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ORIGINAL ARTICLE

# Anti-inflammatory mechanism of taurine against ischemic stroke is related to down-regulation of PARP and NF-κB

**Ming Sun · Yumei Zhao · Yi Gu · Chao Xu**

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**Abstract** Taurine is reported to reduce tissue damage induced by inflammation and to protect the brain against experimental stroke. The objective of this study was to investigate whether taurine reduced ischemic brain damage through suppressing inflammation related to poly (ADP-ribose) polymerase (PARP) and nuclear factor-kappaB (NF-κB) in a rat model of stroke. Rats received 2 h ischemia by intraluminal filament and were then reperfused. Taurine (50 mg/kg) was administered intravenously 1 h after ischemia. Treatment with taurine markedly reduced neurological deficits, lessened brain swelling, attenuated cell death, and decreased the infarct volume 72 h after ischemia. Our data showed the up-regulation of PARP and NF-κB p65 in cytosolic fractions in the core and nuclear fractions in the penumbra and core, and the increases in the nuclear poly (ADP-ribose) levels and the decreases in the intracellular $NAD^+$ levels in the penumbra and core at 22 h of reperfusion; these changes were reversed by taurine. Moreover, taurine significantly reduced the levels of tumor necrosis factor-α, interleukin-1β, inducible nitric oxide synthase, and intracellular adhesion molecule-1, lessened the activities of myeloperoxidase and attenuated the infiltration of neutrophils in the penumbra and core at 22 h of reperfusion. These data demonstrate that suppressing the inflammatory reaction related to PARP and NF-κB-driven expression of inflammatory mediators may be one mechanism of taurine against ischemic stroke.

**Keywords** Experimental stroke · Taurine · PARP · NF-κB · Inflammation

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**Abbreviations**

| | |
|---|---|
| DTT | Dithiothreitol |
| EDTA | Ethylenediaminetetraacetic acid |
| EGTA | Ethyleneglycol bis(2-aminoethyl ether)tetraacetic acid |
| HE staining | Hematoxylin and eosin staining |
| HEPES | *N*-2-Hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid |
| HOCl | Hypochlorous acid |
| IκB | Inhibitory κB |
| ICAM-1 | Intracellular adhesion molecule-1 |
| IL-1β | Interleukin-1β |
| iNOS | Inducible nitric oxide synthase |
| MCAo | Middle cerebral artery occlusion |
| MPO | Myeloperoxidase |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NBT/BCIP | Nitroblue tetrazolium/5-bromo-4-chloro-3-inoloyl-phosphate |
| NF-κB | Nuclear factor-kappaB |
| PAR | Poly (ADP-ribose) |
| PARP | Poly (ADP-ribose) polymerase |
| PMSF | Phenylmethanesulfonyl fluoride |
| ROS | Reactive oxygen species |
| SDS-PAGE | Sodium dodecyl sulfate-polyacrylamide gel electrophoresis |
| Tau-NHCl | Taurine monochloramine |
| Tau-$NCl_2$ | Taurine dichloramine |
| TNF-α | Tumor necrosis factor-α |
| TTC | 2,3,5-Triphenyltetrazolium chlorides |

## Introduction

The progression and extent of brain injury due to experimental stroke are related to several reperfusion mechanisms,

many of which involve post-injury inflammatory response elements. These inflammatory mediators include the rapid activation of resident microglial cells and the infiltration of neutrophils and macrophages into the injured parenchyma. Accompanying the early responses of neutrophil and microglia is a significant accumulation of other inflammatory elements such as cytokines, adhesion molecules, and chemokines (Wang et al. 2007; Jordán et al. 2008; Tuttolomondo et al. 2008; Amantea et al. 2009). Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-1$\beta$ (IL-1$\beta$) are two pleiotropic cytokines with many pro-inflammatory properties. They are expressed by a variety of cell types and play potentially noxious roles during experimental stroke. Intracellular adhesion molecule-1 (ICAM-1), required for neutrophil adhesion and infiltration, is induced in endothelial cells and neutrophils after cerebral ischemia. It is pro-inflammatory in cerebral ischemia, contributing to the no-reflow phenomenon and releasing cytotoxic mediators. Inducible nitric oxide synthase (iNOS) is expressed in inflammatory and vascular cells, and over-production of nitric oxide due to iNOS is detrimental after brain ischemia. These inflammatory elements exacerbate cerebral ischemic injury, which has been demonstrated by the significant neuroprotection observed after inhibition of neutrophil or cytokine actions.

Nuclear factor-kappaB (NF-$\kappa$B), a pivotal transcription factor, is essential for immune and stress responses within brain. It is composed of subunits p65 and p50. In the dormant state, NF-$\kappa$B exists in the cytoplasm as a complex with its inhibitory protein, inhibitory $\kappa$B (I$\kappa$B). When cells are stimulated, I$\kappa$B is phosphorylated, ubiquitinylated, and digested by proteasome, leading to a release of active NF-$\kappa$B. Active NF-$\kappa$B is translocated into the nucleus and stimulates transcriptional activation of potentially deleterious pro-inflammatory genes, such as TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1 (Kumar et al. 2004; Ridder and Schwaninger 2009). In rodents, activation of NF-$\kappa$B occurs after experimental stroke, and inhibiting the NF-$\kappa$B signaling pathway due to pharmacological and genetic approaches has been reported to be neuroprotective in the model of experimental stroke (Schneider et al. 1999; Williams et al. 2006; Ridder and Schwaninger 2009; Wang et al. 2009).

Poly (ADP-ribose) polymerase (PARP) is a nuclear enzyme, which catalyzes the formation of poly (ADP-ribose) (PAR) through transferring ADP-ribose units from nicotinamide adenine dinucleotide ($NAD^+$) to a variety of nuclear proteins under genotoxicity (Virág and Szabó 2002; Moroni 2008). It is involved in DNA repair in response to moderate DNA damage. However, over-activation of PARP due to severe DNA damage depletes $NAD^+$ and ATP stores, eventually leading to necrotic cell death. Recently, PARP is reported to participate in the regulation of gene expression through affecting transcription factors; particularly, increasing evidences highlight the central role of PARP in the regulation of NF-$\kappa$B-driven gene expression (Hassa and Hottiger 2002; Virág and Szabó 2002). It is reported that NF-$\kappa$B-driven transcription of proinflammatory cytokines is reduced in PARP knockout animals and after the administration of PARP inhibitors (Eliasson et al. 1997; Oliver et al. 1999; Ha et al. 2002; Chiarugi and Moskowitz 2003; Koh et al. 2004; Haddad et al. 2006).

