

Original Article



The effects of intrathecal ketamine on improving sensory-motor function in a rat model of compression spinal cord injury

Sajad Fakhri¹ , Fatemeh Abbaszadeh² , Ramin Pouriran³, Masoumeh Jorjani^{2, 4*} 

1. Pharmaceutical Sciences Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Neurobiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Pharmacology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

Introduction: The major secondary complications of spinal cord injury (SCI) are neuropathic pain and motor dysfunction, which remained medical challenges for clinicians. Due to the major role of glutamate in excitotoxicity and central sensitization, the present experiment was to evaluate the effects of ketamine (KET), an n-methyl-D-aspartate (NMDA) receptor blocker, on sensory-motor functions in a rat model of clip compression SCI.

Methods: Wistar rats were divided into sham, SCI and KET-treated groups. The sham group received laminectomy without any compression lesion. KET and SCI groups were subjected to severe compression injury for 1min with an aneurysm clip and then treated with KET (10mg/kg) or 5% dimethyl sulfoxide (as vehicle), respectively. The rats were assessed by pain-related and motor behavioral tests inclusive of von Frey, acetone drop, hot plate, inclined plane and Basso-Beattie-Bresnahan on day 0 prior to the injury and the 7th, 14th, 21st and 28th days following SCI.

Results: KET group compared with the SCI group showed a significant decline in the mechanical allodynia on the 21st and 28th days, and in cold allodynia from the 1st week to the 4th week post-injury. KET treatment improved the motor function but not weight loss and auricle temperature rise during the 4 weeks of follow up from the 1st week until the 4th week.

Conclusion: It was ultimately attained that the advantageous effects of intrathecal KET on sensory-motor dysfunction of SCI rats could provide new approaches for KET as a neuroprotective agent toward clinical applications.

<http://dx.doi.org/10.32598/ppj.24.2.20>

Keywords:

Ketamine;
Spinal cord injury;
Neuropathic pain;
Motor activity;
Rat

* Corresponding author:

M. Jorjani

Email:

msjorjani@sbmu.ac.ir

Tel: +98 (21) 22429768

Received 21 July 2019;

Received in revised form 25

January 2020; Accepted 12

February 2020

Introduction

Spinal cord injury (SCI) is a major complication of

traumatic spinal damage, causing motor disturbance and central neuropathic pain. Neuropathic pain has a prevalence rate of approximately 50% in SCI

patients, which affects the life expectancy, cost burden and causes a diminished quality of life. Development of allodynia and hyperalgesia are clinical hallmarks of post-SCI neuropathic pain (Siddall and Middleton, 2015). Pharmacological treatments are often not useful for post-SCI neuropathic pain and motor dysfunction. Further, these complications are usually resistant to medications. Thus, it is important to develop novel and effective treatments for post-SCI sensory-motor dysfunction.

Following SCI, the elevation of excitatory amino acids leads to prolonged activation of spinal cord neurons causing the spread of nociception and neuropathic pain. According to data, the development and maintenance of neuropathic pain could be influenced by n-methyl-d-aspartate receptors (NMDAR) (Zhou et al., 2011). Ketamine (KET) as a potent sedative, amnestic, analgesic and anesthetic agent blocks NMDAR and in turn its down-stream neuroinflammatory signaling pathways. Thus, it could be considered as a promising neuroprotective agent for SCI complications (Kose et al., 2012). The antinociceptive effects of KET have been shown in several preclinical studies. Ketamine's chronic pain-relieving properties have been reported in neuropathic pain models and inflammatory conditions. The KET antidepressant effects show that KET affects both the sensory-discriminative system and the affective-motivational components of pain (Radvansky et al., 2016).

In clinical situations, pain treatment by KET has been reported on the management of nerve injury in oncologic diseases (Domino, 2010), morphine-resistant cancer pain (Schwartzman et al., 2009), post-herpetic neuralgia (Sawynok and Zinger, 2016) and post-injury pain (Domino, 2010). For conditions with neuropathic pain features with an identifiable nerve injury such as SCI, there is still inconclusive evidence for the effectiveness of KET. At the spinal level, KET has shown an analgesic effect by down-regulating glial fibrillary acidic protein (GFAP) expression and inhibiting astrocyte activation (Mei et al., 2009).

Intravenous (IV) KET reduced post-injury pain and improved allodynia plus hyperalgesia in two randomized, double-blind placebo control studies in patients with neuropathic pain (Mestre et al., 1994). On the other hand, there have not been sufficient

reports to show the effects of intrathecal (IT) KET in pain and particularly motor outcomes in the context of SCI. To the best of our knowledge, this is the first investigation regarding the possible long-lasting motor performance-enhancing and analgesic effects of IT KET on cold, thermal and mechanical pain along with weight and temperature changes as common complications post-SCI. This may lead to the introduction of a therapeutic agent towards clinical applications.

Materials and methods

Animals

In total, 21 adult rats (male Wistar, 250–300g) were provided. The rats were housed in 4–5 per standard cages under a 12 h/12 h light-dark cycle regiment. They were maintained at a $24\pm 2^\circ\text{C}$ temperature-controlled room with free access to food and water for at least 10 days prior to the study. The surgical procedures were performed using aseptic techniques. The entire procedure was approved by the institutional animal care and use committee in Shahid Beheshti University of Medical Sciences (IR.SBMU.REC.1396, 321).

Experimental groups

Randomly, twenty-one animals were divided into 3 experimental groups of sham, SCI and KET ($n=7$ per group). The sham group received only laminectomy. The SCI group and KET group underwent laminectomy and compression injury then treated by IT injection of 5% dimethyl sulfoxide (DMSO) as vehicle and KET 30min after injury, respectively. Sensory and motor function of animals were assessed by von Frey test, acetone drop test, hot plate test, Basso-Beattie-Bresnahan (BBB) test, inclined plane test, as well as body weight and auricle temperature changes on the 0, 7th, 14th, 21st and 28th days following surgery.

