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Original Article

Gap junctional intercellular communication dysfunction mediates the cognitive impairment induced by cerebral ischemia-reperfusion injury: PI3K/Akt pathway involved

Shujun Zhou[1*], Zheng Fang[2*], Gui Wang[1], Song Wu[2]

[1]Department of Critical Care Medicine, The Third Affiliated Hospital of Soochow University, The First People's Hospital of Changzhou, Changzhou, Jiangsu, People's Republic of China; [2]Emergency Department, The Third Affiliated Hospital of Soochow University, The First People's Hospital of Changzhou, Changzhou, Jiangsu, People's Republic of China. [*]Equal contributors.

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Abstract: Purpose: Cerebral ischemia/reperfusion (I/R) injury causes hippocampal apoptosis and cognitive impairment, and the dysfunction of gap junction intercellular communication (GJIC) may contribute to the cognitive impairment. We aim to examine the impact of cerebral I/R injury on cognitive impairment, the role of GJIC dysfunction in the rat hippocampus and the involvement of the phosphatidylinositol 3 kinase (PI3K)/protein kinase B (Akt) pathway. Methods: Rats were subjected to a cerebral I/R procedure and underwent cognitive assessment with the novel object recognition and Morris Water Maze tasks. The distance of Lucifer Yellow dye transfer and the Cx43 protein were examined to measure GJIC. Neural apoptosis was assessed with the terminal deoxynucleotide-transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) method. After rats received inhibitors of the PI3K/Akt pathway, GJIC and cognitive ability were measured again. Results: GJIC promotion by ZP123 significantly reversed cognitive impairment and hippocampal apoptosis induced by cerebral I/R, while the inhibition of GJIC by octanol significantly facilitated cognitive impairment and hippocampal apoptosis. The phosphorylation of Akt was enhanced by cerebral I/R and octanol but inhibited by ZP123. The inhibition of the PI3K/Akt pathway significantly suppressed GJIC and cognitive impairment. Conclusion: The PI3K/Akt pathway is involved in cognitive impairment caused by gap junctional communication dysfunction in the rat hippocampus after ischemia-reperfusion injury.

Keywords: PI3K/Akt pathway, cognitive impairment, gap junctional communication, hippocampus, ischemia-reperfusion injury

Introduction

Cerebral ischemic disease has become the primary factor in disability and the leading cause of death along with cardiovascular accidents and cancer [1, 2]. Transient focal cerebral ischemia refers to regional cerebral blood circulation disorders. The recovery of blood supply to the ischemia sites as soon as possible could reduce the infarct volume and alleviate cognitive dysfunction, but it may cause additional reperfusion injury, namely, ischemia/reperfusion injury. Previous studies demonstrated that the mechanism of ischemia/reperfusion injury was mainly related to oxidative stress, free radical damage, excitatory amino acid toxicity, inflammation, calcium overload and cell apoptosis [3-5]. Searching for tools to combat ischemia/reperfusion injury has been a popular subject in recent years, but no effective tool is available yet.

Intercellular gap junctions are widely present in organs, including heart, liver, skin, muscle and brain. These junctions provide a direct passage between cells, which is important to electrical and chemical signal transmission. It has been shown that gap junction activity plays an import role in cellular homeostasis maintenance, cell growth control and other life processes [6-8]. Gap junction intercellular communication (GJIC) allows the passage of second messenger molecules and other molecular ions smaller than 1.5 KD and directly mediates the exchange of

intercellular information. Large amounts of connexin-mostly connexin 43 (Cx43)-are expressed in astrocytes (AS) and form functional gap junctions [9-11]. Under normal circumstances, astrocytes spread intercellular calcium waves in neurons and protect neurons, but when cerebral ischemia/reperfusion injury occurs, the function of the gap junction is disturbed, leading to other consequences [12, 13].

Some evidence suggests that gap junctions may be involved in cognitive function. Friseh et al used Cx36 gene knockout rats and found that Cx36 gene deletion impaired sensorymotor and learning/memory abilities [14]. Hosseinzadeh et al observed that the application of the gap junction blocker carbenoxolone could impair the spatial learning ability of rats using the Morris Water Maze [15]. Although these studies failed to clarify how gap junction activity was involved in higher cognitive functions, they provided evidence of the important role of gap junctions in cognitive activities.

ZP123 is a novel gap junction channel modifier that is able to promote GJIC between cells [16]. Octanol is a long carbon chain n-alkanol that can inhibit the GJIC by selectively inhibiting Cx43 [17]. To investigate the role of gap junctional intercellular communication in cognitive impairment after ischemia-reperfusion injury, these two chemicals were used in this study. The involvement of the PI3K/Akt pathway in the regulation of GJIC has been reported by several studies, but the results were controversial. It was reported that the homotypic GJIC associated with metastasis suppression was unaffected by PI3K inhibition [18]. Tacheau, however, found that TGF-beta promoted Cx43 gene expression by the activation of the p38 and PI3K/Akt pathways [19]. Hence, in the present study, we first investigated the impact of cerebral ischemia-reperfusion injury on cognitive impairment, and then we examined the role of gap junctional communication dysfunction in the rat hippocampus. Finally, the involvement of PI3K/Akt pathway was investigated to explore the mechanism.

