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Resveratrol attenuates autophagy and inflammation after traumatic brain injury by activation of PI3K/Akt/mTOR pathway in rats

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Abstract

Aim of the study: *Accumulating studies have demonstrated that neuronal autophagy and inflammation are crucial for hippocampus development in rats subjected to traumatic brain injury (TBI). Therefore, we have investigated whether resveratrol is protective against brain damage through the attenuation of neuronal autophagy and inflammation, and explored underlying mechanisms.*

Material and methods: *Rats were injected with resveratrol (50 mg/kg, i.p.), following controlled cortical impact (CCI) injury. Brain water content, behavioral studies, and mNSS score were measured to assess the effects of resveratrol treatment. Autophagy-related proteins and inflammatory cytokines in the hippocampus were detected by Western blotting at 12, 24, and 48 hours after TBI. In addition, spatial distribution of LC3 was evaluated with immunofluorescence analysis 24 hours after injury. Finally, factors related to PI3K/Akt/mTOR signaling pathway were assessed at the same time in the hippocampus.*

Results: *Our results depicted that resveratrol could reduce the cerebral edema caused by TBI and improve the recovery of functional deficits in rats. Resveratrol was also able to remarkably reduce the expression of LC3 II and Beclin-1, while increased the expression levels of P62 in the hippocampus. Moreover, we found that interleukin β (IL-1β) and tumor necrosis factor α (TNF-α) were significantly decreased in resveratrol-treated rats. Indeed, we observed an activation of the PI3K/Akt/mTOR pathway after TBI, which may be related to the neuro-protective effect of resveratrol.*

Conclusions: *Data presented herein support that resveratrol is a potential treatment against TBI through the inhibition of neuronal autophagy and inflammation by activation of PI3K/Akt/mTOR pathway.*

Key words: *resveratrol, traumatic brain injury, autophagy, inflammation, PI3K/Akt/mTOR.*

Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability in young adults, and a major health and socio-economic issue of modern life. Due to a shortage of effective therapeutic agents, TBI survivors often suffer long-term disability in sensorimotor functions, and neurological and cognitive deficits. In TBI, neurological impairment is caused by a direct destruction of brain tissue (primary injury) as well as a series of complex and aggravating pathological processes (secondary injury), which further aggravate the primary neuronal damage [7]. Primary injury can initiate secondary brain injuries, such as a release of neuro-transmitters, formation of oxygen-free radicals, lipid peroxidation of cell

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membranes, mitochondrial dysfunction, calcium-mediated damage, and initiation of inflammatory and immune responses. Therefore, these pathological lesions may lead to neuronal cell death in the form of necrosis and/or apoptosis [24]. It is worth noting that neuronal autophagy and inflammation play a crucial role in the management of TBI, and are depicted as an important source of secondary brain injury in experimental conditions.

Resveratrol (RV, 3,5,40-trihydroxystilbene) is a powerful antioxidant, enriched in vegetables, fruits, grains, grape skin, flowers, nuts, tea, and red wine [4]. Resveratrol was shown to be neuro-protective against various neurological disorders, including Parkinson's disease, Huntington's disease, ischemic stroke, and Alzheimer's disease [32,33,36]. Autophagy is a 'self-eating' phenomenon in cells, and is a highly conserved degradation pathway in several organisms. Moreover, autophagy is a formation of double membrane autophagosomes, in which cells wrap part of their cytoplasm or damaged organelles and transport them to lysosomes for degradation [5,17]. Autophagy is mainly used to remove and degrade damaged cellular structures, senescent organelles, and biological macromolecules that are no longer needed. Several studies have demonstrated that the activation of autophagy can directly cause cell damage or its' death, and is involved in the pathophysiology of various diseases [31,35]. Indeed, Clark *et al*. [6] reported LC3 II and autophagosomes in human brain tissues after TBI. Lai *et al*. [18] demonstrated that autophagy occurs in experimental TBI, while treatment with γ-glutamylcysteine ethyl ester (GCEE) reduced neuronal autophagy and improved neurological outcomes in mice subjected to TBI. More recently, previous reports from our group have suggested that the suppression of neuronal autophagy can be neuro-protective in the rat's hippocampus [10,11]. Interestingly, Lin *et al*. demonstrated in adult rats that resveratrol increased cell survival after TBI through the inhibition of GSK-3β-mediated autophagy and apoptosis [23]. Resveratrol has been reported to regulate several different signaling pathways after brain injury, such as mating-type information regulating 2 homolog 1 (SIRT1), AMP-activated kinase (AMPK), and NF-E2-related factor 2 (NRF2) [22,34,40]. Moreover, a previous study showed that neuronal apoptosis was attenuated by resveratrol treatment in SAH rat brain, which could be partially mediated by the activation of PI3K/Akt signaling pathway [41]. However, it remains unclear whether resveratrol treatment is protective against autophagic neuronal death and inflammation after TBI through PI3K/Akt signaling pathway.

Therefore, in this study, we performed intra-peritoneal injections of resveratrol in rats subjected to brain injury, and evaluated if resveratrol could provide neuro-protection against TBI. In addition, we also investigated a possible underlying mechanism of resveratrol through the activation of PI3K/Akt/mTOR pathway to inhibit neuronal autophagy and inflammatory response.

Material and methods

Animals

In the present study, 150 adult male Sprague-Dawley rats were purchased from the Laboratory Animal Center of Hebei Medical University (aged 12-16 weeks, weight 300-330 g; Shijiazhuang, China). Rats were kept in a standard 12 : 12 hour light/dark cycle environment, with free access to laboratory standard water and food before TBI or sham surgery. All experimental procedures were approved by the Ethics Committee of the Hebei Medical University (Permit number: 20201086). Every effort was employed to minimize animals' suffering during experiments.