Compression spinal cord injury

Deep anesthesia was made by intraperitoneal (IP) injection of 10mg/kg xylazine and 80mg/kg ketamine, before the surgery. The laminectomy of thoracic 8 (T8)-thoracic 9 (T9) was done following the incision of skin and muscle. The spinal cord was exposed using a micro rongeur (Fine Science Tools, USA), then it was compressed by an aneurysm clip (Aesculap, Tuttlingen, Germany, 90g) for 1min (Fakhri et al.,

2018; Fakhri et al., 2019). The dura mater was left intact. After the surgery, the skin and muscle were sutured with 3-0 silk sutures; consequently, rats were allowed to recuperate on a 30°C heating pad. Cefazolin (40mg/kg IP) and 2ml normal saline (subcutaneously, SC) were administrated twice a day to prevent urinary tract infection and attain rehydration, respectively. The bladder in the rats was manually emptied twice a day up to reflex bladder emptying returned. The entire rats were kept under standard conditions in clean cages.

Intrathecal drug injection

KET (Ketalar®, Pfizer Inc., USA) powder was dissolved in a vehicle of 5% DMSO. The modified method of Mestre et al. (1994) was utilized for IT injection of KET and 5% DMSO (as vehicle) following SCI (Burton et al., 1999). A 25-G needle was attached to the Hamilton syringe which was used for IT injection. Needle at a 45° angle was inserted to the subarachnoid space between two adjacent vertebrae in the lumbar 6/5 level. Identifying the needle in the subarachnoid space was based on the existence of an immediate lateral tail movement that occurred following the penetration of the ligament flavum. The dosage of KET (10mg/kg) (Walker et al., 2010) or 5% DMSO (20µl), was injected gradually over 10s into the KET group as well as SCI group, once the needle was in the subarachnoid space. In order to stop the outflow of the medication, the syringe was kept for 10s more in the subarachnoid space.

Behavioral assessment

Mechanical allodynia

Mechanical allodynia was assessed using von Frey filaments. The lateral aspect of the plantar area of both paws was stimulated with an incremental series of 5 von Frey filaments (10, 15, 26, 60 and 100g). Each filament was applied five times with 5s intervals. Paw licking, withdrawal, biting or flinching was expressed as a positive mechanical allodynia response. Positive responses three out of five in repetitive stimuli was considered as an allodynia threshold.

Cold allodynia

The acetone drop test was performed to assess cold allodynia. The animals were placed on the top of a wire mesh grid in acrylic cages to access their paws.

After a 45min acclimatization, approximately 100µl acetone applied from a distance of 2cm to the plantar surface of the hind paw in order to test a cold stimulation reaction. If a rat withdrew its hind paw, a response was recorded, which was classified and scored as follows: no reaction (score 0), startle response without paw withdrawal (score 1), brief withdrawal of the paw (score 2), prolonged withdrawal of 5-30s (score 3), prolonged and repetitive withdrawal of 30s with flinching and/or licking (score 4). This acetone test has been described as composing of chemical, cold and possibly mechanical stimulation. An observer tested cold allodynia in a blinded manner.

Heat hyperalgesia

A hot plate apparatus (Harvard apparatus, America, 45-52°C) was used to measure analgesia. The animals individually were placed on a transparent Plexiglas chamber on a hot plate analgesia meter, then were examined for baseline latency to paw licking or jumping. Each rat was tested three times with an interval of 5min. The average of three replicates was reported as paw withdrawal latency. A 60s cut-off was enforced to stop stimulating and prevent tissue injury.

Locomotor activity

Motor performance was evaluated in rats using an inclined plane and BBB tests. In the first test, each rat was placed on an adjustable wood plane (60×40cm) with changeable angles from 0° (horizontal) to 60° (3 times with 5min intervals). The angle of the plane was increased gradually. Two blinded observers assessed the maximum angle in which the rats stayed stable for 5s and the average angle was considered.

BBB locomotor scale was also used as the second test to evaluate the locomotor activity of rats. Briefly, two blinded examiners observed animals for 4min which were placed in a wooden box (90×10cm). The BBB score was defined in the range of 0 (complete paralysis of both hind limbs) to 21 (standard walking) for all rats, prior to the surgery (day 0) and also on the 1st, 7th, 14th, 21st and 28th days after the surgery. The mean value of the scores for both hind paws was calculated for the rats per time point in order to obtain only one movement value.

Weight difference

In all three groups, the weight changes in rats were calculated using the following formula: weight difference = (animal's weight on the 7th, 14th, 21st and 28th days - weight on day 0 (before the surgery))

Auricle temperature

A quickly responding Microlife infrared thermometer (NC-100) was used to determine rats' auricle temperature. The sensor was held at a distance of 5cm over the dorsal aspect of the auricle surface temperature.

Statistical analysis

The data is indicated as the mean ± SEM. By using Version 6.0 of the GraphPad Prism Program, the

analysis was done. Repeated measures two-way analysis of variance (ANOVA) with Bonferroni post-hoc analysis was performed for the comparison of the groups across all measuring times. A difference at $P < 0.05$ was considered significant in all calculations.

