

Original Article

Baicalein improves sepsis-associated encephalopathy via suppressing oxidative stress and iNOS-mediated NO production and enhancing BDNF/TrkB signaling

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Received October 13, 2015; Accepted January 27, 2016; Epub February 15, 2016; Published February 29, 2016

Abstract: Baicalein is a medicinal herb and has various biological activities. Our present work aimed to evaluate the protective effect of baicalein on sepsis-associated encephalopathy (SAE) and further probe the potential mechanisms. SAE model was prepared using cecal ligation and puncture in mice. Animals were treated with saline or baicalein by doses of 10, 20 and 40 mg/kg for seven consecutive days. Neuronal function was assessed with the Open Field tests, Morris Water Maze task and Y Maze tests. The serum ammonia levels were measured after SAE. Besides, malondialdehyde (MDA) contents and the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and inducible nitric oxide synthase (iNOS) activities were also detected using respective commercial kits. Detection of NO production was conducted using Griess reagent. No significance was found in Open Field test. However, the cognitive deficits were remarkably improved in SAE-induced mice after baicalein treatment based on the results of Morris Water Maze and Y Maze tests. Meanwhile, baicalein didn't alter the serum ammonia levels in SAE-induced mice. But baicalein significantly suppressed oxidative stress, the protein levels of iNOS and NO production while the protein expressions of BDNF was found to be markedly enhanced in mice with SAE after baicalein treatment. It was concluded that baicalein reversed cognitive deficits in a mouse model of SAE via suppressing oxidative stress and iNOS-mediated NO production and activating BDNF/TrkB signaling.

Keywords: Baicalein, sepsis-associated encephalopathy, oxidative stress, inducible nitric oxide synthase, brain-derived neurotrophic factor

Introduction

Sepsis-associated encephalopathy (SAE) is a clinical syndrome that manifests diffuse cerebral dysfunction without direct central nervous system (CNS) infection [1]. It was estimated that there were nearly 70% of patients who had serious systemic infection [2]. The severity of SAE could lead to the more increased mortality. Therefore, it is of desperate need to explore the pathogenesis of SAE and develop the corresponding drugs to reduce the severity of SAE, finally diminishing the death rate of SAE.

Oxidative stress was known to be closely associated with the pathogenesis of SAE. In details, it was previously reported that enhanced production of reactive oxygen species (ROS) caused oxidative stress, leading to injury to sys-

tem tissues and organs [3]. Additionally, excession of oxygen free radicals and/or antioxidant deficiency to different brain regions was reported to trigger evident morphological dysfunction and cognitive deficits [4]. In fact, inhibition of oxidative stress by drugs could indeed reverse cognitive decline in septic rats [3, 5]. Furthermore, high levels of inducible nitric oxide synthase (iNOS) could also promote the generation of SAE [6].

Brain-derived neurotrophic factor (BDNF) is one of the most important members in neurotropic family and is widely distributed in the brain [7, 8]. A previous investigation illustrated that BDNF played a crucial role in neuronal survival and synaptic transmission [9]. Additionally, we previously reported BDNF could prevent neuronal impairments following ischemic insults in

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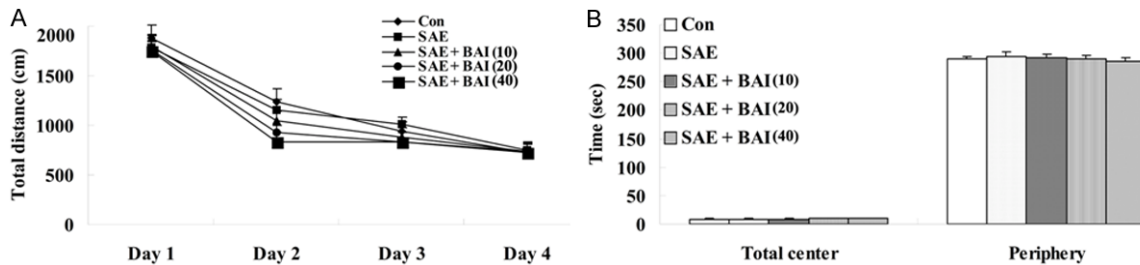