CCI model

CCI procedure was performed according to a previously published procedure. Specifically, rats were anesthetized under intra-peritoneal 10% chloral hydrate (300 mg/kg), with no side effects reported. Their heads were fixed onto a brain stereoscope and body temperature was continuously monitored by a rectal probe to ensure that the temperature was kept at 36.5°C. After cleaning with betadine, the skin at the top of the skull was cut to identify the bregma. An approximate 6 mm bone window was made with a portable drill under aseptic conditions, centered 3.0 mm from the bregma and 2.0 mm lateral (right) from the midline. Rat was impacted with a CCI device using a 3 mm flat-tip impactor (impact velocity = 5 m/s, impact duration = 150 ms, depth = 2.5 mm). Then, the bone window was closed with bone wax and the skin was stitched. In the control group, the bone flap was removed, but CCI was not performed. Humane end points were established following the guideline of assessment for humane end points in animal experiments (People's Republic of China: RB/T 173-2018) in order to minimize pain or distress in experimental animals. Rats were intra-peritoneally deep anesthetized by chloral hydrate (400 mg/kg) prior to

euthanasia by cervical vertebra dislocation. Death was confirmed by cessation of the heartbeat.

Grouping and administration

Rats were randomly divided into three different experimental groups by random number table as described previously [10,11]: sham-operated group (n = 30), TBI group (n = 60), and TBI + resveratrol treatment group (n = 60). Five rats in each sub-group were sacrificed at 12, 24, or 48 hours after TBI. Resveratrol (50 mg/kg body weight; Sigma Aldrich, Yorba Linda, CA, USA) was administered by intra-peritoneal injection immediately after brain injury. Sham and TBI rats were given the same amount of 0.9% saline by intra-peritoneal injection.

Evaluation of brain edema

Brain water content was measured in rat cerebral tissues. Rats (n = 5 in each group) were sacrificed by cervical vertebra dislocation under deep anesthesia. After craniotomy, the brain was removed, the surface moisture was dried, and the brain tissue was weighed immediately (wet weight). The brain was placed in an oven at 100°C for 24 hours, and was re-weighed (dry weight). Finally, the water content was calculated as: brain water content = (wet weight – dry weight)/wet weight × 100%.

Morris water maze

Morris water maze (MWM) was used to examine the spatial learning and memory ability of rats. There were five rats in each group following sham or TBI. Morris water maze consists of heated swimming pool, platform, and computer analysis software. The pool (1.8 m in diameter, 0.5 m high) was filled with water at 25°C, and divided into four equivalent quadrants: East (E), South (S), West (W), and North (N). The platform (12 cm in diameter, 28 cm high) was placed 2 cm below the surface and in the middle of any quadrant. Rats were put in the pool near the edge and facing the wall of the pool, and one of the four starting positions (East, West, South, or North) was randomly selected. Latency (seconds) was recorded as the time taken for rats to find the underwater platform. If the time exceeded 60 seconds, rats were gently put on the platform and allowed to stay on the platform for 20 seconds and returned to the cage for a new trial (time interval, 10 minutes). Movement trajectory of rats in Morris water maze experiment was extracted using video tracking technology (HVS Imaging, Hampton, UK). Rats were tested five times from the same starting point. This test was conducted at 7, 8, 9, and 10 days after trauma or sham surgery.

Evaluation of neurological impairment

The degree of behavioral functional damage after brain injury was evaluated 1 to 5 days after injury. The scoring system included motor tests, sensory tests, reflexes, and balance functions, and the full score was 18 points. Failing to complete a task gave 1 point; thus, 0 = minimum deficit and 18 = maximum deficit. Two double-blinded and independent researchers carried out neuro-behavioral tests.

Immunofluorescence

Brain tissue was collected and immersed in 4% paraformaldehyde for 24 hours, and then transferred to a 30% sucrose solution (0.1 M PBS, pH = 7.4) for 3 days. Brain tissue was embedded in OCT medium (Leica 020106926, Germany) in a cryo-embedding machine and stored at –80°C. Frozen brain tissues were sliced (15 µm) with a frozen slicer, blocked with 0.4% triton X-100 for 40 minutes and 5% donkey serum for 1 hour. Tissue sections were washed with PBS, and incubated with primary antibodies. For double-labeling, polyclonal rabbit anti-LC3 antibody (dilution 1 : 100; MBL) and polyclonal mouse anti-NeuN antibody (dilution 1 : 100; Millipore) were incubated simultaneously overnight at 4°C. Sections were then washed 3 times with fresh PBS and incubated with fluorescent secondary antibodies tagged with FITC 488 (donkey anti-rabbit IgG, 1 : 200, Santa Cruz Biotechnology) or 594 (donkey anti-mouse IgG, 1 : 200, Santa Cruz Biotechnology) for 2 hours at room temperature in dark. All cell nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). After washing with PBS, images were collected using a laser confocal microscope (Olympus FV950).