Results

Mechanical allodynia

A significant result for the main effects was attained by two-way repeated-measures ANOVA which indicated: [group: $F(2, 18) = 21.51$, $P < 0.001$, times: $F(4, 72) = 24.64$, $P < 0.001$ and interaction: $F(8, 72) = 3.808$, $P = 0.0009$] on right paw withdrawal threshold in von Frey test, and also the left paw withdrawal threshold of the groups [$F(2, 18) = 18.82$, $P < 0.001$], times [$F(4, 72) = 27.68$, $P < 0.001$], and interaction [$F(8, 72) = 3.31$, $P = 0.0028$]. Despite the same paw

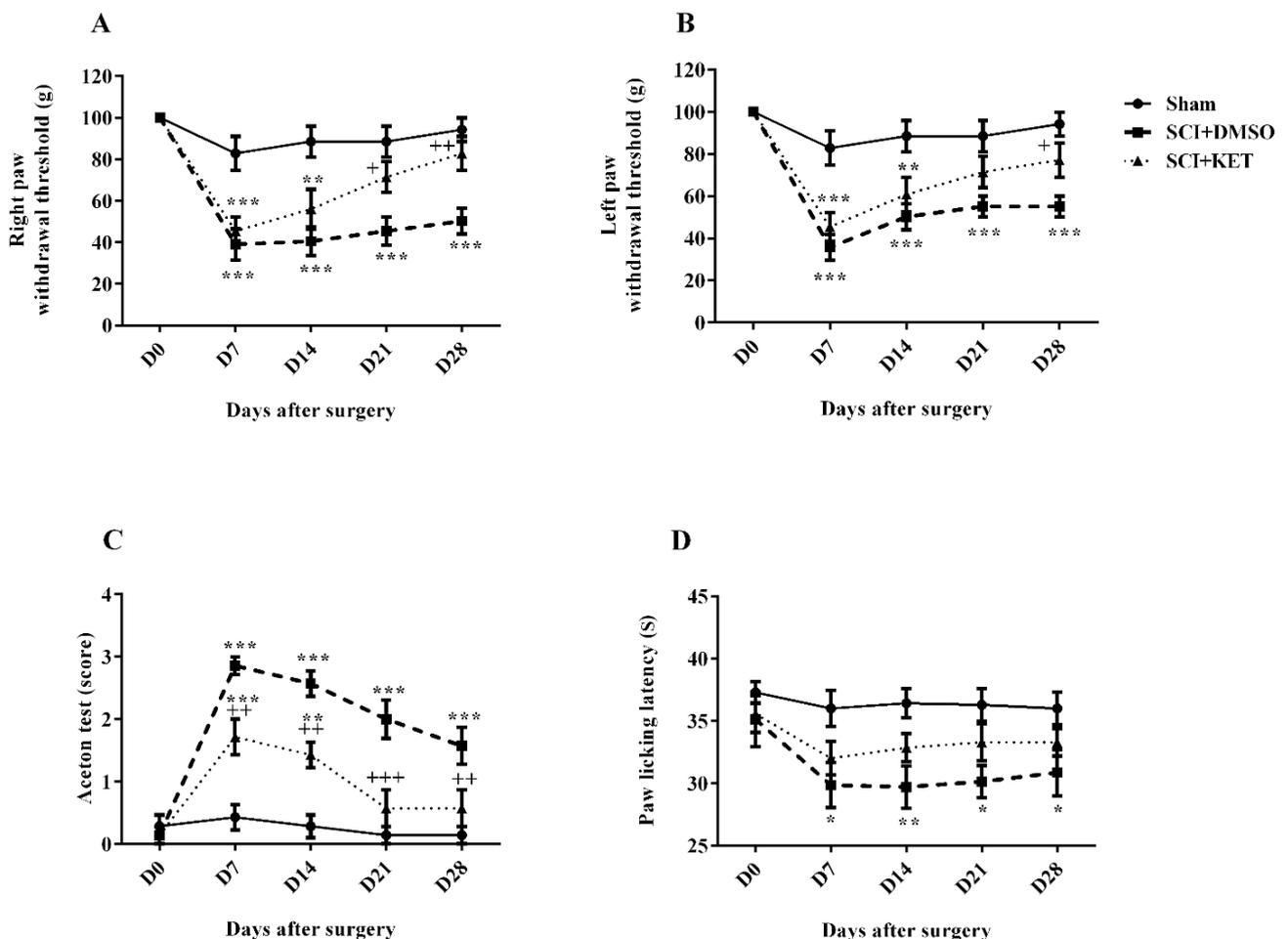


Fig.1. Effects of KET on pain-related behaviors in rats undergoing clip compression SCI. Response thresholds of right hind paw (A) and left hind paw (B) to mechanical stimuli in von Fery test and response threshold to cold and thermal stimuli in the acetone drop test and hot plate test, respectively (C and D). Data are presented as mean ± SEM (n=7). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. sham and + $P < 0.05$, ++ $P < 0.01$ and +++ $P < 0.001$ vs. SCI group. Repeated measures two-way analysis of variance with Bonferroni post-hoc test was used. SCI: spinal cord injury; KET: ketamine (10mg/kg, 30min post-injury); DMSO: dimethyl sulfoxide (as vehicle, 20 μ l).

