INHIBITION OF TRANSFORMING GROWTH FACTOR BETA-ACTIVATED KINASE 1 CONFERS NEUROPROTECTION AFTER TRAUMATIC BRAIN INJURY IN RATS

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Abstract—The transforming growth factor beta-activated kinase 1 (TAK1), a member of the mitogen-activated protein kinase kinase family, is characterized as a key regulator in inflammatory and apoptosis signaling pathways. The aim of the present study was to evaluate the role of the TAK1 pathway in experimental traumatic brain injury (TBI) in rats. Adult male Sprague-Dawley rats were subjected to TBI using a modified Feeney’s weight-drop model. The time course showed that a significant increase of TAK1 and p-TAK1 expression in the cortex after TBI. Moreover, TBI induced TAK1 redistribution both in neurons and astrocytes of the lesion boundary zone. The effects of specific inhibition of the TAK1 pathway by 5Z-7-oxozeaenol (OZ, intracerebroventricular injection at 10 min post trauma) on histopathological and behavioral outcomes in rats were assessed at 24 h post injury. The number of TUNEL-positive stained cells was diminished and neuronal survival and neurological function were improved with OZ treatment. Biochemically, the high dose of OZ significantly reduced the levels of TAK1 and p-TAK1, further decreased nuclear factor-kB and activator protein 1 activities and the release of inflammatory cytokines. In addition, we found that both 10 min and 3 h post-trauma OZ therapies could markedly improve neurological function and neuronal survival after long-term survival. These results revealed that the TAK1 pathway is activated after experimental TBI and the inhibitor OZ affords significant neuro-protection and amelioration of neurobehavioral deficits after experimental TBI, suggesting a potential rationale for manipulating this pathway in clinical practice. © 2013 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: TAK1, traumatic brain injury, 5Z-7-oxozeaenol, NF-kB, AP-1, inflammation.

INTRODUCTION

Traumatic brain injury (TBI) is acknowledged as one of the leading causes of morbidity and mortality, which constitutes a major healthy and socioeconomic problem throughout the world (Maas et al., 2008). The development of the lesion after TBI consists of the primary injury and the secondary injury cascade. The secondary injury leads to ongoing neuronal degeneration within minutes to months after the injury (Morganti-Kossmann et al., 2007). A variety of biochemical and molecular mechanisms are responsible for secondary brain damage including excessive glutamate release, production of free oxygen radicals, lipid peroxidation, and endogenous neuroinflammation mechanisms (McIntosh et al., 1998). Despite intense research, the pathogenesis of the secondary injury following TBI is still not well understood, and no specific pharmacological treatment is available.

Transforming growth factor beta-activated kinase 1 (TAK1), a member of the mitogen-activated protein kinase kinase family, plays an essential role in tumor necrosis factor (TNF), interleukin 1 (IL-1), and Toll-like receptor (TLR) signaling pathways (Ninomiya-Tsuji et al., 1999b; Sato et al., 2005, Shim et al., 2005). TAK1 was recently shown to be activated in rodents after neonatal hypoxic-ischemic injury (Nijboer et al., 2009), in a rodent model of middle cerebral artery occlusion (MCAO), and in vitro after oxygen glucose deprivation (Neubert et al., 2011). Importantly, recent evidences from animal experiments indicated that the inhibition of TAK1 activation decreased infarct volume after focal cerebral ischemia (Neubert et al., 2011). However, to the best of our knowledge, the role of TAK1 in conditions associated with experimental TBI has not yet been elucidated. In the present study, we investigated the time-course of TAK1 activation after TBI. To investigate the role of the TAK1 pathway after TBI, we assessed histologic and behavioral parameters...
after post-traumatic injection of the selective TAK1 inhibitor 5Z-7-oxozeaenol (OZ).

**EXPERIMENTAL PROCEDURES**

**Animals and trauma model**

The male Sprague-Dawley rats weighing 250–300 g were used in this study. Rats were housed in a reversed 12-h light/12-h dark cycle controlled environment with free access to food and water. All procedures were approved by the Nanjing University Animal Care and Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (NIH).