Western blot

Rats were sacrificed by decapitation, and the hippocampus from the injured side of the brain was collected at different time points after TBI. Frozen tissue was placed in complete RIPA buffer, minced, and centrifuged for 15 minutes at 12,000 × g at 4°C. Protein concentration of the supernatant was determined using a bicinchoninic acid (BCA) protein assay kit (Solar-

Bio, Beijing, China), then proteins (50 mg of protein) were separated by 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis (PAGE). Proteins were transferred to a polyvinylidene fluoride (PVDF) membrane with a semi-dry transfer (Bio-Rad, Hercules, CA, USA), and blocked with 5% non-fat dry milk for 2 hours at room temperature. PVDF membranes were incubated with appropriate primary antibodies at 4°C overnight. Primary antibodies were as follows: rabbit polyclonal anti-LC3 (MBL, Japan; diluted 1 : 1000), rabbit polyclonal anti-p62 (Antibody Revolution; diluted 1 : 1000), rabbit polyclonal anti-Beclin-1 (MBL, Japan; diluted 1 : 1000), rabbit polyclonal anti-IL-1β (Affinity; diluted 1 : 500), rabbit polyclonal anti-TNF-α (Affinity; diluted 1 : 500), rabbit polyclonal anti-p-PI3K (MyBioSource; diluted 1 : 500), rabbit polyclonal anti-PI3K (Abcam; diluted 1 : 500), rabbit polyclonal anti-p-Akt (Invitrogen; diluted 1 : 500), rabbit polyclonal anti-Akt (Invitrogen; diluted 1 : 500), mouse monoclonal anti-p-mTOR (Invitrogen; diluted 1 : 500), rabbit polyclonal anti-mTOR (Abcam; diluted 1 : 500), and rabbit monoclonal anti-β-actin (Affinity; diluted 1 : 1000). On the following day, membranes were rinsed with PBS, and goat anti-rabbit or anti-mouse secondary antibodies conjugated with horseradish peroxidase were incubated for 2 hours at 37°C. Grey intensity of each protein was observed using an enhanced chemiluminescence (ECL) system (Pierce, Rockford, USA). Band intensities were quantified using Image J (Image Lab 4.1, Bio-Rad Laboratories), and β-actin was used as an internal control.

Statistical analysis

SPSS v. 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the data. All experimental data were expressed as mean ± standard deviation (SD). Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Student and Newman-Keuls post-hoc tests. $P < 0.05$ was considered statistically significant.

Results

Resveratrol treatment attenuated brain edema

We evaluated the effect of resveratrol on brain edema after injury by dry-wet specific weight method. Compared with the sham operation group, the brain water content induced by TBI was dramatically increased at 12, 24, and 48 hours (Fig. 1), whereas the level of brain water content was sig-

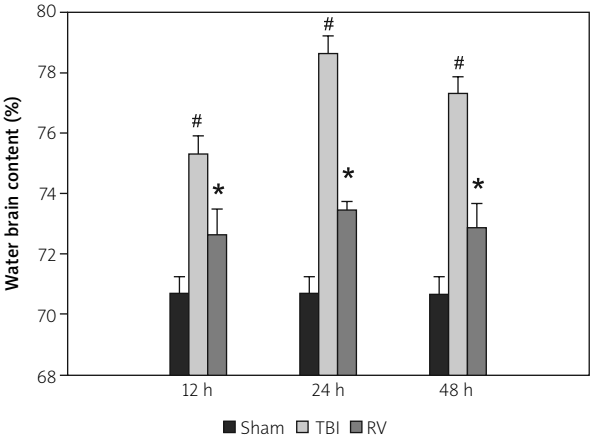

Fig. 1. Resveratrol treatment attenuates brain edema. Brain water content was measured by dry/wet ratio method in rats subjected to TBI. The brain water content increased significantly at 12, 24, and 48 hours after brain injury ($^{\#}p < 0.01$ vs. sham group). Resveratrol significantly reduced brain edema ($^{*}p < 0.05$ vs. TBI group), which is reflected by a decrease in brain water content. Bars represent mean ± SD ($n = 5$ per group).

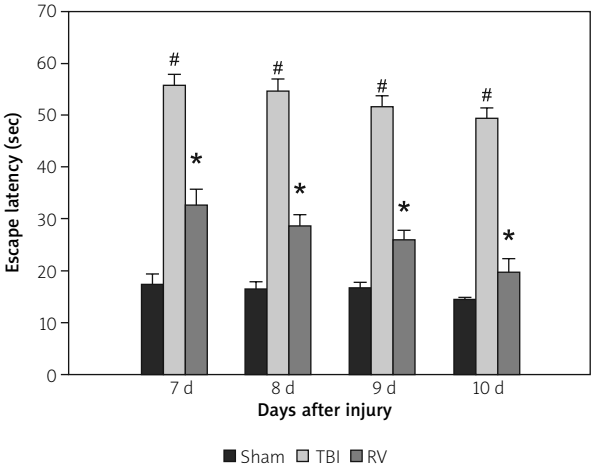

Fig. 2. Effects of resveratrol on Morris water maze test. The escape latency (seconds) increased significantly at 7, 8, 9, and 10 days after TBI (#p < 0.01 vs. sham group). Resveratrol treatment reduced the time taken by rats to find the platform, which indicated that the escape latency was significantly shortened ($^{*}p < 0.01$ vs. TBI group). Bars represent mean ± SD ($n = 5$ per group).

nificantly reduced after TBI by treatment with resveratrol.

Resveratrol treatment ameliorated the cognitive dysfunction

Morris water maze was designed to test behavioral changes of laboratory animals. We used this approach to investigate whether resveratrol could improve spatial memory deficits in rats at 7, 8, 9, and 10 days after brain injury. As shown in Figure 2, compared with the sham operation group, the escape latency was increased in the TBI group at days 7, 8, 9, and 10, depicting significant spatial learning deficits. Of note, the escape latency was significantly reduced in resveratrol-treated rats subjected to TBI.

Resveratrol treatment attenuated TBI-induced sensorimotor deficits

Sensorimotor function was evaluated by mNSS scores. As presented in Figure 3, the mNSS scores were significantly increased in rats after 1-4 days of TBI when compared with those of the sham group. Interestingly, treatment with resveratrol reduced the mNSS scores, suggesting a recovery of sensorimotor function in injured rats.