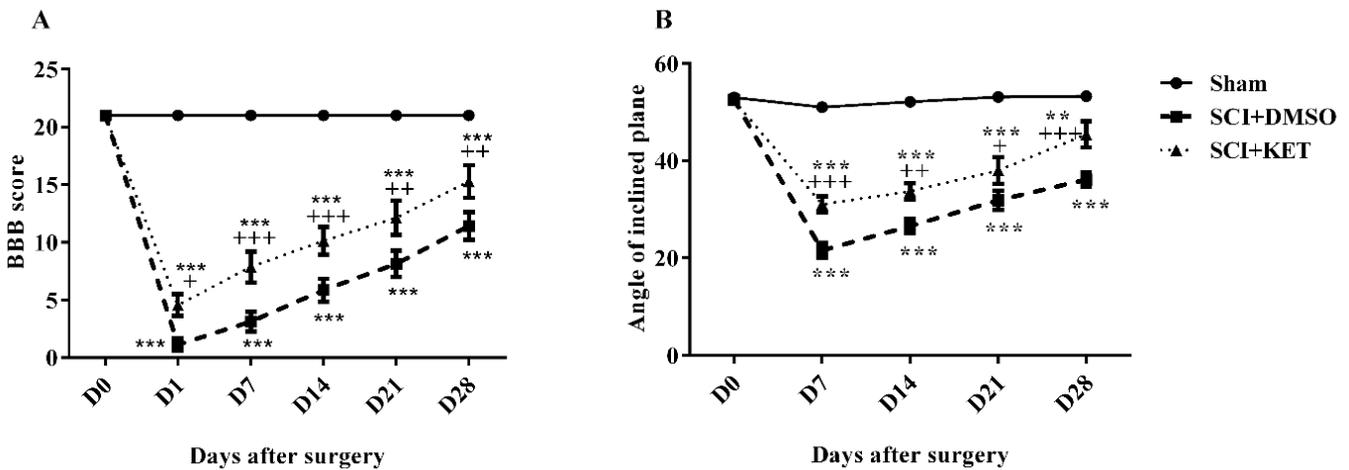


Fig.2. Effects of KET on motor performance of rats undergoing clip compression SCI. BBB motor score (A) and inclined plane test (B). Data are presented as mean±SEM (n=7). ** $P<0.01$, *** $P<0.001$ vs. sham and + $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. SCI group. Repeated measures two-way analysis of variance with Bonferroni post-hoc test was used. SCI: spinal cord injury; KET: ketamine (10mg/kg, 30min post-injury), DMSO: dimethyl sulfoxide (as vehicle, 20µl).

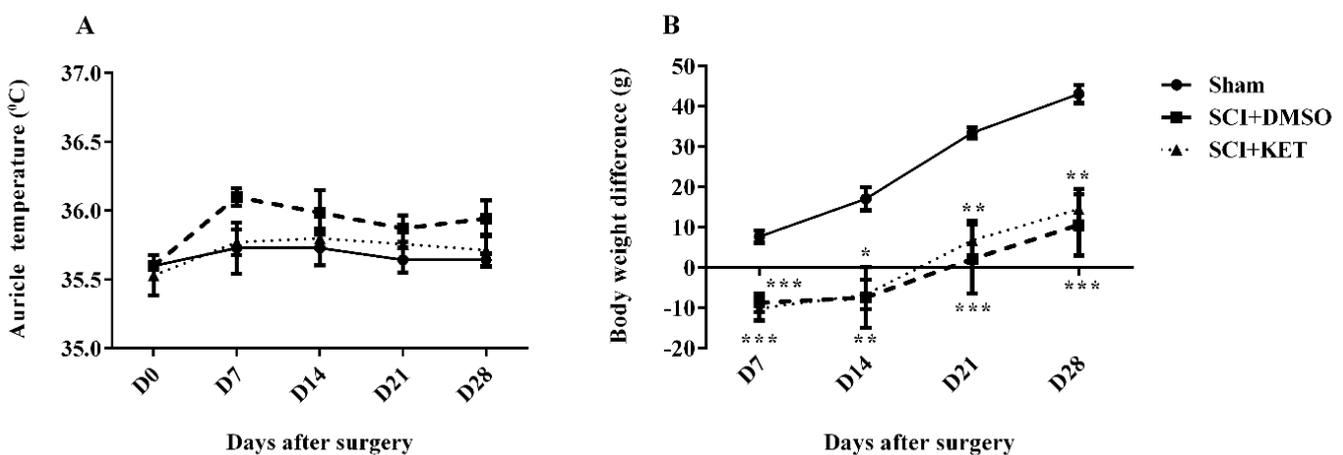


Fig.3. Effects of KET on body weight and temperature in rats undergoing clip compression SCI. Auricle temperature (A) and body weight differences (B) were measured over a course of 28 days post-injury. Data are presented as mean±SEM (n=7). ** $P<0.01$, *** $P<0.001$ vs. sham group. Repeated measures two-way analysis of variance with Bonferroni post-hoc test was used. SCI: spinal cord injury; KET: ketamine (10mg/kg, 30min post-injury); DMSO: dimethyl sulfoxide (as vehicle, 20µl).

withdrawal response in the sham group in 4 weeks follow-up, SCI elicited a significant and long-lasting reduction, from day 7 post-injury, in the right and left paw withdrawal threshold which is a sign of mechanical allodynia ($P<0.001$). The KET treatment group significantly reduced the sign induced by SCI; therefore, from day 21, there was no significant difference in the paw withdrawal threshold between the sham and KET groups (Figs. 1A and B).

Cold allodynia

In the evaluation of the cold pain threshold in acetone drop test, two-way repeated-measures ANOVA indicated significant effects of group and time [group:

$F(2, 18)= 25.93$, $P<0.001$, times: $F(4, 72)= 32.56$, $P<0.001$, and interaction: $F(8, 72)= 9.208$ $P<0.001$]. In 4 weeks follow up, rats continued to undergo the normal cold hypersensitivity in the sham group. SCI caused a significant cold hypersensitivity as compared to the sham group. KET improved response to cold stimulation compared to the SCI group. Indeed, from the 3rd week, there was no considerable difference between the sham and KET-treated groups (Fig. 1C).