Experimental TBI was performed with modification of Feeney's weight-drop model as previously described in our laboratory (Han et al., 2005). Briefly, under intraperitoneal anesthesia with Phenobarbital sodium (50 mg/kg) and spontaneous breathing, a 20-mm midline incision was made over the skull, the skin and fascia were reflected, and a bone window of right parietal was opened by dental drill, 5 mm in diameter, with the center positioned 1.5 mm posterior and 2.5 mm lateral to the bregma. The dura was intact during the operation. A steel weight (40 g with a flat-end) fell on a little pillar (4 mm in diameter, 5 mm long) on the dura from a height of 25 cm along a stainless steel rod. The pillar was allowed to compress the cortex in a maximum depth of 5 mm. The sham operated animals were also anesthetized and the right parietal was made a bone window without cortex contusion. Rectal and temporalis muscle thermistors are used to maintain core and body temperature around 37.0°C. The rats were placed on the heating pad to maintain their parameters within normal physiological ranges. Because there was no significant difference of all measurements in vehicle- and OZ-treated rats at 24 h, 72 h and 7 days by analysis of variance (ANOVA) followed by a Student-Newman-Keuls test.

**OZ treatment protocol**

The TAK1 inhibitor OZ was purchased from Tocris Bioscience. OZ was dissolved in pure DMSO just before use (Bersudsky et al., 1997). OZ solution (5 μl) was injected into the left lateral ventricle 10 min post-injury, using a 10 μl Hamilton microsyringe. For the intracerebroventricular (ICV) injections, each animal was positioned in a stereotactic frame under phenobarbital sodium anesthesia. Coordinates for the injection placement were 1.0 mm posterior to bregma, 1.4 mm lateral to midline, and 4.4 mm below the skull surface (Paxinos and Watson, 2007) and the injection duration was 10 min.

**Beam-walking score**

The behavioral functional tests were performed in animals by an observer who was blinded to the experimental conditions. This score evaluates motor coordination of animals. The test was performed as described earlier (Su et al., 2011). The beam-walking performance was scored in a blinded fashion using a grading scale from 1 to 7 as follows: 1, the rat is unable to place the affected hind limb on the horizontal surface of the beam; 2, it places the affected hind limb on the horizontal surface of the beam and maintains balance but is unable to traverse the beam; 3, the rat traverses the beam dragging the affected hind limb; 4, it traverses the beam and once places the affected hind limb on the horizontal surface of the beam; 5, the rat crosses the beam and places the affected hind limb on the horizontal surface of the beam to aid less than half its steps; 6, the rat uses the affected hind limb to aid more than half its steps, and 7, the rat traverses the beam with no more than two foot slips. All rats were trained to traverse the beam 24 h before and on the day of injury immediately before anesthesia. The maximum was seven for non-operated rats. Beam-walking score was assessed after injury in vehicle- and OZ-treated rats at 24 h, 72 h and 7 days by an investigator who was blinded with regard to the study groups.

**Biochemical assays**

The surrounding brain tissue of the injured cortex was dissected from the region that was less than 3 mm from the margin of the contusion site on ice as described in our previous study (Wang et al., 2012), which were stored at −80 °C until homogenization. Brain tissue segment was homogenized on ice in 10 mM Tris–HCl buffer (pH 7.4), 10 mM EDTA, containing 3% Triton-100, 1% SDS and 200 mM NaCl using a homogenizer. The homogenized samples were then centrifuged at 13,000 g for 10 min at 4 °C. Following centrifugation, protein concentration in each sample was assayed with BCA reagent (Sigma). Samples were then diluted (1:1) in a sample buffer (82.5 mM Tris–HCl, pH 6.8, 2% SDS, 5 mM β-mercaptoethanol, 10% glycerol), boiled for 5 min, and stored at −20 °C. Proteins were fractionated by 10% SDS–PAGE, transferred to a polyvinylidene difluoride (PVDF; Bio-Rad, Shanghai, China) membrane, and incubated for 1 h in blocking solution (3% milk, 150 mM NaCl, 50 mM Tris–HCl, pH 7.5, 0.05% Tween 20) at room temperature. Blots were incubated with specific antibodies overnight at 4 °C. The primary antibodies, diluted according to manufacturer's recommendations, were directed against TAK1, phosphorylated TAK1 (p-TAK1, Cell Signaling, Danvers, MA, USA), Antibody against β-actin (1: 1000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was incubated for 1 h at room temperature. Blots were washed extensively in TBST (150 mM NaCl, 50 mM Tris–HCl, pH 7.5, 0.05% Tween 20), then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 1 h at room temperature. The blotted protein bands were visualized by enhanced chemiluminescence (ECL) Western blot detection reagents (Amersham, Arlington Heights, IL, USA) and were exposed to X-ray film. Developed films were digitized using an
Epson Perfection 2480 scanner (Seiko Corp, Nagano, Japan). Optical densities were obtained using Glyko Bandscan software (Glyko, Novato, CA, USA).

