Treatment with harmine ameliorates functional impairment and neuronal death following traumatic brain injury

ZEQI ZHONG[1*], YUAN TAO[2*] and HUI YANG[1]

[1]Department of Neurosurgery, Xinqiao Hospital, The Third Military Medical University, Chongqing 400037;
[2]Department of Neurology, Research Institute of Field Surgery, Daping Hospital,
The Third Military Medical University, Chongqing 400042, P.R. China

Received October 21, 2014; Accepted July 17, 2015

DOI: 10.3892/mmr.2015.4437

Abstract. Traumatic brain injury (TBI) is a leading cause of mortality in young individuals, and results in motor and cognitive deficiency. Excitotoxicity is an important process during neuronal cell death, which is caused by excessive release of glutamate following TBI. Astrocytic glutamate transporters have a predominant role in maintaining extracellular glutamate concentrations below excitotoxic levels, and glutamate transporter 1 (GLT-1) may account for >90% of glutamate uptake in the brain. The β-carboline alkaloid harmine has been demonstrated to exert neuroprotective actions *in vivo*, and the beneficial effects were specifically due to elevation of GLT-1. However, whether harmine provides neuroprotection following TBI remains to be elucidated. The present study performed intraperitoneal harmine injections in rats (30 mg/kg per day for up to 5 days), in order to investigate whether harmine treatment attenuates brain edema and improves functional recovery in a rat model of TBI. The neuronal survival ratio and the protein expression of apoptosis-associated caspase 3 were also assessed in the hippocampus of the rat brain. Furthermore, the expression levels of GLT-1 and inflammatory cytokines were detected, in order to determine the underlying mechanisms. The results of the present study demonstrated that administration of harmine significantly attenuated cerebral edema, and improved learning and memory ability. In addition, harmine significantly increased the protein expression of GLT-1, and markedly attenuated the expression levels of interleukin-1β and tumor necrosis factor-α, thereby attenuating apoptotic neuronal death in the hippocampus. These results provided *in vivo* evidence that harmine may exert neuroprotective effects by synergistically reducing excitotoxicity and inflammation following TBI.

Correspondence to: Mr. Hui Yang, Department of Neurosurgery, Xinqiao Hospital, The Third Military Medical University, 183 Xinqiao Road, Chongqing 400037, P.R. China
E-mail: huiyang24k@163.com

*Contributed equally

Abbreviations: TBI, traumatic brain injury; GLT-1, glutamate transporter 1; NSS, neurologic severity score; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; NeuN, neuron-specific nuclear protein

Key words: traumatic brain injury, harmine, glutamate transporter 1, inflammatory, neuronal death, functional recovery

Introduction

Traumatic brain injury (TBI) is one of the leading causes of mortality and morbidity in adults and children worldwide, which results in immediate and delayed motor and cognitive deficiency (1). TBI-induced tissue loss and cell death occur following primary injury (direct physical disruption of the tissue) and secondary injury (delayed molecular pathophysiological changes) (2). Numerous pharmacological interventions are currently being investigated to attempt to reduce the severity of secondary injury following primary injury; however, current therapies are limited in their utility and efficacy (1).

Excitotoxicity is widely recognized as an important process in nervous cell death, produced by excessive release of glutamate following acute brain injury. In order to protect neurons from glutamate-mediated excitotoxicity, astrocytes transmit excessive glutamate from the extracellular space (3,4). Previous data confirmed that excitatory amino acid transporters are important in maintaining extracellular glutamate concentrations below excitotoxic levels (3). In total, five subtypes of sodium-dependent glutamate transporters have been identified: Glutamate aspartate transporter (GLAST; EAAT1), glutamate transporter-1 (GLT-1; EAAT2), EAAT3, EAAT4 and EAAT5 (5). Of these transporters GLT-1 has a principal role, and accounts for >90% of glutamate uptake in the brain (6). Previous studies have demonstrated that GLT-1 dysfunction is associated with the pathogenesis of multiple neurological disorders, including Alzheimer's disease, amyotrophic lateral sclerosis, stroke, several forms of epilepsy and TBI (7-11). Therefore, drugs and agents that increase glutamate transport activity may be significant in the treatment of neurological diseases.

Harmine is a β-carboline alkaloid, which was isolated >100 years ago (12). Harmine exerts a series of pharmacological actions, including antioxidative, antigenotoxic, antidepressant-like and antiplasmodial actions (13-16). Previously, harmine was observed to exert neuroprotective

actions in an *in vivo* investigation, and the beneficial effects were specifically due to the elevation of GLT-1 (17). However, no experiments have been performed to determine whether harmine provides neuroprotection following TBI.

In the present study, intraperitoneal (i.p) injection of harmine (30 mg/kg per day, for up to 5 days) into rats was performed in order to investigate whether harmine attenuates brain edema and improves functional recovery in a rat model of TBI. Furthermore, to determine whether the mechanism underlying the neuroprotective effects of harmine are associated with glutamate transport and inflammatory factors, the expression levels of GLT-1 and inflammatory cytokines were examined in the hippocampus of the rat brain. These investigations aimed to investigate a potential novel therapeutic modality for the treatment of TBI.

Materials and methods

Animals and a controlled TBI model. All experimental procedures were approved by The Third Military Medical University Committee (Chongqing, China) for the use and care of animals in research, and were performed in accordance with the The Third Military Medical University's guidelines for the care and use of laboratory animals. A total of 150 male Sprague-Dawley rats (age, 10-12 weeks; weighing, 280-320 g; obtained from The Third Military Medical University Animal Center) were used in the present study. All the rats had free access to food and water, and were housed under a standard 12-h light/dark cycle. The housing temperature was 25-28˚ and the rats were housed in groups.