Materials and methods

Animal model preparation

Adult male Sprague-Dawley (SD) rats (250±20 g) were bought from the Shanghai Experimental Animal Center. Animal procedures were carried out following the Guidelines for Care and Use of Laboratory Animals. Rats were induced with cerebral I/R as previously described [20]. Animals were randomly divided into the Control, Sham, Cerebral I/R, LY294002, Triciribine, ZP123 and Octanol groups with 10 rats per group. All the chemicals were purchased form Sigma (St. Louis, MO). Rats in the Control group received no treatment, rats in the Sham group received a sham cerebral I/R surgery, the chemicals LY294002 (100 mg/kg, i.p.) or triciribine (2 mg/kg, i.p.) were given 30 minutes before the cerebral I/R in their respective groups, and the chemicals ZP123 and octanol was intracerebrally injected 30 minutes before the cerebral I/R according to the methods of Haugan et al and Bostanci et al [16, 21] in their respective groups. Cognitive performance was examined using the Morris Water Maze one week after the cerebral I/R procedure.

Cerebral I/R procedure

Similar to Jia et al [22], the rats were first injected with sodium pentobarbital (100 mg/kg, i.p.) to obtain full anesthesia. After a midline ventral incision was made in the neck of the rats, the right common carotid artery was exposed. A nylon suture (size: 4-0) with a blunted tip was prepared. The suture was carefully inserted into the anterior cerebral artery so the blood supply to the brain was blocked. Two h later, the 4-0 nylon suture was removed and blood flow was restored. Rats in the Sham group received a similar surgical procedure, but the filament was not actually inserted to block the blood supply.

Evaluation of novel object recognition

Twenty-four h after brain blood flow was restored, the rats were tested for novel object recognition with the method described in Zhang et al [23]. The experiment was divided into two sessions: the training session and the test session. In the training session, two of the same objects were placed in 2 opposite corners of the apparatus. The rats were exposed to them for 10 min, and 90 min after the training session was over, 1 familiar and 1 novel object was put in the open field. Animals were free to explore the open field with these two objects. The amount of time the rats explored the familiar and novel objects were recorded. Location preference = the amount of time the rats explored one of the identical objects/the

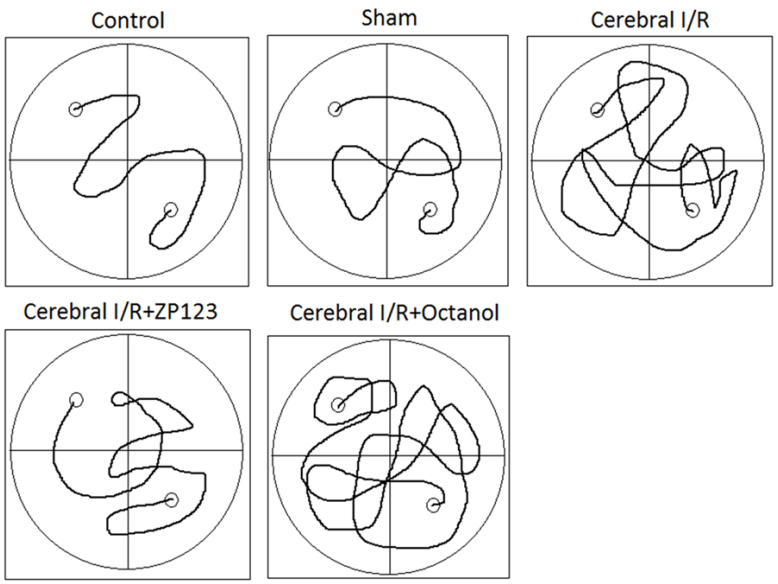

Figure 1. The travel path of rats in the Morris Water Maze. Rats in the Cerebral I/R group traveled longer than rats in the Control and Sham groups, while rats in the Cerebral I/R+ZP123 group traveled less distance than the Cerebral I/R group. When rats were treated with octanol, they traveled longer than the Cerebral I/R group. Control: rats received no treatment; Sham: rats received sham MCAO procedure. Cerebral I/R: Cerebral ischemia reperfusion injury.

amount of time the rats explored the identical object pairs × 100%. Recognition index (RI) = the amount of time the rats explored the novel object/(the amount of time the rats explored the novel object + the amount of time the rats explored the familiar object) × 100%.

Morris water maze

Seven days after the cerebral I/R procedure, rats were tested with the Morris Water Maze with the method described by Morris [24]. Rats were exposed to four trials per day. The escape latency was recorded as the time rats spent swimming from the start point to the target platform; the travel length was recorded as the distance rats traveled from the start point to the platform. Both parameters were consecutively recorded for five days. In the probe trials, the escape platform was removed after the acquisition period. One day later, the memory consolidation of the rats was tested. The numbers of platform-site crossovers within 1 min were recorded. More crossovers indicated better memory consolidation.

Measurement of GJIC

Twenty-four h after blood flow was restored, the measurement of GJIC was performed according to the method described by da Silva et al [25], which used Lucifer Yellow dye that can diffuse through gap junctions. Briefly, the hippocampal tissue was excised, submerged in oxygenated artificial cerebrospinal fluid (ACSF) at 25°C for 60 min and put in a glass tube. Four incisions were cut on the surface of each specimen. The fluorescent dyes were mixed with 0.5% LY and 0.5% RhD in PBS. At room temperature (25°C), the incisions were incubated with the fluorescent dye mixture for five minutes. After the incubation, the incisions were washed with PBS three times, then fixed in 10% formalin for 12 h. Slices were cut the next day and then observed and analyzed with a microscope (Nikon Eclipse-800, Japan). The net area stained with Lucifer Yellow dye and the length of the incision were recorded and then calculated with Image-Pro-Plus software (version 4.5, Media Cybernetics) to obtain the mean value. GJIC capacity was calculated from the ratio of the distance that the Lucifer Yellow dye transferred and the incision line (area/length). Values are expressed as a fraction compared to the control.

