TAURINE ATTENUATES HIPPOCAMPAL AND CORPUS CALLOSUM DAMAGE, AND ENHANCES NEUROLOGICAL RECOVERY AFTER CLOSED HEAD INJURY IN RATS

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Abstract—The protective effects of taurine against closed head injury (CHI) have been reported. This study was designed to investigate whether taurine reduced white matter damage and hippocampal neuronal death through suppressing calpain activation after CHI in rats. Taurine (50 mg/kg) was administered intravenously 30 min and 4 h again after CHI. It was found that taurine lessened the corpus callosum damage, attenuated the neuronal cell death in hippocampal CA1 and CA3 subfields and improved the neurological functions 7 days after CHI. Moreover, it suppressed the over-activation of calpain, enhanced the levels of calpastatin, and reduced the degradation of neurofilament heavy protein, myelin basic protein and βII-spectrin in traumatic tissue 24 h after CHI. These data confirm the protective effects of taurine against gray and white matter damage due to CHI, and suggest that down-regulating calpain activation could be one of the protective mechanisms of taurine against CHI. © 2014 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: taurine, closed head injury, calpain, corpus callosum, hippocampus.

INTRODUCTION

Traumatic brain injury (TBI) can result in neurological impairment because of immediate tissue disruption in the central nervous system (primary injury), but, additionally, surviving cells may be secondarily damaged by complex mechanisms triggered by the primary event, leading to further damage and disability. The critical mechanisms of secondary injury after brain trauma include inflammation, oxidative stress, ionic imbalance, increased vascular permeability, mitochondrial dysfunction, and excitotoxic damage. This combination of cellular and physiologic disturbances causes increased neuronal cell death, lesion enlargement, and the impairment of neurological behavior, motor, and cognition (Saatman et al., 2010; Sande and West, 2010; McAllister, 2011).

Taurine (2-aminoethanesulfonic acid) is the major intracellular free β-amino acid present in most mammalian tissues. It is not involved in primary metabolism, and neither is incorporated into proteins. It possesses a number of cytoprotective properties through its actions as a neurotransmitter, neuromodulator, osmoregulator, modulator of intracellular calcium homeostasis, antioxidant, membrane stabilizer, and anti-inflammatory factor, and is reported to protect against a variety of pathological conditions including hypoxia, glutamate-induced neurotoxicity, and inflammation (Huxtable, 1992; Schuller-Levis and Park, 2004; Louzada et al., 2004). Under cell-damaging conditions, the release of taurine is increased; meanwhile, the uptake of taurine is inhibited. The increase in the extracellular levels of taurine in cell-damaging conditions may be an important endogenous protective mechanism (Saransaari and Oja, 2000). These reports suggest that taurine may act as an endogenous neuroprotectant to block multiple targets of detrimental cascade after TBI.

Marmarou’s weight drop model is one of the most frequently used constrained rodent models of acceleration closed head injury (CHI) as it is inexpensive and easy to perform, although the biomechanics of the impact produced by this model is not fully and strictly controlled. Although this model cannot produce supratentorial focal brain lesion, it induces widespread damage of the neurons, axons, dendrites, and microvasculature. It is noteworthy that this model causes massive diffuse axonal injury, particularly in the corpus callosum, internal capsule, optic tracts, cerebral and cerebellar peduncles, and the long tracts in the...
Experimental procedures

Ethical statement

The current study was reviewed and approved by Animal Ethics Committee of Beijing Neurosurgical Institute.