A previously described TBI procedure (18) was performed in the present study. Briefly, the rats were anesthetized with sodium pentobarbital [i.p, 50 mg/kg; Bio-Rad Laboratories (Shanghai) Co., Ltd., Shanghai, China], and a 5-mm craniotomy was performed over the left parietal cortex, which was centered on the coronal suture [Bio-Rad Laboratories (Shanghai) Co., Ltd.] and 3 mm lateral to the sagittal suture. The TBI was performed using a pneumatic piston [Bio-Rad Laboratories (Shanghai) Co., Ltd.] with a rounded metal tip (2.5 mm diameter), which was angled 22.5˚ to vertical to ensure that the tip was perpendicular with the brain surface at the center of the craniotomy. A velocity of 4 m/s and a deformation depth of 2 mm below the dura were used to generate the TBI in the procedure. The bone flap was immediately replaced and sealed, and the scalp was closed using sutures. Body temperature was monitored throughout the surgery, to ensure it was maintained at 37.0±0.5˚C. Whilst the rats were recovering from anesthesia, they were placed in a heated cage to maintain body temperature.

Group and drug administration. The rats were randomly divided into three groups: Sham-operated group (sham; n=15); the TBI group (TBI; n=35) and the TBI + harmine (Beijing Aoboxing Biotechnology Co., Ltd., Beijing, China)-treated group (Harmine; n=35). Harmine was administered immediately following TBI (i.p, 30 mg/kg per day) for up to 5 days. The sham and TBI groups received equal volumes of 0.9% saline solution (i.p.).

Measurement of brain edema. Brain edema was evaluated by analyzing the brain water content, as described previously (19). Following anesthetization, as described above, and sacrifice by exsanguination, the rat brains were separated and weighed immediately using a chemical balance, in order to obtain the wet weight (WW). Following drying in a desiccating oven for 24 h at 100˚C, the dry tissues were weighed to obtain the dry weight (DW). The water percentage in the brain was calculated according to the following formula Brain water % = (WW - DW) / WW) x 100.

Behavioral recovery. The neurobehavioral status of the rats was evaluated using a set of 10 tasks, collectively termed the neurologic severity score (NSS) (20), which assesses reflexes, alertness, coordination and motor abilities. A score of one is awarded for failure to perform a particular task; therefore, a score of 10 reflects maximal impairment, whereas a normal rat scores 0. The rats were grouped as follows for examination of behavioral recovery: Sham, n=3; TBI, n=7; and Harmine, n=7. Following TBI, the NSS was evaluated at 1, 3 and 5 days. Each rat was assessed by an observer who was blinded to the animal treatment. The difference between the initial NSS and that at any later time-point was calculated for each rat, and this value (ΔNSS) reflects the spontaneous or treatment-induced recovery of motor function. Furthermore, the spatial learning ability of the rats was assessed using a Morris water maze, as previously described (21). Briefly, the Morris water maze consisted of a black circular pool (diameter, 180 cm; height, 45 cm) filled with water (depth, 30 cm; temperature, 26˚C) and divided into four equal quadrants: North (N), west (W), south (S) and east (E). An escape platform (diameter, 12 cm; height, 28 cm, made opaque with paint and submerged 2 cm) was placed in the center of one of the quadrants, equidistant from the sidewall and the center of the pool. Rats were trained to locate the platform prior to TBI or undergoing the sham surgery. For each trial, a rat was randomly placed into a quadrant start point (N, S, E or W) facing the wall of the pool and was allowed ≤60 sec to escape to the platform. Rats that failed to escape within 90 sec were placed on the platform for ≤20 sec and returned to the cage for a new trial (intertrial interval, 20 sec). The maze performance was recorded using a video camera suspended above the maze and interfaced with a video tracking system (HVS Imaging; Hampton, UK). The mean escape latency of a total of five trials was calculated. This assessment was performed 1, 3 and 5 days following TBI or sham surgery.

Hematoxylin and eosin (H&E) staining. At 24 h post-surgery, the rats (sham, n=3; TBI, n=7; and Harmine, n=7) were anesthetized, as described above, and perfused intracardially with isotonic sodium chloride solution, followed by 4% (w/v) paraformaldehyde [Bio-Rad Laboratories (Shanghai) Co., Ltd.] in 0.1 M sodium phosphate buffer [pH 7.4; Bio-Rad Laboratories (Shanghai) Co., Ltd.]. The rats were sacrificed via exsanguination. The brains were subsequently removed and fixed for 48 h in 4% (w/v) paraformaldehyde. Following fixation, the brains were embedded in paraffin [Bio-Rad Laboratories (Shanghai) Co., Ltd.], sliced into 4 μm coronal sections at the bregma, and stained with H&E [Bio-Rad Laboratories (Shanghai) Co., Ltd.]. The surviving and dying neurons in the CA1 area per 1 mm were subsequently quantified under an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan).

