCI treatment. Many studies have demonstrated the neuroprotective effect of ketamine (Horvath et al., 2007; Labombarda et al., 2008), but the mechanism of neuroprotection remains unclear. In this study, we evaluated the impact of ketamine on the inflammatory cytokines TNF- $\alpha$  and IL-6 in rat serum, malondialdehyde (MDA) content in spinal cord, and apoptosis following ASCI. Our results are critical for understanding the neuroprotective mechanism of ketamine, and may serve as a theoretical basis for clinical work.

## MATERIAL AND METHODS

### Experimental animals and groups

Healthy, specific pathogen free, male Sprague Dawley rats (N = 70; 6-8-weeks-old; weight 170-210 g; purchased from Experimental Animal Center of Shandong University) were randomly divided into three groups: sham-operated (N = 10), control (N = 30), and treatment (N = 30) groups. Based on the time of spinal cord injury, the control and treatment groups were divided into three groups each (24, 72, and 144 h). In the treatment and control groups, 10 rats were killed at each time point for analysis, and sham-operated rats were killed 72 h later. This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shandong University Qilu Hospital.

### Establishment of animal models

After anesthetization, spinal cord of the rats was moderately injured by using the modified Allen method. Hair of the rats was shaved, and by using the thirteenth rib and the eighth thoracic vertebra as markers, the vertebral plates were removed through midline incisions on the back to expose the chest 7-11 of the spinal cord, maintaining an intact dura. ASCI was

induced in rats at a height of 2.5 cm, vertical drop of weight with 10 g, and 2.5 mm diameter. The emergences of tail wagging reflexes, lower limbs and body retraction flutter, and flaccid paralysis of the lower limbs indicated the efficiency of the model. The sham-operated group only underwent laminectomy, without damage, drugs, or saline. In the ketamine treatment group, intraperitoneal injection of ketamine (120 mg/kg) was initiated 1 h post-operation, and administered every 12 h thereafter. In the control group, intraperitoneal physiological saline injections were administered, and all models were sampled within the scheduled time. Any dead rats were replenished randomly.

### **Determination of TNF- $\alpha$ and IL-6**

Punctured blood from the left ventricle was centrifuged at 5000 rpm for 8 min. After the serum was separated, analysis was performed in accordance with the kit instructions (Thermo Fisher Scientific, Inc., MA, USA). The optical densities OD of TNF- $\alpha$  and IL-6 in serum were estimated by a microplate reader by using a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) and the concentrations were calculated by standard curve.

### **Measurement of MDA**

Rat spinal cord tissue (0.5 g) was taken at each time point, added into saline solution to obtain a 10% homogenate, and centrifuged at low temperatures. The supernatants were stored at -70°C. The MDA levels were measured by the thiobarbituric acid method, the operation conformed to the instructions of kit (Nanjing Jiancheng Biological Engineering Co., Ltd, Nanjing, China).

### **Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)**

Paraformaldehyde (200 mL) was infused into the hearts rapidly at each time point. Spinal cord tissues were then fixed in 4% paraformaldehyde for 2 h, followed by alcohol dehydration and dimethylbenzene treatment. Samples were embedded in paraffin and cut into coronal slices (6  $\mu$ m). All sections were dewaxed, treated with 0.25% trypsin (37°C for 60 min), and washed twice with phosphate buffered saline (PBS). TUNEL reaction mixture (50  $\mu$ L) was added to each section (37°C for 60 min), followed by the addition of 50  $\mu$ L of conversion agent peroxidase (POD) (37°C for 30 min), staining with 3,3'-diaminobenzidine (DAB) for 5 min, and counterstaining with hematoxylin. An optical microscope was used to observe the apoptotic cells, which were stained brown. Apoptotic cells were counted at 400X, from 5 fields in each section. The number of TUNEL-positive cells among 2500 cells was used to obtain the average.

### **Statistical analysis**

The SPSS 11.5 software was used. Experimental data are reported as means  $\pm$  standard deviation (SD). Groups were compared by using the Student *t*-test and a P value less than 0.05 was considered to be statistically significant.