CHI

The experimental designs and all procedures were in accordance with the ARRIVE Guidelines (NC3Rs Reporting Guidelines Working Group, 2010). Every effort was made to minimize the number of animals used and their suffering. Male adult Sprague-Dawley rats (3 months old, specific pathogen free, weighing 290–330 g, Beijing Vital River experimental animals Technology Ltd., Beijing, China) were kept under controlled light conditions with a 12-h/12-h light/dark cycle. Food and water were provided ad libitum. All the animal experiments were performed in SPF laboratory. With the rats under chloral hydrate anesthesia (400 mg/kg, i.p.), experimental CHI was induced using a weight drop device described previously (Sun et al., 2014). Briefly, the skull of the rat was exposed by a longitudinal incision of the skin. A metal disk of 0.45 cm in diameter and 2 mm in thickness was firmly fixed by quick adhesive to the right skull vault of the rat 1 mm lateral to the midline, just in front of the coronal suture. The rat was placed on a foam bed in the prone position right under a 25-cm-tall Plexiglas tube. A 200-g weight inside the tube was allowed to precisely strike the disk cemented to the skull face. The foam bed together with the rat was then moved away from underneath the tube immediately after the impact to insure a single hit. The rat was placed on the operating table for close observation to determine whether the skull vault was fractured. The scalp was then sutured and the rat was allowed to recover from anesthesia. Rats that died on impact and those with skull fractures were excluded. In sham-operated rats, the surgical procedure was prepared for impact in the same way as above, but the animals were not subjected to head trauma. Rectal temperature was continuously monitored and maintained at 37 ± 0.5 °C by a negative-feedback-controlled heating pad during the whole experiment.

Experimental protocols

Referring to the doses of taurine used in experimental TBI and stroke, 50 mg/kg of taurine was used in this study (Sun and Xu, 2008; Sun et al., 2014). According to the Latin square design, rats were randomly allocated to three groups treated with taurine or vehicle: (1) Taurine (Nanjing Pharmaceutical Factory Co., Ltd., Jiangsu, China), 50 mg/kg; (2) vehicle, normal saline (2 ml/kg), and (3) sham, normal saline (2 ml/kg). Taurine was administered intravenously twice, in a volume of 2 ml/kg, 30 min and again 4 h after induction of CHI. Traumatic control and sham animals were given vehicle (normal saline). Neurological Severity Score (NSS) was evaluated at 24 h, 48 h, and 7 days after CHI (n = 10 per group). The activities of calpain were determined, and the levels of calpastatin, neurofilament heavy protein (NF-H), myelin basic protein (MBP) and βII-spectrin were measured by Western blot analysis on the same animals as the neurological scoring. The histopathology of corpus callosum and hippocampus was observed 7 days after CHI (n = 10 per group).

Neurobehavioral evaluation

In all animals, a battery of neurobehavioral tests was performed before CHI and at 24 h, 48 h, and 7 days after CHI by an investigator blinded to the experimental groups. Neurological function was measured in terms of the NSS, an 18-point scale that assesses functional neurological status based on the presence of certain reflexes and the ability to perform motor and behavioral tasks such as beam walking, beam balance, and spontaneous locomotion (Chen et al., 2001). All the animals were subjected to neurological scoring, and histology was done on the same animals as the neurological scoring. The NSS results of animals for calpain assay and Western blot analysis were recorded but not presented in Fig. 1.

Sample collection and preparation

The tissues of right hemisphere were dissected according to the experimental protocols at 4 °C, and samples were prepared as described previously (Sun et al., 2011).

![Fig. 1. Effects of taurine on the neurological deficits after closed head injury. Vehicle or taurine was administered by intravenous injection over 1 min, twice 30 min and again 4 h after induction of closed head injury. Data were presented as scatterplots, with bar as the median. n = 10. *P < 0.001 vs. sham-operated rats. **P < 0.01 and ***P < 0.01 vs. vehicle-treated rats.](image)
Briefly, the tissue was homogenized in five volumes of homogenization buffer (20 mM HEPES, 1.5 mM MgCl₂, 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 250 mM sucrose, 1 mM dithiothreitol, and 10 μg/ml of each of aprotinin, and leupeptin, pH 7.5). Sample was centrifuged at 750 × g at 4 °C for 15 min to separate the sample into supernatant and pellet, and the pellet was discarded. The supernatant was further centrifuged at 16,000 × g at 4 °C for 20 min, and the separated supernatant was used as the cytosolic fraction. The protein concentrations of cytosolic fractions were determined by method of Bradford (Bradford, 1976).

