Astrocytic p-connexin 43 regulates neuronal autophagy in the hippocampus following traumatic brain injury in rats

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Abstract. Gap junctions are conductive channels formed by membrane proteins termed connexins, which permit the intercellular exchange of metabolites, ions and small molecules. Previous data demonstrated that traumatic brain injury (TBI) activates autophagy and increases microtubule-associated protein 1 light chain 3 (LC3) immunostaining predominantly in neurons. Although previous studies have identified several extracellular factors that modulate LC3 expression, knowledge of the regulatory network controlling LC3 in health and disease remains incomplete. The aim of the present study was to assess whether gap junctions control the *in vivo* expression of LC3 in TBI. Using a modified weight-drop device, adult male Sprague-Dawley rats (weight, 350-375 g) were subjected to TBI. Phosphorylated gap junction protein levels and LC3-II levels were quantified using western blot analysis. The spatial distribution of immunoreactivity for phosphorylated connexin 43 (p-CX43) and LC3-II was analyzed by immunofluorescence. The results showed that p-CX43 expression in the hippocampus reached a maximum level 6 h following injury. In addition, the immunoreactivity of p-CX43 was localized in the astrocytes surrounding pyramidal neurons. The LC3-II protein content remained at high levels 24 h following injury. Double immunolabeling demonstrated that LC3-II dots colocalized with the hippocampus pyramidal neurons. Furthermore, inhibition of p-CX43 reduced TBI-induced autophagy, according to western blot analysis. As astrocytic gap junction coupling is affected in various forms of brain injury, the results suggest that point gap junctions/connexins are important regulators of autophagy in the hippocampal neurons following TBI.

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Abbreviations: CX43, connexin 43; CBX, carbenoxolone; TBI, traumatic brain injury; LC3, light chain 3; GFAP, glia fibrillary acidic protein; NeuN, neuronal nuclei; GJIC, gap junctional intercellular communication

Key words: CX43, CBX, autophagy, LC3, TBI

Introduction

Traumatic brain injury (TBI) is the leading cause of mortality in the young aged population and is a predominant reason for hospital admission in modern life (1). Mechanical disruption of neurons triggers a cascade of events leading to neuronal cell death following TBI (2). Apoptosis has been attributed to programmed neuronal cell death in TBI (3). Notably, previous studies have also shown that autophagy is increased following TBI (4). Autophagy is an evolutionarily conserved pathway that leads to the degradation of proteins and entire organelles in cells undergoing stress (5). The increased LC3 immunostaining was located predominantly in neurons 24 h post-TBI (6). However, few studies have addressed how the autophagy pathway is regulated following traumatic damage.

Previous studies demonstrated that astrocytes release ATP, at least in part, by opening connexin 43 (CX43) hemichannels (7). Connexins are a family of proteins with dual channel functions (8) involved in forming gap junctions, which are composed of two docked hemichannels linking the cytosol of two neighboring cells. Gap junctions allow cell-to-cell passage of ions and small molecules, including Ca^{2+}, cyclic adenosine monophosphate, inositol triphosphate, ATP, glutamate and glucose. In addition, the intracellular condition and phosphorylation state of Cx affect the intercellular permeability via gap junctions (9). Furthermore, TBI results in the rapid loss of astrocytes and thereafter induces reactive astrocytes in the hippocampus (10). Glial fibrillary acidic protein (GFAP)-positive astrocytes exist more extensively in $CX43^{+/+}$ than in $CX43^{+/-}$ mice.

Gap junctions were shown to provide neurons, through astrocytic gap junction channels, with energy-producing compounds, such as ATP, glucose, glucose-6-phosphate and lactate (11). Conversely, gap junctions also propagate death signals in astrocytic networks, which may affect neuronal fate (12). Based on the results of previous studies, it was hypothesized that CX43 regulates autophagy in TBI-induced damage. To confirm this hypothesis, using the relatively

selective CX43 inhibitor, the present study aimed to determine whether astrocytic gap junction-dependent modulation of neuronic LC3 expression occurs under TBI conditions.