## **RESULTS**

SERUM TNF- $\alpha$  and IL-6 levels in the control group 24, 72, and 144 h after injury

were significantly higher than the corresponding levels in the sham-operated group ( $P < 0.05$ ). Further, MDA levels in the injured spinal cord tissue were significantly higher ( $P < 0.05$ ) and an increase in the number of TUNEL-positive cells was observed ( $P < 0.05$ ). In contrast, treatment with ketamine decreased serum TNF- $\alpha$  and IL-6, MDA levels, and number of TUNEL-positive cells. Compared with the control group at the same phase, the difference was statistically significant ( $P < 0.05$ , Table 1).

**Table 1.** Impacts of ketamine on rats with spinal cord injury (serum TNF- $\alpha$ , IL-6, MDA in spinal cord tissue and neuronal apoptosis).

Group	N	TNF- $\alpha$ (ng/mL)	IL-6 (ng/mL)	MDA (mmol/g)	TUNNEL (apoptosis)
Sham	10	2.87 $\pm$ 0.91	30.17 $\pm$ 10.05	1.16 $\pm$ 1.02	6.78 $\pm$ 2.23
Control					
24 h	10	6.01 $\pm$ 1.37*	68.78 $\pm$ 13.55*	4.68 $\pm$ 1.32*	34.08 $\pm$ 7.08*
72 h	10	8.02 $\pm$ 1.50*	71.56 $\pm$ 13.08*	4.36 $\pm$ 1.09*	56.01 $\pm$ 7.99*
144 h	10	8.12 $\pm$ 1.27*	70.62 $\pm$ 10.08*	2.10 $\pm$ 1.17*	28.67 $\pm$ 6.36*
Treatment					
24 h	10	4.68 $\pm$ 1.05**	50.98 $\pm$ 10.16**	2.12 $\pm$ 1.20**	25.96 $\pm$ 6.09**
72 h	10	5.86 $\pm$ 1.12**	54.96 $\pm$ 11.89**	2.21 $\pm$ 0.67**	46.28 $\pm$ 8.02**
144 h	10	6.20 $\pm$ 1.17**	57.02 $\pm$ 10.45**	1.62 $\pm$ 1.37**	18.89 $\pm$ 4.21**

Compared with the sham group (\* $P < 0.05$ ); with the same period of the control group (\*\* $P < 0.05$ ).

## DISCUSSION

Ketamine is a commonly used anesthetic in clinics that is known to have anti-inflammatory, antioxidative, anti-apoptotic, and neuroprotective effects. Ketamine inhibits peroxide formation, thereby inhibiting neutrophil activation. Further, ketamine is an N-methyl-D-aspartate (NMDA) receptor antagonist that binds to a regulatory site on the receptor channel complex, thereby affecting the structure of the complex. As a result, the function of the receptor recognition site is decreased, thereby interfering with its normal activation by excitatory amino acids and reducing the NMDA receptor-mediated  $\text{Ca}^{2+}$  influx. These effects can reduce the cell damage induced by glutamate and intracellular  $\text{Ca}^{2+}$  overload (Yon et al., 2006).

ASCI is a common clinical disease with high morbidity and mortality rates. The results of this study confirmed that following ASCI, TNF- $\alpha$ , IL-6, and other inflammatory cytokines in plasma and cerebrospinal fluid are significantly increased, leading to the expression of cell adhesion molecules (Pasarica et al., 2005). High TNF- $\alpha$  and IL-6 levels can promote the accumulation and activation of inflammatory cells and enhance the adhesion of neutrophils and monocytes, leading to structural damage of the spinal vasculature and exacerbation of spinal cord damage (Whitehead et al., 2010). TNF- $\alpha$  and other cytokines, including IL-1, IL-2, IL-6, and platelet activating factor, form a signaling network that impacts nearly all immune and endothelial cells, leading to inflammation and tissue injury (Pasarica et al., 2005). IL-6-mediated inflammatory responses play an important role in spinal cord injury, and some scholars believe that IL-6 is produced immediately after tissue damage, varying with the degree of injury (Marklund et al., 2005). Ketamine treatment can significantly reduce the overexpression of inflammatory cytokines in serum, indicating that ketamine plays a protective role during spinal cord injury in rats through its anti-inflammatory activity.