Resveratrol treatment ameliorated the neuronal autophagy in the hippocampus

A previous study has shown that the expression of LC3-autophagy-associated proteins was significantly increased 24 hours after brain injury [35]; therefore, we assessed the co-localization of NeuN and LC3 by immunofluorescence analysis 24 hours after TBI. Images presented in Figure 4A show that neuronal autophagy occurred after brain trauma, and the co-localization of NeuN and LC3 was significantly increased in the TBI group. Then, we detected proteins expression levels of LC3 II, Beclin-1, and P62 in the hippocampus using Western blot (Fig. 4B, C, Fig. 5). As expected, LC3 II and Beclin-1 were significantly increased in the TBI group compared with the sham group, while resveratrol significantly down-regulated these proteins at 12, 24, and 48 hours after TBI. In addition, P62 protein expression was significantly reduced in the injured hippocampus, while resveratrol treatment restored P62 levels.

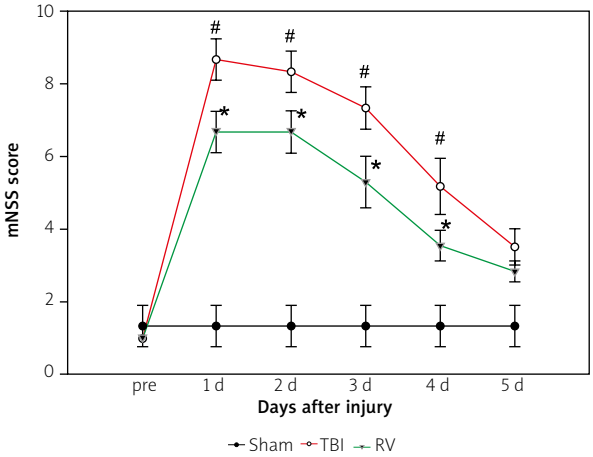

Fig. 3. Resveratrol attenuates TBI-induced sensorimotor deficits. The mNSS of TBI rats was significantly increased at 1-4 days when compared with that of the sham group ($^{\#}p < 0.05$ vs. sham group), while resveratrol treatment significantly lowered mNSS scores. This shows an improved recovery of sensorimotor function ($^{*}p < 0.05$ vs. TBI group). Data represent mean ± SD ($n = 5$ per group).

Resveratrol treatment reduced the levels of inflammatory factors in the hippocampus

The expression levels of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) in rat hippocampal tissues were detected using Western blot at 12, 24, and 48 hours after brain injury. As shown in Figure 6, the expression of TNF-α and IL-1β were significantly increased in the TBI group when compared with those of the sham group at every time point. Compared with the TBI group, TNF-α and IL-1β were significantly down-regulated at their corresponding times in resveratrol-treated rats. These results suggest that resveratrol can significantly reduce the production of inflammatory cytokines in the hippocampus of rats subjected to TBI.

Resveratrol treatment activated PI3K/Akt/mTOR signaling pathway in the hippocampus

Finally, to elucidate the potential effects of resveratrol in ameliorating brain injury, the PI3K/Akt/mTOR signaling pathway in the hippocampus was determined. We detected the phosphorylation lev-