Western blot

Twenty-four h after blood flow was restored, the hippocampal tissue was collected. After centrifugation of hippocampal tissue, the supernatant was separated and stored at -80°C. The supernatants were first separated by SDS-PAGE and then transferred to PVDF at 100 V for two hours. After that, the membranes were blocked in 5% milk in PBS-Tween 20 at 25°C for 1 h and incubated at 4°C overnight with corresponding antibodies (anti-Cx43, anti-p-Akt, anti-Akt and anti-β-actin, Santa Cruz, CA, USA). Next, the membrane was rinsed with PBST, incubated with peroxidase-labelled secondary antibodies at 25°C for 2 h. Protein blots were detected with an enhanced chemiluminescence detection kit (Invitrogen, Carlsbad, CA, USA). The protein concentration was calculated

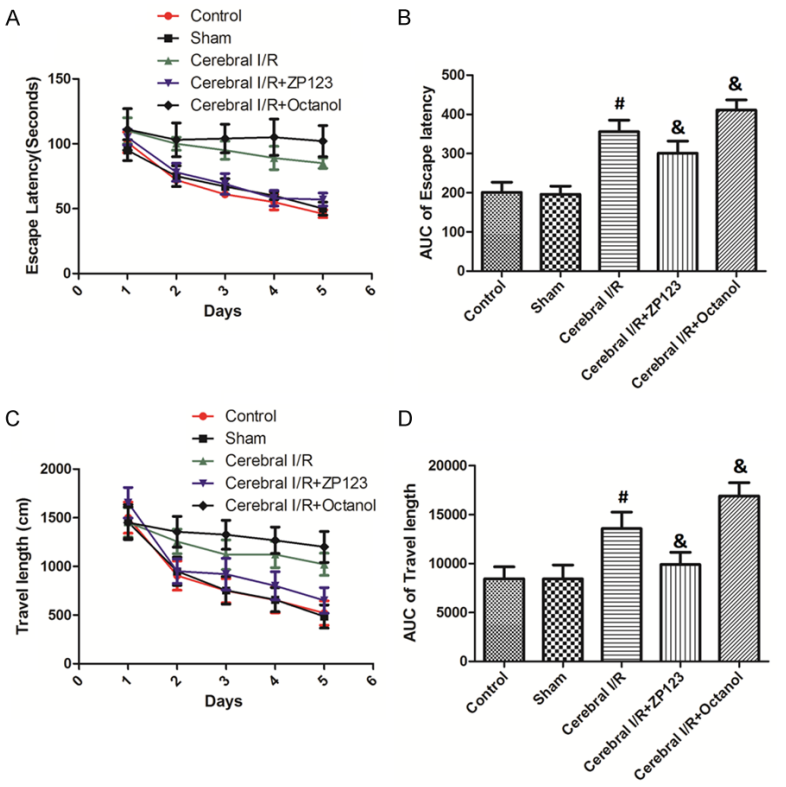

Figure 2. The effects of cerebral I/R, ZP123 and octanol on escape latency and travel length in the Morris Water Maze test. As shown in A and B, rats with cerebral I/R showed a significant deficit in locating the submerged escape platform. ZP123 decreased the escape latencies significantly, while octanol significantly increased the escape latencies. C and D. Show the AUC of escape latency and travel length. Control: rats received no treatment; Sham: rats received sham MCAO procedure. Cerebral I/R: Cerebral ischemia reperfusion injury. AUC: area under the curve. #: $p<0.05$ compared to Sham; &: $p<0.05$ compared to Cerebral I/R.

with a Bradford reagent (Bio-Rad Co., Hercules, CA, USA).

Terminal deoxynucleotide-transferase-mediated dUTP-digoxigenin nick end labeling (TUNEL) assay

Twenty-four h after blood flow was restored, detection of apoptosis was conducted with the method described by Walsh et al using an immunofluorescent TUNEL assay (Kit S7110, ApopTag, Intergen Company, NY, USA) [26]. Briefly, after sections were de-paraffinized, they were digested with Proteinase K (20 μg/mL in PBS) at 25°C for 15 min and then soaked in PBS for 5 min. Afterwards,they were incubated with Tdt enzyme at 37°C for 1 h. Sections were then washed with stop/wash buffer and then rinsed with PBS three times. Fluorescein isothiocyanate-labeled anti-digoxigenin conjugate was then applied to the sections and incubated at 25°C for 30 min. They were then mounted with DABCO, which contains propidium iodide. Finally, they were visualized with a fluorescence microscope using an excitation wavelength of 460-490 nM, and the percentage of TUNEL-positive cells was determined.

Statistical analysis

All the statistical analyses were performed with SPSS 17.0 software (SPSS Inc., Chicago, USA). For comparison, one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used. $P<0.05$ was considered statistically significant.

Results

Effects of GJIC promotion or inhibition on cognitive impairment caused by cerebral I/R

Figure 1 shows the travel path of rats in the Morris Water Maze. It shows that rats in the Cerebral I/R group traveled longer than rats in the Control and Sham groups. Rats in the Cerebral I/R+ZP123 group, however, traveled less distance than the Cerebral I/R group before they reached the platform. Rats in the Cerebral I/R+Octanol group, on the other hand, traveled a longer distance than the Cerebral I/R group. **Figures 2** and **3** show the effects of ZP123 and octanol on cognitive impairment caused by cerebral I/R in rats. As shown in **Figure 2A** and **2B**, rats with cerebral I/R showed a significant deficit in locating the submerged escape platform and showed increased escape latencies compared with control and sham rats. The GJIC promotion by ZP123 decreased the escape latencies significantly ($P<0.05$), while the GJIC inhibition by octanol significantly increased the escape latencies ($P<0.05$). Consistently, as shown in **Figure 2C** and **2D**, rats with cerebral I/R had longer swimming distance compared to that of