Materials and methods

Animals and TBI model. All experimental procedures were conducted in accordance with the guidelines of the Chinese Council on Animal Protection and were approved by the Hebei Medical University Animal Care and Use Committee (Hebei, China). A total of 126 male Sprague-Dawley rats (age, 12-16 weeks; weight, 350-375 g; Tangshan, China) were used in the present study. The rats were housed with a standard of 12 h light/dark cycle and access to water and food *ad libitum* prior to and following surgery or sham operation. The rat model of TBI was induced using a modified weight-drop device (Tangshan Railway Vehicle Co., Ltd., Tangshan, China), as described previously by Marmarou *et al* (13). Briefly, the rats were anaesthetised with sodium pentobarbital (Nembutal 60 mg/kg). A midline incision was made to expose the skull between bregma and lambda suture lines and a steel disc (10 mm in diameter and 3 mm in thickness) was adhered to the skull using dental acrylic (Tangshan Railway Vehicle Co., Ltd., Tangshan, China). Animals were moved onto a foam mattress underneath a weight-drop device where a weight of 450 g fell freely through a vertical tube from 1.5 m onto the steel disc. Sham-operated animals underwent the same surgical procedure without weight-drop impact. Rats were housed in individual cages following surgery and placed on heat pads (37˚C) for 24 h to maintain normal body temperature during the recovery period.

Groups and drug administration. The rats were randomly divided into the sham, TBI and TBI treated with carbenoxolone (CBX) groups. Each sub-group was composed of five rats and the rats were killed 3, 6, 24 or 48 h following TBI. CBX (50 μg/kg body mass; Sigma-Aldrich, Yorba Linda, CA, USA) was administered by right ventricle injection 30 min prior to sham operation or TBI induction (14). For intracerebroventricular injection, the animals were fixed in a stereotaxic apparatus (RWD68025; RWD Life Science Co., Ltd., Shenzhen, China), a midline incision was made in the skin and a small hole was induced in the cranial region. Using a Hamilton syringe (RWD62201; RWD Life science Co., Ltd.), CBX in 5 μl saline was injected into the right cerebral ventricle according to the following coordinates: bregma: AP -0.8 mm, L +1.6 mm (midline) and deep 3.4 mm form dura (15).

Western blot analysis. Western blot analysis was conducted as described previously (16). Briefly, the rats were deeply anesthetized and underwent an intracardiac perfusion with 0.1 mol/l phosphate-buffered saline (PBS; pH 7.4). The hippocampal CA1 was rapidly isolated, total proteins were extracted and the protein concentration was determined by the bicinchoninic acid reagent (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) method. Samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis. Separated proteins on the gel were transferred onto polyvinylidene fluoride membranes (Roche Diagnostics, Mannheim, Germany). Blots were blocked with 5% fat-free dry milk for 1 h at room temperature. Subsequently, blots were incubated overnight at 4˚C with the following primary antibodies: Rabbit anti-p-CX43 polyclonal antibodies, rabbit anti-LC3 polyclonal antibody and mouse anti-β-actin monoclonal antibody (dilution, 1:500; Santa Cruz Biotechnology, Inc.). The blots were then incubated with horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG (dilution,1:5000; Cell Signaling Technology, Inc., Danvers, MA, USA) for 2 h at room temperature. Subsequent to incubation with a properly titrated secondary antibody, the immunoblot on the membrane was visible following development with an enhanced chemiluminescence (ECL) detection system (ChemiDoc XRS; Bio-Rad, Hercules, CA, USA) and the densitometric signals were quantified using an imaging program (Image Lab 4.1; Bio-Rad). Immunoreactive bands of the protein expression were normalized to the intensity of the corresponding bands for β-actin. The western blot analysis results were analyzed with National Institutes of Health Image 1.41 software (Bethesda, MD, USA).

Immunofluorescence analyses. The brain tissues were fixed in 4% paraformaldehyde for 24 h, and transferred to a 30% sucrose solution (0.1 M PBS, pH 7.4). When the tissues had sunk to the bottom of the solution, sections, 200 μm apart from anterior to posterior hippocampus (bregma -1.90 to -3.00) were made from TBI animal and embedded in OCT. Frozen sections (15 μm) were sliced with a frozen slicer, treated with 0.4% Triton X-100 for 10 min, and blocked in normal donkey serum for 1 h. For double labeling, the frozen sections were incubated with a mixture of rabbit anti-p-CX43 polyclonal antibody (dilution, 1:100) and mouse anti-GFAP monoclonal antibody (dilution, 1:100; Santa Cruz Biotechnology), or rabbit anti-LC3 polyclonal antibody (dilution, 1:100) and mouse anti-neuronal nuclei (NeuN; dilution, 1:100; Santa Cruz Biotechnology) overnight at 4˚C. The following day, the sections were incubated with a mixture of fluorescein-conjugated anti-rabbit IgG and anti-mouse IgG (dilution 1:1000; Santa Cruz Biotechnology) for 2 h at 37˚C in the dark. Images were captured in a laser scanning confocal microscope (Olympus Fluoview™ FV1000; Olympus, Tokyo Japan). Primary antibodies were replaced with PBS in the negative control group.