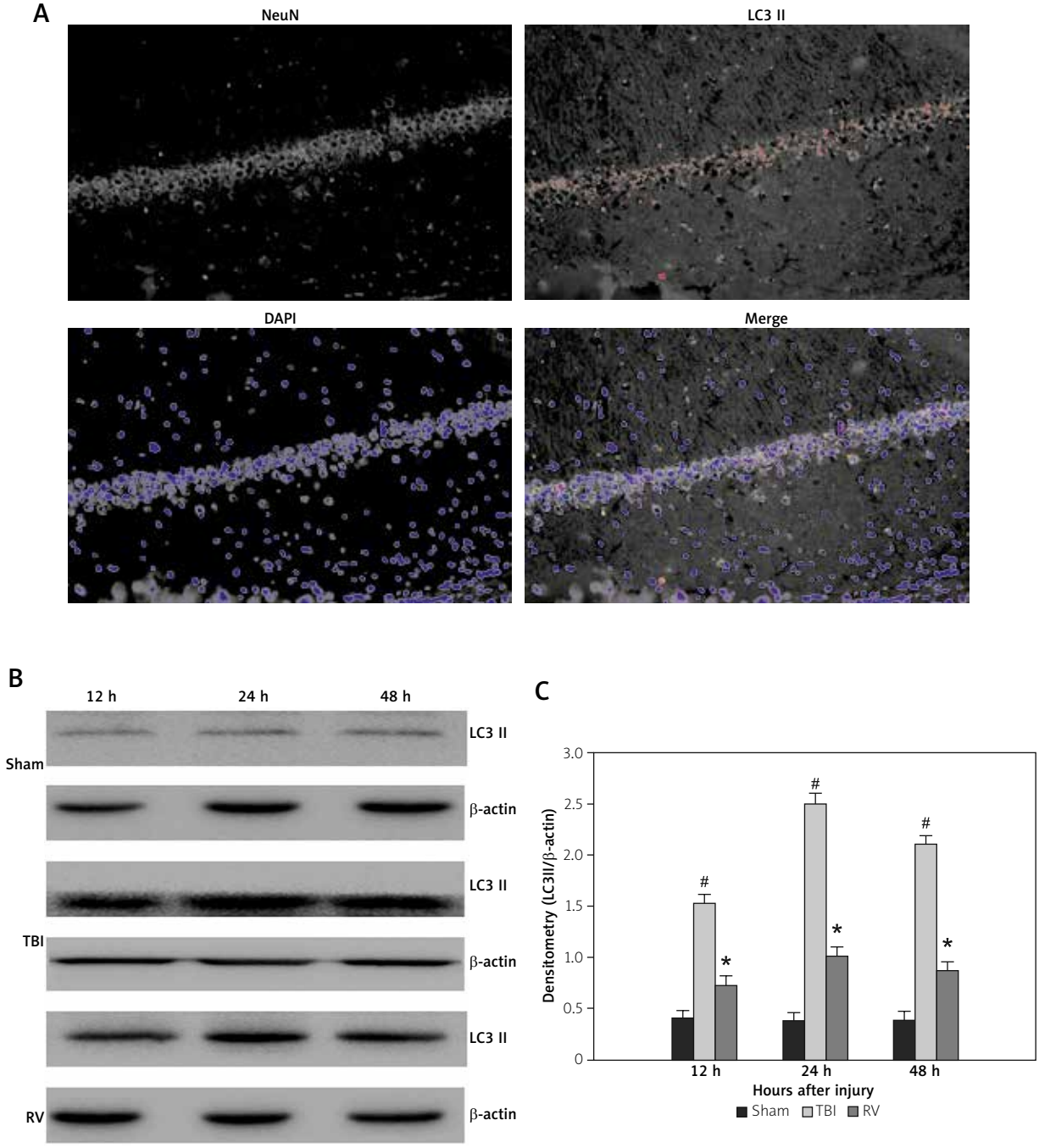

Fig. 4. A) Co-localization of NeuN and LC3 was determined by immunofluorescence analysis of the hippocampus 24 hours after TBI. The co-localization of NeuN (neuronal marker, green) and LC3 (red) was significantly increased in the TBI group. Cell nuclei were counterstained by DAPI (blue). Scale bar = 50 µm. **B**) The protein expression of LC3 II in the hippocampus was analyzed at 12, 24, and 48 hours using Western blot. **C**) Densitometry of the LC3 II band was corrected for the β-actin band. Results revealed that TBI induced LC3 II activation ($^{\#}p < 0.01$ vs. sham group), while resveratrol treatment markedly decreased LC3 II protein levels at 12, 24, and 48 hours after TBI ($^{*}p < 0.05$ vs. TBI group). Bars represent mean ± SD ($n = 5$ per group).

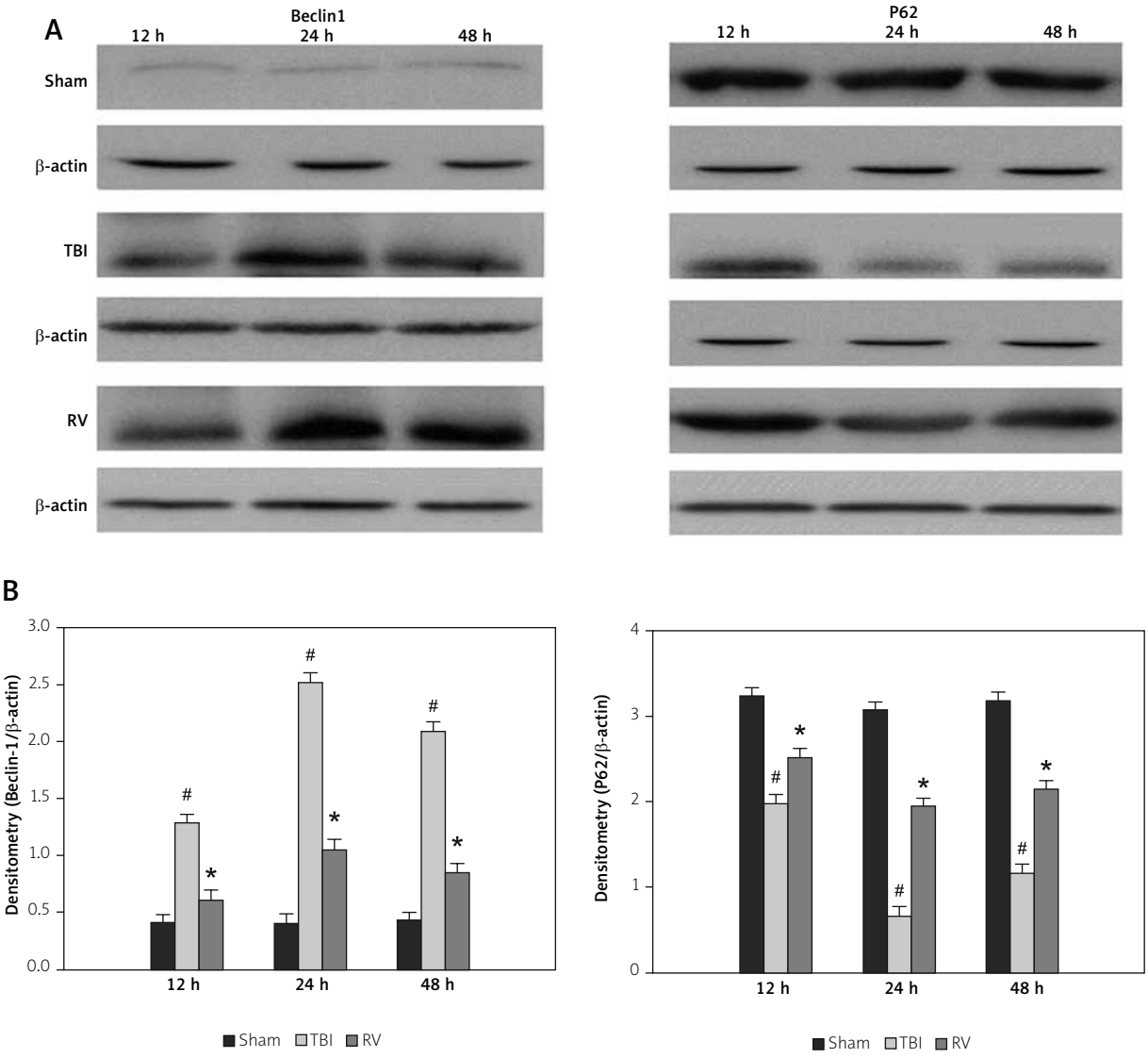

Fig. 5. A) Western blot analysis demonstrates the levels of Beclin-1 and P62 in the hippocampus at 12, 24, and 48 hours after TBI. **B**) Densitometry of Beclin-1 and P62 bands were corrected for β-actin. Results revealed that TBI induced Beclin-1 activation and P62 decrease ($^{\#}p < 0.01$ vs. sham group), while resveratrol treatment markedly decreased Beclin-1 protein levels and maintained P62 levels at 12, 24, and 48 hours after TBI ($^{*}p < 0.05$ vs. TBI group). Bars represent mean ± SD ($n = 5$ per group).