Taurine, a major intracellular free $\beta$-amino acid present in leukocytes (Fukuda et al. 1982; Huxtable 1992), is reported to protect against tissue damage in a variety of models that share inflammation as a common pathogenic feature (Schuller-Levis and Park 2003, 2004). One possibility is that taurine can react with hypochlorous acid (HOCl) generated by the myeloperoxidase (MPO) pathway to produce the more stable and less toxic taurine monochloramine (Tau-NHCl). Tau-NHCl is a powerful regulator of inflammation. Specifically, it has been reported to down-regulate the production of proinflammatory mediators in inflammatory cells, such as TNF-$\alpha$, IL-1$\beta$, ICAM-1, and iNOS (Schuller-Levis and Park 2003, 2004). In addition, taurine can protect against a variety of pathological conditions including hypoxia, neurotoxicity, oxidative stress, and cardiomyocyte ischemia (Schurr et al. 1987; Hagar 2004; Takatani et al. 2004; El Idrissi 2008). Recently, we had reported on the dose-dependent neuroprotection of taurine against experimental stroke, and taurine at 5–50 mg/kg had significant protection (Sun and Xu 2008). Therefore, we hypothesized that taurine could reduce ischemic brain injury through suppressing the inflammatory reaction after experimental stroke. This study was designed to evaluate the effects of taurine at a dose of 50 mg/kg on the activation and expression of PARP and NF-$\kappa$B, the levels of TNF-$\alpha$, IL-1$\beta$, ICAM-1, and iNOS, and the infiltration of neutrophils in the penumbra and core in a rat model of ischemic stroke.

## Materials and methods

### Rat model of focal cerebral ischemia

All animal procedures were in accordance with the Guidelines for Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee. Under chloral hydrate anesthesia (400 mg/kg, i.p.), male adult Sprague–Dawley rats (weighing 315–340 g, Beijing Vital River Experimental Animals Technology Ltd.) were subjected to middle cerebral artery occlusion (MCAo) using an intraluminal filament as described previously (Sun et al. 2009). Reperfusion was accomplished

by withdrawing the filament 2 h after MCAo. Sham-operated animals were subjected to the same surgical procedure without MCAo.

### Experimental protocols

For evaluating the effects of taurine on neurological deficits, brain swelling, neutrophil infiltration, and infarct volume, rats were randomly assigned to two groups treated with taurine (Shanghai Chemical Reagents Company; dissolved in 0.9% saline, 50 mg/kg) or vehicle (0.9% saline). In the experiments of assay of the $NAD^+$ levels and MPO activities, Western blot analysis, and histopathology, rats were randomly assigned to three groups treated with taurine or vehicle: (1) taurine (50 mg/kg); (2) vehicle (0.9% saline); and (3) sham (0.9% saline). Vehicle or taurine (1 ml/kg) was administered intravenously 1 h after MCAo. Rat neurological deficits, brain swelling, infarct volume, and histopathology ($n = 7$ per group) were evaluated at 70 h of reperfusion after 2 h MCAo (R 70 h), and the infiltration of neutrophils ($n = 8$ per group) was determined at 22 h of reperfusion after 2 h MCAo (R 22 h). In the experiments of assay of the $NAD^+$ levels and MPO activities, and Western blot analysis, the tissues of penumbra and core in vehicle- or taurine-treated rats were dissected at R 22 h, and regions from the right hemispheres that corresponded to the penumbra and core in sham-operated rats were dissected 24 h after operation. The activities of MPO and the levels of $NAD^+$, PARP, PAR, NF-$\kappa$B p65, IL-1$\beta$, TNF-$\alpha$, iNOS, and ICAM-1 were determined ($n = 5$ per group).

### Evaluation of neurological deficits

The rat neurological deficits, including postural reflex, forelimb placement, and beam balance, were tested at R 70 h by a person who was blind to the treatment conditions. The postural reflex of rat was evaluated using a 6-point score described by Schmid-Elsaesser et al.: 0, normal; 1, contralateral forelimb flexion; 2, lowered resistance to lateral push (and forelimb flexion) without circling; 3, circling if pulled by tail; 4, spontaneous circling; and 5, no spontaneous motor activity (Schmid-Elsaesser et al. 1998). The limb placement test was employed to examine sensorimotor integration in the forelimb placing responses to visual, tactile, and proprioceptive stimuli. The scores were as follows: 0, complete immediate placing; 1, incomplete and/or delayed placing (<2 s); and 2, absence of placing (De Ryck et al. 1989). The modified beam balance test examined vestibulomotor activity as the animal balanced on a narrow beam (1,750 × 19 mm) for 60 s (Clifton et al. 1991). Scoring was as follows: 0, steady posture with paws on top of beam; 1, paws on the side of beam or wavering; 2, one or two limbs slip off the beam; 3, three limbs slip off the beam; 4, rat attempts to balance with paws on the beam but falls; 5, rat drapes over the beam, then falls; and 6, rat falls off the beam without attempting to stay on.

### Measurement of volumes of infarction and brain swelling

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated at R 70 h. The brains were rapidly removed and sliced into 2 mm-thick coronal sections. The sections were immediately immersed in 1% 2,3,5-triphenyltetrazolium chlorides (TTC) (Sigma Co., St Louis, MO, USA) at 37°C for 15 min in the dark, and then fixed by 4% formaldehyde in phosphate-buffered solution. The unstained area of the brain section was defined as infarction. The infarct volume was measured using an image analysis program (Beijing Konghai Co., China). Since brain edema might significantly affect the accuracy of infarct estimation, the corrected infarct volume was calculated (Lin et al. 1993; Swanson et al. 1990). Brain swelling was determined by subtracting the total volume of the nonischemic hemisphere from that of the ischemic hemisphere (Lin et al. 1993).

### Sample collection and preparation

The tissues of penumbra and core were dissected according to the experimental protocols at 4°C (Ashwal et al. 1998; Sun et al. 2009). The designation of these core and penumbral regions was based on the thresholds of the cerebral blood flow, biochemical changes, and the studies of pharmacology and histopathology (Ginsberg 1997; Lipton 1999; Graham and Chen 2001). For the measurement of cellular $NAD^+$ levels, the tissue was weighed and homogenized in 0.5 ml of 0.4 M hyperchloric acid containing 1 mM ethylenediaminetetraacetic acid (EDTA) and then neutralized with 80 µl of 2.5 M $KHCO_3$. Cellular debris was removed by centrifugation at 10,000$g$ at 4°C for 10 min. The supernatant was used to determine the intracellular $NAD^+$ levels (Bernofsky and Swan 1973; Nagayama et al. 2000). For determining the activities of MPO, the tissue was weighed and homogenized using the method provided by the MPO activity assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and the homogenate was used to measure the activities of MPO.