Statistical analysis. Experiments were repeated three times and similar results were obtained. Statistical analysis was performed using the SPSS 16.0 statistics software (SPSS, Chicago, IL, USA). Data were expressed as the mean ± SE. Statistical analysis was performed using analysis of variance, followed by the Student-Newman-Keuls post hoc tests or Student's t-test (two means comparison). $P<0.05$ was considered to indicate a statistically significant difference.

Results

General. There were no significant differences in body weight or temperature between the TBI and sham-injured groups, and no differences in injury levels among the 3-, 6-, 24- or 48-h TBI groups.

P-CX43 colocalizes with astrocyte markers. To assess the effect of connexins on astrocytic gap junctions evoked by

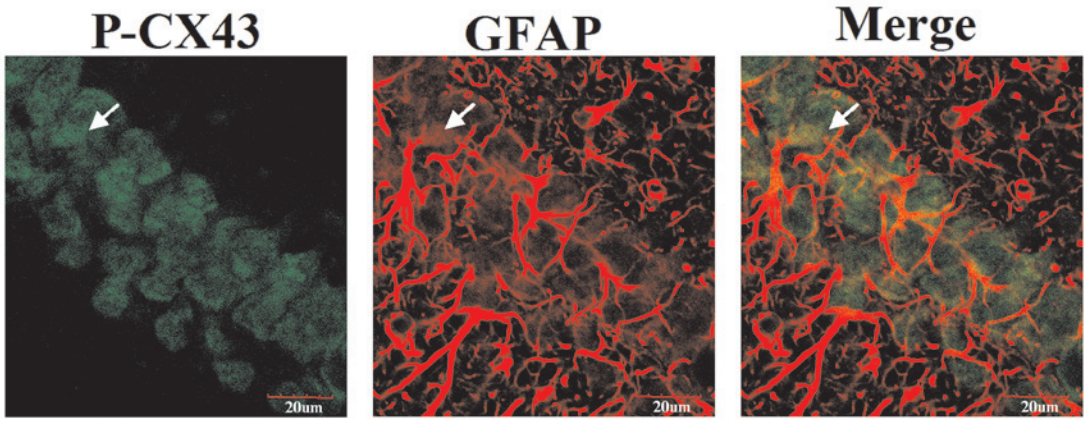

Figure 1. Phosphorylated-conncexin 43 (P-CX43) colocalizes with astrocyte glial fibrillary acidic protein (GFAP). Corresponding immunofluorescence images obtained using anti-p-CX43 and anti-GFAP antibodies in hippocampal astrocytes 6 h after traumatic brain injury. The overlap is shown between anti-p-CX43 and anti-GFAP immunoreactivities. Arrows show cytoplasmic p-CX43 immunoreactive structures and GFAP immunoreactivity at the cytoplasm is indicated by arrowheads. All microphotographs were visualized by confocal laser scanning microscopy. Scale bar, 20 μm.

