ONO-2506 alleviates lipopolysaccharide-induced hippocampal neuroinflammation and apoptosis

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Research article

Keywords: Sepsis associated encephalopathy, S100β, neuroinflammation, apoptosis, arundic acid

Posted Date: November 12th, 2019

DOI: https://doi.org/10.21203/rs.2.17161/v1

Abstract

Background Sepsis associated encephalopathy has high mortality rate, but there is no targeted therapy to reduce brain damage in septic patients. In our previous study, we found that S100β concentration was higher in patients with SAE. At high concentration, S100β exerts neurotoxic effects through receptor for advanced glycation end products (RAGE). RAGE-activated signalling could induce the inflammatory response. And neuroinflammation is an important mechanism of SAE. So inhibiting S100β expression may be a potential treatment of SAE. ONO-2506 can inhibit the production and release of S100 protein from astrocytes. In this study, we administered ONO-2506 to mice in order to evaluate its effectiveness on neuroinflammation and apoptosis in hippocampus induced by lipopolysaccharides.

Results We found administration with lipopolysaccharides increased the levels of S100β, RAGE, IL-β, TNF-α and the TUNEL positive brain cells in hippocampus tissue. The ONO-2506 30mg/kg and 90mg/kg could reduce the levels of neuroinflammation (IL-β and TNF-α), and alleviate the apoptosis in hippocampus.

Conclusions ONO-2506 could reduce the neuroinflammation and alleviate brain cell apoptosis in hippocampus of LPS mice models. Moreover, the RAGE expression was inhibited after ONO-2506 treatment.

1. Background

Organ injury is common in sepsis. Brain function is an important parameter in both Sequential Organ Failure Assessment Scores and quick Sequential Organ Failure Assessment Scores [1]. It is commonly called sepsis associated encephalopathy (SAE) when brain injury occurs in sepsis. The symptoms of SAE can vary from delirium to coma [2]. Our previous study showed the mortality of SAE was above 50 % [3]. In addition, survivors may have long-term cognitive impairment and low health-related quality of life [4]. In spite of the high mortality rate associated with SAE, there is no targeted therapy to reduce brain damage in septic patients. Clinical management is limited to the resolution of the underlying sepsis and treatment of the symptomatic treatment, using sedation medications to control the delirium symptom for example [5]. In an animal study, rapamycin was found to have protective effect on sepsis induced cognitive impairment in mouse [6], but rapamycin is essentially an immunosuppressive drug.

In our previous study, we found that S100β concentration was higher in patients with SAE and better for both diagnosing SAE and predicting the outcome of sepsis compared to neuron-specific enolase [7]. S100β exerts neurotrophic effects at low concentrations, but has neurotoxic effects at high concentrations through receptor for advanced glycation end products (RAGE) activation [8] [9]. RAGE-activated signalling could induce the inflammatory response. And neuroinflammation is an important mechanism of SAE [10]. So inhibiting S100β expression may be a potential treatment of SAE. Arundic acid (ONO-2506) can inhibit the production and release of S100 protein from astrocytes [11]. While numerous studies have assessed the effect of ONO-2506 in the diseased nervous system [12-21], to the best of our knowledge, no study has examined the effect of ONO-2506 in SAE. In this study, we

administered ONO-2506 to mice in order to evaluate its effectiveness on neuroinflammation and histological injury induced by lipopolysaccharides (LPS).

2. Results

There were no adverse events caused by ONO-2506 infusion. All animals were included in the analysis.

2.1 Western Blot Analysis

We found in hippocampus tissue, administration with LPS increased the levels of S100β, RAGE, IL-β and TNF-α. With the treatment of ONO-2506, the S100β, RAGE and TNF-α in LPS model were lower than LPS model without treatment of ONO-2506 (P□0.05). Moreover, it showed a dose-dependent relationship. There was no difference in IL-β between LPS group and LPS+ONO-2506 (10mg/kg) group (P□0.05). But IL-β in LPS+ONO-2506 (30mg/kg) and LPS+ONO-2506 (90mg/kg) groups was lower than LPS group (P□ 0.05). In addition, IL-β was lower in the group with administration of LPS+ONO-2506 with 90 mg/kg than that in LPS+ONO-2506 with 30mg/kg group (P□0.05). In addition, S100β, RAGE, IL-β and TNF-α expression were not significantly changed in Normal+ONO-2506 mice compared to Normal group (P□ 0.05) (Fig. 1 and Fig. 2).

Fig. 1 Representative Western blots of IL-1β, TNF-α, RAGE and S100β in groups

□Normal group; □LPS group; □LPS+ONO-2506 10mg/kg group; □LPS+ONO-2506 30mg/kg group; □LPS+ONO-2506 90mg/kg group; □Normal+ONO-30mg/kg group

Fig. 2 Quantitative densitometry analysis of Western blots for the ratio of S100β/β-actin, RAGE/β-actin, IL-1β/β-actin and TNF-α/β-actin

1 Normal group; 2 LPS group; 3 LPS+ONO-2506 10mg/kg group; 4 LPS+ONO-2506 30mg/kg group; 5 LPS+ONO-2506 90mg/kg group; 6 Normal+ONO-30mg/kg group

*P□0.05, versus the Normal group; #P□0.05, versus the LPS group

2.2 TUNEL staining

The number of TUNEL-positive cells in the hippocampus increased significantly in the LPS group compared with the Normal group (P□0.05) (Fig. 3 and Fig. 4). The increased brain cell apoptosis in LPS model was partially decreased by treatment of ONO-2506 30mg/kg and ONO-2506 90mg/kg (P□0.05) (Fig. 3 and Fig. 4).