Figure 1. Effects of baicalein on the total distance (A) and time spent in the open field (B) ($n = 10$, mean \pm SD). Con, control group; SAE, septic-associated encephalopathy; SAE + BAI (10), baicalein (10 mg/kg)-treated group; SAE + BAI (20), baicalein (20 mg/kg)-treated group; SAE + BAI (40), baicalein (40 mg/kg)-treated group.

gerbils [10]. In a mouse model of SAE, it was found that activation of BDNF by valproic acid could remarkably improve cognitive deficits [7], suggesting that BDNF might be served as the useful therapeutic target for alleviation of SAE.

Baicalein is the biological ingredients purified from *Scutellaria baicalensis* George. Various investigations have disclosed that it has anti-inflammatory, anti-oxidative, anti-tumor and cardiovascular potentials [11-13]. Some investigations has reported that baicalein reverse learning and memory dysfunction in beta-amyloid-induced amnesia [14] and chronic cerebral hyperfusion in animals [15]. Besides, our previous study also observed that baicalein could alleviate cognitive deficits caused by epilepsy in experimental animals [16]. However, whether baicalein can improve cognitive performance in septic mice remains elusive. Therefore, our present work aimed to evaluate the protective effect of baicalein on SAE and figure out the potential mechanisms.

Materials and methods

Preparation of SAE and drug treatment

The SAE model was induced by cecal ligation and puncture (CLP) as previously described [17]. Briefly, after anesthesia, the cecum was carefully isolated and ligated with 4.0 silk below the ileocecal junction. A sterile 22-gauge needle was used to perforate the cecum and the cecum was gently squeezed to extrude a small amount of feces from the puncture site. Animals were randomly assigned to the following five groups: (1) control group (Con) ($n = 10$), control group with physiological saline (0.1 ml/100 g) intraperitoneally (i.p.); (2) SAE group ($n = 10$), CLP with physiological saline (0.1 ml/100 g)

intraperitoneally (i.p.); (3) SAE + BAI (10) group ($n = 10$), CLP with baicalein (10 mg/kg); (4) SAE + BAI (20) group ($n = 10$), CLP with baicalein (20 mg/kg); (5) SAE + BAI (40) group ($n = 10$), CLP with baicalein (40 mg/kg). Baicalein (Sigma, with a purity of 95%) was dissolved in physiological saline and intraperitoneally injected once a day for 7 consecutive days.

Open field test

Open Field test was firstly employed to study the spontaneous explorative activity as previously described [7]. The total distance traveled (cm) and time spent in the center were calculated.

Morris Water Maze task

Morris Water Maze task was conducted to investigate spatial learning and memory function. For spatial training experiments, mice were given three trials per day over 4 consecutive days. The escape latency (s) and path length (cm) were calculated in each trial and averaged over three trials for each mouse. Swimming speed was also analyzed by dividing the path length by the escape latency. For the probe trial, the percentage of time spent in target quadrant and number of times of crossing platform within 60 s were determined.

Y Maze experiment

Memory function was also confirmed by Y Maze experiment according to the previous study [18]. The number of learning trials was recorded from each group.

Serum ammonia assay

The level of serum ammonia was measured by an autoanalyzer (Hitachi 7080, Tokyo, Japan).