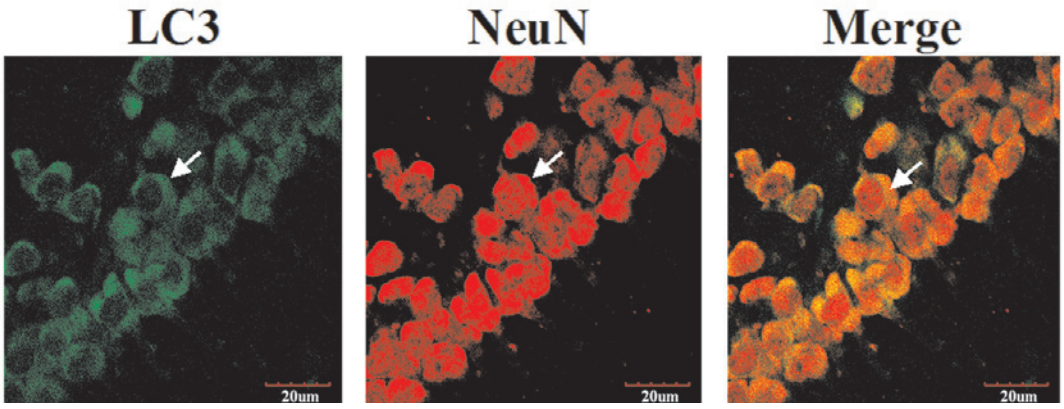

Figure 2. Confocal images of microtubule-associated protein 1 light chain 3 (LC3) and neuronal nuclei (NeuN). LC3 immunoreactivity (green) was present in NeuN-positive cells (red) 24 h following traumatic brain injury. Overlay of LC3 (green) and NeuN (red) showed specificity of LC3 for neuron cells. Arrows show ring- or cup-shaped LC3-immunopositive structures and NeuN immunoreactivity in the hippocampal neurons is indicated by arrowheads. The microphotographs were visualized by confocal laser scanning microscopy. Scale bar, 20 μm.

TBI, double immunofluorescence staining was performed to investigate co-localization of p-CX43 and GFAP expression. As shown in Fig. 1, p-CX43 was stained with rabbit anti-p-CX43 antibody and secondary antibodies labeled with green fluorescence. Astrocytes were stained with mouse anti-GFAP antibodies and secondary antibody labeled with red fluorescence. The images were merged and yellow was observed under a laser scanning confocal microscope. These results suggest that the majority of p-CX43 colocalizes with astrocytes.

LC3 colocalizes with neuron markers. Experiments were performed to investigate the involvement of autophagy in TBI-induced brain damage. LC3 expression in hippocampal neurons 24 h following TBI was detected using immunohistochemistry and confocal microscopy (indicated by arrows, Fig. 2). As shown in Fig. 2, LC3 was stained with rabbit anti-LC3 antibody and secondary antibodies labeled with green fluorescence. The LC3-immunoreactive structures appeared cup-shaped or circular, which may reflect the different stages of autophagosome formation (the isolation of membranes prior to and following closure to form autophagosomal vesicles). In addition, neurons were stained with mouse anti-NeuN antibody and secondary antibody labeled with red fluorescence. The images were merged and yellow was observed under a laser scanning confocal microscope. These results clarified alterations in the LC3 proteins in neurons in the hippocampal region following TBI.

CBX treatments suppress p-CX43 protein expression. P-CX43 protein expression was analyzed by western blot analysis (Fig. 3A). The p-CX43 protein expression was identified at low levels in the hippocampus in the sham group. The immunoreactivity of p-CX43 in the hippocampus was significantly induced 3 h following injury, persisted at a high level until 24 h after injury and thereafter, gradually decreased. In addition, the p-CX43 protein content reached a maximum level 6 h following injury. As demonstrated in Fig. 3B, the p-CX43 protein band intensity was quantified and the results demonstrated that CBX pretreatment significantly inhibited the upregulation of p-CX43 protein levels compared with that of the TBI groups at 3, 6 and 24 h.