For Western blot analysis, protein samples ($n = 5$ for each group) were prepared as described previously (Solaroglu et al. 2006). Briefly, the tissue was homogenized in 5 volumes of homogenization buffer A (20 Mm *N*-2-hydroxyethylpiperazine-*N*′-2′-ethanesulfonic acid (HEPES), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM EDTA, 1 mM ethyleneglycol bis(2-aminoethyl ether)tetraacetic

acid (EGTA), 250 mM sucrose, 0.1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT), and 10 μg/ml of each of aprotinin, pepstatin A, and leupeptin, pH 7.9). The sample was centrifuged at 750*g* at 4°C for 15 min to separate the sample into supernatant A and pellet A. Pellet A, containing the nuclear fraction, was resuspended in 90 μl of buffer B (20 mM HEPES, 1.5 mM $MgCl_2$, 20 mM KCl, 0.2 mM EDTA, 0.5 mM EGTA, 0.2 mM PMSF, 0.5 mM DTT, and 10 μg/ml of each of aprotinin, pepstatin A, and leupeptin, pH 7.9) and mixed with 30 μl of buffer C (20 mM HEPES, 1.2 M KCl, 0.2 mM EDTA, 0.2 mM PMSF, 0.5 mM DTT, and 10 μg/ml of each of aprotinin, pepstatin A, and leupeptin, pH 7.9). The sample was placed on ice for 30 min during the extraction and then centrifuged at 12,000*g* for 30 min at 4°C. The supernatant containing the nuclear fraction was transferred and stored at −70°C. Supernatant A, containing the cytosolic/mitochondrial protein, was further centrifuged at 16,000*g* for 30 min at 4°C to separate supernatant B from pellet B. Supernatant B was used as the cytosolic fraction and pellet B was discarded. The protein concentrations in cytosolic and nuclear fractions were determined by the method of Bradford (Bradford 1976).

### Measurement of intracellular $NAD^+$ levels

Supernatant (50 μl) was added to 1,125 μl buffer (0.105 M bicine, 0.527 M ethanol, 1.755 mM phenazine ethosulfate, and 0.439 mM 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, pH 7.8), and the mixture was incubated at 37°C for 30 min after addition of 16 U alcohol dehydrogenase. The reaction was stopped by addition of 1 ml of 12 mM sodium iodoacetate, and the absorbance was measured at 570 nm (Bernofsky and Swan 1973; Nagayama et al. 2000). The results were expressed as percentage of the levels in sham-operated rats.

### Western blot analysis

The proteins in the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as described previously (Sun et al. 2008, 2009). As much as 30 μg of proteins were separated by SDS-PAGE, and molecular weight markers (New England Biolabs Inc., Ipswich, MA, USA) were loaded on each gel for protein band identification. The proteins on the gel were subsequently transferred onto a PVDF membrane. The membrane was then probed with antibody reactive with PARP (1:400; Chemicon International Inc., Temecula, CA, USA), PAR(1:3,000; Calbiochem, San Diego, CA, USA), NF-$\kappa$B p65 (1:1,000; Calbiochem), TNF-$\alpha$ (1:400; R&D Systems Inc., Minneapolis, MN, USA), IL-1$\beta$ (1:500; R&D Systems Inc.), ICAM-1 (1:400; R&D Systems Inc.), or iNOS (1:100; Santa Cruz Biotechnology, CA, USA) at 4°C overnight and subsequently incubated with alkaline phosphatase-conjugated secondary antibody for 1.5 h at room temperature. The color reaction was observed by incubation of membrane with nitroblue tetrazolium/5-bromo-4-chloro-3-inoloyl-phosphate (NBT/BCIP) (Amresco, Solon, OH, USA), and the integrated optical densities of the protein bands were analyzed by gel image analyzer (Aalpha Innotech Co.). The membrane was then washed and probed with antibody reactive with $\beta$-actin (1:400; ProteinTech Group, Inc., Chicago, IL, USA) or histone H2A.X (1:400; Signal way Antibody Co., Ltd., Pearland, TX, USA), and the color reaction was observed by the method described above. $\beta$-actin and histone H2A.X were used as an internal control for the cytosolic and nuclear fractions, respectively. The results were expressed as percentage of the levels in sham-operated rats.

### MPO activity assay

The MPO activity in the homogenate was determined by MPO activity assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). One unit (U) of MPO activity is defined as the amount that degrades 1 μmol hydrogen peroxide at 37°C, and was normalized to the wet tissue weight (U/g wet tissue). The results were expressed as percentage of the levels in sham-operated rats.

### Analysis of neutrophil infiltration

Animals were anesthetized with chloral hydrate at R 22 h, and transcardially perfused with 100 ml heparinized normal saline followed by 200 ml 4% paraformaldehyde. Brains were removed, fixed in 4% paraformaldehyde, embedded in paraffin, and 8 μm-thick coronal sections were collected through the anterior commissure. The sections were stained with hematoxylin and eosin (HE), and the anatomical distribution of penumbra and core after experimental stroke was demarcated (Ashwal et al. 1998; Sun et al. 2009). The infiltration of neutrophils into the penumbra and core was determined. Briefly, neutrophils were counted in 12 random fields within the penumbra and core under light microscopy at 400× magnification, and only intact, extravascular neutrophils were included (Phillips et al. 2000).

### Analysis of cell death

At 72 h after MCAo, animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). The brains were removed, fixed, embedded in paraffin, and 8 μm-thick coronal sections were collected through the anterior commissure, as in the above-mentioned method. The sections

were stained with HE or cresyl violet (Sun et al. 2009) and examined with light microscopy. Pictures were taken with a digital camera. The anatomical distribution of penumbra and core after experimental stroke was demarcated (Ashwal et al. 1998). Cell death showed the disappearance of Nissl's body in the cytoplasma, chromatolysis, nuclear pyknosis, eosinophilic cytoplasm (red neuron), or lack of cellular structure (ghost neuron). The following 5-point score was used to evaluate the necrotic neurons in penumbra and core: 0, normal; 1, damaged neurons were <25%; 2, damaged neurons were 25–50%; 3, damaged neurons were 50–75%; and 4, damaged neurons were >75% (Sun et al. 2009).

### Data expression and statistical analysis

Data were presented as mean ± SEM. Comparisons between groups were statistically evaluated by Student's *t* test (infarct volume and brain swelling volume) or one-way ANOVA with a post hoc Fisher's test (the levels of $NAD^+$, PARP, PAR, NF-$\kappa$B p65, TNF-$\alpha$, IL-1$\beta$, ICAM-1, and iNOS). Neurological deficits (postural reflex, forelimb placement test, and beam balance test) and the cell death were analyzed with a nonparametric Mann–Whitney *U* test. A probability of <0.05 was considered to be statistically significant.