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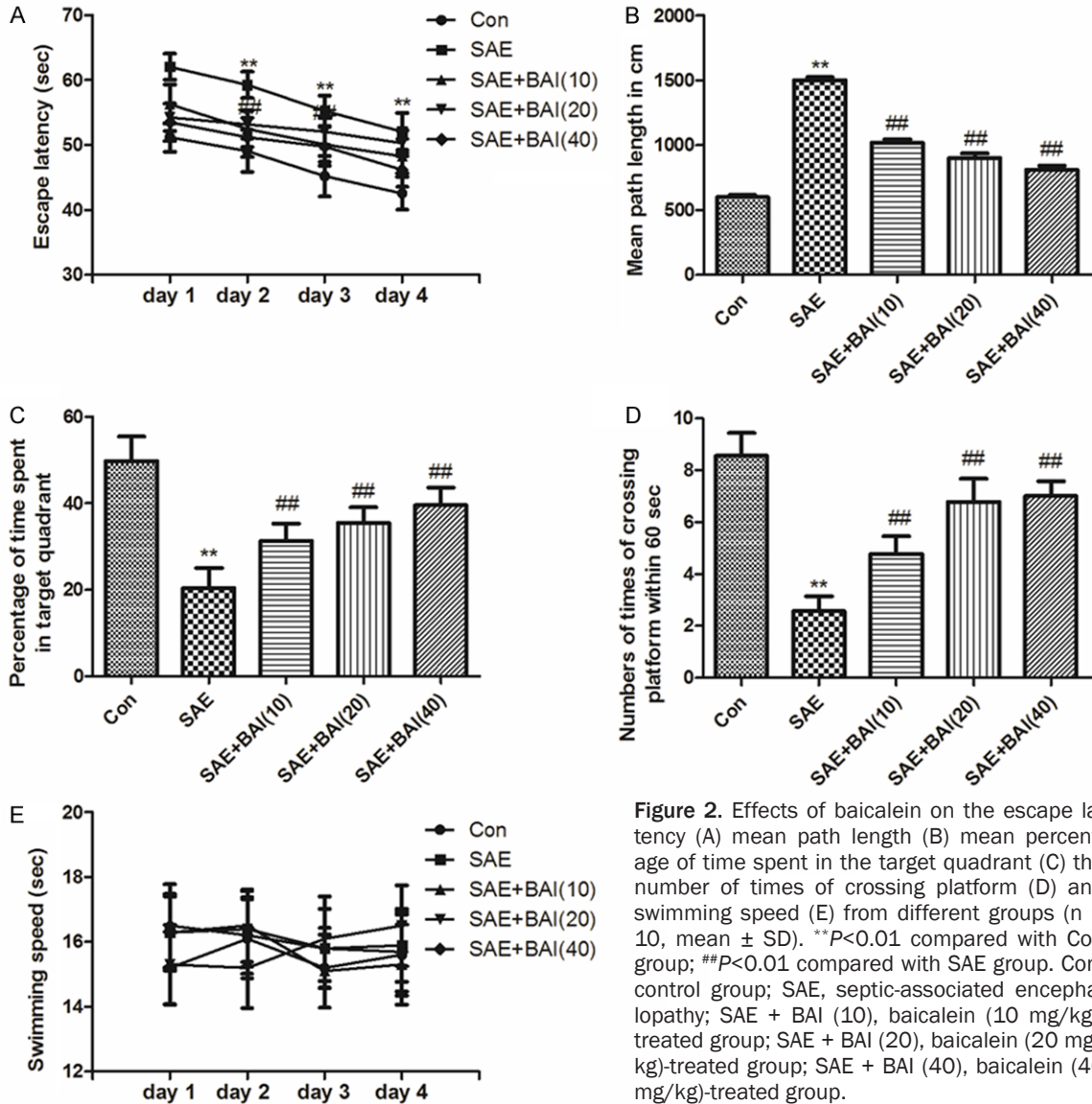


Figure 2. Effects of baicalein on the escape latency (A) mean path length (B) mean percentage of time spent in the target quadrant (C) and the number of times of crossing platform (D) and swimming speed (E) from different groups (n = 10, mean ± SD). **P<0.01 compared with Con group; ##P<0.01 compared with SAE group. Con, control group; SAE, septic-associated encephalopathy; SAE + BAI (10), baicalein (10 mg/kg)-treated group; SAE + BAI (20), baicalein (20 mg/kg)-treated group; SAE + BAI (40), baicalein (40 mg/kg)-treated group.