**Calpain spectrophotometric assay**

Calpain activity was estimated by the spectrophotometric assay that uses azocasein as the substrate for endogenous calpain, as described previously (Moss et al., 1991; Yoshida et al., 1993). The endogenous calpain activity in tissue homogenates was obtained by measuring the amount of azo chromophore released into solution after the addition of azocasein and calcium. Briefly, a 20-μl aliquot of the cytosolic fraction was added to 200 μl of assay buffer (100 mM HEPES, 0.02% β-mercaptoethanol, 10 mM KCl, pH 7.5). Next, 30 μl of an azocasein (Sigma–Aldrich Co., St. Louis, MO, USA) stock solution (20 mg/ml) was added, and the assay was initiated by adding 30 μl of 10 mM CaCl₂. The samples were incubated at 37 °C for 2 h and were placed on ice before adding 130 μl of 20% trichloroacetic acid. The samples were maintained at ~20 °C for 5 min and then at 4 °C for 15 min. The samples were centrifuged for 10 min at 16,000 × g, and the 130-μl supernatant was placed in a separate tube to which 130 μl of 1 N NaOH was added to maximize absorbance of the azo chromophore. The absorbance of the supernatant at 440 nm was determined and compared with that of supernatants from homogenates that were not incubated with calcium. The result was expressed as absorption value/h/mg protein.

**Western blot analysis**

The tissues were collected and the cytosolic fractions were prepared by the methods used in the section of calpain assay. Calpastatin, NF-H, MBP and αⅣ-spectrin were determined from cytosolic fraction separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Forty-microgram proteins were separated by SDS–PAGE, and the proteins on the gel were transferred onto a nitrocellulose membrane. The membrane was then probed with antibody reactive with calpastatin (1:1000; Chemicon International Inc., Temecula, CA, USA), NF-H (1:800; Chemicon), MBP (1:500; Chemicon), or αⅣ-spectrin (1:500; Chemicon) at 4 °C overnight, and subsequently incubated with alkaline phosphatase-conjugated secondary antibody (1:5000; Chemicon) for 2 h at room temperature. The color reaction was observed by incubation of membrane with Nitroblue tetrazolium/5-Bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) (Chemicon), and the integrated optical densities (IODs) of the protein bands were analyzed by Western blot analysis combined with gel image analyzer (AlphalmagerTM 2200, Alpha Innotech Co., San Leandro, CA, USA). β-Actin (1:2000; Abcam Inc., Cambridge, MA, UK) was used as internal control, and the IODs of the protein bands were normalized to β-actin immunoreactivity.

**Histopathological examination**

Animals were anesthetized with chloral hydrate, and transcardially perfused with heparinized normal saline followed by 4% paraformaldehyde 7 days after CHI. Brains were removed, fixed, embedded in paraffin, and the 8-μm-thick coronal sections through the hippocampus were collected. Hematoxylin eosin (HE) staining was performed following the procedures described in our previous paper (Sun et al., 2009). The sections were examined with light microscopy and pictures were taken with a digital camera. Quantification of neurons in the CA1 and CA3 subfields of dorsal hippocampus was performed in two adjacent HE-stained coronal sections of the dorsal hippocampus for each animal. Clearly defined pyramidal neurons (cell body and nucleus) in the CA1 and CA3 hippocampus were counted in two high-power fields (HPFs), and hippocampal neuronal survival in CA1 and CA3 subfields was expressed as neurons per HPF.