## Results

### Effects of taurine on neurological deficits, infarct volume, and brain swelling

Before ischemia, all animals showed no neurological deficits and performed normally in postural reflex, limb placement, and beam balance tests. The vehicle-treated rats displayed significant neurological dysfunction at R 70 h. The abnormalities of postural reflex, limb placement, and beam balance in taurine-treated rats were markedly reduced versus vehicle-treated rats (Fig. 1a, b, c; $P < 0.05$, 0.01, and 0.01, respectively). Representative coronal brain sections from vehicle- and taurine-treated rats stained with 1% TTC at R 70 h are shown in Fig. 1d. Two-hour ischemia following 70 h reperfusion resulted in an infarct of 188 ± 23 $mm^3$ and brain swelling of 108 ± 14 $mm^3$ in

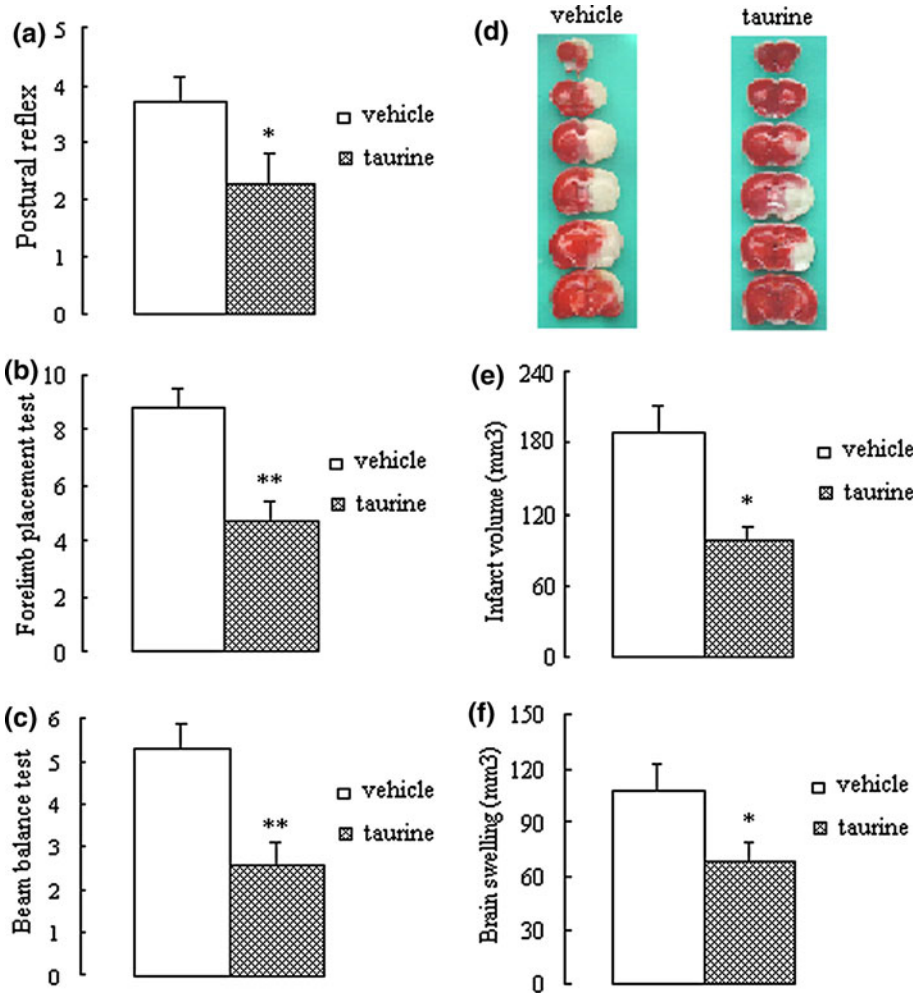


**Fig. 1** Effects of taurine on the neurological deficits, brain swelling, and infarct volume at 70 h of reperfusion after 2 h focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. **a** Postural reflex. **b** Forelimb placement. **c** Beam balance. **d** The infarct zone was displayed by TTC staining in vehicle- or taurine-treated rats. **e**, **f** The *bar* graph reflects the volume of infarct zone and brain swelling from TTC staining in vehicle- or taurine-treated rats, respectively. Data are presented as mean ± SEM. $n = 7$. $^*P < 0.05$ and $^{**}P < 0.01$ versus vehicle

vehicle-treated rats (Fig. 1e, f). Treatment with taurine decreased the infarct volume and lessened the brain swelling significantly (both $P < 0.05$ vs. vehicle-treated rats).

Effect of taurine on the PARP expression and activation

For determining the PARP expression, the protein levels of PARP in the cytosolic and nuclear fractions in the penumbra and core were investigated after experimental stroke, and the results are illustrated in Fig. 2a, b, c. Compared with sham-operated rats, the PARP levels in the cytosolic fractions in the penumbra in vehicle-treated rats had no significant change, while those in the cytosolic fractions in the core and in the nuclear fractions in the penumbra and core were increased significantly ($P < 0.01$, 0.05 and 0.01, respectively). Taurine treatment markedly reduced the PARP levels in the cytosolic fractions in the core and in the nuclear fractions in the penumbra and core (Fig. 2b, c; $P < 0.05$, 0.05 and 0.01 vs. vehicle-treated rats, respectively), although it had no significant effect on the PARP levels in the cytosolic fractions in the penumbra.

The activation of PARP was determined through assaying the levels of nuclear PAR and intracellular $NAD^+$. Western blot analysis showed the significant increases in the nuclear PAR levels in the penumbra and core in vehicle-treated rats (Fig. 2a, d; $P < 0.05$ and 0.01 vs. sham-operated rats, respectively). Concomitantly, the intracellular levels of $NAD^+$ in the penumbra and core were reduced significantly following ischemia and reperfusion (Fig. 2e; both $P < 0.01$). Taurine treatment markedly reduced the nuclear PAR levels, and enhanced the intracellular $NAD^+$ levels in the penumbra and core versus vehicle-treated rats (PAR: both $P < 0.05$; $NAD^+$: $P < 0.01$ and 0.05, respectively). These data demonstrated the down-regulation of taurine on the induction and activation of PARP in the penumbra and core during experimental stroke.