Detections of malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX)

The MDA level and the activities of CAT, SOD and GSH-PX in hippocampal homogenates were determined by respective commercial kits (Jiancheng Bioengineering Institute, Nanjing, China).

Determination of inducible nitric oxide synthase (iNOS)

The activity of iNOS was measured using the iNOS assay kit (Jiancheng Bioengineering Institute, Nanjing, China).

Assay of plasma NO production

The NO production was also detected by the colorimetric analysis using the Griess reagent.

Western blot analysis

The hippocampal samples were collected after baicalein treatment. The membranes were incubated with the following primary antibodies: anti-iNOS (1:1000, Santa Cruz, USA), anti-GAPDH (1:2000, Kangcheng, China), anti-phosphorylated TrkB (1:200, Santa Cruz, USA), anti-TrkB (1:300, Santa Cruz, USA) and anti-β-actin (1:500, Sigma, USA). Immunodetected protein bands were analyzed using Quantity One software (BioRad, USA).

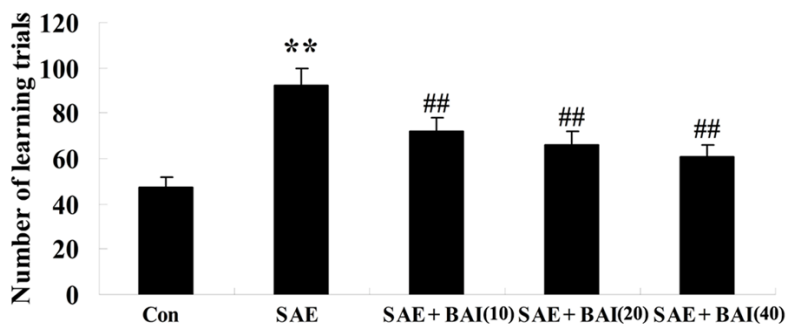


Figure 3. Effects of baicalein on the number of learning trials by Y Maze test from different groups (n = 10, mean ± SD). **P<0.01 compared with Con group; ##P<0.01 compared with SAE group. Con, control group; SAE, septic-associated encephalopathy; SAE + BAI (10), baicalein (10 mg/kg)-treated group; SAE + BAI (20), baicalein (20 mg/kg)-treated group; SAE + BAI (40), baicalein (40 mg/kg)-treated group.

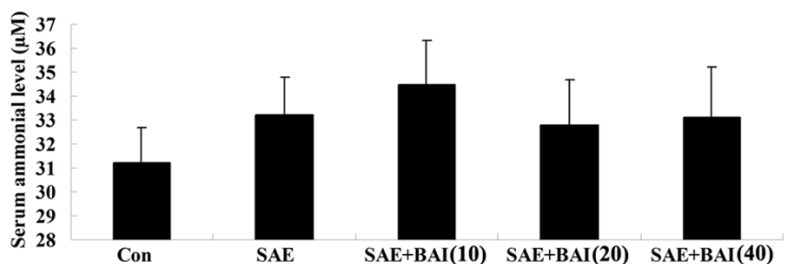


Figure 4. Effects of baicalein on the serum ammonia level from different groups (n = 10, mean ± SD). Con, control group; SAE, septic-associated encephalopathy; SAE + BAI (10), baicalein (10 mg/kg)-treated group; SAE + BAI (20), baicalein (20 mg/kg)-treated group; SAE + BAI (40), baicalein (40 mg/kg)-treated group.

Statistical analysis

All values were expressed as mean ± SD. Comparison between different groups was carried out using one-way ANOVA followed by Dunnett’s test. A p value less than 0.05 was considered statistically significant.

Results

Effects of baicalein on locomotor activity and cognitive function in SAE-induced mice

Figure 1A and **1B** showed the results of Open Field test from different groups. It was noteworthy that there were no significant differences for the total distance and time spent in the open field among groups. Cognitive function was assessed by Morris Water Maze test and Y maze experiments. Septic mice treated with baicalein by different doses exhibited reduced escape latency (P<0.01, **Figure 2A**) and mean

path length (P<0.01, **Figure 2B**) while enhanced percentage of time spent in target quadrant (P<0.01, **Figure 2C**) and number of times of crossing the platform (P<0.01, **Figure 2D**). The swimming speed was similar among different groups (P<0.01, **Figure 2E**). Y maze test disclosed that baicalein significantly diminished the number of learning trials in SAE-induced mice (P<0.01, **Figure 3**).