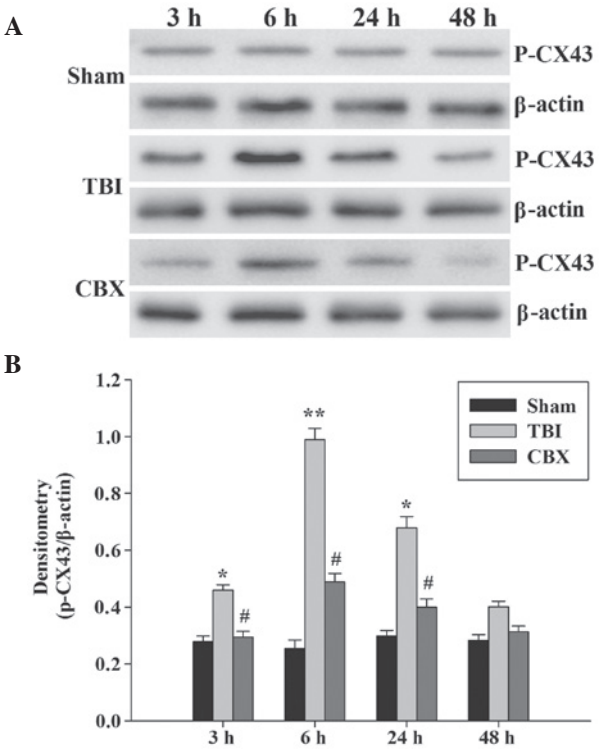

Figure 3. Pretreatment with carbenoxolone (CBX) prevented traumatic brain injury (TBI)-induced p-CX43 activation. Animals were sacrificed at various time points following TBI as indicated. Extracts from injured hippocampus were separated on sodium-dodecyl sulfate polyacrylamide gel electrophoresis. (A) Western blot analysis of p-CX43 in the hippocampus. (B) Densitometry analysis of p-CX43 band corresponding to β-actin. Optical densities of respective protein bands were analyzed with ImageJ. The quantitative results of p-CX43 protein are expressed as the mean of the ratio of densitometries of p-CX43 to β-actin bands ±standard error for 5 different experiments. Results demonstrated that p-CX43 protein increased markedly at 3, 6 and 24 h following TBI (*P<0.05 and **P<0.01 vs. sham group). Treatment with CBX significantly decreased the level of p-CX43 protein expression at 3, 6 and 24 h following TBI. $^{\#}$P<0.05 vs. the TBI group at the same time point).

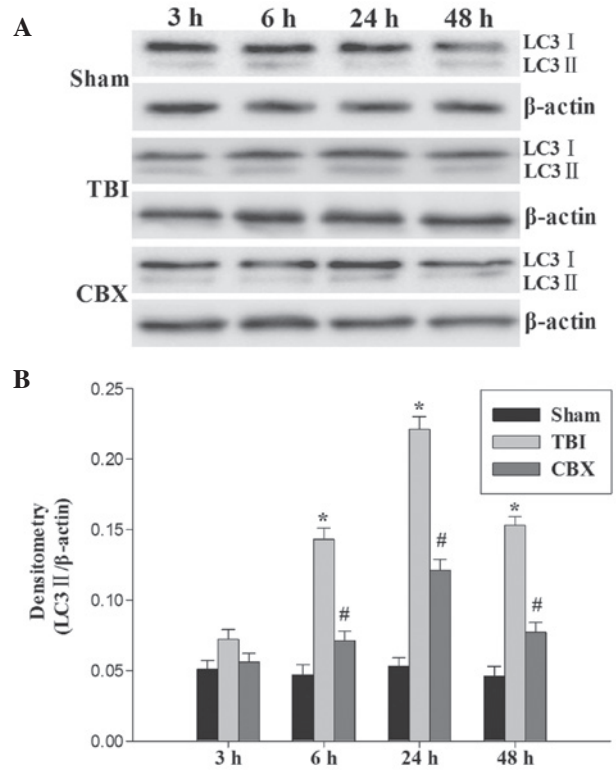

Figure 4. Pretreatment with phosphorylated-carbenoxolone (p-CBX) prevented traumatic brain injury (TBI)-induced microtubule-associated protein 1 light chain 3 (LC3)-II activation. Protein levels of LC3-II in the hippocampus were detected with (A) immunoblotting and (B) densitometry analysis of p-CX43 band corresponding to β-actin. Results demonstrated that LC3 protein levels increased markedly at 6, 24 and 48 h following TBI. Pretreatment with CBX resulted in marked reduction of LC3-II expression. Data are expressed as the mean ± SE (n=5). $^{\#}$P<0.05 vs. the TBI group at the same time point; *P<0.05 vs. sham group.

CBX treatments suppress LC3-II protein expression. To determine how autophagic activity is altered following TBI and to confirm the ability of CBX to inhibit autophagy, the protein levels of LC3-II were determined (Fig. 4A). The expression of LC3-II protein in the hippocampus was significantly upregulated 6 h following TBI and persisted at a high level until 48 h following injury. As demonstrated in Fig. 4B, pretreatment with CBX significantly reduced the relative protein expression of LC3-II in the hippocampus.