Effects of taurine on the levels of NF-$\kappa$B p65 in the cytosolic and nuclear fractions

For determining the activation of NF-$\kappa$B, we assayed the levels of p65 in the cytosolic and nuclear fractions, as p65 is an active subunit of NF-$\kappa$B. The results are shown in

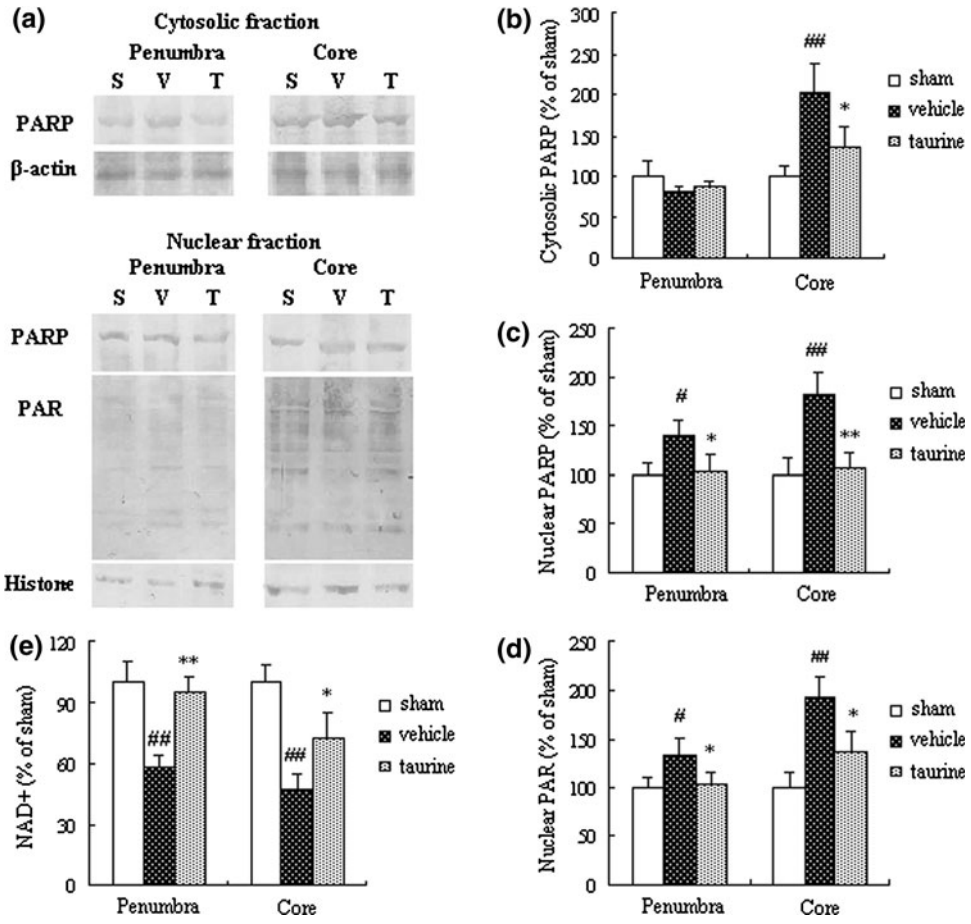


**Fig. 2** Effects of taurine on the protein levels and activation of PARP in the penumbra and core at 22 h of reperfusion after 2 h focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. **a** Western blot analysis using PARP or PAR antibody. *S* sham, *V* vehicle, *T* taurine. **b, c** The *bar* graphs reflect the densitometric data from the experiment of PARP Western blot in the cytosolic and nuclear fractions, respectively. **d** The *bar* graph reflects the densitometric data from the experiment of PAR Western blot in the nuclear fractions. **e** The levels of intracellular $NAD^+$. The results are expressed as percentage of the levels in sham-operated rats (mean ± SEM. $n = 5$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. sham. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle)

Fig. 3. The levels of p65 in the cytosolic fractions in the penumbra in vehicle-treated rats had no significant change compared with sham-operated rats. However, the levels of p65 in cytosolic fractions in the core and in nuclear fractions in the penumbra and core were increased significantly (Fig. 3; $P < 0.01$, 0.01 and 0.05, respectively). Treatment with taurine markedly reduced the levels of p65 in cytosolic fractions in the core and in nuclear fractions in the penumbra and core ($P < 0.01$, 0.05 and 0.05 vs. vehicle-treated rats, respectively), although it had no significant effect on the levels of p65 in cytosolic fractions in the penumbra. These data confirmed the suppression of taurine on induction and activation of NF-$\kappa$B in the penumbra and core after experimental stroke.

## Effects of taurine on the levels of inflammatory mediators

Western blot analysis was used to assay the protein levels of TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1. The representative protein bands of TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1 are displayed in Fig. 4a. The protein levels of TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1 in the penumbra and core increased significantly following experimental stroke versus sham-operated rats (Fig. 4b, c, d, e; TNF-$\alpha$ or ICAM-1: $P < 0.05$ and 0.01, respectively; IL-1$\beta$ or iNOS: both $P < 0.01$). Taurine treatment markedly reduced the protein levels of TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1 in the penumbra and core compared with vehicle-treated rats (TNF-$\alpha$ or iNOS: $P < 0.05$ and 0.01, respectively; IL-1$\beta$ or ICAM-1: both $P < 0.05$).

## Effects of taurine on neutrophil infiltration

For determining the infiltration of neutrophils into the penumbra and core, we measured the activities of MPO and counted the number of neutrophils. As showed in Fig. 5, the activities of MPO in the penumbra and core were markedly increased at 22 h of reperfusion after 2 h of ischemia ($P < 0.05$ and 0.01 in the penumbra and core, respectively). Administration of taurine reduced the activities of MPO in the penumbra and core significantly (both $P < 0.05$ vs. vehicle). Figure 6 describes the neutrophil infiltration in the penumbra and core. There is rare infiltration of neutrophils in the contralateral hemispheres of vehicle- or taurine-treated rats. Rats treated with vehicle showed significant infiltration of neutrophils in the penumbra and core. Treatment with taurine markedly reduced the number of neutrophils in the penumbra and core ($P < 0.01$ and 0.05 vs. vehicle, respectively).

## Effect of taurine on ischemic cell death

HE staining and cresyl violet staining were used to investigate the morphology of cell death, and the representative photographs are shown in Fig. 5b. In sham-operated rats, neurons in the cortex displayed intact morphology. In ischemic core in vehicle-treated rats, most neurons showed