Baicalein did not alter serum ammonia level in SAE-induced mice

No significance was observed for the serum ammonia levels among different groups (**Figure 4**).

Baicalein suppressed the oxidative stress, iNOS activity and NO production SAE-induced mice

It was indicated the increased oxidative stress (the high level of MDA, increased activities of CAT, SOD and GSH-PX) (P<0.01, **Figure 5A-D**), elevated iNOS activity (P<0.01, **Figure 5E**) and excessive generation of NO (P<0.01, **Figure 5F**) in septic mice. However, these indices was markedly reversed in SAE-induced mice when treatment with baicalein in a dose-dependent manner.

Baicalein increased the protein expressions of BDNF and phosphorylated TrkB (p-TrkB) SAE-induced mice

We further investigated whether baicalein influenced the protein levels of BDNF and its specific receptor, namely, TrkB in septic mice. It was noted that the protein expressions of BDNF and p-TrkB (the active form of TrkB) were both evidently augmented in mice subjected to SAE after baicalein treatment (P<0.01, **Figure 6A, 6B**). Nevertheless, there were no significance for the total level of TrkB among different groups.

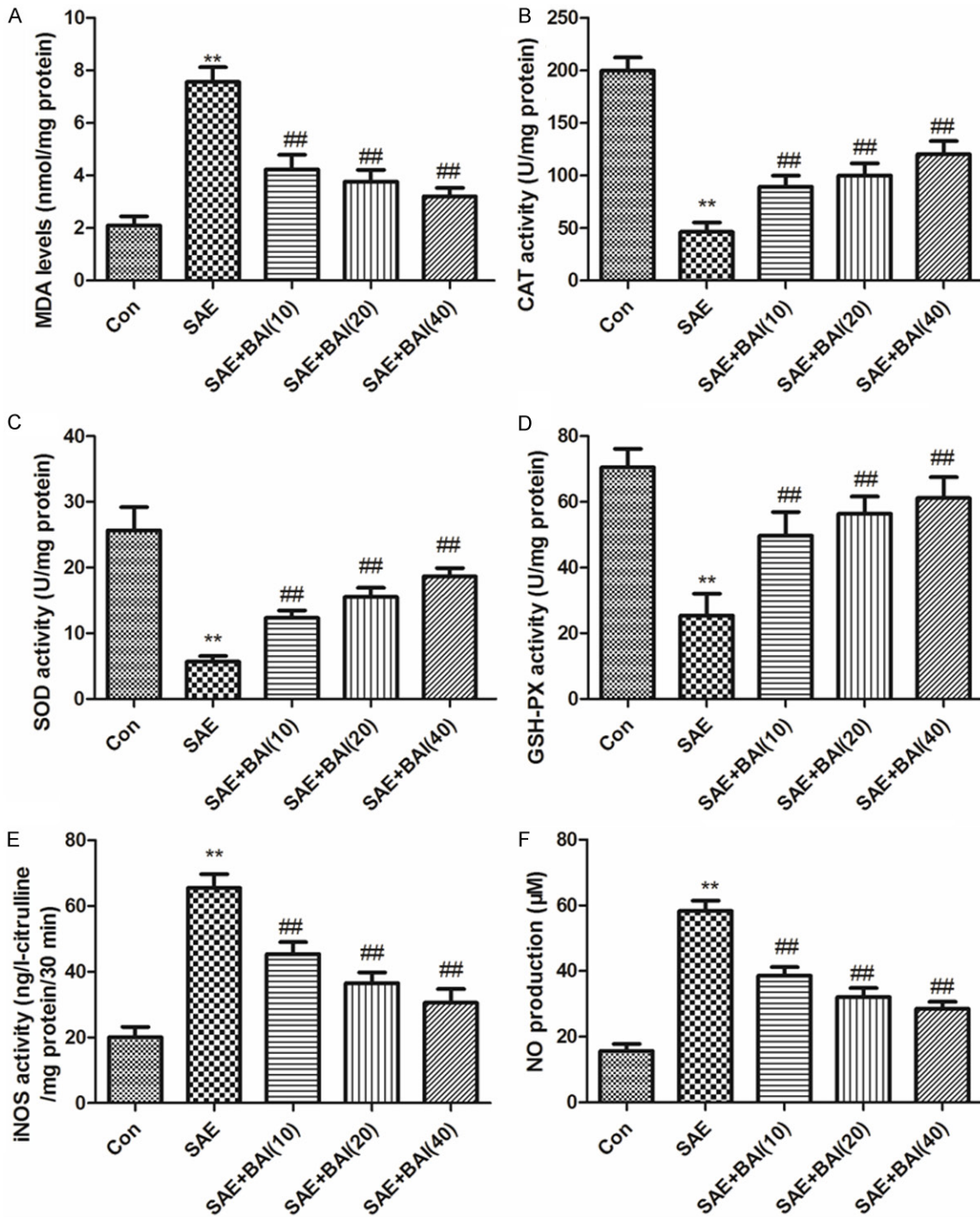


Figure 5. Effects of baicalein on MDA (A), CAT (B), SOD (C), GSH-PX (D), iNOS activity (E) and NO production (F) from different groups (n = 10, mean ± SD). **P<0.01 compared with Con group; ##P<0.01 compared with SAE group. Con, control group; SAE, septic-associated encephalopathy; SAE + BAI (10), baicalein (10 mg/kg)-treated group; SAE + BAI (20), baicalein (20 mg/kg)-treated group; SAE + BAI (40), baicalein (40 mg/kg)-treated group.

Discussion

The major findings of our present work illustrated that baicalein has protective effects against

SAE in mice and its neuroprotection might be linked with suppressing oxidative stress, inhibiting iNOS-related NO generation and enhancing BDNF/TrkB signaling.