Following ASCI, oxygen free radicals and lipid peroxidation induce oxidative stress, contributing to the pathogenesis of secondary spinal cord injury (Vural et al., 2010). Spinal cord tissue edema can cause severe pathological damage after ASCI, and is closely related to

free radical oxidative damage (Ohta et al., 2005). MDA, an end metabolic product of biofilm unsaturated fatty acid peroxidation induced by oxygen free radicals, may reflect the level of lipid peroxidation and the degree of injury after free radical exposure (Karlidag et al., 2005). Our study indicates that MDA levels in the spinal cord tissue were significantly higher post ASCI than in the sham-operated group, indicating that ASCI affects the oxidant-antioxidant balance. Consumption or suppression of the free radical scavenging system can affect oxidative stress. However, dynamic changes in levels of MDA in the spinal cord of the treatment group were consistent with the control group; however, the values were lower at each time point. These data suggested that ketamine mitigates secondary spinal cord injury through scavenging of free radicals.

Neuronal apoptosis, which is a secondary injury after ASCI, is of great interest (Torres et al., 2010). In the terminal stages of injury, hematomas formed due to mechanical compression, reduction of local blood flow, release of blood components, an increase in various cytotoxic substances, and the generation of oxygen free radicals, ultimately initiates apoptosis (Emery et al., 1998). Apoptosis is a gene-regulated mechanism of delayed neuronal death that can be induced by internal and external environmental stimuli (Friedman, 2010). The results of this experiment suggest that apoptosis during spinal cord injury was significantly higher in the control group than in the sham-operated group. Further, ketamine treatment significantly decreased apoptosis, indicating that apoptosis was involved in secondary injury after acute injury. Thus, this study confirmed the anti-apoptotic effects of ketamine.

In summary, ketamine reduced MDA content in the spinal cord by scavenging oxygen free radicals, downregulated water content in the spinal cord, exerted antiinflammatory and anti-apoptotic effects, thereby alleviating spinal cord injury to achieve neuroprotection.

### Conflicts of interest

The authors declare no conflict of interest.

### ACKNOWLEDGMENTS

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### REFERENCES

- Carmen J, Rothstein JD and Kerr DA (2009). Tumor necrosis factor-alpha modulates glutamate transport in the CNS and is a critical determinant of outcome from viral encephalomyelitis. *Brain Res.* 1263: 143-154.
- Carvajal CA, Herrada AA, Castillo CR, Contreras FJ, et al. (2009). Primary aldosteronism can alter peripheral levels of transforming growth factor beta and tumor necrosis factor alpha. *J. Endocrinol. Invest.* 32: 759-765.
- Emery E, Aldana P, Bunge MB, Puckett W, et al. (1998). Apoptosis after traumatic human spinal cord injury. *J. Neurosurg.* 89: 911-920.
- Esposito E, Paterniti I, Mazzon E, Genovese T, et al. (2011). MK801 attenuates secondary injury in a mouse experimental compression model of spinal cord trauma. *BMC Neurosci.* 12: 31.
- Friedman WJ (2010). Proneurotrophins, seizures, and neuronal apoptosis. *Neuroscientist.* 16: 244-252.
- Ha KY, Carragee E, Cheng I, Kwon SE, et al. (2011). Pregabalin as a neuroprotector after spinal cord injury in rats: biochemical analysis and effect on glial cells. *J. Korean Med. Sci.* 26: 404-411.
- Horvath G, Kekesia G, Tuboly G and Benedek G (2007). Antinociceptive interactions of triple and quadruple combinations of endogenous ligands at the spinal level. *Brain Res.* 1155: 42-48.
- Jamous M, Barbarawi M, Samrah S, Khabaz MN, et al. (2010). Emergency decompressive craniectomy for trauma patients