Heat hyperalgesia

In the sham group, paw licking latency was almost the same in the entire experiment. Even though SCI

caused a significant reduction in paw licking latency on the 7th ($P=0.0123$) and 28th days ($P=0.0465$) as well, it was seen on the 14th ($P=0.0053$) and 21st days ($P=0.0123$). Additionally, in between-group comparison, no considerable difference was observed between SCI and KET-treated groups (Fig. 1D).

Locomotor activity

Motor behavior was evaluated by BBB scale and inclined plane test following the SCI. Generally, two-way repeated-measures ANOVA for BBB score showed significant effects in groups [$F(2, 18)= 94.77, P<0.001$], times [$F(5, 90)= 155.2, P<0.001$] and interaction [$F(10, 90)= 40.84, P<0.001$]. Rats in the laminectomy sham group continued to the score 21 showing that no functional impairments occurred during the surgery intrinsically. SCI resulted in average scores of 0 and 1 on the 1st day post-injury and continued to be different from the sham animals up to day 28th post-injury ($P<0.001$). The KET treatment enhanced motor function in SCI rats, especially from the 1st ($P=0.0174$) to the 28th day ($P=0.0060$) post-injury, compared to the SCI group (Fig. 2A).

Two-way repeated measures of ANOVA specified significant effects in group [$F(2, 18)= 62.30, P<0.001$], time [$F(4, 72)= 129.9, P<0.001$] and interaction [$F(4, 24)= 98.57, P<0.001$] in the inclined plane test. The same angle stays on the inclined plane apparatus for all intervals in the sham group confirmed that no motor impairments were observed after laminectomy. SCI caused a significant reduced in the mean angle of stay on the inclined-plane apparatus as compared to the sham (Fig. 2B). Though, as shown in the BBB test, SCI rats indicated a trend of continuous improvement of locomotion during the 4 weeks follow up. Apparently, rats treated with KET significantly caused an increase in the recovery promoting after SCI; therefore, from the 1st week, there was a significant difference between SCI and KET-treated groups.

Auricle temperature

In total, the two-way repeated measures of ANOVA indicated significant effects of the groups [$F(2, 18)= 7.451, P=0.0044$], times [$F(4, 72)= 3.095, P=0.0208$] and interaction [$F(8, 72)=.4648, P=0.8769$] on auricular body temperature. SCI elicited a slight

increase in the body temperature measured on the 7th day post-injury (which was not significant) in comparison to day 0, before the injury. Nevertheless, between-group comparisons presented no significant differences between the sham, SCI and KET-treated animals (Fig. 3A).

Weight differences

Based on monitoring animals' weight alterations during 4 weeks post-surgery/injury follow up, two-way repeated-measures ANOVA showed significant effects (groups: [$F(2, 18)= 11.64, P<0.001$], times [$F(3, 54)= 61.88, P<0.001$] and interaction [$F(6, 54)= 2, P=0.0816$]). There was a pattern of normal weight gain in the sham group through the 4 weeks follow up. Weight loss was observed in SCI at the 1st two weeks post-injury and significantly disrupted weight gain compared to the sham group on the 14th ($P=0.0078$), 21th and 28th days ($P<0.001$). The KET treatment did not prevent weight loss post-injury (Fig. 3B).

Discussion

In our data, KET attenuated the mechanical and cold pain-related behavior, which were evaluated by von Frey and acetone drop test and provoked a better recovery in the motor function of SCI animals examined by BBB and inclined plane tests. These effects of KET which started within the first week and continued into the 4 weeks, were long-lasting. KET also led to a mild (not significant) fall in the body temperature with no significant changes in the SCI-induced weight loss.

Contusion, hemisection and compression are mostly used in animal models of SCI-induced sensory-motor dysfunctions. They are considered as the clinical situations of SCI. Clip compression is usually preferred to assess SCI-induced neuropathic pain since it could precisely control the injury rate (Cheriyian et al., 2014). Chronic pain, as a problem in clinical practice for SCI, has a great importance in clinical research. In the current study, post-injury IT KET treatment significantly ameliorated mechanical and cold allodynia. On the other hand, it could not improve heat hyperalgesia which was in line with a randomized, double-blind, crossover study, revealing that IV KET reduced evoked and continuous pain; nonetheless, it could not relieve heat pain following SCI (Eide et al., 1995). In another randomized

double-blind study, IV KET showed a dose-dependent reduction in stroking pain scores where a significant decline was observed in the von Frey hyperalgesia (Leung et al., 2001). Apparently, IV KET also reduced allodynia (Kim et al., 2013) and showed a significant analgesic effect assessed via a visual analogue scale in patients with post-SCI neuropathic pain (Kvarnström et al., 2004). In complex regional pain syndrome type 1, KET infusion reduced pain significantly in the patients (Sigtermans et al., 2009). Bennet et al. (2000) reported that IT administration of non-NMDA or NMDA glutamate receptor antagonist decreased mechanical allodynia; however, it could not mitigate thermal hyperalgesia in a rat model of SCI-induced pain, which is similar to our data.

In addition to IT and IV routes, KET may be administrated by other routes including SC, transdermal, intranasal, IP, oral and topical (Bell and Kalso, 2018). Oral and topical usage of KET was also effective in reducing various neuropathic pain syndromes (Rigo et al., 2017). In a rat model of segmental spinal nerve ligation, IT compared to IP KET revealed a more and long-lasting withdrawal threshold assessed by von Frey and acetone drop tests (Burton et al., 1999). Further, a meta-analysis showed that KET analgesic effect persists for at least 4 weeks (Niesters et al., 2014), which was in line with the long-lasting impact of KET in our study. Despite the long-lasting effects of IT KET on sensory-motor dysfunction, Doncheva et al. (2019) found that 14-day IP injection of KET showed an early and short-lasting analgesic effect in a rat model of sciatic nerve ligation as evaluated by formalin, analgesia-meter and hot plate tests. In the current study, pain and motor performance were assessed from the 7th day to 28th day post-injury. Thus, the behavioral changes are completely attributed to the IT KET.