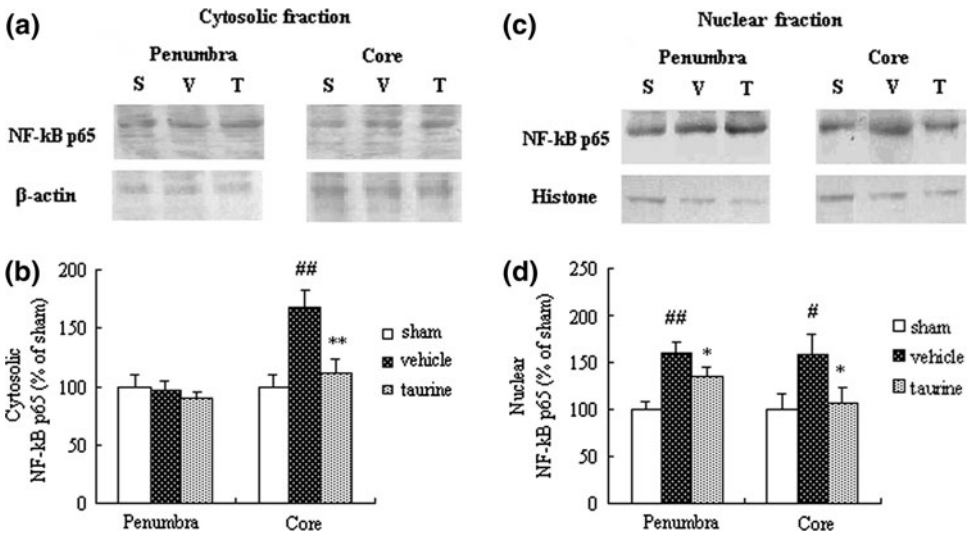


**Fig. 3** Effects of taurine on the protein levels of NF-$\kappa$B p65 in the penumbra and core at 22 h of reperfusion after 2 h of focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. **a**, **c** Western blot analysis using NF-$\kappa$B p65 antibody in the cytosolic and nuclear fractions, respectively. *S* sham, *V* vehicle, *T* taurine. **b**, **d** The *bar* graphs reflect the densitometric data from the experiment of NF-$\kappa$B p65 Western blot in the cytosolic and nuclear fractions, respectively. The results are expressed as percentage of the levels in sham-operated rats (mean ± SEM. $n = 5$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. sham. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle)

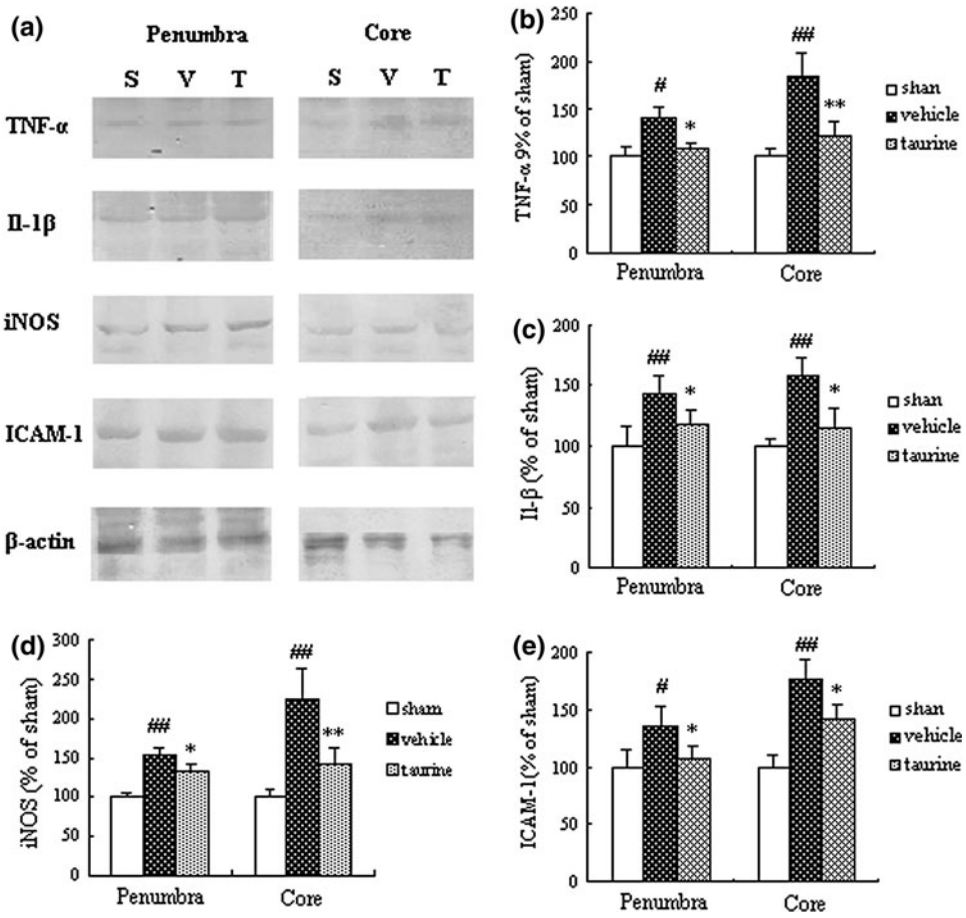


**Fig. 4** Effects of taurine on the protein levels of inflammatory mediators in the penumbra and core at 22 h of reperfusion after 2 h of focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. **a** Western blot analysis using TNF-$\alpha$, IL-1$\beta$, iNOS, or ICAM-1 antibody in the cytosolic fractions. *S* sham, *V* vehicle, *T* taurine. **b**, **c**, **d**, **e** The *bar* graphs reflect the densitometric data from the experiment of TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1 Western blot, respectively. The results are expressed as percentage of the levels in sham-operated rats (mean $\pm$ SEM. $n = 5$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. sham. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle)

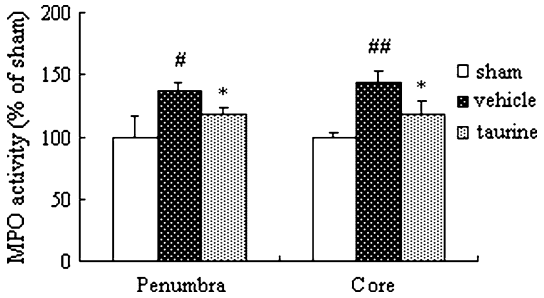


**Fig. 5** Effects of taurine on the activities of MPO in the penumbra and core at 22 h of reperfusion after 2 h of focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. The results are expressed as percentage of the levels in sham-operated rats (mean $\pm$ SEM. $n = 5$. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. sham. $^{*}P < 0.05$ vs. vehicle)

disappearance of Nissl's body in the cytoplasma, chromatolysis, nuclear pyknosis, eosinophilic cytoplasm (red neuron), or lack of cellular structure (ghost neuron). Although it was not as severe as in the core, cell death changes were also observed in some cells in the penumbra in vehicle-treated rats. The cell death scores in the penumbra Fig. 7 and core were reduced significantly in taurine-treated rats versus vehicle-treated rats (Fig. 5c; $P < 0.01$ and 0.05, respectively).