- with Glasgow Coma Scale of 3 and bilateral fixed dilated pupils. *Eur. J. Trauma Emer. Surg.* 36: 465-469.
- Jiao JJ, Jiang JN, Du B, Mo Y, et al. (2010). Extract of *Ginkgo biloba* EGB 761 inhibits cell apoptosis following spinal cord injury. *Neural Regeneration Res.* 5: 1745-1749.
- Karlidag T, Iihan N, Laygusus L, Keles E, et al. (2005). Roles of free radicals, nitric oxide, and scavenging enzymes in nasal polyp development. *Ann. Otol. Rhinol. Laryngol.* 114: 122-126.
- Kato K, Kikuchi S, Shubayev VI and Myers RR (2009). Distribution and tumor necrosis factor-alpha isoform binding specificity of locally administered etanercept into injured and uninjured rat sciatic nerve. *Neuroscience.* 160: 492-500.
- Labombarda F, Coronel MF, Villar MJ, Nicola AF, et al. (2008). Neuropathic pain and temporal expression of preprodynorphin, protein kinase C and N-methyl-D-aspartate receptor subunits after spinal cord injury. *Neurosci. Lett.* 447: 115-119.
- Lemcke J, Ahmadi S and Meier U (2010). Outcome of patients with severe head injury after decompressive craniectomy. *Acta. Neurochir.* (Suppl. 106): 231-233.
- Margaryan NV, Kirschmann DA, Lipavsky A, Bailey CM, et al. (2010). New insights into cathepsin D in mammary tissue development and remodeling. *Cancer Biol. Ther.* 10: 457-466.
- Marklund N, Keck C, Hoover R, Soltesz K, et al. (2005). Administration of monoclonal antibodies neutralizing the inflammatory mediators tumor necrosis factor alpha and interleukin -6 does not attenuate acute behavioral deficits following experimental traumatic brain injury in the rat. *Restor. Neurol. Neurosci.* 23: 31-42.
- Ohta S, Iwshita Y, Takada H, Kuno S, et al. (2005). Neuroprotection and enhanced recovery with edaravone after acute spinal cord injury in rats. *Spine (Phila. Pa. 1976).* 30: 1154-1158.
- Pasarica D, Gheorghiu M, Toparceanu F, Bleotu C, et al. (2005). Neurotrophin-3, TNF-alpha and IL-6 relations in serum and cerebrospinal fluid of ischemic stroke patients. *Roum. Arch. Microbiol. Immunol.* 64: 27-33.
- Torres BB, Caldeira FM, Gomes MG, Serakides R, et al. (2010). Effects of dantrolene on apoptosis and immunohistochemical expression of NeuN in the spinal cord after traumatic injury in rats. *Int. J. Exp. Pathol.* 91: 530-536.
- van Neerven S, Mey J, Joosten EA, Steinbusch HW, et al. (2010). Systemic but not local administration of retinoic acid reduces early transcript levels of pro-inflammatory cytokines after experimental spinal cord injury. *Neurosci. Lett.* 485: 21-25.
- Vural M, Arslantaş A, Yazihan N, Köken T, et al. (2010). NMDA receptor blockage with 2-amino-5-phosphonovaleric acid improves oxidative stress after spinal cord trauma in rats. *Spinal Cord.* 48: 285-289.
- Whitehead KJ, Smith CG, Delaney SA, Curnow SJ, et al. (2010). Dynamic regulation of spinal pro-inflammatory cytokine release in the rat *in vivo* following peripheral nerve injury. *Brain Behav. Immun.* 24: 569-576.
- Yon JH, Carter LB, Reiter RJ and Jevtovic-Todorovic V (2006). Melatonin reduces the severity of anesthesia-induced apoptotic neurodegeneration in the developing rat brain. *Neurobiol. Dis.* 21: 522-530.