Immunofluorescence. At 24 h post-TBI, coronal sections were incubated with 10% normal donkey serum [Bio-Rad Laboratories (Shanghai) Co., Ltd. for 30 min at room temperature in phosphate-buffered saline (PBS) containing 0.1% Triton X-100 [Bio-Rad Laboratories (Shanghai) Co., Ltd.]. The sections were then incubated at 4˚C overnight with the following primary antibodies: Goat anti-rat neuron-specific nuclear protein [NeuN; 1:200 (cat. no. sc-31154); Santa Cruz Biotechnology, Inc., Dallas, TX, USA] and rabbit anti-rat caspase 3 [1:50; (cat. no. sc-7148); Santa Cruz Biotechnology, Inc.]. The sections were then washed with PBS four times at room temperature, followed by an incubation with appropriate fluorescent-labeled secondary antibodies [1:200 (cat. nos. sc-2342 and sc-2341) Santa Cruz Biotechnology, Inc.] for 1 h at room temperature. The sections were incubated with DAPI (1 ng/Nl; Beijing Aoboxing Biotechnology Co., Ltd.) to counterstain the nucleus. Subsequently, the sections were washed with PBS and mounted using water-based mounting medium containing anti-fading agents (Biomeda; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Confocal images were captured using a laser scanning confocal microscope (Olympus FV1000; Olympus Corporation, Tokyo, Japan) and digital imaging software (FV10-ASW 1.5 Viewer; Olympus Corporation).

Western blot analysis. The rats (sham, n=3; TBI, n=7; and Harmine, n=7) were anesthetized and underwent intracardiac perfusion with 0.1 mol/l PBS (pH 7.4). The cortex region of the brain was rapidly isolated and the brain tissues were homogenized using a homogenizer, total proteins were extracted using protein extraction reagent [both Bio-Rad Laboratories (Shanghai) Co., Ltd.] and protein concentration was determined using a bicinchoninic acid reagent (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) method. The protein samples (5 mg) were separated by 20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis [Bio-Rad Laboratories (Shanghai) Co., Ltd.] and were subsequently transferred onto polyvinylidene fluoride membranes (Roche Diagnostics Deutschland GmbH, Mannheim, Germany). The blots were then blocked with 5% fat-free dry milk for 1 h at room temperature. Following blocking, the membranes were incubated with the following primary antibodies overnight at 4˚C: Rabbit anti-GLT-1 polyclonal antibody (cat. no. sc-15317), rabbit anti-interleukin (IL)-1β polyclonal antibody (cat. no. sc-7884), rabbit anti-tumor necrosis factor (TNF)-α polyclonal antibody (cat. no. sc-7895), rabbit anti-caspase 3 polyclonal antibody (cat. no. sc-7148) and mouse anti-β-actin monoclonal antibody [(cat. no. sc-7210) all 1:500; Santa Cruz Biotechnology, Inc.). The membranes were then incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG; cat. no. sc-2027) and anti-mouse IgG (cat. no. sc-2025) for 2 h at room temperature (1:5,000; Santa Cruz Biotechnology, Inc.). Following incubation with the secondary antibody, the immunoblots were then visualized following development with an enhanced chemiluminescence detection system [Bio-Rad Laboratories (Shanghai) Co., Ltd.], and densitometric signals were quantified using an enhanced chemiluminescence detection system [Bio-Rad Laboratories (Shanghai) Co., Ltd.]. The western blotting results were analyzed using ImageJ 1.41 software (National Institutes of Health, Bethesda, MD, USA).

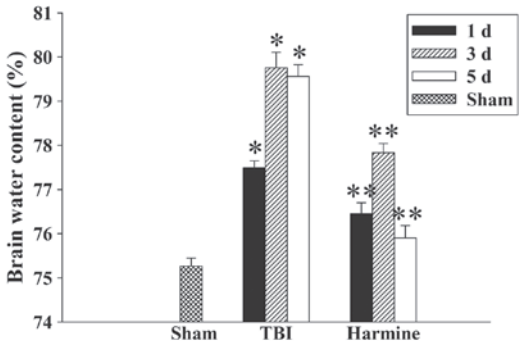

Figure 1. Effects of harmine on brain edema. Brain water content was determined after 1, 3 and 5 days (d). Data are presented as the mean ± standard error of the mean (n=5/group). Brain water content was markedly increased 1, 3 and 5 days following TBI. Administration of harmine significantly decreased brain edema, reflected by a decrease in brain water content. *P<0.01, vs. the sham group;**P<0.05, vs. the TBI group. TBI, traumatic brain injury.

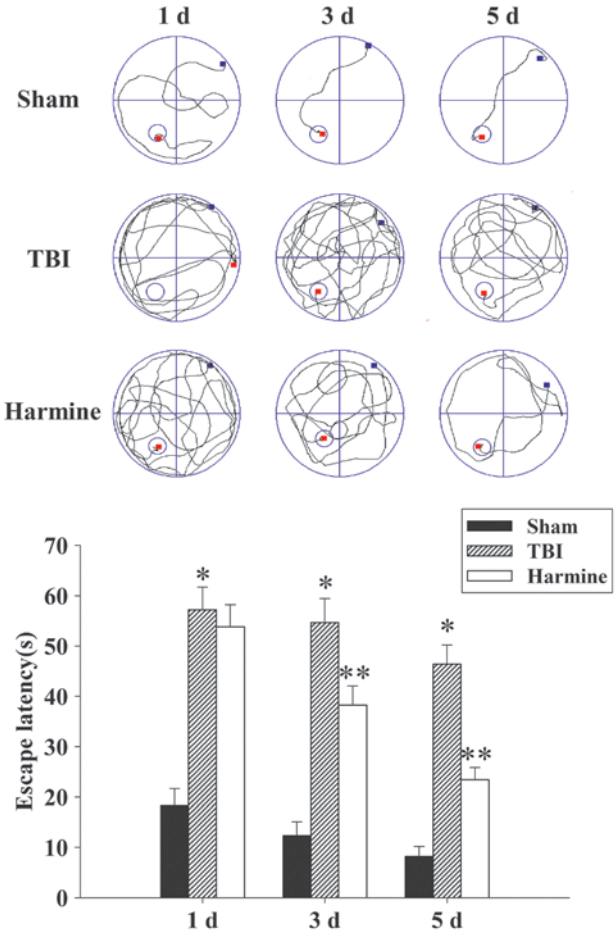

Figure 2. Effects of harmine on escape latency performance at 1, 3 and 5 days (d). The lines represent the movement locus of rats; red dots represent the starting point and the blue dots represent the final point. Data are presented as the mean ± standard error of the mean (n=5/group). The escape latency was markedly increased 1, 3 and 5 days following TBI, whereas treatment with harmine significantly reduced the time taken to identify the platform at 3 and 5 days. *P<0.01, vs. the sham group;**P<0.05, vs. the TBI group. TBI, traumatic brain injury.