## Discussion

MPO is a tetrameric, glycosylated, and heme-containing enzyme. It is abundant in primary azurophilic granules of neutrophils. It has also been localized in monocytes/macrophages and microglia (Yap et al. 2007; Lau and Baldus 2006; Deby-Dupont et al. 1999). MPO can catalyze the reaction of hydrogen peroxide ($H_2O_2$) with chloride to generate HOCl. HOCl is a major oxidant formed by inflammatory cells. It not only reacts with ferrous and superoxide anion to generate hydroxy radical in the brain, but also reacts with nitrite (breakdown product of nitric oxide metabolism) to form nitryl chloride. Therefore, HOCl possesses oxidizing, chlorinating, and nitrating abilities. It can react with a variety of critical bio-molecules including heme proteins and porphyrins, thiol, iron sulfur centers, nucleotides, DNA, unsaturated lipids, amines, and amino acids, resulting in the dysfunction of bio-molecules, tissue destructure, cell growth arrest, and cell damage and

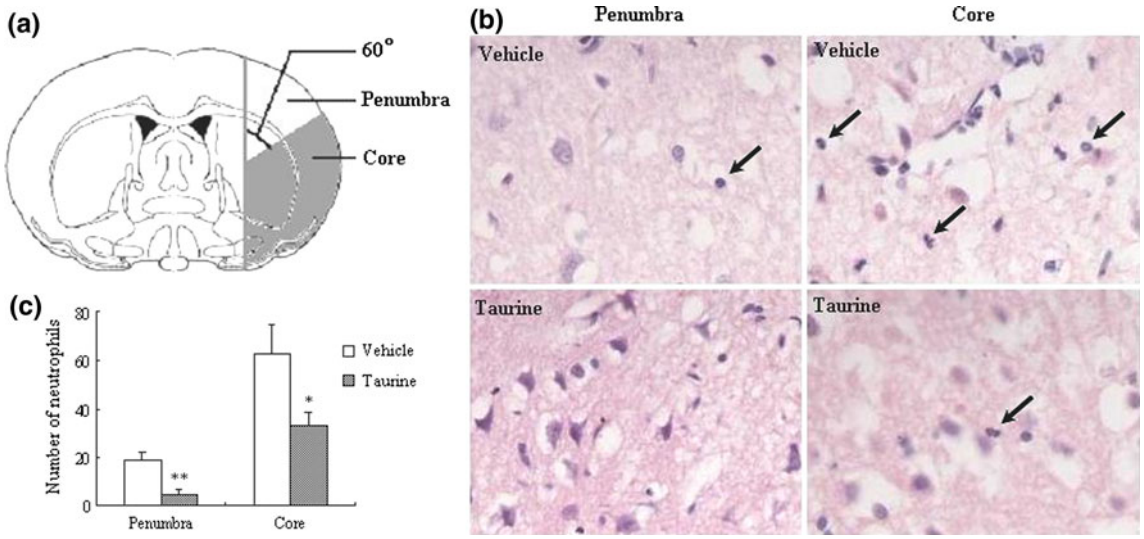


**Fig. 6** Effects of taurine on the infiltration of neutrophils in the penumbra and core at 22 h of reperfusion after 2 h of focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. **a** Anatomical distribution of penumbra and core. **b** Representative photographs showing neutrophils in the ischemic region by HE staining (original magnification, 400x). *Black arrows* indicate typical neutrophils. **C** The *bar* graph reflects the neutrophil counts in the penumbra and core in each group (mean ± SEM. $n = 8$. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle)

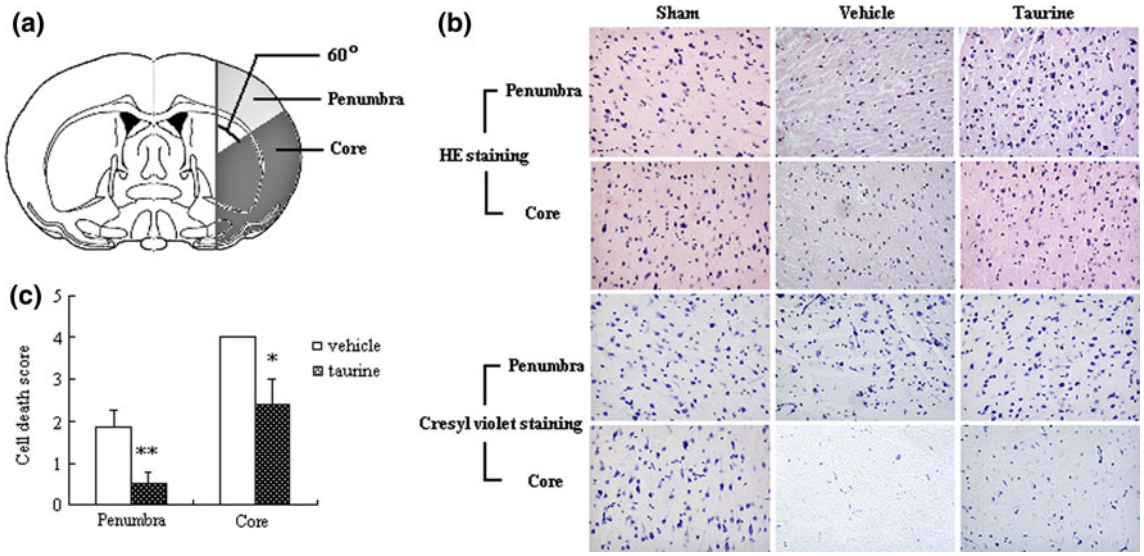


**Fig. 7** Effects of taurine on the cell death in the penumbra and core at 70 h of reperfusion after 2 h of focal cerebral ischemia. Vehicle or taurine was injected intravenously 1 h after ischemia. **a** Anatomical distribution of penumbra and core. **b** Representative photographs showing cell death by H&E staining and cresyl violet staining (original magnification 200×). **c** The *bar* graph reflects the cell death score in the penumbra and core in each group (mean ± SEM; $n = 7$; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. vehicle)

death (Yap et al. 2007; Lau and Baldus 2006; Deby-Dupont et al. 1999).

Inflammatory reaction plays a critical role in propagating tissue damage during experimental stroke (Wang et al. 2007; Tuttolomondo et al. 2008; Amantea et al. 2009). After the interruption of cerebral blood flow, tissue damage begins with an inflammatory reaction that requires the infiltration of neutrophils and monocytes/macrophages, activation of microglia, and production of inflammatory mediators. Once neutrophils penetrate into the ischemic brain, tissue damage is incited through their release of reactive oxygen species (ROS) and proteolytic enzymes. Among all of the ROS in the ischemic brain parenchyma, superoxide anion is a major one. Once generated, superoxide anion is converted to $H_2O_2$ both spontaneously and by various forms of superoxide dismutases. MPO could catalyze the reaction of $H_2O_2$ with chloride to generate HOCl (Yap et al. 2007; Lau and Baldus 2006;

Deby-Dupont et al. 1999). HOCl has been reported to induce neuronal death and endothelial dysfunction in vitro (Yap et al. 2006; Radovits et al. 2007). Moreover, MPO activation is detrimental during experimental stroke (Miljkovic-Lolic et al. 2003), and it is used to evaluate the infiltration of neutrophils in the rodent model of ischemic stroke (Barone et al. 1991; Matsuo et al. 1994). These data suggest that the triad of MPO, chloride, and $H_2O_2$ could involve in ischemic brain damage through producing cytotoxic HOCl.