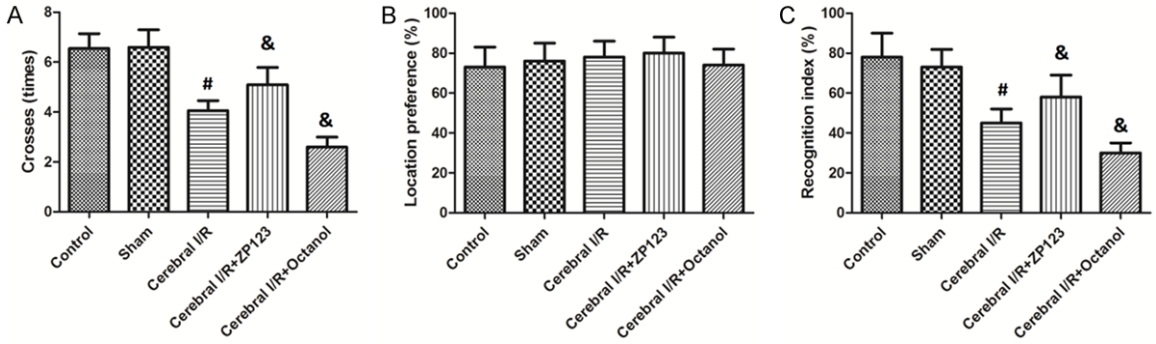

Figure 3. The effects of cerebral I/R, ZP123 and octanol on crosses in the Morris Water Maze and recognition tests. Rats with cerebral I/R exhibited impaired memory retention as they crossed over the target site fewer times than control and sham rats (A). Rats treated with ZP123 crossed more often, while rats treated with octanol crossed less often. As shown in (B), there was no significant differences in location preference, which indicates that the location of the objects did not affect the exploratory behavior of rats. (C) showed that ZP123 significantly increased the recognition index (RI) compared to the Cerebral I/R group, while octanol significantly decreased it. Control: rats received no treatment; Sham: rats received sham MCAO procedure. Cerebral I/R: Cerebral ischemia reperfusion injury. AUC: area under the curve. #: $p<0.05$ compared to Sham; &: $p<0.05$ compared to Cerebral I/R.

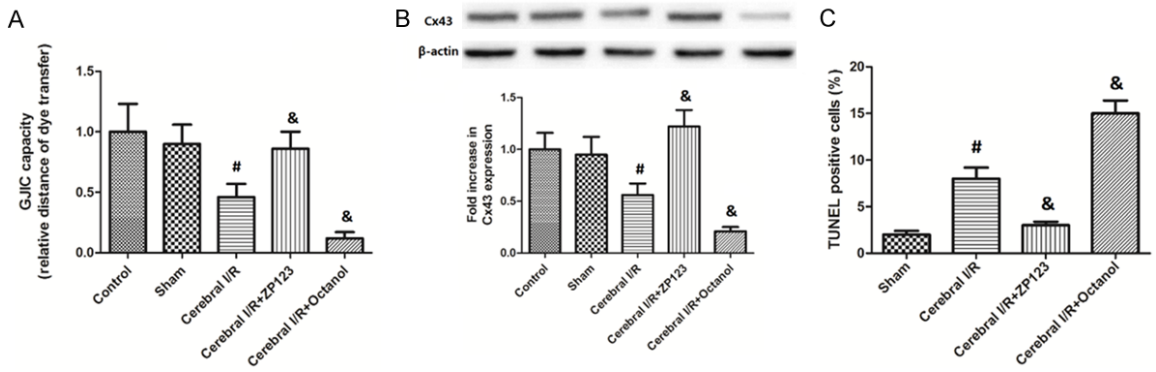

Figure 4. The effects of cerebral I/R, ZP123 and octanol on GJIC and hippocampal apoptosis. The expression of a representative GJIC protein Cx43 was examined by Western blot. Rats with cerebral I/R showed a significant decrease in GJIC capacity, which was increased by ZP123 but decreased by octanol. The expression of Cx43 was increased by ZP123, but decreased by octanol. The TUNEL assay showed that cerebral I/R induced neuronal apoptosis in the hippocampus, which was reversed by ZP123 but facilitated by octanol. Control: rats received no treatment; Sham: rats received sham MCAO procedure. Cerebral I/R: Cerebral ischemia reperfusion injury. Cx43: connexin 43. #: $p<0.05$ compared to Sham; &: $p<0.05$ compared to the Cerebral I/R group.

control and sham rats, which was decreased by ZP123 but increased by octanol ($P<0.05$). In the probe trial experiment, rats with cerebral I/R exhibited impaired memory retention, as they crossed over the target site fewer times than control and sham rats (**Figure 3A**). Rats treated with ZP123 crossed more often, while rats treated with octanol crossed less often ($P<0.05$ compared to the Cerebral I/R group). In the evaluation of novel object recognition, as shown in **Figure 3B**, there was no significant difference in location preference. The location of the objects did not affect the exploratory behavior of rats in different groups. **Figure 3C** shows that the recognition index (RI) of rats with cerebral I/R was worse than the control and sham rats, which suggests a deficit in their memory for the familiar object. ZP123 significantly increased the RI compared to the Cerebral I/R group, while octanol significantly decreased it ($P<0.05$).