Discussion

Management of traumatic brain injury poses considerable challenges to healthcare services (17). Brain injury may result in an energy crisis and oxidative stress, which induces cell death (18). Cell death is broadly classified into three types: necrosis, apoptosis [type 1 programmed cell death (PCD)] and autophagy (type 2 PCD) (19). Autophagy is a process that is regulated and is key in numerous diseases (20-22). TBI causes pathophysiologic responses leading to autophagy activation, cell membrane breakdown, cell loss and motor and cognitive outcome deficits. Pretreatment with 3-methyladenine (3-MA), a relatively selective autophagy inbihitor, attenuates TBI-induced cell death, lesion volume and behavioral outcome deficits, thereby indicating that inhibition of autophagy may be a therapeutic target for TBI treatment (23). However, the way in which to manipulate the autophagy pathway is not clear, therefore, the probable regulatory mechanism of autophagy in TBI has been hypothesized based on the studies.

Previous studies using LC3 as an autophagic biomarker showed that autophagy is detected in the human brain following trauma and critical illness (24). LC3, an autophagosomal ortholog of yeast Atg8, is one of the most reliable markers in the study of autophagy induction (25). LC3 is synthesized as pro-LC3, which is cleaved by ATG4 protease to form the 16-18 kDa LC3-I. On activation of autophagy, LC3-I is conjugated with phosphatidylethanolamine (lipidated). The lipidated form is referred to as LC3-II (26). Another study has demonstrated autophagosomal vacuole formation by the observation of a shift from LC3-I to -II in hippocampal neurons following TBI and has demonstrated a marked increase in LC3-II levels from 1 to 48 h post-TBI. Pretreatment with a specific autophagy inhibitor 3-MA partially inhibited traumatic-elicited induction of LC3-II (23). Previous studies have demonstrated that astrocytes, the predominant cell type in the brain, receive signals from neurons and also release neuroactive substances (27), provide energy substrates to neurons (28) and are important in neuronal support in normal and pathological conditions. In the central nervous system, astrocytes established a glial syncytium through intercellular connections via gap junctions (29). CX43 is the primary component protein in astrocytic gap junctions (30). Gap junctional intercellular communication (GJIC) mediates electronic coupling and permits rapid propagation among cell networks (31). GJIC between astrocytes may regulate the concentration of extracellular K^+ and distribute neurotransmitters (32). Certain studies have suggested that astroglial cells may participate in neuronal apoptosis through their gap junctions under ischemic conditions (33). However, no studies have focused on astrocytic gap junction-dependent modulation of neuronal autophagy following TBI *in vivo*.

In the present study, coupling of labeled LC3 with NeuN and p-CX43 with GFAP was conducted to demonstrate the correlation between p-CX43 and autophagy following TBI. P-CX43 was colocalized with GFAP immunoreactivity in the hippocampal astrocytes (Fig. 1). Thus, p-CX43 is expressed by astrocytes. LC3 immunoreactivity was located predominantly in living hippocampal neurons under confocal microscopy (Fig. 2). Autophagy may occur in cells other than astrocytes, particularly in neurons. Astrocytes may communicate through their gap junctions and be able to affect neurons (27). In the present study, astrocytic gap junction proteins were phosphorylated in the hippocampus following TBI. An increase in p-CX43 protein expression was observed in the hippocampus of injured brain at 3, 6 and 24 h, with peak relative abundance at 6 h (Fig. 3). These results suggested that phosphorylation of CX43 induces cell injury in the post-traumatic region. Therefore, it was hypothesized that phosphorylation of CX43 may contribute to hippocampal dysfunction through astrocytic gap junction communication in the early phases following TBI. The results also indicated that TBI activates autophagy, which may begin at 6 h or earlier, and lasts at least 48 h in the hippocampus following TBI (Fig. 4). This result is concurrent with that of previous studies (23). Carbenoxolone is related to glycyrrhetinic acid and is thought to bind directly to connexins, inducing a conformational change and results in a closure of gap junctions (34). Pretreatment with a specific CX43 inhibitor, CBX, partially inhibited traumatic-elicited induction of p-CX43 (Fig. 3). The results of the present study demonstrated that inhibition of p-CX43 suppressed TBI-induced autophagy (determined by the expression of LC3-II detected by western blot analysis) (Fig. 4). Thus, this identified astrocytic gap junctions/connexins as part of the regulatory network controlling *in vivo* autophagy in neurons of the hippocampus.