Taurine is one of the most abundant free amino acids in mammalian tissue including leukocytes (Huxtable 1992; Fukuda et al. 1982). It is reported to play an important role as a regulatory molecule of the inflammatory reaction (Schuller-Levis and Park 2003, 2004). Taurine can act as a trap for HOCl forming the long-lived oxidant Tau-NHCl, which is more stable and less toxic than HOCl. Moreover, Tau-NHCl down-regulates the generation of proinflammatory mediators by phagocytic cells, such as TNF-$\alpha$, IL-1$\beta$, and iNOS (Marcinkiewicz et al. 1998; Park et al. 1995; Kim et al. 1996; Schuller-Levis and Park 2003, 2004). This process suppresses the inflammatory reaction and protects cells from the cytotoxic and cytolytic actions of HOCl.

During experimental stroke, the extracellular taurine is significantly increased due to the release of intracellular taurine (Lo et al. 1998), which could constitute an important endogenous protective mechanism against neuronal damage (Saransaari and Oja 2000). Meanwhile, the depletion of intracellular taurine may result in the disruption of intracellular homeostasis or enantiostasis, leading to neuronal damage (Huxtable 1992; Michalk et al. 1996). Hence, the release of taurine may be an obligatory self-protective mechanism under ischemic stress. Taurine either in the extracellular space or in inflammatory cells could scavenge HOCl generated in the ischemic brain, producing Tau-NHCl (Thomas 1979; Weiss et al. 1982). Tau-NHCl might then down-regulate the expression of proinflammatory mediators (Marcinkiewicz et al. 1998; Park et al. 1995; Kim et al. 1996), suppressing the inflammatory reaction and protecting the brain against experimental stroke. The present study shows that taurine depresses the activation of MPO, reduces the production of TNF-$\alpha$, IL-1$\beta$, iNOS, and ICAM-1, attenuates the infiltration of neutrophils in the penumbra and core, improves neurological functions, lessens brain swelling, and decreases the infarct volumes in the rat model of experimental stroke. These data suggest that taurine may protect the brain against experimental stroke through suppressing MPO activation, subsequently reducing the formation of HOCl, scavenging HOCl, down-regulating the expression of inflammatory mediators, and attenuating the infiltration of neutrophils.

On the other hand, Tau-NHCl exerts prolonged oxidative and chlorinating effects long after the initiation of inflammation and at some distance from the cell of origin (Zgliczyński et al. 1971). It is reported that Tau-NHCl mediates HOCl-induced apoptosis (Englert and Shacter 2002). Moreover, Tau-NHCl can be oxidized to taurine dichloramine (Tau-$NCl_2$), which is more toxic than Tau-NHCl (Stelmaszyńska and Zgliczyński 1978). These data suggest that Tau-NHCl and Tau-$NCl_2$ produced at the site of inflammation could suppress neutrophil-mediated inflammation through inducing apoptotic cell death of neutrophils, which may be protective during experimental stroke. Contrarily, it may aggravate ischemic brain damage through triggering neuronal apoptotic cell death in the ischemic region. It is reported that a molar excess of taurine increases the formation of Tau-NHCl, decreases the generation of Tau-$NCl_2$, and protects tissue against the cytotoxicity of HOCl (Cantin 1994). Hence, it is possible that exogenous administration of adequate amount of taurine could reduce the release of intracellular taurine and increase the levels of extracellular taurine, which would contribute to maintaining intracellular homeostasis, scavenging HOCl, reducing the formation of Tau-$NCl_2$, enhancing Tau-NHCl, depressing the inflammatory reaction, and finally leading to the reduction of ischemic brain injury. This speculation has been supported by our results in this study. Although in vivo, the generation of Tau-$NCl_2$ may be marginal, further study is necessary to evaluate the production and the toxicity of Tau-NHCl and Tau-$NCl_2$ during experimental stroke, as Tau-NHCl at the concentration of 1 mM and higher is cytotoxic, and Tau-$NCl_2$ is more toxic than Tan-NHCl (Cantin 1994; Kim and Kim 2005).

One mechanism for reducing inflammation after injury is to block the inflammatory gene response of cells. Activation of the transcription factor NF-$\kappa$B is largely responsible for up-regulation of inflammatory genes after ischemic brain injury (Kumar et al. 2004; Ridder and Schwaninger 2009). Moreover, PARP is reported to act as a coactivator of NF-$\kappa$B, thereby contributing to the expression of NF-$\kappa$B-driven inflammatory gene (Koh et al. 2004, 2005; Haddad et al. 2006). The present study shows that taurine down-regulates the activation and expression of PARP and NF-$\kappa$B in the penumbra and core after experimental stroke. Moreover, the activation of PARP is related well to the expression of NF-$\kappa$B-driven inflammatory mediators. These data indicate that suppression of inflammatory reaction involved PARP and NF-$\kappa$B is one mechanism of taurine against experimental stroke. Our results are supported by previous studies that PARP-1 knockout or PARP inhibitors suppress the expression of NF-$\kappa$B-driven genes (Chiarugi and Moskowitz 2003; Koh et al. 2004, 2005; Haddad et al. 2006). The possible mechanisms concerning the effects of taurine on PARP and NF-$\kappa$B during experimental stroke may be explained by the

following: (1) taurine can down-regulate PARP-induced NF-κB activation, as taurine can reduce the over-activation of PARP due to ROS-induced DNA damage through scavenging ROS or reducing ROS formation (Messina and Dawson 2000; Virág and Szabó 2002; Chiarugi and Moskowitz 2003; Saito et al. 2005); (2) taurine can reduce oxidative stress-induced NF-κB activation, since oxidative stress can induce the nuclear translocation of NF-κB without degradation of IκB or promote the degradation of IκB through phosphorylating IκB in the serine reside (Redmond et al. 1996; Hanna et al. 2004; Traenckner et al. 1995; Canty et al. 1999); and (3) Tau-NHCl generated by the reaction of taurine with HOCl can suppress NF-κB activation through affecting NF-κB signal pathway, including inhibiting the phosphorylation of IκB (Barua et al. 2001; Kim and Kim 2005) or directly oxidizing IκB (Kanayama et al. 2002).

## Conclusions

This study demonstrates that treatment with taurine down-regulates PARP and NF-κB, inhibits the expression of NF-κB-driven inflammatory mediators, suppresses the infiltration of neutrophils in the penumbra and core, and reduces ischemic brain damage in the rat model against experimental stroke. These data suggest that taurine may protect the brain against experimental stroke through suppressing inflammatory reaction, which may be related to its capacity for scavenging HOCl and down-regulating PARP and NF-κB-driven expression of inflammatory mediators. Further study is needed to elucidate the detailed anti-inflammatory mechanism of taurine against experimental stroke.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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