els of PI3K, Akt, and mTOR by using Western blot. The results showed in Figure 7A, compared with the sham group, phosphorylation levels of PI3K, Akt, and mTOR were raised in the TBI group ($^{\#}p < 0.05$ vs. sham group). Interestingly, the expression levels of p-PI3K, p-Akt, and p-mTOR were further increased by resveratrol treatment (Fig. 7B; $^{*}p < 0.05$ vs. TBI group), which corroborate that the PI3K/Akt/mTOR signaling pathway was activated upon treatment with resveratrol.

Discussion

Traumatic brain injury is the major cause of disability and death among young individuals in developed countries, and is a predominant medical problem. Therefore, investigation of the pathogenesis of TBI as well as the identification of effective interventions and/or treatments has been a topic of intense scientific output in the field of neuro-medicine. Although some mechanisms of brain injury

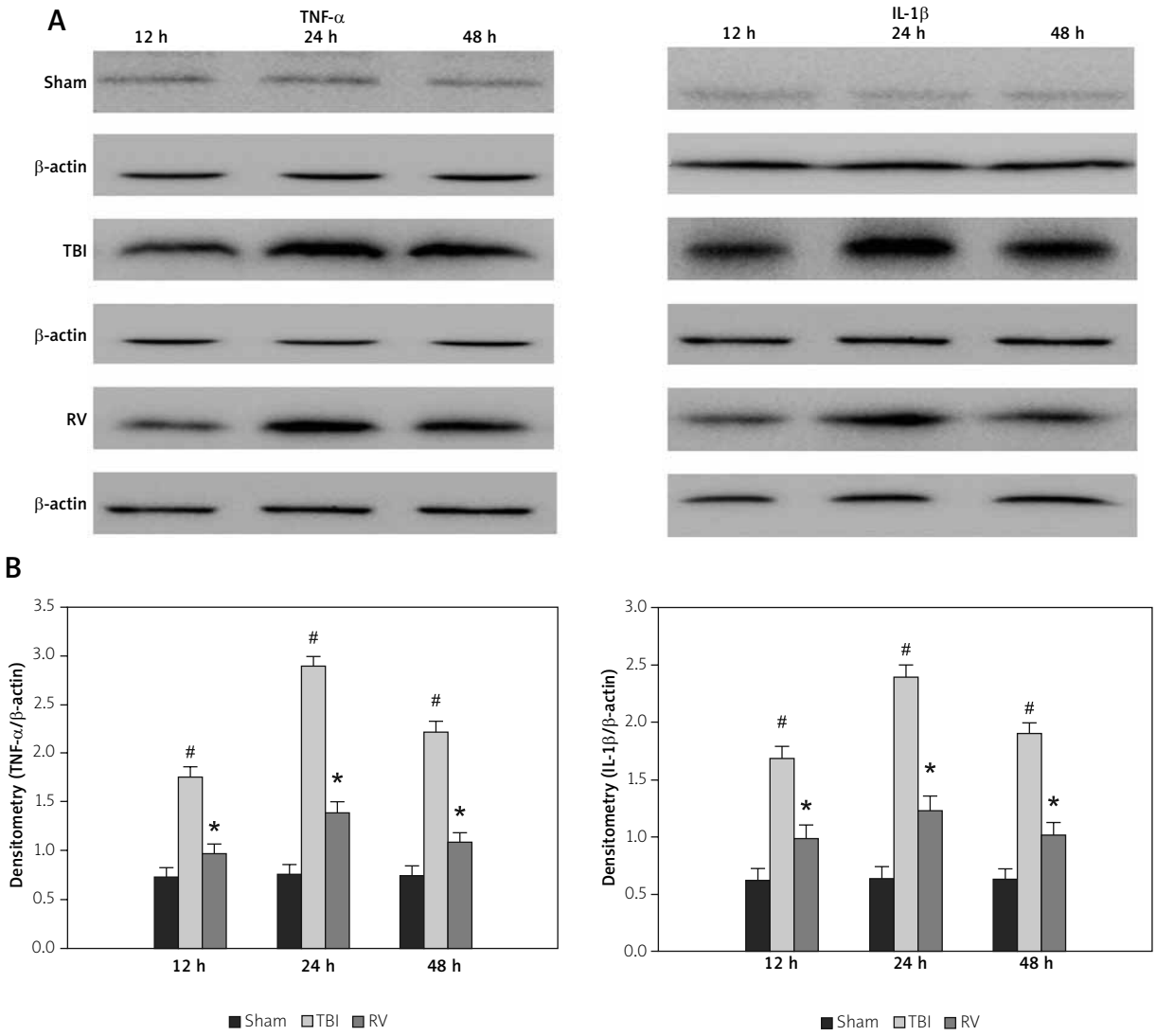

Fig. 6. The effect of resveratrol on IL-1β and TNF-α expression. **A**) The protein levels of IL-1β and TNF-α were determined by Western blot in the hippocampus of rats at 12, 24, and 48 hours after TBI or sham surgery. **B**) Densitometry of IL-1β and TNF-α bands was corrected for β-actin. Results showed that the expression of IL-1β and TNF-α increased significantly in the TBI group ($^{\#}p < 0.01$ vs. sham group). Resveratrol treatment significantly down-regulated the expression of IL-1β and TNF-α at 12, 24, and 48 hours after TBI ($^{*}p < 0.05$ vs. TBI group). Data were expressed as mean ± SD ($n = 5$ per group).