For the study of the white matter injury, the coronal sections (8-μm thickness) through the hippocampus were stained with Luxol fast blue–periodic acid Schiff (LFB–PAS) and Bielschowsky’s silver impregnation (Pantoni et al., 1996; Cheng et al., 1998). The LFB–PAS and Bielschowsky’s silver stains were used to measure optical densities (ODs) of myelin (LFB–PAS) and axons (Bielschowsky’s stain) in the corpus callosum. The measured OD values reflect the stainability of white matter, and a decreased OD value indirectly reflects destruction of white matter because of loss of stainability. For Bielschowsky’s silver stain, slices were rinsed in distilled water after deparaffination, and then transferred to a 20% solution of silver nitrate for 30 min at 37 °C. The slices were washed with distilled water, differentiated in 10% formaldehyde. After rinsing in distilled water, slices were stained by ammoniacal silver solution for 30 s. After washing in distilled water, slices were incubated with 0.1% gold chloride for 3 min and immersed in 5% sodium thiosulfate in distilled water. Finally, slices were rinsed, dehydrated, cleared, mounted, and stored in 4 °C for a few days until evaluation and photo acquisition. For LFB–PAS stain, paraffin-embedded 8-μm-thick slices were rinsed in distilled water after deparaffination, and then transferred through 95% ethanol to a 0.1% solution of Luxol fast blue (LFB; Sigma–Aldrich Co., St. Louis, MO, USA) in 95% ethanol and 0.05% acetic acid. After staining for 16 h at 60 °C, slices were washed with distilled water, differentiated in 0.05% aqueous lithium carbonate followed by 70% ethanol. After rinsing in distilled water, slices were oxidized in 0.5% periodic acid and then stained in 0.5% Schiff’s solution for 15 min. Slices were finally counterstained with hematoyxlin. The slices stained with Bielschowsky’s silver and LFB–PAS–hematoxylin were examined with light microscopy and pictures were taken with a digital camera, and the average ODs of corpus callosum were measured using the image analysis program (Beijing Konghai Co., Beijing, China).
Statistical analysis

Data from neurological score are presented as scatterplots, with bar as the median. Other data are presented as mean ± SEM. Comparisons between groups were statistically evaluated by a one-way analysis of variance (ANOVA) with a post hoc Least Significant Difference (LSD) test (the activities of calpain and the levels of calpastatin, NF-H, MBP and αII-spectrin, the neuronal numbers in CA1 and CA3 subfields, and the average optical density of Bielschowsky’s silver stain and LFB–PAS–hematoxylin stain). NSS was analyzed with a nonparametric Mann–Whitney U test. A probability of <0.05 was considered statistically significant.

RESULTS

Effects of taurine on the neurological score

Total 60 rats were used in this study. Three of the 60 rats were skull fracture, and the six other rats died before sample collection. They were excluded from the study. Before induction of CHI, all animals showed no significant neurological deficits. The vehicle-treated rats showed significant neurological deficits at 24 h, 48 h and 7 days after CHI versus sham-operated rats (all \( P < 0.001 \)). Treatment with taurine markedly reduced the NSS at 24 h, 48 h and 7 days after CHI versus sham-operated rats (all \( P < 0.05 \)). The NSS of taurine-treated rats was still markedly impaired compared to sham-operated rats at 24 h, 48 h and 7 days after CHI.

Effects of taurine on calpain activities

The results are shown in Fig. 2. The calpain activities in traumatic brain tissue in vehicle-treated rats increased significantly (\( P < 0.05 \)) versus sham-operated rats. Treatment with taurine markedly reduced the activities of calpain in traumatic brain tissue (\( P < 0.05 \)) versus vehicle-treated rats.

Effects of taurine on calpastatin

Calpastatin is well-known as an endogenous calpain inhibitor. The results of taurine on the protein levels of calpastatin in traumatic brain tissue are illustrated in Fig. 3. The protein levels of calpastatin in traumatic brain tissue 24 h after CHI in vehicle-treated rats decreased significantly (\( P < 0.01 \)) versus sham-operated rats. Treatment with taurine markedly enhanced the calpastatin protein levels in traumatic brain tissue 24 h after CHI (\( P < 0.01 \)) versus vehicle-treated rats.