Effects of GJIC promotion or inhibition on GJIC and hippocampal apoptosis

To examine the effects of cerebral I/R on GJIC, we measured the distance of dye transfer and calculated the relative value compared to the

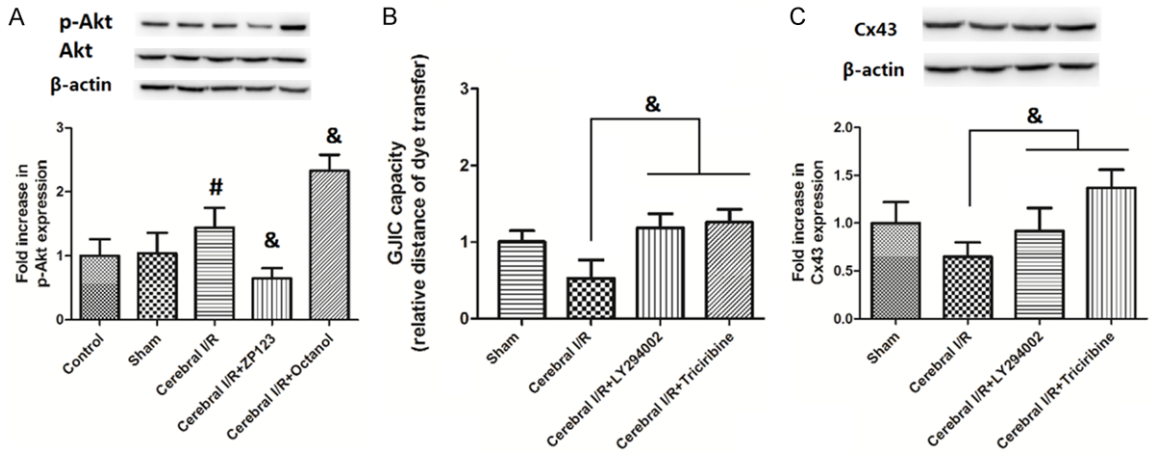

Figure 5. The effects of cerebral I/R, ZP123 and octanol on Akt phosphorylation and the effects of PI3K/Akt inhibition on GJIC. A. Shows the effects of cerebral I/R, ZP123 and octanol on Akt phosphorylation. B. Shows the PI3K/Akt inhibition of GJIC capacity. C. Shows the PI3K/Akt inhibition of Cx43 levels. The phosphorylation of Akt was significantly increased in the Cerebral I/R group. The phosphorylation of Akt was decreased by ZP123 but increased by octanol. LY294002 and triciribine both significantly suppressed the decrease in GJIC capacity induced by cerebral I/R. Similarly, the expression of Cx43 was significantly increased after rats were treated with LY294002 or triciribine. Control: rats received no treatment; Sham: rats received sham MCAO procedure. Cerebral I/R: Cerebral ischemia reperfusion injury. Cx43: connexin 43. #: $p<0.05$ compared to Sham; &: $p<0.05$ compared to the Cerebral I/R group.

control group. The expression of a representative GJIC protein, Cx43, was examined by Western blot. As shown in **Figure 4A**, rats with cerebral I/R showed a significant decrease in GJIC capacity, which was increased by ZP123 but decreased by octanol ($P<0.05$. Similarly, the expression of Cx43 was significantly decreased in the Cerebral I/R group. Cx43 expression was increased by ZP123 but decreased by octanol ($P<0.05$). As shown in the TUNEL assay (**Figure 4C**), cerebral I/R induced neuronal apoptosis in the hippocampus, which was reversed by ZP123 but facilitated by octanol ($P<0.05$).

Effects of GJIC promotion or inhibition of Akt phosphorylation

To examine the effects of cerebral I/R or GJIC on the PI3K/Akt pathway, we measured the phosphorylation of the Akt protein by Western blot. As shown in **Figure 5A**, the phosphorylation of Akt was significantly increased in the Cerebral I/R group. It was decreased by ZP123 but increased by octanol ($P<0.05$).

PI3K/Akt pathway inhibitors suppressed GJIC capacity and Cx43 expression

To examine the involvement of the PI3K/Akt pathway in the decrease of GJIC caused by cerebral I/R, we measured the changes in GJIC capacity and Cx43 expression in the presence of PI3K/Akt pathway inhibitors LY294002 and triciribine. As shown in **Figure 5B**, LY294002 and triciribine both significantly suppressed the decrease in GJIC capacity induced by cerebral I/R ($P<0.05$). Similarly, as shown in **Figure 5C**, the expression of Cx43 was significantly increased after rats were treated with LY294002 or triciribine ($P<0.05$).

Effects of inhibitors of PI3K/Akt pathway on cognitive impairment caused by cerebral I/R

To examine the role of the PI3K/Akt pathway in cognitive impairment caused by cerebral I/R, we treated rats with PI3K/Akt pathway inhibitors LY294002 or triciribine, and then we measured the cognitive ability of the rats. As shown in **Figure 6A** and **6C**, rats that were given LY294002 or triciribine showed a significant improvement when locating the submerged escape platform. Similarly, rats in the LY294002 or triciribine groups had shorter swimming distances compared to that of Cerebral I/R rats, as shown in **Figure 6B** and **6D**. The number of crosses in the LY294002 and triciribine groups were also much fewer than that in the Cerebral I/R group (**Figure 6E**). As shown in **Figure 6F**, rats in the LY294002 or triciribine groups had