Nuclear extracts from cortical tissue were prepared as described previously (Hang et al., 2005). Protein concentration was determined using the Bradford method (Bradford, 1976). The binding activities to the oligonucleotides with an active protein 1 (AP-1) or nuclear factor-kappaB (NF-κB) binding-consensus sequence were detected by electrophoretic mobility shift assay (EMSA) kits (Viagene Biotech, Changzhou, China) according to the manufacturer’s instructions. Briefly, 4 μg of nuclear extract was incubated at 25 °C for 30 min with labeled or unlabeled competitor oligonucleotides in binding buffer. The sequences of the oligonucleotide probes are as follows: AP-1, 5′-CGCTTGATGAGTCAGCCGGAA-3′; NF-κB, 5′-AGTTGAGGGACTTTCCCAGGC-3′. Reaction products were separated on 6% polyacrylamide gels, transferred to nylon membranes and visualized by chemiluminescence using streptavidin–HRP conjugate. Excess of cold DNA probe eliminated the signal, showing specificity of the EMSA. NF-κB and AP-1 activities were quantified by computer-assisted densitometric analysis.

IL-1β and TNF-α levels were analyzed by enzyme-linked immunosorbent assay (ELISA). The ELISA kit was purchased from Neobioscience Technology Company (Beijing, China). The procedure was performed according to the instructions provided by the company. Equal amounts of lysates were used for TNF-α and IL-1β analysis. Values were expressed as pg/mg protein.

Histological assays

Immunohistochemistry (IHC) was performed to ascertain the immunoreactivity of TAK1. Briefly, the tissue sections (4 μm) were deparaffinized and rehydrated in gradient alcohol. And then the sections were incubated with anti-TAK1 monoclonal antibody (diluted 1: 50, Santa Cruz, CA, USA) overnight at 4 °C, followed by a 15-min wash in phosphate-buffered saline (PBS). After that the sections were incubated with HRP-conjugated IgG (diluted 1: 500, Santa Cruz, CA) for 60 min at room temperature. 3,3′-Diaminobenzidine–H2O2 solution was used to visualize TAK1. The specificity of the IHC reaction was evaluated by replacement of the primary antibody with rabbit IgG.

For immunofluorescence (IF) microscopy, frozen tissue sections (6 μm thick) of normal adult brains were blocked by treatment with appropriate preimmune serum. Tissue sections were rinsed with 0.1 M PBS (pH 7.4). Sections were incubated overnight at 4 °C with the primary antibodies: rabbit anti-TAK1 (1: 50, Santa Cruz, CA, USA), mouse anti-neuronal nuclei (NeuN) (1:1000; Chemicon International, CA, USA), mouse anti-glial fibrillary acidic protein polyclonal (GFAP) antibody (1:1000; BD Biosciences, San Diego, CA, USA), DyLight 594 AffiniPure Goat Anti-Rabbit IgG (1:200; EarthOx, CA, USA) was used to detect TAK1, and DyLight 488 AffiniPure Goat Anti-Mouse IgG (1:200; EarthOx, CA, USA) was prepared to show the immunoreactivity of NeuN and GFAP. In addition, 4′,6-diamidino-2-phenylindole (DAPI) was used to show cell nuclei. The specificity of IF reaction was evaluated by replacement of the primary antibody with rabbit IgG.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) was performed using a TUNEL detection kit (Roche, Indianapolis, IN, USA). The procedures were according to the protocol of the kit and our previous study (Hang et al., 2004; Zhuang et al., 2012). The positive cells were identified, counted, and analyzed under the light microscope by an investigator blinded to the grouping. The extent of brain damage was evaluated by the apoptotic index, defined as the average percentage of TUNEL-positive cells in

Fig. 1. Time course of TAK1 and p-TAK1 expression in the lesion boundary zone after TBI in rats. Expression levels of TAK1 and p-TAK1 have been normalized against β-actin. Since there was no significant difference observed between samples collected from 1 to 24 h within the control group in the preliminary study, animals of the control group were killed for sample collection at 6 h after being sham operated. Both TAK1 and p-TAK1 showed substantial and persistent increases following TBI. (A) A typical Western blot result. (B) Quantification data of (A). Data are represented as mean ± SEM. (*P, #P < 0.05 vs. control, n = 4).