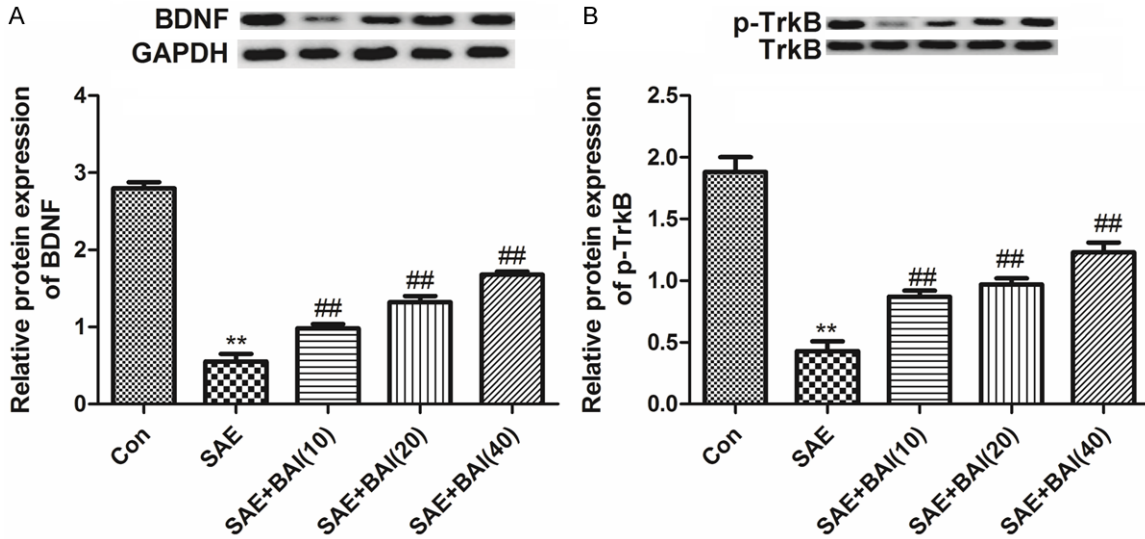


Figure 6. Effects of baicalein on the protein expressions of BDNF and phosphorylated TrkB (p-TrkB) from different groups (n = 10, mean ± SD). The relative protein expression of BDNF was normalized to GAPDH (A) and p-TrkB was normalized to TrkB (B). ***P*<0.01 compared with Con group; ##*P*<0.01 compared with SAE group. Con, control group; SAE, septic-associated encephalopathy; SAE + BAI (10), baicalein (10 mg/kg)-treated group; SAE + BAI (20), baicalein (20 mg/kg)-treated group; SAE + BAI (40), baicalein (40 mg/kg)-treated group.

SAE is a clinical syndrome that manifests serious cognitive deficits together with systemic infection in the absence of direct brain infection [2]. Especially, it has high morbidity and mortality rates for the ICU patients [19]. It was previously reported that SAE often had long-term memory dysfunction that could affect nearly 70% of patients [2]. Granger et al found that CLP model was a very useful model to study the cognitive deficits after sepsis [7]. Besides, CLP mice were also shown to exhibit serious cognitive decline after seven days [7]. Consistent with the previous findings, our present work confirmed the memory loss in CLP mice and depicted that baicalein improved memory functions in SAE-induced mice according to the results of Morris Water Maze and Y Maze tests. Meanwhile, our study indicated a similar tendency for the results in the Open Field among different groups, suggesting baicalein did not influence anxiety-related behaviors.

Oxidative stress and iNOS signaling were the important mechanisms in the pathogenesis of SAE. Under normal condition, oxidative stress can be manipulated by various endogenous anti-oxidative enzymes including CAT, SOD and GSH-PX. However, the deficiencies of these anti-oxidative enzymes are taken place under the state of disease. The high content of MDA is