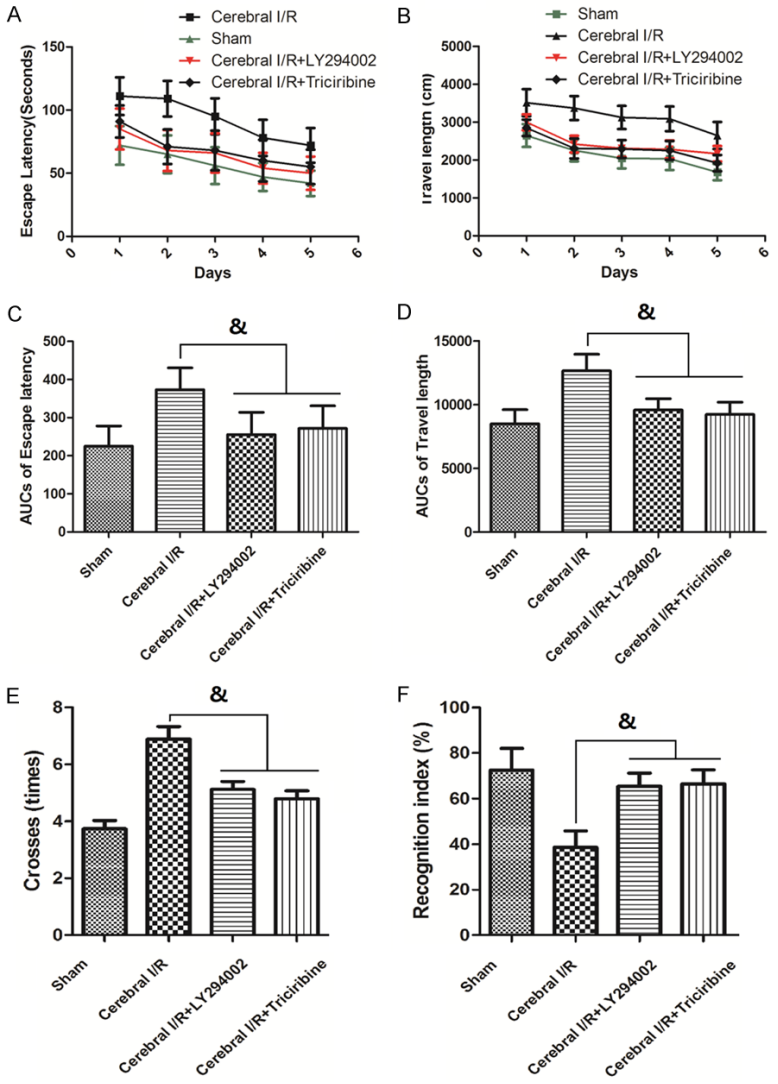

Figure 6. The effects of PI3K/Akt inhibition on cognitive impairment caused by cerebral I/R. Sham: rats received a sham MCAO procedure. Rats that were given LY294002 or triciribine showed a significant improvement when locating the submerged escape platform, a shorter swimming distance, and fewer crosses compared to Cerebral I/R rats. Rats in LY294002 and triciribine groups had higher RIs than Cerebral I/R rats, indicating an improvement in their memory for the familiar object. Cerebral I/R: Cerebral ischemia reperfusion injury. Cx43: connexin 43. #: $p<0.05$ compared to Sham; &: $p<0.05$ compared to Cerebral I/R.

higher RIs than Cerebral I/R rats, indicating an improvement in their memory for the familiar object.

Discussion

Gap junctions are widely distributed in the central nervous system, primarily between neurons and glial cells [27, 28]. However, the role of GJIC in cerebral ischemia pathological processes is not fully understood yet. It has been widely accepted that cerebral I/R causes cognitive impairment, but the participation of gap junction communication dysfunction in the process has not been studied in depth yet. Here, we showed that GJIC capacity was greatly impaired by cerebral I/R in rats. GJIC promotion by ZP123 significantly reversed the cognitive impairment caused by cerebral I/R in rats, while GJIC inhibition by octanol significantly facilitated the cognitive impairment, demonstrating the important role of GJIC in the cognitive impairment caused by cerebral I/R.

Under normal conditions, GJIC plays an important role in many cellular activities [29]. The rapid flow of small molecules, such as inorganic ions, amino acids and glucose, through the gap junctions between cells is important to maintain cellular homeostasis. It is believed that gap junctions could communicate the electrical signals between neurons in the brain [30]. Chemical signals or metabolites can also be transferred between glial cells by gap junctions to bolster the activities of the neuronal, glial and vascular cells [31]. It was shown that after a cerebral ischemia/reperfusion procedure in a rat model, obvious neurological deficits occurred with decreased Cx43 expression in the cortex and hippocampus [32], indicating an inhibition of GJIC. The present study showed cognitive impairment caused by cerebral I/R, which was accompanied by the inhibition of GJIC and reduced Cx43 protein level. As gap junctions may protect cells by supplying essential nutrients and metabolites [33], the inhibition of GJIC may prevent the spatial buffering of potas-

sium and glutamate as well as the transmission of energy between the astrocytic syncytium and neurons [34], which may be the one of the many reasons for neural death after I/R injury.

Hippocampal activity is directly related to cognitive function and plays an important role in the pathophysiology of focal cerebral ischemia. In addition to direct ischemic insult, neuronal apoptosis in the hippocampus may be one of the mechanisms. In the present study, we found that cerebral I/R induced neuronal apoptosis in the hippocampus. The treatment of gap junction modifier ZP123 significantly inhibited apoptosis, while the GJIC inhibition by octanol worsened the apoptosis, which reveals that GJIC plays an important role in apoptosis in the hippocampus. The cerebral I/R may cause deactivation of GJIC and lead to neuronal apoptosis. The successful inhibition of neuronal apoptosis by ZP123 suggests a possible strategy to mitigate neural injury caused by I/R.

Gap junction communication is the main means of communication in astrocytes, and the main connexin expressed in the cell membrane is connexin 43. GJIC may contribute to the expansion of injured lesions after cerebral I/R and distant neuronal death [35, 36]. Our results revealed that the expression of Cx43 was significantly decreased in the cerebral I/R group. Cx43 expression was increased by ZP123 but decreased by octanol, indicating the effectiveness of these two chemicals in GJIC modification.

In the presence of LY294002 or triciribine, the decrease of GJIC capacity was significantly suppressed, and the expression of Cx43 was significantly increased, indicating the participation of the PI3K/Akt pathway in the regulation of GJIC capacity. Ito et al suggested that Akt was critical for the disruption of GJIC, while the inhibition of Akt restored GJIC after it was suppressed [37]. A study by Geletu et al, however, revealed that the pharmacological inhibition of PI3k eliminated GJIC [38]. Taken together, PI3k/Akt may act differently on GJIC in different disease models and cells. It is possible that activation of the PI3K/Akt pathway promotes nuclear accumulation of β-catenin, which in turn stimulates Cx43 expression [39].