Beyond post-SCI pain and the effects of IT KET on pain, motor dysfunction is another SCI-induced complication that is originally associated with the loss of motor neurons. Thus, neuroprotective treatments have been striving to recover SCI-induced motor dysfunction (Pomeroy et al., 2010). In their study, motor dysfunction was recovered slightly over time among SCI rats which is consistent with some other reports (Hama and Sagen, 2007). The long-lasting results of a single dose of IT KET proposed that only a short time blocking of the NMDA receptor, reduced glutamate-induced central pain (Burton et al., 1999).

In the mechanistic perspective, NMDAR is widely distributed throughout the spinal cord and performs an essential role in the development of pain. KET is an NMDAR antagonist that has anti-inflammatory functions in traumatic injury models (Loix et al., 2011). Glutamatergic mechanisms are involved in the progress of neuropathic pain, post-SCI pain and neurodegenerative diseases; thus confirming the crucial role of glutamate (Samandari et al., 2019). Apparently, it was responsible for the central sensitization of nociceptive neurons existing in the spinal cord and activation of astrocytes by elevating intracellular ion concentration $[Ca^{2+}]$ (Sheldon and Robinson, 2007). On the other hand, glutamate induced neuronal apoptosis through attenuating the activation of caspase-3 and the expression of Bcl-2 family proteins (Zhang and Bhavnani, 2006).

At the spinal level, KET showed an analgesic effect by down-regulating GFAP expression and inhibiting astrocyte activation (Mei et al., 2009). At high doses, KET also played different roles in the channels and receptors such as antagonism at calcium, potassium and sodium channels. Further, KET played an agonistic role at γ -aminobutyric acid receptor and affected dopamine D2 receptors to provide an analgesic effect (Bell and Kalso, 2018). KET also affected other channels/receptors, including kainate, γ -aminobutyric acid, μ -opioid, muscarinic and monoaminergic receptors, L-type calcium channels, as well as NMDARs (Himmelseher and Durieux, 2005). Glutamatergic signaling and nitric oxide metabolism are interrelated. In this line KET prevented nitric oxide-dependent cyclic guanosine monophosphate and nitric oxide synthesis formation stimulated by glutamate (Sleigh et al., 2014). Also, it interferes with the inflammatory responses in ischemia to reduce the intensity of cerebral injury. In an *in vitro* study, KET suppressed lipopolysaccharide-induced production of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6) and IL-8, which are downstream signaling pathways of NMDA (Kawasaki et al., 1999). It has also been suggested that KET reduced NMDAR type 1 and blocked intracellular protein kinase A, PKC or interconnected pathways to show analgesic effects (Mei et al., 2010).

From the stereochemical point of view, KET has two isomers of (R) and (S). An *in vitro* study on rat hippocampal-glutamate exposed neurons demonstrated that (S) and racemic attenuated neuron

injury; however, (R)-KET could not attenuate neuronal damage. Neuroregenerative activities were attained with (S), but not with racemic or (R)-KET (Himmelseher et al., 1996). KET reduced the amount of hemorrhagic necrosis and improved the neurologic severity score following a head trauma in rats (Shapira et al., 1992). In a rabbit model with spinal cord ischemia-reperfusion injury, KET indicated a potent protective effect through reducing malondialdehyde and elevating superoxide dismutase in the spinal cord tissue. The data for the treatment of neuropathic pain by KET has been also derived from clinical reports on the management of nerve injury in oncologic diseases (Domino, 2010). For instance, KET induced a dose-dependent analgesic effect in patients who suffered from morphine-resistant cancer pain. Further, studies have investigated the effects of KET on complex regional pain syndrome (Schwartzman et al., 2009). In a clinical trial, KET also mitigated post-herpetic neuralgia (Sawynok and Zinger, 2016). Besides, IV KET reduced post-injury pain and improved allodynia plus hyperalgesia in two randomized double-blind placebo control studies, in patients with neuropathic pain (Backonja et al., 1994). KET also showed a neuroprotective effect on the injured spinal cord via lowering IL-6, TNF- α and malondialdehyde levels in rats (Tang et al., 2015). A decline in cytokines possibly results in a slight reduction in the term that KET compared to the SCI group caused a slight (but not significant) decrease in the temperature of the body, as observed. (S)-KET is histopathologically as productive as methylprednisolone in preventing secondary SCI. It has been found that KET is even more effective than methylprednisolone in reducing lipid peroxidation levels in the subacute stage of SCI in rats (Kose et al., 2012). As Hijazi et al. (2003) reported, epidural injected KET was considered as a safe adjunct in SCI-induced chronic pain patients limited to 30 days post-injection which highlighted the importance of the routes of KET administration to show anti-nocifensive effects.

As another complication post-SCI, weight loss occurs due to bone mineral lost, muscle atrophy and metabolism changes in humans (Bauman and Cardozo, 2015). In rodent SCI models, homeostatic mechanisms in later stages, permanent changes in the gastrointestinal tract and hypoplasia at early stages were the main reasons for weight loss (Gefen,

2014). In our study, KET did not attenuate the SCI-induced weight loss during 4 weeks in the context of SCI. This may be due to the reason that KET did not affect the mechanisms involved in weight gain.

Conclusion

Conclusively, the findings of the present study suggest that KET can be applied as an option in clinical applications to improve sensory-motor dysfunction following SCI. Further investigations focusing on the dose-response and molecular effects of KET are required to show the exact mechanism, safety and the therapeutic value of intrathecal KET in SCI models.