Statistical analysis. Data are expressed as the mean ± standard error of the mean. SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. Statistical analysis

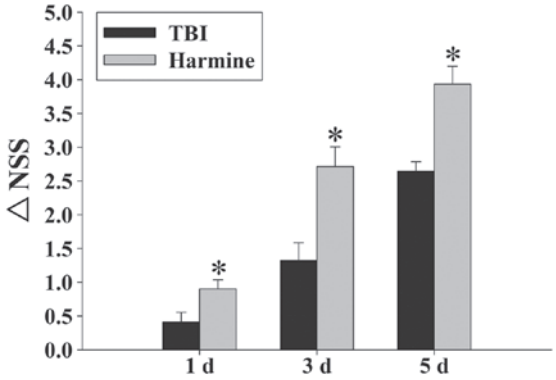

Figure 3. Effects of harmine on TBI-induced motor deficits. The temporal changes in motor recovery of the rats were determined at 1, 3 and 5 days (d) following TBI and calculated as the NSS. Data are presented as the mean ± standard error of the mean (n=5/group). Administration of harmine significantly improved motor function 1, 3 and 5 days following TBI, as reflected by an increase of ΔNSS. *P<0.01, vs. the TBI group. TBI, traumatic brain injury; NSS, neurologic severity score.

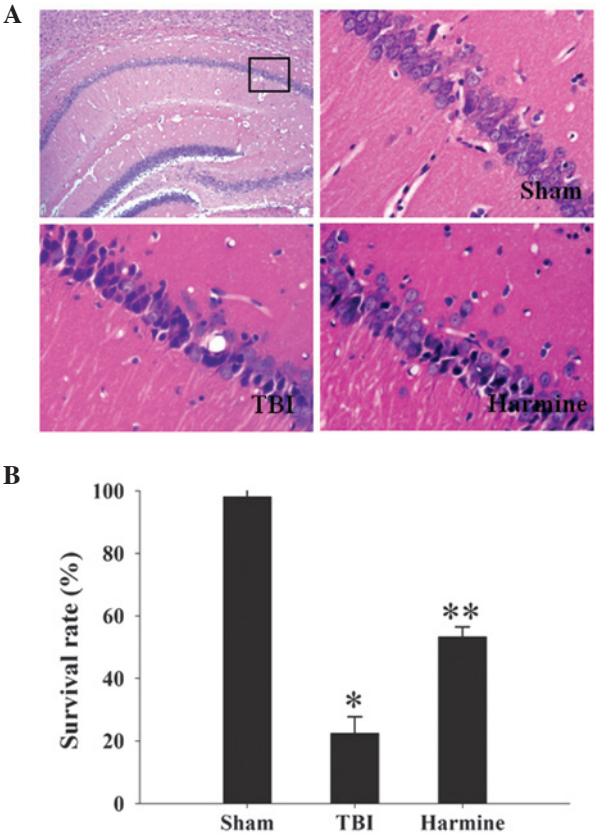

Figure 4. Effects of harmine on the survival rates of neurons in the hippocampal region at 24 h. (A) Representative hematoxylin and eosin staining of hippocampal neurons from the sham, TBI and harmine groups (magnification, x400). (B) Percentage of surviving neurons in the sham, TBI and harmine groups. Data are presented as the mean ± standard error of the mean (n=5/group). TBI resulted in significant neuronal loss, whereas neuronal survival rate was significantly increased in the harmine-treated group. *P<0.01, vs. the sham group; **P<0.01, vs. the TBI group. TBI, traumatic brain injury.

was performed using one-way analysis of variance followed by the Student-Newman-Keuls post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