the indicator that can reflect serious cellular damage. Results from our current study showed that baicalein increased the activities of CAT, SOD and GSH-PX as well as decreased MDA level in the hippocampus of SAE-induced mice, suggesting the anti-oxidative effects of baicalein on SAE. In line with our study, prior work also found that baicalein ameliorated epilepsy-associated cognitive deficits via the anti-oxidative potential [16]. It was reported that iNOS played a crucial role in the development of SAE [20]. Preconditioning of LPS-induced septic mice by heat shock was observed to decrease the severity of SAE accompanied with down-regulation iNOS [6], indicating that reduced iNOS by drugs might have protective role against SAE. We presently found that baicalein could decrease iNOS activity and NO production in SAE-induced mice, implicating that the protection of baicalein might be related to inhibiting iNOS-mediated NO generation.

BDNF/TrkB signaling is demonstrated to be involved in the modulation of learning and memory function and synaptic transmission [9]. Prior work revealed that enhanced BDNF expression could attenuate ischemic impairments in gerbils [10]. Besides, activation of BDNF by valproic acid was previously shown to have protection against cognitive deficits in a mouse model of SAE [7]. It is known that BDNF

exert protective role via binding and activating its specific receptor TrkB [21]. Wu et al also reported that valproic acid reversed cognitive decline in SAE-induced mice via increasing p-TrkB (the active form of TrkB) [7]. Similarly, our present investigation revealed that baicalin significantly increased the protein levels of BDNF and p-TrkB after sepsis, implying that its protection against cognitive deficits might be associated with activating BDNF/TrkB signaling.

Conclusions

In conclusion, our investigation disclosed that baicalin improved cognitive deficits in SAE-induced mice and this neuroprotection might be associated with suppressing oxidative stress and iNOS-mediated NO production together with enhancing BDNF/TrkB signaling.

Acknowledgements

This work was partly supported by National Natural Science Foundation of China (Grant number: 81202025).

Disclosure conflict of interest

None.

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