Acknowledgments

The current research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors stated no conflicts of interest

References

- Backonja M, Arndt G, Gombar KA, Check B, Zimmermann M. Response of chronic neuropathic pain syndromes to ketamine: a preliminary study. *Pain* 1994; 56: 51-7. [https://doi.org/10.1016/0304-3959\(94\)90149-X](https://doi.org/10.1016/0304-3959(94)90149-X)
- Bauman WA, Cardozo CP. Osteoporosis in individuals with spinal cord injury. *PM R* 2015; 7: 188-201. <https://doi.org/10.1016/j.pmrj.2014.08.948>
- Bell RF, Kalso EA. Ketamine for pain management. *Pain Rep* 2018; 3: e674. <https://doi.org/10.1097/PR9.0000000000000674>
- Bennett AD, Everhart AW, Hulsebosch CE. Intrathecal administration of an nmda or a non-nmda receptor antagonist reduces mechanical but not thermal allodynia in a rodent model of chronic central pain after spinal cord injury. *Brain Res* 2000; 859: 72-82. [https://doi.org/10.1016/S0006-8993\(99\)02483-X](https://doi.org/10.1016/S0006-8993(99)02483-X)
- Burton AW, Lee DH, Saab C, Chung JM. Preemptive intrathecal ketamine injection produces a long-lasting decrease in neuropathic pain behaviors in a rat model. *Reg Anesth Pain Med* 1999; 24: 208-13. <https://doi.org/10.1097/00115550-199924030-00005>
- Cheriyian T, Ryan DJ, Weinreb JH, Cheriyian J, Paul JC, Lafage V, et al. Spinal cord injury models: a

- review. *Spinal cord* 2014; 52: 588-95. <https://doi.org/10.1038/sc.2014.91>
- Domino EF. Taming the ketamine tiger. 1965. *Anesthesiology* 2010; 113: 678-84. <https://doi.org/10.1097/ALN.0b013e3181ed09a2>
- Doncheva ND, Vasileva L, Saracheva K, Dimitrova D, Getova D. Study of antinociceptive effect of ketamine in acute and neuropathic pain models in rats. *Adv Clin Exp Med* 2019; 28: 573-579. <https://doi.org/10.17219/acem/94143>
- Eide PK, Stubhaug A, Stenehjem AE. Central dysesthesia pain after traumatic spinal cord injury is dependent on n-methyl-d-aspartate receptor activation. *Neurosurgery* 1995; 37: 1080-7. <https://doi.org/10.1227/00006123-199512000-00007>
- Fakhri S, Dargahi L, Abbaszadeh F, Jorjani M. Astaxanthin attenuates neuroinflammation contributed to the neuropathic pain and motor dysfunction following compression spinal cord injury. *Brain Res Bull.* 2018; 143: 217-224. <https://doi.org/10.1016/j.brainresbull.2018.09.011>
- Fakhri S, Dargahi L, Abbaszadeh F, Jorjani M. Effects of astaxanthin on sensory-motor function in a compression model of spinal cord injury: involvement of erk and akt signalling pathway. *Eur J Pain* 2019; 23: 750-764. <https://doi.org/10.1002/ejp.1342>
- Gefen A. Tissue changes in patients following spinal cord injury and implications for wheelchair cushions and tissue loading: a literature review. *Ostomy Wound Manag* 2014; 60: 34-45.
- Hama A, Sagen J. Behavioral characterization and effect of clinical drugs in a rat model of pain following spinal cord compression. *Brain Res* 2007; 1185: 117-28. <https://doi.org/10.1016/j.brainres.2007.09.013>
- Hijazi Y, Bodonian C, Bolon M, Salord F, Bouliou R. Pharmacokinetics and haemodynamics of ketamine in intensive care patients with brain or spinal cord injury. *Br J Anaesth* 2003; 90: 155-60. <https://doi.org/10.1093/bja/aeg028>
- Himmelseher S, Durieux ME. Ketamine for perioperative pain management. *Anesthesiology* 2005; 102: 211-20. <https://doi.org/10.1097/0000542-200501000-00030>
- Himmelseher S, Pfenninger E, Georgieff M. The effects of ketamine-isomers on neuronal injury and regeneration in rat hippocampal neurons. *Anesth Analg* 1996; 83: 505-12. <https://doi.org/10.1213/0000539-199609000-00011>
- Kawasaki T, Ogata M, Kawasaki C, Ogata J, Inoue Y, Shigematsu A. Ketamine suppresses proinflammatory cytokine production in human whole blood in vitro. *Anesth Analg* 1999; 89: 665-9. <https://doi.org/10.1213/0000539-199909000-00024>
- Kim K, Mishina M, Kokubo R, Nakajima T, Morimoto D, Isu T, et al. Ketamine for acute neuropathic pain in patients with spinal cord injury. *J Clin Neurosci* 2013; 20: 804-7. <https://doi.org/10.1016/j.jocn.2012.07.009>
- Kose EA, Bakar B, Ayva SK, Kilinc K, Apan A. Neuroprotective effects of racemic ketamine and (s)-ketamine on spinal cord injury in rat. *injury* 2012; 43: 1124-30. <https://doi.org/10.1016/j.injury.2012.02.022>
- Kvarnström A, Karlsten R, Quiding H, Gordh T. The analgesic effect of intravenous ketamine and lidocaine on pain after spinal cord injury. *Acta Anaesthesiol Scand* 2004; 48: 498-506. <https://doi.org/10.1111/j.1399-6576.2003.00330.x>
- Leung A, Wallace MS, Ridgeway B, Yaksh T. Concentration-effect relationship of intravenous alfentanil and ketamine on peripheral neurosensory thresholds, allodynia and hyperalgesia of neuropathic pain. *Pain* 2001; 91: 177-87. [https://doi.org/10.1016/S0304-3959\(00\)00433-4](https://doi.org/10.1016/S0304-3959(00)00433-4)
- Loix S, De Kock M, Henin P. The anti-inflammatory effects of ketamine: state of the art. *Acta Anaesthesiol Belg* 2011; 62: 47-58.
- Mei X, Wang W, Wang W, Li Y, Zhang H, Wu S, et al. Inhibiting astrocytic activation: a novel analgesic mechanism of ketamine at the spinal level? *J Neurochem* 2009; 109: 1691-700. <https://doi.org/10.1111/j.1471-4159.2009.06087.x>
- Mei XP, Wang W, Wang W, Zhu C, Chen L, Zhang T, et al. Combining ketamine with astrocytic inhibitor as a potential analgesic strategy for neuropathic pain. Ketamine, astrocytic inhibitor and pain. *Mol Pain* 2010; 6: 50. <https://doi.org/10.1186/1744-8069-6-50>
- Mestre C, Pélissier T, Fialip J, Wilcox G, Eschalier A. A method to perform direct transcutaneous intrathecal injection in rats. *J Pharmacol Toxicol Methods* 1994; 32: 197-200. [https://doi.org/10.1016/1056-8719\(94\)90087-6](https://doi.org/10.1016/1056-8719(94)90087-6)
- Niesters M, Martini C, Dahan A. Ketamine for chronic pain: risks and benefits. *Br J Clin Pharmacol* 2014; 77: 357-67. <https://doi.org/10.1111/bcp.12094>
- Pomeroy IM, Jordan EK, Frank JA, Matthews PM, Esiri MM. Focal and diffuse cortical degenerative changes in a marmoset model of multiple sclerosis. *Mult Scler* 2010; 16: 537-48.