Effects of taurine on the levels of MBP

MBP is a major constituent of the myelin sheath in the nervous system, which is a marker of demyelination due to neurological diseases (Massaro et al., 1990; Bartosik-Psujek and Stelmasiak, 2001). We analyzed the effects of taurine on the levels of MBP in traumatic brain tissue after CHI, and the results are shown in Fig. 4. Compared with sham-operated rats, the levels of MBP in traumatic brain tissues in vehicle-treated rats were lessened significantly (\( P < 0.01 \)). Taurine treatment markedly enhanced the levels of MBP compared with vehicle-treated rats (\( P < 0.05 \)).

Effects of taurine on the levels of NF-H

Neurofilament protein consists of three subunits termed neurofilament light protein (68 kDa), neurofilament medium protein (160 kDa), and NF-H (200 kDa) that assembled to build filaments. They are substrates of calpain (Schlaepfer et al., 1985; Maxwell et al., 1997).
The levels of NF-H were determined in traumatic brain tissue after CHI, and the results are shown in Fig. 5. The levels of NF-H were reduced significantly in vehicle-treated rats versus sham-operated rats ($P < 0.01$). Treatment with taurine markedly enhanced the levels of NF-H after CHI ($P < 0.05$ vs. vehicle-treated rats).

**Effects of taurine on the degradation of αII-spectrin**

Endogenous αII-spectrin (240 kDa), a well-characterized calpain substrate, can be cleaved into 150 and 145-kDa fragments (Wang, 2000), and 145-kDa αII-spectrin breakdown fragment is indicative of the activation of calpain. As illustrated in Fig. 6, the levels of αII-spectrin in traumatic brain tissue following CHI were decreased significantly ($P < 0.01$ vs. sham-operated rats), and the levels of 145-kDa fragment of αII-spectrin were increased ($P < 0.01$). Treatment with taurine markedly enhanced the levels of αII-spectrin, and reduced the levels of 145-kDa fragment of αII-spectrin in traumatic brain tissue ($P < 0.05$ vs. vehicle-treated rats, respectively). These data indicated the inhibition of αII-spectrin degradation and calpain activation by taurine after experimental CHI, which was consistent with the result of calpain spectrophotometric assay.

**Effects of taurine on corpus callosum damage**

LFB–PAS–hematoxylin and Bielschowsky’s silver stain were used to investigate the morphology of corpus callosum after CHI. Representative photomicrographs chosen from the right corpus callosum are shown in Fig. 7. The myelin sheaths and axons were moderately damaged. Myelin sheaths lost their LFB–PAS stainability and appeared as empty spaces (vacuoles) separating myelin sheaths in the lesion areas of white matter. Axons appeared as irregular, twisted profiles and showed segmental fragmentation with Bielschowsky’s stain. Moreover, increased cellular reactions occurred in the injured corpus callosum. Taurine treatment decreased the damage of myelin sheaths and axons after CHI. Overall statistical analysis demonstrated that the axonal injury and damage to the myelin sheath in the corpus callosum in vehicle-treated rats ($P < 0.001$ and $0.01$ vs. sham-operated rats, respectively), and taurine markedly reduced the axonal injury and damage to the myelin sheath in the corpus callosum ($P < 0.01$ and $0.05$ vs. vehicle-treated rats, respectively).

**Effects of taurine on and neuronal death in the CA1 and CA3 hippocampus**

HE staining was used to investigate the morphology of dead cells in hippocampal CA1 and CA3 subfields after CHI; the results are illustrated in Fig. 8. CA1 and CA3 neurons in sham-operated rats were normal, with a clearly rounded appearance and intact well-defined membranes, a clear nucleus, distinct nucleoli, and a clear cytoplasm. In vehicle-treated rats, some neurons showed shrunken and distorted shape, pyknosis and dark staining, and the number of normal neurons in the hippocampal CA1 and CA3 subfields were significantly decreased versus sham-operated rats (Fig. 8; both $P < 0.001$ vs. sham-operated rats). Treatment with
taurine markedly enhanced the numbers of normal neurons in CA1 and CA3 subfields (Fig. 8; both $P < 0.01$ vs. vehicle-treated rats).