Fig. 2. Representative photomicrographs of TAK1 immunohistochemistry in the normal and TBI groups. TAK1 was mainly expressed around nuclei in the cortex of normal rats (A, arrows). The enhanced immunoreactivity of TAK1 was detected in the cytoplasm of the stained cells in the border zone area adjacent to the cortical lesion at 24 h after TBI (B, arrowheads). Scale bar = 200 μm.
each section counted in 10 cortical microscopic fields (at 400× magnification). A total of four sections from each animal were used for quantification. The final average percentage of TUNEL-positive cells of the four sections was regarded as the data for each sample.

Tissue sections were stained with Cresyl Violet (Nissl) as described (Zhuang et al., 2012). Normal neurons have relatively big cell bodies, are rich in cytoplasm, with one or two big round nuclei. In contrast, damaged cells show shrunken cell bodies, condensed nuclei, dark cytoplasm, and many empty vesicles. Cell counting was restricted to the lesion boundary zone. Ten random high-power fields (400×) in each coronary section were chosen, and the mean number of surviving neurons in the ten views was regarded as the data of each section. A total of four sections from each animal were used for quantification. The final average number of the four sections was regarded as the data for each sample. Data were presented as the number of neurons per high-power field. All the processes were conducted by two pathologists blinded to the grouping.

Fig. 3. Representative photomicrographs of fluorescent double staining of TAK1 (red), NeuN (green) or GFAP (green) in the sham and 24 h post-TBI groups. Nuclei were counterstained with DAPI (blue). Scale bar = 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Western blot analysis of TAK1 and p-TAK1 in cortex samples of the sham and TBI ± OZ groups at 24 h after TBI. Expression levels of TAK1 and p-TAK1 have been normalized against β-actin. The expressions of p-TAK1 and TAK1 prominently increased in the vehicle-treated rats compared with sham-operated rats. OZ (20 μg) treatment significantly prevented the TBI-induced up-regulation of p-TAK1 and TAK1. (A) A representative Western blot result. (B) Quantification data. Data are represented as mean ± SEM. (⁎⁎P, ⋆⋆P < 0.05 vs. sham-operated rats; ⋆P, ⋆⋆P < 0.05 vs. vehicle-treated rats, n = 5).
Statistical analysis

Data are expressed as mean ± SEM. One-way ANOVA followed by Tukey’s test was used to analyze differences between groups except for the neurobehavioral scores, which were analyzed with nonparametric tests (Kruskal–Wallis, followed by Dunn’s post hoc test). *P < 0.05 was considered statistically significant. SPSS 16.0 was used for the statistical analysis (SPSS, Inc., Chicago, IL, USA).

RESULTS

Western blot analysis of TAK1 and p-TAK1 expression after TBI

Previous study demonstrated that stroke-induced loss of activated TAK1 (p-TAK1) at 4 h after the induction of ischemia (White et al., 2012). To investigate whether TBI resulted in the activation of TAK1 signaling pathway, we first investigated the temporal expression of TAK1 and p-TAK1 in the cortex of the control and traumatized rats. As shown in Fig. 1, both p-TAK1 and TAK1 were significantly increased at 24 h after TBI (*P < 0.05).

TAK1 IHC after TBI

We then performed IHC on sections of the rat brain. TAK1 was mainly expressed around nuclei in the cortex of sham-operated rats (Fig. 2A). Consistent with the results obtained by western blot analysis, the expression of TAK1 was increased 24 h after TBI, with the enhanced immunoreactivity of TAK1 in the cell cytosol in the border zone adjacent to the cortical lesion (Fig. 2B).

To gain a better understanding of the physiologic functions of TAK1 in the brain, we performed double immunofluorescent staining to evaluate the distribution of TAK1 (Fig. 3). TAK1 immunostaining was colocalized with NeuN in the cortex, indicating that TAK1 is expressed in neurons in the normal adult rat brain. Compared with the sham group, TBI group showed loss of intensity of NeuN staining neurons, and an increase in TAK1 expression in cortex neurons (Fig. 3, upper TBI panel). To determine whether TAK1 was expressed in astrocytes, sections were double labeled with antibody against TAK1 and GFAP. The number of GFAP-positive astrocytes was significantly increased in the lesion boundary zone by 24 h post-injury, and more prominent immunoreactivity of TAK1 was detected in the astrocytes of the injured group than that of the sham group (Fig. 3, bottom panel).