- <https://doi.org/10.1177/1352458509360362>
Radvansky BM, Puri S, Sifonios AN, Eloy JD, Le V. Ketamine-a narrative review of its uses in medicine. *Am J Ther* 2016; 23: e1414-e1426. <https://doi.org/10.1097/MJT.0000000000000257>
- Rigo FK, Trevisan G, Godoy MC, Rossato MF, Dalmolin GD, Silva MA, et al. Management of neuropathic chronic pain with methadone combined with ketamine: a randomized, double blind, active-controlled clinical trial. *Pain Physician* 2017; 20: 207-215.
- Samandari R, Hassanpour-Ezatti M, Fakhri S, Abbaszadeh F, Jorjani M. Sex differences and role of gonadal hormones on glutamate level after spinal cord injury in rats: A microdialysis study. *Basic Clin Neurosci* 2019; 10: 225-34. <https://doi.org/10.32598/bcn.9.10.260>
- Sawynok J, Zinger C. Topical amitriptyline and ketamine for post-herpetic neuralgia and other forms of neuropathic pain. *Expert Opin Pharmacother* 2016; 17: 601-9. <https://doi.org/10.1517/14656566.2016.1146691>
- Schwartzman RJ, Alexander GM, Grothusen JR, Paylor T, Reichenberger E, Perreault M. Outpatient intravenous ketamine for the treatment of complex regional pain syndrome: a double-blind placebo controlled study. *PAIN* 2009; 147: 107-15. <https://doi.org/10.1016/j.pain.2009.08.015>
- Shapira Y, Artru AA, Lam AM. Ketamine decreases cerebral infarct volume and improves neurological outcome following experimental head trauma in rats. *J Neurosurg Anesthesiol* 1992; 4: 231-40. <https://doi.org/10.1097/00008506-199210000-00001>
- Sheldon AL, Robinson MB. The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. *Neurochem Int* 2007; 51: 333-55. <https://doi.org/10.1016/j.neuint.2007.03.012>
- Siddall PJ, Middleton JW. Spinal cord injury-induced pain: mechanisms and treatments. *Pain Manag* 2015; 5: 493-507. <https://doi.org/10.2217/pmt.15.47>
- Sigtermans MJ, Van Hilten JJ, Bauer MC, Arbous MS, Marinus J, Sarton EY, et al. Ketamine produces effective and long-term pain relief in patients with complex regional pain syndrome type 1. *Pain* 2009; 145: 304-11. <https://doi.org/10.1016/j.pain.2009.06.023>
- Sleigh J, Harvey M, Voss L, Denny B. Ketamine-More mechanisms of action than just NMDA blockade. *Trends Anaesth Crit care* 2014; 4: 76-81. <https://doi.org/10.1016/j.tacc.2014.03.002>
- Tang SH, Yu JG, Li JJ, Sun JY. Neuroprotective effect of ketamine on acute spinal cord injury in rats. *Genet Mol Res* 2015; 14: 3551-6. <https://doi.org/10.4238/2015.April.17.4>
- Walker SM, Westin BD, Deumens R, Grafe M, Yaksh TL. Effects of intrathecal ketamine in the neonatal rat: evaluation of apoptosis and long-term functional outcome. *Anesthesiology* 2010; 113: 147-59. <https://doi.org/10.1097/ALN.0b013e3181dcd71c>
- Zhang Y, Bhavnani BR. Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (aif) and this process is inhibited by equine estrogens. *BMC Neurosci* 2006; 7: 49. <https://doi.org/10.1186/1471-2202-7-49>
- Zhou HY, Chen SR, Pan HL. Targeting n-methyl-d-aspartate receptors for treatment of neuropathic pain. *Expert Rev Clin Pharmacol* 2011; 4: 379-88. <https://doi.org/10.1586/ecp.11.17>