Astrocytes exchange inositol 1,4,5-triphosphate, lactate, glutamate and other smaller molecules through gap junctions and provide energy substrates such as ATP to neurons (35). It is well known that astrocytes also modulate extracellular glutamate concentrations, thereby contributing to extracellular neurotransmitter homeostasis and astrocyte-neuron signaling (36). Disturbance of extracellular glutamate levels, acting on N-methyl-D-aspartate receptors, is a primary cause of neuronal cell death following acute damage, which is observed following stroke, trauma and seizure (37). Furthermore, Rami *et al* (38) demonstrated that the inhibition of gap junction permeability effectively decreases neuronal death. A large number of intracellular/extracellular stimuli, including amino acid starvation and invasion of microorganisms, are able to induce the autophagic response (39). The autophagy pathway triggers a cascade of events leading to tissue edema, neuronal cell death and impaired motor and cognitive functions following TBI (23). Elucidation of the molecular mechanisms by which astrocytic gap junctions regulate neuronic autophagy is an important area of investigation and aims to develop novel therapeutic interventions for the prevention of autophagy formation following traumatic brain injury.

In conclusion, the correlation between gap junctions and autophagy following TBI was shown and it was determined that astrocytic gap junctions/connexins act as a regulatory factor controlling neuronal autophagy in the hippocampus. As

connexin expression and/or astrocytic gap junction coupling is affected in various forms of brain injury (40), this regulatory mechanism may be prominent in the diseased brain.