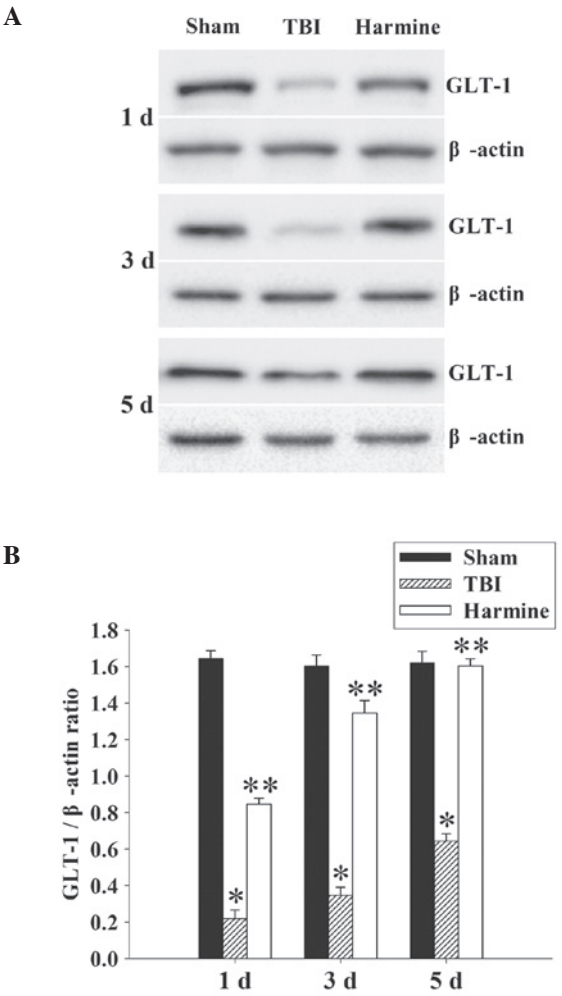

Figure 5. Protein expression of GLT-1. (A) Western blot analysis was used to detect the protein expression levels of GLT-1 in the hippocampus at 1, 3 and 5 days (d). (B) Quantitative results of GLT-1 are presented as the ratio of densitometries of GLT-1 to β-actin. Data are presented as the mean ± standard error of the mean (n=5/group). There was a significant reduction in the expression of GLT-1 in the TBI group at 1, 3 and 5 days. Harmine treatment resulted in a significant elevation of the protein expression of GLT-1 at 1, 3 and 5 days. *P<0.01, vs. the sham group; **P<0.05, vs. TBI group. GLT-1, glutamate transporter 1; TBI, traumatic brain injury.

Results

Treatment with harmine attenuates cerebral edema. The wet-dry weight method was used to evaluate brain edema. As shown in Fig. 1, brain water content was significantly increased in the TBI group, compared with the sham group at 1, 3 and 5 days following trauma. Treatment with harmine significantly reduced the tissue water content at 1, 3 and 5 days, a compared with the TBI group.

Treatment with harmine attenuates TBI-induced learning and spatial memory dysfunction. Since treatment with harmine successfully attenuated brain edema, the present study examined whether harmine also improved spatial learning function, which was assessed using a Morris water maze at 1, 3 and 5 days post-TBI or sham surgery. As shown in Fig. 2, TBI resulted in a significant spatial learning deficit at 1, 3 and 5 days, compared with the sham group, and harmine treatment

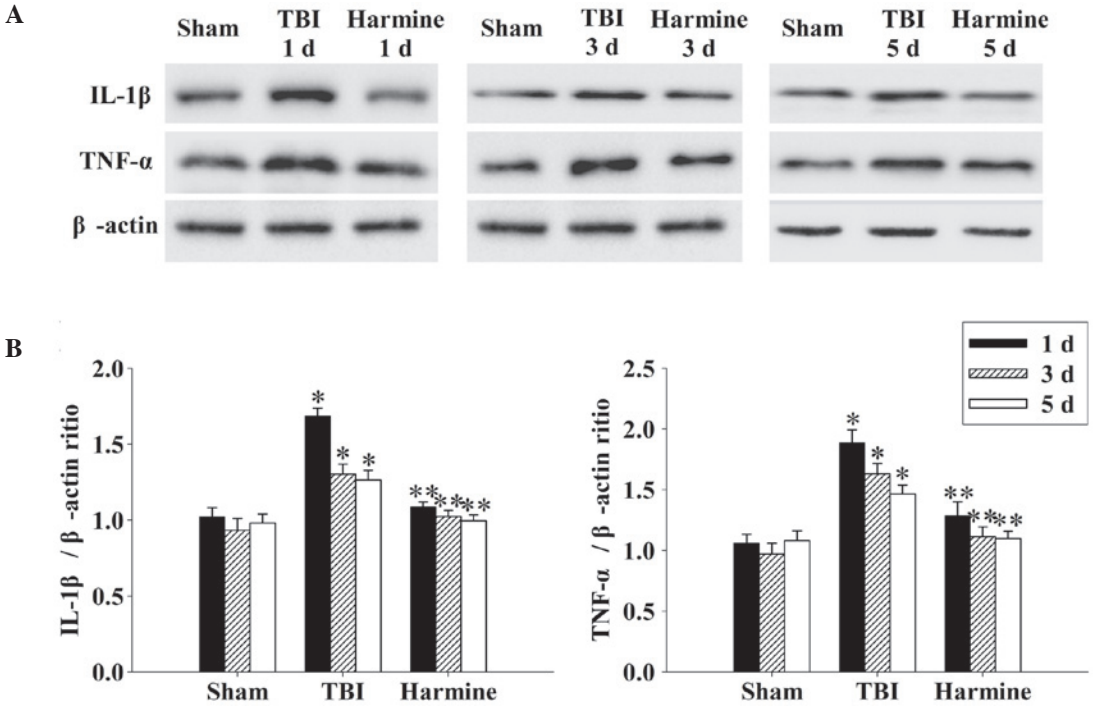

Figure 6. Protein expression levels of IL-1β and TNF-α. (A) Western blot analysis was used to detect the protein expression levels of IL-1β and TNF-α in the hippocampus at 1, 3 and 5 days (d). (B) Quantitative results of IL-1β and TNF-α are presented as the ratio of densitometries of IL-1β and TNF-α to β-actin. Data are presented as the mean ± standard error of the mean (n=5/group). A significant increase in the expression levels of IL-1β and TNF-α were detected in the TBI group at 1, 3 and 5 days, whereas treatment with harmine resulted in a significant downregulation of the expression levels of IL-1β and TNF-α at 1, 3 and 5 days. *P<0.05, vs. the sham group; **P<0.05, vs the TBI group. TBI, traumatic brain injury; IL, interleukin; TNF, tumor necrosis factor.