Effects of the OZ on the expression of TAK1 and p-TAK1 in the brain after TBI

To investigate the role of OZ in the activation of TAK1 following TBI, we evaluated the expression of TAK1 and p-TAK1. In the injured cortex homogenates at 24 h after TBI a significant increase of TAK1 and p-TAK1 levels was observed in TBI-operated rats (Fig. 4). Treatment with OZ 10 min after TBI suppressed the expression of TAK1 and p-TAK1 in a dose-dependent manner. A significant decrease of the expression of TAK1 and p-TAK1 was exhibited when a high dosage of OZ (20 µg)
was administrated \((P < 0.05)\). Our data demonstrated that the high expression levels of TAK1 and p-TAK1 after TBI was reversed by OZ.

**Blockage of TBI-induced NF-κB and AP-1 activation mediated by TAK1 inhibition**

To determine whether TAK1 inhibition downregulates the DNA binding activity of NF-κB and AP-1 following TBI, nuclear extracts were used for EMSA using oligonucleotides corresponding to the NF-κB and AP-1 binding sites. TBI-induced activation of AP-1 (Fig. 5A) and NF-κB (Fig. 5B) was markedly reduced by OZ treatment at the dosage of 20 μg at 24 h post injury. The data suggested that TBI-induced activation of NF-κB and AP-1 was abolished via TAK1 inhibition by OZ.

**Suppression of cytokine expression mediated by TAK1 inhibition**

To test whether OZ downregulates the expression of proinflammatory cytokines, we analyzed the levels of TNF-α and IL-1β in the injured cortex. A substantial increase of IL-1β and TNF-α production was revealed in the injured cortex collected from rats 24 h after injury when compared to the sham group (Fig. 6A, B). ICV injection of a higher dosage (20 μg) of OZ significantly reduced cytokine levels \((P < 0.05)\). The data demonstrate that increased cytokine expression induced by TBI can be reversed through TAK1 inhibition by OZ.

**Protection against TBI-induced neuronal apoptosis via TAK1 inhibition**

Previous study showed that the inhibition of TAK1 shortly before oxygen-glucose deprivation protects neurons from apoptosis *in vitro* (Neubert et al., 2011). To test whether the neuroprotective effect of OZ is through preventing neurons from apoptosis *in vivo*, we measured TUNEL-positive cells in the rat brain. A few TUNEL-positive cells were detected in the brain of sham-operated rats (Fig. 7A). The apoptotic index in the lesion boundary cortex was significantly increased in the TBI group (Fig. 7B). Treatment with OZ significantly reduced the apoptotic index (Fig. 7C). These findings suggested that OZ protected neurons from apoptosis in the lesion boundary zone after TBI.

**Influence of OZ on neuronal survival and neurological function**

To further see the protection of OZ, neuronal cells were directly examined. A large proportion of neurons in the TBI group (Fig. 8B, F) were damaged, exhibiting extensive degenerative changes including sparse cell arrangements, loss of integrity, shrunken cytoplasm, oval or triangular nucleus, and swollen cell bodies, whereas the sham group was full of clear and intact neurons (Fig. 8A, E). Both 10 min (Fig. 8C, G) and 3 h (Fig. 8D, H) post-trauma therapies, however, could evidently alleviate the severity of neuronal degeneration. To see whether the protected neurons by OZ are functional, beam-walking scoring was performed. As
shown in Fig. 8, a significant decrease in the beam-walking score was observed in the TBI group when compared to the sham group. The lower dosage (2 or 10 μg) of OZ used in our study failed to improve the beam-walking score (not shown). However, rats given 20 μg of OZ exhibited a marked increase of the neurological score. To determine whether the effect of these agents was durable and could lead to long-term neuroprotection, we subjected a separate cohort of rats to survive for 7 days. During these periods, functional recovery of the OZ-treated rats was kept consistently improved compared to rats treated with vehicle (Fig. 8J). Delayed treatment with OZ (3 h after TBI) showed a very similar effect to treatment 10 min post injury.

**DISCUSSION**

There are some primary findings in the present study as follows: TBI increased the expression of TAK1 and p-TAK1 and induced TAK1 redistribution in neurons and astrocytes of the lesion boundary zone; the specific TAK1 inhibitor OZ suppressed the expression of TAK1 and p-TAK1 in a dose-dependent manner, significantly reduced the activation of AP-1 and NF-κB, and reduced the elevation of IL-1β and TNF-α following TBI; consequently, OZ effectively reduced apoptotic cell death in the lesion boundary zone. Finally, OZ treatment 10 min or 3 h post TBI significantly improved rat cortical neuron survival and functional outcome at 7 d post-injury.