Finally, we found that both LY294002 and triciribine produced a significant improvement in cognitive impairment from cerebral I/R. Rats that were given LY294002 or triciribine showed a significant shorter escape latency and swimming distance and fewer crosses. Rats in the LY294002 or triciribine groups had higher RI, indicating an improvement in their memory for the familiar object. These results indicate the cognitive impairment induced by cerebral I/R and the involvement of the PI3K/Akt pathway. As gap junction communication dysfunction plays an important role in the cognitive impairment in the cerebral I/R model, and it can be regulated via the PI3K/Akt pathway, it is likely that the PI3K/Akt pathway is involved in the cognitive impairment caused by GJIC dysfunction in the rat hippocampus after cerebral I/R injury.

Disclosure of conflict of interest

None.

Address correspondence to: Song Wu, Emergency Department, The Third Affiliated Hospital of Soochow University, The First People's Hospital of Changzhou, No.185 Juqian Street, Changzhou 213003, Jiangsu, People's Republic of China. Tel: 86-0519-68870-000; E-mail: songwu_changzhou@sina.com

References

[1] WRITING GROUP MEMBERS, Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, Go A, Greenlund K, Haase N, Hailpern S, Ho PM, Howard V, Kissela B, Kittner S, Lackland D, Lisabeth L, Marelli A, McDermott MM, Meigs J, Mozaffarian D, Mussolino M, Nichol G, Roger VL, Rosamond W, Sacco R, Sorlie P, Roger VL, Thom T, Wasserthiel-Smoller S, Wong ND, Wylie-Rosett J; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. Circulation 2010; 121: e46-e215.

[2] Simerabet M, Robin E, Aristi I, Adamczyk S, Tavernier B, Vallet B, Bordet R, Lebuffe G. Preconditioning by an in situ administration of hydrogen peroxide: involvement of reactive oxygen species and mitochondrial ATP-dependent potassium channel in a cerebral ischemia-reperfusion model. Brain Res 2008; 1240: 177-84.

[3] Cai F, Li C, Wu J, Min Q, Ouyang C, Zheng M, Ma S, Yu W, Lin F. Modulation of the oxidative stress and nuclear factor kappaB activation by

theaflavin 3,3'-gallate in the rats exposed to cerebral ischemia-reperfusion. Folia Biol (Praha) 2007; 53: 164-72.
[4] Ozbal S, Erbil G, Koçdor H, Tuğyan K, Pekçetin C, Ozoğul C. The effects of selenium against cerebral ischemia-reperfusion injury in rats. Neurosci Lett 2008; 438: 265-9.
[5] Yousuf S, Atif F, Ahmad M, Hoda N, Ishrat T, Khan B, Islam F. Resveratrol exerts its neuroprotective effect by modulating mitochondrial dysfunctions and associated cell death during cerebral ischemia. Brain Res 2009; 1250: 242-53.
[6] Abrams CK, Scherer SS. Gap junctions in inherited human disorders of the central nervous system. Biochim Biophys Acta 2012; 1818: 2030-47.
[7] Giaume C, Orellana JA, Abudara V, Sáez JC. Connexin-based channels in astrocytes: how to study their properties. Methods Mol Biol 2012; 814: 283-303.
[8] Vinken M, Vanhaecke T, Papeleu P, Snykers S, Henkens T, Rogiers V. Connexins and their channels in cell growth and cell death. Cell Signal 2006; 18: 592-600.
[9] Giaume C, Theis M. Pharmacological and genetic approaches to study connexin-mediated channels in glial cells of the central nervous system. Brain Res Rev 2010; 63: 160-76.
[10] Homkajorn B, Sims NR, Muyderman H. Connexin 43 regulates astrocytic migration and proliferation in response to injury. Neurosci Lett 2010; 486: 197-201.
[11] Márquez-Rosado L, Solan JL, Dunn CA, Norris RP, Lampe PD. Connexin43 phosphorylation in brain, cardiac, endothelial and epithelial tissues. Biochim Biophys Acta 2012; 1818: 1985-92.
[12] de Pina-Benabou MH, Szostak V, Kyrozis A, Rempe D, Uziel D, Urban-Maldonado M, Benabou S, Spray DC, Federoff HJ, Stanton PK, Rozental R. Blockade of gap junctions in vivo provides neuroprotection after perinatal global ischemia. Stroke 2005; 36: 2232-7.
[13] Talhouk RS, Zeinieh MP, Mikati MA, El-Sabban ME. Gap junctional intercellular communication in hypoxia-ischemia-induced neuronal injury. Prog Neurobiol 2008; 84: 57-76.
[14] Frisch C, De Souza-Silva MA, Söhl G, Güldenagel M, Willecke K, Huston JP, Dere E. Stimulus complexity dependent memory impairment and changes in motor performance after deletion of the neuronal gap junction protein connexin36 in mice. Behav Brain Res 2005; 157: 177-85.
[15] Hosseinzadeh H, Asl MN, Parvardeh S, Tagi Mansouri SM. The effects of carbenoxolone on spatial learning in the Morris water maze task in rats. Med Sci Monit 2005; 11: BR88-94.
[16] Haugan K, Marcussen N, Kjølbye AL, Nielsen MS, Hennan JK, Petersen JS. Treatment with the gap junction modifier rotigaptide (ZP123) reduces infarct size in rats with chronic myocardial infarction. J Cardiovasc Pharmacol 2006; 47: 236-42.
[17] Manjarrez-Marmolejo J, Franco-Pérez J. Gap junction blockers: an overview of their effects on induced seizures in animal models. Curr Neuropharmacol 2016; 14 : 759-71.
[18] Bodenstine TM, Vaidya KS, Ismail A, Beck BH, Cook LM, Diers AR, Landar A, Welch DR. Homotypic gap junctional communication associated with metastasis suppression increases with PKA activity and is unaffected by PI3K inhibition. Cancer Res 2010; 70: 10002-11.
[19] Tacheau C, Fontaine J, Loy J, Mauviel A, Verrecchia F. TGF-beta induces connexin43 gene expression in normal murine mammary gland epithelial cells via activation of p38 and PI3K/AKT signaling pathways. J Cell Physiol 2008; 217: 759-68.
[20] Zhang P, Guo ZF, Xu YM, Li YS, Song JG. N-Butylphthalide (NBP) ameliorated cerebral ischemia reperfusion-induced brain injury via HGF-regulated TLR4/NF-kappaB signaling pathway. Biomed Pharmacother 2016; 83: 658-666.
[21] Bostanci MO, Bağirici F. The effects of octanol on penicillin induced epileptiform activity in rats: an in vivo study. Epilepsy Res 2006; 71: 188-94.
[22] Jia D, Han B, Yang S, Zhao J. Anemonin alleviates nerve injury after cerebral ischemia and reperfusion (i/r) in rats by improving antioxidant activities and inhibiting apoptosis pathway. J Mol Neurosci 2014; 53: 271-9.
[23] Zhang MY, Zheng CY, Zou MM, Zhu JW, Zhang Y, Wang J, Liu CF, Li QF, Xiao ZC, Li S, Ma QH, Xu RX. Lamotrigine attenuates deficits in synaptic plasticity and accumulation of amyloid plaques in APP/PS1 transgenic mice. Neurobiol Aging 2014; 35: 2713-25.
[24] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11: 47-60.
[25] da Silva TC, Cogliati B, da Silva AP, Fukumasu H, Akisue G, Nagamine MK, Matsuzaki P, Haraguchi M, Górniak SL, Dagli ML. Pfaffia paniculata (Brazilian ginseng) roots decrease proliferation and increase apoptosis but do not affect cell communication in murine hepatocarcinogenesis. Exp Toxicol Pathol 2010; 62: 145-55.
[26] Walsh DS, Lane JE, Abalos RM, Myint KS. TUNEL and limited immunophenotypic analyses of apoptosis in paucibacillary and multibacillary leprosy lesions. FEMS Immunol Med Microbiol 2004; 41: 265-9.