**DISCUSSION**

We have reported the dose-dependent protection of taurine against CHI in rats (Sun et al., 2014). The main purpose of this study is to investigate whether taurine protects brain against white and gray matter damage through suppressing the over-activation of calpain during CHI, as calpain is located in both neuronal bodies and axonal processes (Hamakubo et al., 1986; Perlmutter et al., 1990), and a growing body of literature has emerged, demonstrating that calpain plays a key role in neuropathologic events following TBI (Saatman et al., 2010). This study reports that treatment with taurine suppresses the over-activation of calpain, enhances the levels of calpastatin, reduces the degradation of NF-H, MBP, and $\alpha$II-spectrin, lessens the corpus callosum damage, improves the neurologic function, and attenuates the neuronal death in hippocampal CA1 and CA3 subfields after experimental CHI. These data confirm the neuroprotection of taurine against CHI again, and suggest that blocking calpain-mediated white and gray matter damage may be one mechanism of taurine against CHI.

It is believed that intracellular calcium overload is induced through the opening of ionic channels gated by receptors and voltage, the release of calcium from intracellular calcium storage pools, and other mechanisms during TBI. Subsequently, it initiates a series of intracellular events that impact the development of tissue damage profoundly, such as activation of proteolytic enzymes and endonucleases, production of reactive oxygen species, release of excitatory amino acids (EAAs), and apoptosis (Saatman et al., 2010; Weber, 2012). Calpains, one family of cysteine proteases, are activated by calcium and autolytic processing, and regulated reversibly by calcium and calpastatin, an endogenous inhibitor of calpain. The physiological functions of calpains are not yet fully appreciated, but transient calpain activation is involved in essential functions such as cell signaling transduction, gene transcription, synaptic plasticity, and protein turnover (Goll et al., 2003; Wu and Lynch, 2006). Calpain is activated during TBI, which has been confirmed through investigating the proteolysis of substrates and the protection of calpain inhibitors (Saatman et al., 2010; McAllister, 2011). Moreover, some reports have shown the involvement of calpain in the damage of white matter after TBI through causing the degradation of substrates such as neurofilament, spectrin and MBP (Liu et al., 2006; McGinn et al., 2009; Saatman et al., 2010; Reeves et al., 2010).

A number of reports have demonstrated the regulation of taurine on intracellular calcium homeostasis through: (1) suppressing EAA-induced excitotoxicity through acting upon nerve terminals to reduce the release of EAAs (Kamisaki et al., 1996), and acting upon GABA-A receptors to enhance chloride currents (El Idrissi and Trenkner, 2004), as membrane depolarization due to EAAs is contributed to the calcium influx through opening the ionic channels (Arundine and Tymianski, 2003; Salinska et al., 2005); (2) improving mitochondrial function and modulating mitochondrial calcium homeostasis, by which, reducing the release of calcium from intracellular calcium storage pools and enhancing the sequestration of mitochondrial calcium (Palmi et al., 1999; Foos and Wu, 2002; El Idrissi and Trenkner, 2003); (3) inhibiting the reversal of sodium/calcium exchangers (Foos and Wu, 2002); and (4) affecting the calcium influx through voltage-gated calcium channels (Huxtable, 1992). Therefore, it is logical that taurine may block the calpain-mediated neuronal cell death and white matter damage through suppressing the intracellular calcium overload during TBI, since the increases in intracellular calcium can directly activate calpain (Goll et al., 2003). This hypothesis has been demonstrated by our
data that taurine down-regulates calpain activation, although we do not directly investigate the effects of taurine on intracellular calcium in this study.