After documenting the activation of TAK1 following TBI, we explored the relevance of this pathway in the pathogenesis of secondary brain injury. Expansion of brain damage into the surrounding healthy tissue occurs 6–24 h after the initial impact (Zweckberger et al., 2006), and the secondary brain damage reaches its maximum extent at 24 h post TBI (Zweckberger et al., 2003). NF-κB binding activity in the part of the brain adjacent to the injured site increased significantly by 24 h after TBI, which contributes to the post-injury inflammatory gene expression and thus brain damage (Hang et al., 2004, 2005). Furthermore, our previous study showed that concentrations of TNF-α and IL-1β in the injured brain were significantly increased by 6 h after TBI with a maximum on 24 h post-injury (Li et al., 2011). Based on the above data, we chose 24 h as a time
point to perform biochemical analysis and evaluate behavioral outcomes following TBI with/without OZ treatment.

Accumulating evidences showed that pharmacological strategies aiming at reducing the activity of NF-kB attenuated tissue damage in models of brain trauma (Chen et al., 2007a, 2008a). Attenuation of AP-1 activation was associated with a more favorable outcome in animal models of acute CNS insults including focal ischemia (Dai et al., 1999), spinal cord injury (Xu et al., 2001) and surgical TBI (Beni et al., 2004). In view of the important role of NF-kB and AP-1 signaling pathways in neuronal apoptosis following TBI, it is interesting to note that they share a common upstream activator TAK1 (Ninomiya-Tsuji et al., 1999a; Takaesu et al., 2003; Dey et al., 2011). Thus, the blockade of both TAK1/NF-kB and AP-1 activation may provide a more effective means to reduce secondary neuronal damage after TBI. Our data confirm that ICV administration of the TAK1 inhibitor resulted in blockade of the NF-kB and AP-1 signaling pathway to protect neurons from apoptosis.

Besides the role of NF-kB and AP-1 signaling pathways in apoptosis, both pathways are also important in inflammation (Hailer et al., 2005; Chen et al., 2009; Shojo et al., 2010). Maas pharmacological intervention targeting at reducing cerebral inflammation could ameliorate secondary brain damage (Chen et al., 2007b, 2008b; Lloyd et al., 2008). Targeting inflammation is, therefore, a promising approach to prevent secondary post-traumatic brain damage. TAK1 has become one of the major target kinases in inflammatory disorders (Ninomiya-Tsuji et al., 2003; Omori et al., 2006; Kajino-Sakamoto et al., 2008; Ma et al., 2011). In this study, the results demonstrated that TAK1 inhibition was associated with downregulation of inflammatory cytokines TNF-α and IL-1β, which are regulated by AP-1 and NF-kB (Lee et al., 2003; Hang et al., 2005; Serrkolla and Hurme, 2005). The revealed protective effect of OZ against brain injury is likely attributable, at least in part, to its anti-inflammatory properties.

Taken together, we propose a mechanism that TAK1 might function as a key regulator of neuronal apoptosis, as reported by Neubert et al. (2011), and inflammatory injury in response to TNF-α and IL-1β combined stimuli released by glias both through activating AP-1 and NF-kB signaling pathways. In addition, TAK1 also plays a key role in transactivating genes encoding matrix metalloproteinase-1 (MMP-1), MMP-9, cox-2 and iNOS (Hou et al., 2007; Safina et al., 2007). Accordingly, the mechanisms of OZ-induced neuro-protection in the rat brain may be far more complicated than the down-regulation of TNF-α and IL-1β release, thus the potential mechanisms in neuroprotection remain to be further investigated.

### CONCLUSION

In conclusion, this is the first time, to our knowledge, to report that TAK1 is upregulated after TBI and the inhibition of TAK1 can protect neurons from apoptosis. Although more work is required to determine the role of other potential downstream targets of TAK1 in TBI, current data provide a potential rationale for manipulating this pathway in clinical practice and support OZ as a possible therapeutic agent in the treatment of TBI.

Acknowledgements—We would like to thank Dr. Zong Zhuang, Jiawei Wang and Lin Zhu for their technical assistance. This study was supported by the National Natural Science Foundation, China (No. 81171170) and the Nature Science Foundation of the Jiangsu Province, China (BK2010459).

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