[27] Orthmann-Murphy JL, Freidin M, Fischer E, Scherer SS, Abrams CK. Two distinct heterotypic channels mediate gap junction coupling between astrocyte and oligodendrocyte connexins. J Neurosci 2007; 27: 13949-57.
[28] Hidaka S. Conflicting effects by antibodies against connexin36 during the action of intracellular Cyclic-AMP onto electrical synapses of retinal ganglion cells. J Integr Neurosci 2016; 15: 571-591.
[29] Thomas MA, Zosso N, Scerri I, Demaurex N, Chanson M, Staub O. A tyrosine-based sorting signal is involved in connexin43 stability and gap junction turnover. J Cell Sci 2003; 116: 2213-22.
[30] Pereda AE, Electrical synapses and their functional interactions with chemical synapses. Nat Rev Neurosci 2014; 15: 250-263.
[31] Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N. Astroglial networks: a step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 2010; 11: 87-99.
[32] Li Z, Lin XM, Gong PL, Zeng FD, Du GH. Effects of Gingko biloba extract on gap junction changes induced by reperfusion/reoxygenation after ischemia/hypoxia in rat brain. Am J Chin Med 2005; 33: 923-34.
[33] Decrock E, De Vuyst E, Vinken M, Van Moorhem M, Vranckx K, Wang N, Van Laeken L, De Bock M, D'Herde K, Lai CP, Rogiers V, Evans WH, Naus CC, Leybaert L. Connexin 43 hemichannels contribute to the propagation of apoptotic cell death in a rat C6 glioma cell model. Cell Death Differ 2009; 16: 151-163.
[34] Rossi DJ, Brady JD, Mohr C. Astrocyte metabolism and signaling during brain ischemia. Nat Neurosci 2007; 10: 1377-1386.
[35] Das S, Lin D, Jena S, Shi A, Battina S, Hua DH, Allbaugh R, Takemoto DJ. Takemoto. Protection of retinal cells from ischemia by a novel gap junction inhibitor. Biochem Biophys Res Commun 2008; 373: 504-8.
[36] Saltman AE, Aksehirli TO, Valiunas V, Gaudette GR, Matsuyama N, Brink P, Krukenkamp IB. Gap junction uncoupling protects the heart against ischemia. J Thorac Cardiovasc Surg 2002; 124: 371-6.
[37] Ito S, Hyodo T, Hasegawa H, Yuan H, Hamaguchi M, Senga T. PI3K/Akt signaling is involved in the disruption of gap junctional communication caused by v-Src and TNF-alpha. Biochem Biophys Res Commun 2010; 400: 230-5.
[38] Geletu M, Guy S, Greer S, Raptis L. Differential effects of polyoma virus middle tumor antigen mutants upon gap junctional, intercellular communication. Exp Cell Res 2015; 336: 223-31.
[39] Xia X, Batra N, Shi Q, Bonewald LF, Sprague E, Jiang JX. Prostaglandin promotion of osteocyte gap junction function through transcriptional regulation of connexin 43 by glycogen synthase kinase 3/beta-catenin signaling. Mol Cell Biol 2010; 30: 206-19.