αII-spectrin is a major structural component of the cortical membrane cytoskeleton located in axons, presynaptic terminals and cell bodies. It is reported to crosslink with the cytoskeleton surface of neuronal membrane, synaptic vesicles and cytoskeletal elements, where it is thought to form a latticework involved in the maintenance of cell shape, membrane structure stability, neurite extension, integrity of myelinated axons, and axonal transport (Goodman and Zagon, 1986; Pinder and Baines, 2000; Voas et al., 2007). The degradation of αII-spectrin mediated by calpain leads to the formation of 150- and 145-kDa fragments (Wang, 2000). Neurofilament is present in perikarya and dendrites, particularly abundant in axons. As a skeletal protein and as a facilitator of axonal transport, neurofilament plays a critical role in neuronal function, including the radial growth of axons during development, the maintenance of axon caliber and neuronal structure, and the velocity of nerve conduction (Lee and Cleveland, 1994; Maxwell et al., 1997). MBP is the major protein component in myelin sheath that encases axons (Massaro et al., 1990; Bartosik-Psujek and Stelmasiak, 2001). It contains clusters of positively charged amino acid residues, suggesting that MBP could interact with negatively charged lipids and stabilize the major dense line of myelin in the central nervous system.
by which, facilitating myelin sheath compaction, and maintaining myelin integrity (Stys, 1998; Richter-Landsberg, 2000; Harauz et al., 2004). The cleavage of αII-spectrin, NF-H and MBP is shown during TBI (McCracken et al., 1999; Posmantur et al., 2000; Liu et al., 2006; McGinn et al., 2009; Reeves et al., 2010). Our results suggest that CHI-induced activation of calpain could promote degradation of αII-spectrin, NF-H and MBP, leading to disruption of structural integrity and stability of neurons and myelin sheath, initiation of demyelination, disturbances of axonal transport, neuronal and axonal dysfunction, and neuronal cell death. Taurine inhibition of CHI-induced activation of calpain could protect the brain against CHI through suppressing degradation of αII-spectrin and MBP due to calpain over-activation, reducing damage of the corpus callosum and neuronal death in the hippocampus, and promoting recovery of neurological function.

Calpastatin is an endogenous calpain inhibitor. It has been shown that calpastatin can be degraded by calpain and caspase-3 during apoptosis or cerebral hypoxia–ischemia (Wang et al., 1998; Blomgren et al., 1999), and an increase in expression of calpastatin is neuroprotective to several neurological diseases, such as cerebral ischemia and TBI (Rami et al., 2003; Schoch et al., 2012). On the other hand, taurine is an endogenous neuroprotectant (Wu et al., 1994; Saransaari and Oja, 2000), and it reduces traumatic brain
damage, as shown in the present study. Therefore, we are particularly interested in the effects of taurine on calpastatin during CHI. Our study reveals that taurine treatment increases calpastatin protein levels in traumatic tissue 24 h after CHI, suggesting that exogenous administration of taurine could strengthen the endogenous protective mechanism of calpastatin, which is supported by the study that the expression of calpastatin mRNA is induced by TBI in rats (Ringger et al., 2004), and over-expression of calpastatin protects the brain against TBI (Schoch et al., 2012). The mechanism by which taurine increases calpastatin actions is unclear, which might be related to the up-regulation of gene expression by taurine (Park et al., 2006), or down-regulation of calpain and caspase-3 activation by taurine, as showed in this study and our previous study (Sun and Xu, 2008). Further study is needed to determine the mechanism by which taurine up-regulates calpastatin actions during CHI.

CONCLUSIONS
This study reports that taurine suppresses the activation of calpain, enhances the levels of calpastatin, lessens the degradation of αII-spectrin, NF-H and MBP, attenuates corpus callosum damage and neuronal cell death in the hippocampus, and promotes the neurological recovery after CHI. These results confirm the protective effects of taurine against white matter and gray matter damage due to CHI, and suggest that suppressing calpain over-activation may be one of the mechanisms of taurine against CHI.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have no conflict of interest.

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