have been proposed, the mechanical destruction of neurons may lead to a series of pathological events that can potentiate neuronal damage and cell death upon brain injury [25]. Extensive pre-clinical animal research has been conducted to develop effective treatments for TBI. Despite these efforts, no treatment was demonstrated to improve the prognosis of patients with TBI. This may be due to biological differences between animals and humans, and/or pathophysiological differences in patients with severe brain injury. In this regard, studies have demonstrated that resveratrol present neuro-protective proprieties against a variety of diseases, including Parkinson's disease, Alzheimer's disease, and Huntington's disease [32,33].

In the present study, we further evaluated the neuro-protective effect of resveratrol on a rat model of TBI. Our results showed that resveratrol treatment (50 mg/kg) could decrease brain edema, improve learning and memory ability, and improve neuro-

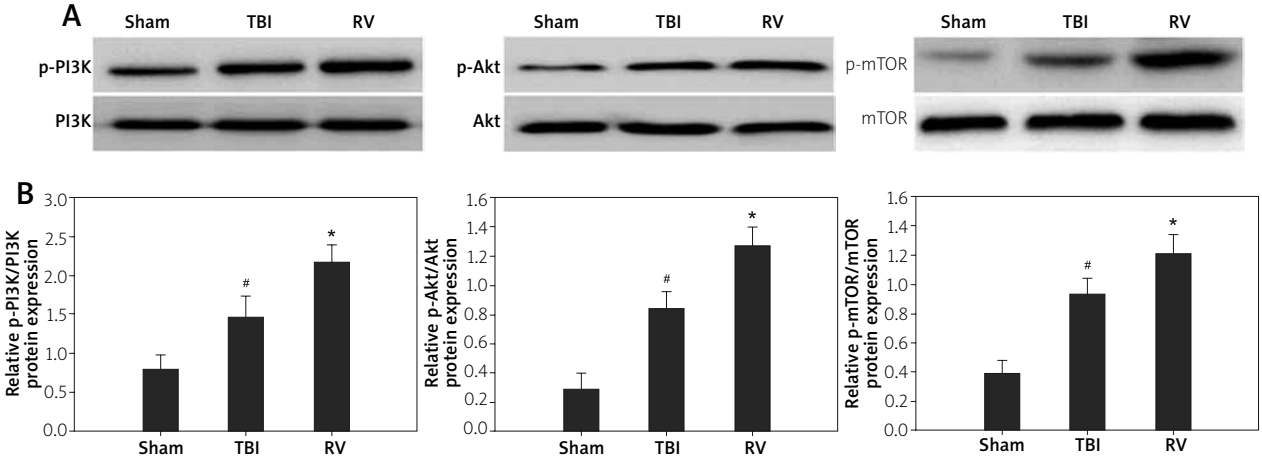

Fig. 7. Resveratrol activated the PI3K/Akt/mTOR signaling pathway in the injured rat hippocampus. **A**) Western blot analysis shows the expression levels of p-PI3K, p-Akt, and p-mTOR in the hippocampus at 24 hours following TBI or sham surgery. **B**) Quantitative analyses of p-PI3K, p-Akt, and p-mTOR in the hippocampus at 24 hours after TBI. Results demonstrated a significant elevation of p-PI3K, p-Akt, and p-mTOR expression in the TBI group ($^{\#}p < 0.01$ vs. sham group). Resveratrol treatment further enhanced the expression levels of p-PI3K, p-Akt, and p-mTOR following TBI at 24 hours ($^{*}p < 0.05$ vs. TBI group). Data were expressed as mean ± SD ($n = 5$ per group).

logical deficits in TBI rats. MWM test is a hippocampus-dependent memory task that is frequently used for examining cognitive deficits, and demonstrating permanent spatial learning ability and reference memory in rodents [20]. A finding that severity of post-mTBI deficits correlates with endogenous opioid system activity, and mTBI resulted in a significant deterioration of spatial memory performance and severity of depressive-like behavior in low analgesia (LA) mouse line [19]. In the present study, we confirmed that administration of resveratrol resulted in a significant reduction in escape latency, enhanced cognitive performance, and amelioration of the memory deficits associated with TBI. It is unclear, however, if the beneficial effects of resveratrol on memory deficits are related to enhanced endogenous opioid system activity after TBI in rats. Previous studies have found that the administration of resveratrol alleviated the degree of brain injury, since rats treated with this compound presented reduced brain water content and attenuated cognitive dysfunction after TBI [10,34], which is consistent with our findings. Moreover, since resveratrol can cross the blood-brain barrier and prevent brain damage caused by cerebral ischemia, we speculate that this compound is a potentially promising therapeutic option for TBI patients [3,37].