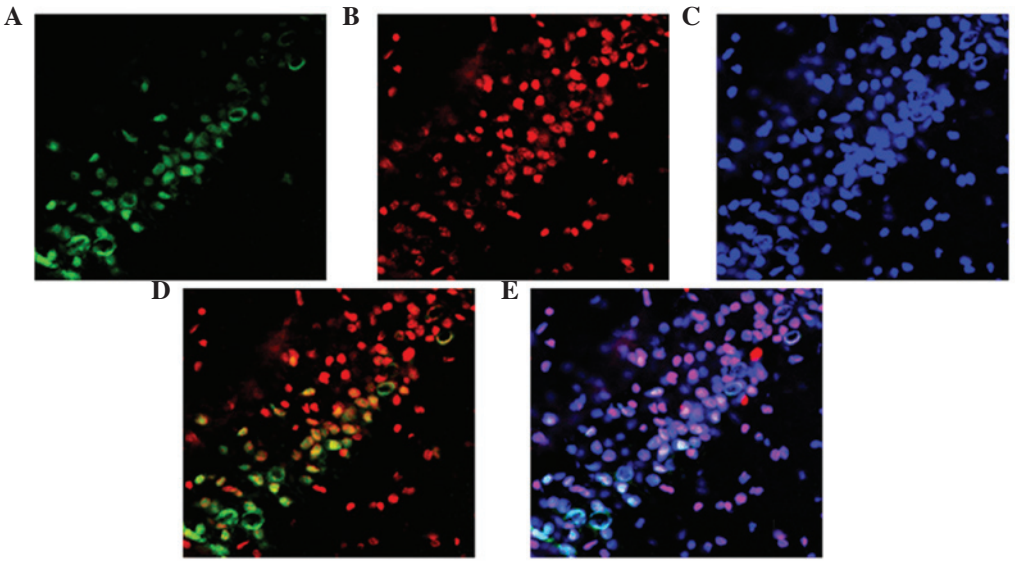

Figure 7. Co-localization of NeuN and caspase 3 24 h post-TBI was determined using immunofluorescent staining (magnification, x400). Cell nuclei were counterstained with DAPI. (A) Caspase 3; (B) NeuN; (C) DAPI; (D) A and B merge; (E) A, B and C merge. NeuN, neuron-specific nuclear protein; TBI, traumatic brain injury.

significantly reduced the escape latency at 3 and 5 days, compared with the TBI group.

Treatment with harmine attenuates TBI-induced motor deficits. Temporal variations in the functional recovery of the TBI rats are demonstrated in Fig. 3 and expressed as ΔNSS. Post-TBI administration of harmine significantly improved the motor function recovery of the rats at 1, 3 and 5 days following TBI, compared with the TBI group without harmine treatment.

Treatment with harmine suppresses neuronal death in the hippocampal region following TBI. Neuronal survival was assessed at 24 h using H&E staining. Morphologically, the nuclei of the normal neurons were round and poorly stained, whereas the nuclei of the dying neurons were pyknotic and

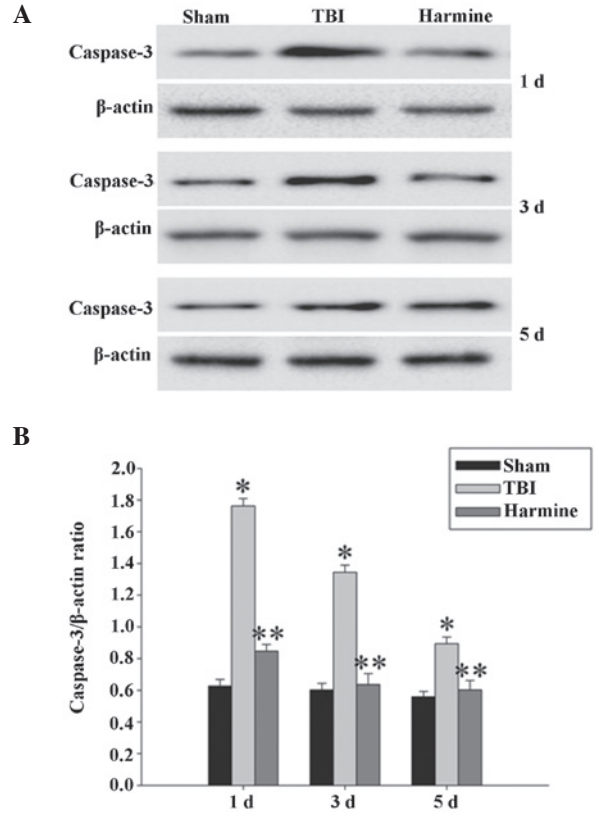

Figure 8. Protein expression levels of caspase 3 and β-actin. (A) Western blot analysis was used to detect the protein expression levels of caspase 3 and β-actin in the hippocampus at 1, 3 and 5 days (d) following injury. (B) Densitometry of caspase 3 relative to β-actin. Data are presented as the mean ± standard error of the mean (n=5/group). Expression of caspase 3 was significantly increased in the TBI group at 1, 3 and 5 days, whereas administration of harmine significantly decreased the protein expression of caspase 3. *P<0.01, vs. the sham group; **P<0.05, vs. the TBI group. TBI, traumatic brain injury.

darkly stained (Fig. 4A). As shown in Fig. 4B, compared with the sham group, TBI resulted in a marked decrease in the survival rate of the neurons. However, the neuronal survival rate in the harmine-treated group was significantly increased, compared with the TBI group.

Treatment with harmine increases the expression of GLT-1 in the hippocampus following TBI. The protein expression levels of GLT-1 in the hippocampus were analyzed using western blot analysis. As shown in Fig. 5, at 1, 3 and 5 days post-surgery, there was a significant downregulation in the expression of GLT-1 in the TBI group, compared with the sham group. Administration of harmine resulted in marked elevation in the expression of GLT-1, compared with the TBI group.