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References

1. Mammis A, McIntosh TK and Maniker AH: Erythropoietin as a neuroprotective agent in traumatic brain injury Review. Surgical Neurol 71: 527-531, 2009.
2. Luo CL, Chen XP, Yang R, *et al*: Cathepsin B contributes to traumatic brain injury-induced cell death through a mitochondria-mediated apoptotic pathway. J Neurosci Res 88: 2847-2858, 2010.
3. Tehranian R, Rose ME, Vagni V, *et al*: Disruption of Bax protein prevents neuronal cell death but produces cognitive impairment in mice following traumatic brain injury. J Neurotrauma 25: 755-767, 2008.
4. Lai Y, Hickey RW, Chen Y, *et al:* Autophagy is increased after traumatic brain injury in mice and is partially inhibited by the antioxidant gamma-glutamylcysteinyl ethyl ester. J Cereb Blood Flow Metab 28: 540-550, 2008.
5. Pozuelo-Rubio M: Regulation of autophagic activity by 14-3-3ζ proteins associated with class III phosphatidylinositol-3-kinase. Cell Death Differ 18: 479-492, 2011.
6. Liu CL, Chen S, Dietrich D and Hu BR: Changes in autophagy after traumatic brain injury. J Cereb Blood Flow Metab 28: 674-683, 2008.
7. Cotrina ML and Nedergaard M: Physiological and pathological functions of P2X7 receptor in the spinal cord. Purinergic Signal 5: 223-232, 2009.
8. Bennett MV, Contreras JE, Bukauskas FF and Sáez JC: New roles for astrocytes: gap junction hemichannels have something to communicate. Trends Neurosci 26: 610-617, 2003.
9. Cottrell GT, Lin R, Warn-Cramer BJ, Lau AF and Burt JM: Mechanism of v-Src-and mitogen-activated protein kinase-induced reduction of gap junction communication. Am J Physiol Cell Physiol 284: C511-C520, 2003.
10. Zhao X, Ahram A, Berman RF, Muizelaar JP and Lyeth BG: Early loss of astrocytes after experimental traumatic brain injury. Glia 44: 140-152, 2003.
11. Giaume C, Tabernero A and Medina JM: Metabolic trafficking through astrocytic gap junctions. Glia 21: 114-123, 1997.
12. Lin JH, Weigel H, Cotrina ML, *et al*: Gap-junction-mediated propagation and amplification of cell injury. Nature Neurosci 1: 494-500, 1998.
13. Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H and Demetriadou K: A new model of diffuse brain injury in rats. J Neurosurg 80: 291-300, 1994.
14. Khorasani MZ, Hosseinzadeh SA and Vakili A: Effect of central microinjection of carbenoxolone in an experimental model of focal cerebral ischemia. Pak J Pharm Sci 22: 349-354, 2009.
15. Milad MR, Vidal-Gonzalez I and Quirk GJ: Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner. Behav Neurosci 118: 389-394, 2004.
16. Song SX, Gao JL, Wang KJ, *et al*: Attenuation of brain edema and spatial learning deficits by the inhibition of NADPH oxidase activity using apocynin following diffuse traumatic brain injury in rats. Mol Med Rep: 327-331, 2012.
17. Eghwrudjakpor PO and Allison AB: Oxidative stress following traumatic brain injury: enhancement of endogenous antioxidant defense systems and the promise of improved outcome. Niger J Med 19: 14-21, 2010.
18. Uryu K, Laurer H, McIntosh T, *et al*: Repetitive mild brain trauma accelerates Abeta deposition, lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. J Neurosci 22: 446-454, 2002.
19. Werner C and Engelhard K: Pathophysiology of traumatic brain injury. Br J Anaesth 99: 4-9, 2007.
20. Geng J and Klionsky DJ: The Atg8 and Atg12 ubiquitin-like conjugation systems in macroautophagy. 'Protein modifications: beyond the usual suspects' review series. EMBO Rep 9: 859-864, 2008.
21. Mizushima N, Levine B, Cuervo AM and Klionsky DJ: Autophagy fights disease through cellular self-digestion. Nature 451: 1069-1075, 2008.
22. Meijer AJ and Codogno P: Autophagy: regulation and role in disease. Crit Rev Clin Lab Sci 46: 210-240, 2009.
23. Luo CL, Li BX, Li QQ, *et al*: Autophagy is involved in traumatic brain injury-induced cell death and contributes to functional outcome deficits in mice. Neuroscience 184: 54-63, 2011.
24. Clark RS, Bayir H, Chu CT, Alber SM, Kochanek PM and Watkins SC: Autophagy is increased in mice after traumatic brain injury and is detectable in human brain after trauma and critical illness. Autophagy 4: 88-90, 2008.
25. Maiuri MC, Criollo A, Tasdemir E, *et al*: BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). Autophagy 3: 374-376, 2007.
26. Kabeya Y, Mizushima N, Yamamoto A, Oshitani-Okamoto S, Ohsumi Y and Yoshimori T: LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. J Cell Sci 117: 2805-2812, 2004.
27. Araque A, Parpura V, Sanzgiri RP and Haydon PG: Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 22: 208-215, 1999.
28. Tsacopoulos M and Magistretti PJ: Metabolic coupling between glia and neurons. J Neurosci 16: 877-885, 1996.
29. Dermietzel R and Spray DC: From neuro-glue ('Nervenkitt') to glia: a prologue. Glia 24: 1-7, 1998.
30. Giaume C, Fromaget C, el Aoumari A, Cordier J, Glowinski J and Gros D: Gap junctions in cultured astrocytes: single-channel currents and characterization of channel-forming protein. Neuron 6: 133-143, 1991.
31. Paul DL: New functions for gap junctions. Curr Opin Cell Biol 7: 665-672, 1995.
32. Hansson E, Muyderman H, Leonova J, *et al*: Astroglia and glutamate in physiology and pathology: aspects on glutamate transport, glutamate-induced cell swelling and gap-junction communication. Neurochem Int 37: 317-329, 2000.
33. Nakase T, Fushiki S and Naus CC: Astrocytic gap junctions composed of connexin 43 reduce apoptotic neuronal damage in cerebral ischemia. Stroke 34: 1987-1993, 2003.
34. Rozental R, Srinivas M and Spray DC: How to close a gap junction channel. Efficacies and potencies of uncoupling agents. Methods Mol Biol 154: 447-476, 2001.
35. Magistretti PJ: Cellular bases of functional brain imaging: insights from neuron-glia metabolic coupling. Brain Res 886: 108-112, 2000.
36. Anderson CM and Swanson RA: Astrocyte glutamate transport: review of properties, regulation, and physiological functions. Glia 32: 1-14, 2000.
37. Hardingham GE and Bading H: The Yin and Yang of NMDA receptor signalling. Trends Neurosci 26: 81-89, 2003.
38. Rami A, Volkmann T and Winckler J: Effective reduction of neuronal death by inhibiting gap junctional intercellular communication in a rodent model of global transient cerebral ischemia. Exp Neurol 170: 297-304, 2001.
39. Zhang YB, Li SX, Chen XP, *et al*: Autophagy is activated and might protect neurons from degeneration after traumatic brain injury. Neurosci Bull 24: 143-149, 2008.
40. Chew SS, Johnson CS, Green CR and Danesh-Meyer HV: Role of connexin43 in central nervous system injury. Exp Neurol 225: 250-261, 2010.