To further explore the molecular mechanisms of the neuro-protective effect of resveratrol, we evaluated the levels of autophagy markers with Western blotting. Autophagy is a deleterious process, in which cells dispose of misfolded proteins and damaged organelles into vesicles fused with lysosomes to form autophagy lysosomes, to accomplish metabolic needs of cells and renewal of organelles [30]. Interestingly, the exact functional role of autophagy after TBI has not been fully elucidated. Erlich *et al.* [8] showed that rapamycin, which induces neuronal autophagy by inhibiting its' mammalian target protein, improved neurological prognosis after TBI. On the other hand, accumulating evidence suggests that the activation of the neuronal autophagy pathway is related to the pathological mechanism of post-traumatic brain injury, and inhibition of this pathway can alleviate brain injury and functional defects [10,15,21]. In our current study, treatment with resveratrol suppressed the expression levels of LC3 II and Beclin-1, and increased P62 in the hippocampus at 12, 24, and 48 hours after injury. Armour *et al.* [2] demonstrated that rapamycin-induced autophagy in multiple cell lines (NIH/3T3 cells or HEK293 cells) can be dramatically reduced by resveratrol treatment. Lin *et al*. [23] shown that TBI leads to glutamate-induced astrocyte death through the activation of

a ROS-mediated GSK-3β signaling pathway. These authors reported that resveratrol improved cell survival against TBI by inhibiting GSK-3β-mediated autophagy and apoptosis. Recently, Fan *et al*. [9] observed that resveratrol could activate autophagy and inhibit apoptosis of neurons by the PI3K signaling pathway; thus, promoting the recovery of neuronal function in spinal cord injury rats. Taken together, it seems reasonable to assume that the neuro-protection of resveratrol in TBI-subjected rats may be related to a decreased neuronal autophagy.

Furthermore, increasing evidence suggests that neuro-inflammatory responses mediated by inflammatory mediators may play an important role in the establishment or secondary injury development of multiple brain conditions [26]. Our data showed that resveratrol reduced the expression levels of TNF-α and IL-1β in the hippocampus of rats at 12, 24, and 48 hours after injury. Indeed, previous studies indicated that resveratrol reduced protein levels of TNF-α and Iba-1 in the hippocampus of high-fat diet (HFD)-fed mice, reversed obesity-related neuroinflammation and metabolic derangements, and enhanced cognitive function [13,29]. Another study has observed that resveratrol attenuated the inflammatory response and alleviated the degree of TBI in rats, which may occur through the reduction of reactive oxygen species and inhibition of NLRP3 [42]. Moreover, there is evidence that TNF-α induces the up-regulation of macro-autophagy *in vitro* in human skeletal muscle cells and murine macrophages [1,16]. In particular, in human atherosclerotic vascular smooth cells, the expression of autophagy genes can be influenced by TNF-α, in a process mediated by Jun kinase (JNK) or Akt pathways [14]. However, the mechanism of autophagy induced by TNF-α is still not fully understood, and there may be differences among various cell types or injury models. We hypothesized that resveratrol might influence autophagy pathways by down-regulating inflammatory cytokines to some extent, and we will investigate other pathways in the future.

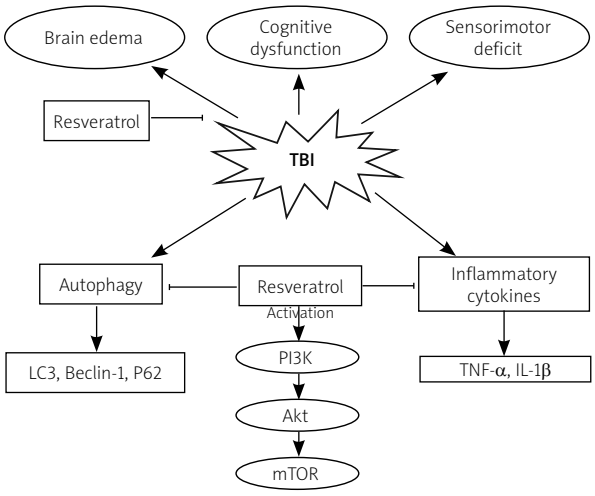

Fig. 8. A simplified schematic diagram representing neuro-protective mechanisms of resveratrol in rats subjected to TBI.

Many studies have shown that activation of the PI3K/AKT signaling pathway is neuro-protective in TBI through the induction of anti-apoptosis and autophagy signaling [12,27]. Previous evidence indicated that mTOR signaling pathway is the key to improving the internal regeneration ability of neurons, while mTOR activation can effectively promote the regeneration of nerve fibers [28]. Wang *et al*. reported that the PI3K/Akt/mTOR signaling pathway was closely associated with the initiation of autophagy, and the inhibition of this pathway by LY294002 (a PI3K-specific inhibitor) could significantly promote autophagy, which protects against mitochondrial-related apoptosis [39]. Another recent study has shown that homer1a expression is protective against neuronal injury in a traumatic neuronal injury model *in vitro*, acting through increased neuron autophagy and decreased expression of pro-apoptosis proteins, which may be involved in the PI3K/AKT/mTOR signaling pathway [38]. Our results showed that treatment with resveratrol induced the activation of PI3K/AKT/mTOR pathway, in which the protein expression levels of p-PI3K, p-AKT, and p-mTOR were further increased when compared with those of the TBI group. Therefore, we hypothesized that TBI might induce neuronal autophagy, aggravating inflammatory responses, while resveratrol treatment weakens TBI-induced autophagy and inflammation, thus being neuro-protective through the activation of PI3K/AKT/mTOR signal pathway.

In conclusion, the experimental data presented herein corroborates that resveratrol treatment decreases brain edema, improves spatial learning and memory, and improves the recovery of sensorimotor function of rats subjected to TBI. At the molecular level, resveratrol attenuates neuronal autophagy and inflammation in the injured hippocampus, and the activation of the PI3K/Akt/mTOR

signaling pathway is involved in this neuro-protective effect (Fig. 8). Therefore, we propose that resveratrol is a promising therapeutic agent, which might provide a clinical alternative for the treatment of TBI.

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Ethics approval and consent to participate

The present study was approved by the Animal Ethics Committee of Hebei Medical University Animal Center.

Disclosure

The authors report no conflict of interest.

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