Treatment with harmine attenuates the expression of inflammatory cytokines in the hippocampus following TBI. The expression levels of IL-1β and TNF-α were detected in the hippocampus 1, 3 and 5 days following TBI. As shown in Fig. 6, the expression levels of the inflammatory cytokines in the sham group were low; however, the expression levels of IL-1β and TNF-α were markedly elevated at each of the time-points in the TBI group. Furthermore, TBI-induced expression of IL-1β and TNF-α was significantly reduced following administration of harmine.

Caspase 3 and NeuN are co-localized in the hippocampus following TBI. The co-localization of NeuN and caspase 3 was detected using immunofluorescent staining 24 h post-TBI. As shown in Fig. 7, the majority of TBI-induced apoptosis occurred in the neurons.

Treatment with harmine reduces the expression of caspase 3 in the hippocampus following TBI. The expression of caspase 3 in the hippocampus were detected using western blot analysis. As shown in Fig. 8, at 1, 3 and 5 days post-TBI, the expression of caspase 3 was significantly increased in the TBI group, compared with the sham group. However, the administration of harmine significantly reduced the expression of caspase 3, compared with the TBI group.

Discussion

The present study demonstrated that administration of harmine (30 mg/kg per day) for up to 5 days immediately following TBI had neuroprotective potential in the rats. Treatment with harmine attenuated post-traumatic cerebral edema, and improved learning and memory abilities. Furthermore, the present study investigated the mechanisms underlying the protective effects of harmine on TBI. At the molecular level, the protein expression levels of GLT-1 and inflammatory cytokines in the hippocampus of the rat brain were detected using western blot analysis. Treatment with harmine resulted in a marked elevation in the expression of GLT-1, as well as significant reductions in the expression levels of IL-1β and TNF-α, suggesting that harmine attenuated apoptotic neuronal death in the hippocampus. Previous studies have reported that treatment with harmine significantly attenuate glutamate-induced and oxidative stress-induced neuronal death in neuronal cell cultures (15,17). In addition, a previous *in vivo* study demonstrated that harmine exerts neuroprotective effects against glutamate-mediated excitotoxicity in a rat model of amyotrophic lateral sclerosis disease (17). These findings, together with the findings of the present study, improve current understanding of harmine-mediated neuroprotection in neurological disorders.

Previous *in vivo* studies have demonstrated that GLT-1 dysfunction causes acute and chronic brain injury, and that glutamate-mediated excitotoxicity is considered to be an important process in neurological disorders (22,23). The accumulation of excess extracellular glutamate and subsequent overstimulation of glutamatergic receptors increases the production of reactive and excitotoxic oxygen/nitrogen species, which may induce oxidative stress leading to neuronal death (24). Previous studies have reported that elevated activity and expression of GLT-1 induces demonstrated in rat models of various acute and chronic neurologic diseases (7-11), including a rat model of TBI, in which the overexpression of GLT-1 was observed to significantly reduce glutamate overflow, decrease cell death and improve behavioral recovery (20). The results of the present study confirmed that treatment with harmine markedly elevated the expression of GLT-1 in the hippocampus following trauma. These findings may demonstrate one of the predominant mechanisms by which harmine exerts its neuroprotective effects in TBI.

Treatment with harmine not only protects neurons against glutamate insult via elevating the expression level of GLT-1, but it also led to the exertion of anti-inflammatory effects following TBI. Previous studies have demonstrated that inflammatory cytokines can directly and indirectly attenuate the expression of glutamate transporters in astrocytes, which has a significant role in TBI-induced glutamate excitotoxicity, and it has been reported that IL-1β and TNF-α regulate glutamate transmission directly through elevation of the expression of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (25-27). In the present study, the expression levels of inflammatory cytokines in the hippocampus of the rat brain were suppressed following treatment with harmine. Although the mechanism underlying the neuroprotective effects of harmine on TBI remain to be fully elucidated, the data of the present study suggested that attenuation of inflammation may synergistically reduce extracellular glutamate. This suggests that the recruitment of attenuated inflammation occurs a physiological action, which acts synergically with the GLT-1 response to protect neurons from TBI-induced insult and death, and further investigation of this is required.

The present study performed only preliminarily examination of the neuroprotective effects induced by harmine on TBI rats; therefore, further investigations are required to establish the appropriate routes of administration, the exact time-frame, and whether these effects are observed following different injury magnitudes.

The results of the present study demonstrated that administration of harmine significantly attenuated cerebral edema, and improved learning and memory abilities following experimental TBI. Furthermore, treatment with harmine markedly elevated the expression of GLT-1 and significantly attenuated the expression levels of IL-1β and TNF-α, thereby attenuating apoptotic neuronal death in the hippocampus. These findings indicated that administration of harmine (30 mg/kg per day, up to 5 days) following TBI can be neuroprotective, via anti-inflammatory and anti-excitotoxicity effects following experimental TBI in rats. These findings may represent a potential novel therapeutic modality for the treatment of TBI.

References

1. Wu X, Hu J, Zhuo L, Fu C, Hui G, Wang Y, Yang W, Teng L, Lu S and Xu G: Epidemiology of traumatic brain injury in eastern China, 2004: A prospective large case study. J Trauma 64: 1313-1319, 2008.
2. Werner C and Engelhard K: Pathophysiology of traumatic brain injury. Br J Anaesth 99: 4-9, 2007.
3. Johnston MV: Excitotoxicity in perinatal brain injury. Brain Pathol 15: 234-240, 2005.
4. Palmer AM, Marion DW, Botscheller ML, Swedlow PE, Styren SD and DeKosky ST: Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. J Neurochem 61: 2015-2024, 1993.
5. Amara SG and Fontana AC: Excitatory amino acid transporters: Keeping up with glutamate. Neurochem Int 41: 313-318, 2002.
6. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, *et al*: Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. Science 276: 1699-1702, 1997.
7. Lauderback CM, Hackett JM, Huang FF, Keller JN, Szweda LI, Markesbery WR and Butterfield DA: The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: The role of Abeta1-42. J Neurochem 78: 413-416, 2001.
8. Rothstein JD, Van Kammen M, Levey AI, Martin LJ and Kuncl RW: Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 38: 73-84, 1995.
9. Rao VL, Dogan A, Todd KG, Bowen KK, Kim BT, Rothstein JD and Dempsey RJ: Antisense knockdown of the glial glutamate transporter GLT-1, but not the neuronal glutamate transporter EAAC1, exacerbates transient focal cerebral ischemia-induced neuronal damage in rat brain. J Neurosci 21: 1876-1883, 2001.
10. Samuelsson C, Kumlien E, Flink R, Lindholm D and Ronne-Engström E: Decreased cortical levels of astrocytic glutamate transport protein GLT-1 in a rat model of posttraumatic epilepsy. Neurosci Lett 289: 185-188, 2000.
11. Rao VL, Dogan A, Bowen KK, Todd KG and Dempsey RJ: Antisense knockdown of the glial glutamate transporter GLT-1 exacerbates hippocampal neuronal damage following traumatic injury to rat brain. Eur J Neurosci 13: 119-128, 2001.
12. Cao R, Peng W, Wang Z and Xu A: beta-Carboline alkaloids: Biochemical and pharmacological functions. Curr Med Chem 14: 479-500, 2007.
13. Réus GZ, Stringari RB, de Souza B, Petronilho F, Dal-Pizzol F, Hallak JE, Zuardi AW, Crippa JA and Quevedo J: Harmine and imipramine promote antioxidant activities in prefrontal cortex and hippocampus. Oxid Med Cell Longev 3: 325-331, 2010.
14. Moura DJ, Richter MF, Boeira JM, Pêgas Henriques JA and Saffi J: Antioxidant properties of beta-carboline alkaloids are related to their antimutagenic and antigenotoxic activities. Mutagenesis 22: 293-302, 2007.
15. Fortunato JJ, Réus GZ, Kirsch TR, Stringari RB, Fries GR, Kapczinski F, Hallak JE, Zuardi AW, Crippa JA and Quevedo J: Effects of beta-carboline harmine on behavioral and physiological parameters observed in the chronic mild stress model: Further evidence of antidepressant properties. Brain Res Bull 81: 491-496, 2010.
16. Astulla A, Zaima K, Matsuno Y, Hirasawa Y, Ekasari W, Widyawaruyanti A, Zaini NC and Morita H: Alkaloids from the seeds of *Peganum harmala* showing antiplasmodial and vasorelaxant activities. J Nat Med 62: 470-472, 2008.
17. Li Y, Sattler R, Yang EJ, Nunes A, Ayukawa Y, Akhtar S, Ji G, Zhang PW and Rothstein JD: Harmine, a natural beta-carboline alkaloid, upregulates astroglial glutamate transporter expression. Neuropharmacology 60: 1168-1175, 2011.
18. Chen SF, Hsu CW, Huang WH and Wang JY: Post-injury baicalein improves histological and functional outcomes and reduces inflammatory cytokines after experimental traumatic brain injury. Br J Pharmacol 155: 1279-1296, 2008.
19. Täuber MG, Khayam-Bashi H and Sande MA: Effects of ampicillin and corticosteroids on brain water content, cerebrospinal fluid pressure, and cerebrospinal fluid lactate levels in experimental pneumococcal meningitis. J Infect Dis 151: 528-534, 1985.
20. Lu D, Sanberg PR, Mahmood A, Li Y, Wang L, Sanchez-Ramos J and Chopp M: Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. Cell Transplant 11: 275-281, 2002.
21. Cui C, Cui Y, Gao J, Sun L, Wang Y, Wang K, Li R, Tian Y, Song S and Cui J: Neuroprotective effect of ceftriaxone in a rat model of traumatic brain injury. Neurol Sci 35: 695-700, 2014.
22. Namura S, Maeno H, Takami S, Jiang XF, Kamichi S, Wada K and Nagata I: Inhibition of glial glutamate transporter GLT-1 augments brain edema after transient focal cerebral ischemia in mice. Neurosci Lett 324: 117-120, 2002.
23. Han F, Shioda N, Moriguchi S, Qin ZH and Fukunaga K: Downregulation of glutamate transporters is associated with elevation in extracellular glutamate concentration following rat microsphere embolism. Neurosci Lett 430: 275-280, 2008.
24. Rauen T and Kanner BI: Localization of the glutamate transporter GLT-1 in rat and macaque monkey retinae. Neurosci Lett 169: 137-140, 1994.
25. Chu K, Lee ST, Sinn DI, Ko SY, Kim EH, Kim JM, Kim SJ, Park DK, Jung KH, Song EC, *et al*: Pharmacological induction of ischemic tolerance by glutamate transporter-1 (EAAT2) upregulation. Stroke 38: 177-182, 2007.
26. Pickering M, Cumiskey D and O'Connor JJ: Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. Exp Physiol 90: 663-670, 2005.
27. Lai AY, Swayze RD, El-Husseini A and Song C: Interleukin-1 beta modulates AMPA receptor expression and phosphorylation in hippocampal neurons. J Neuroimmunol 175: 97-106, 2006.