Fig. 3 Representative TUNEL staining images in CA1 regions of hippocampus. Scale bar=50 μm

Fig. 4 The mean percentages of TUNEL positive cells in CA1 regions of hippocampus.

1 Normal group; 2 LPS group; 3 LPS+ONO-2506 10mg/kg group; 4 LPS+ONO-2506 30mg/kg group; 5 LPS+ONO-2506 90mg/kg group; 6 Normal+ONO-30mg/kg group

*P□0.05, versus the Normal group; #P□0.05, versus the LPS group

3. Discussion

In this study, ONO-2506 could reduce the neuroinflammation and alleviate brain cell apoptosis in hippocampus of LPS mouse model. It demonstrates that ONO-2506 is effective on lipopolysaccharide-induced brain injury.

Previous studies proved that the neuroprotective effect of ONO-2506. Administration of ONO-2506 improved motor function, inhibited expansion of secondary injury in spinal cord injury rats and reduced the neuropathic pain [12, 13]. Ohtani R et al found that ONO-2506 had protective effect on the rat brain in chronic cerebral hypoperfusion [14]. Higashino H et al found ONO-2506 can prevent hypertension-induced stroke, and may inhibit the enlargement of the stroke lesion by preventing the inflammatory changes caused by overproduction of the S100β protein in the astrocytes [15]. In Parkinson's disease and acute subdural hematomas rat model, ONO-2506 protected against brain injury and apoptosis via suppression of S100 protein expression in ischemic lesions [16] [17]. Moreover, delayed treatment with ONO-2506 also reduced the MPTP-induced Neurotoxicity in Mice [18]. In addition, the agent was found have a wide therapeutic time window [19]. In clinical studies, ONO-2506 therapy also showed benefits in patients with acute ischemic stroke [20, 21]. However, to our knowledge, there was no study about effects of ONO-2506 on SAE.

Neuroinflammation is an important mechanism in SAE [22]. In this study, we found that some inflammation in brain, such as TNF-α and IL-1β, increased in LPS mouse models. After we used ONO-2506 to inhibit the S100β expression in LPS model, the TNF-α and IL-1β expression generally reduced with the increasing ONO-2506 dose. Cognitive impairment is an important clinical manifestations in SAE, and hippocampus is an important brain injury area in SAE [22]. The magnetic resonance imaging of hippocampus can change at 6h after a murine sepsis model [23]. In addition, apoptosis in hippocampus have been observed in the brain in SAE rat model [24]. In this study, we also found the hippocampal apoptosis in LPS model after 24h. Middle and high dose of ONO-2506 could alleviate the apoptosis in hippocampus. So the pretreatment of ONO-2506 in LPS model could reduce neuroinflammation and brain cell apoptosis.

ONO-2506 can inhibit the production and release of S100 protein. S100B is a dual action protein. At nanomolar concentrations, it could stimulate neurite outgrowth and enhances survival of neurons. At micromolar concentrations, it could stimulate expression of proinflammatory cytokines and induces apoptosis [8] [9]. In this study, the S100β expression and neuroinflammation increased apparently after LPS administration. After the treatment of ONO-2506, S100β expression and neuroinflammation in hippocampus reduced. Which signaling pathway that S100β affects the neuroinflammation in SAE is worthy of studying. RAGE-activated signaling induces the transcription of proinflammatory cytokines [26]. And blockade of RAGE was able to inhibit inflammatory responses induced by LPS [27]. In addition, RAGE activation in brain was associated to memory impairment [28]. RAGE is a member of the immunoglobulin superfamily and can be bound by several ligands, such as S100β. It is confirmed that S100 Proteins can play trophic and toxic effects through RAGE activation [9]. Villarreal A et al found that S100β/RGAE-mediated NF-κb signaling played a role in cortical neurons of the ischemic penumbra [29]. In our study, we found that RAGE was overexpressed by LPS. After inhibiting S100β by ONO-2506, the RAGE expression reduced, and the TNF-α and IL-1β level also reduced. So the ONO-2506 may reduce the neuroinflammation by S100β/RAGE signaling pathway.

There are some limits in our study. Firstly, apart from inflammation, glutamate, a major neurotransmitter, also plays a role in SAE [5]. The glutamate concentration can be influenced by ONO-2506 which can increase expression and function of glutamate transporter EAAT1 via Akt, ERK, and NF-κB signaling pathways [30]. So we can not exclude the effect of glutamate for SAE. Secondly, although S100β/RAGE signaling pathway was confirmed of playing role in some diseases. But we did not make an exact conclusion that ONO-2506 reduce the neuroinflammation by this S100β/RAGE signaling pathway because we did not specifically inhibit the RAGE expression. Advanced studies were needed to confirm it. Thirdly, behavioral test, such as Morris water maze, was not performed. So we did not confirm whether the effect of reducing inflammation and apoptosis by ONO-2506 could result in improving cognitive ability or not. Lastly, it needs clinical studies to confirm the effect of ONO-2506 on patients with SAE.

4. Conclusions

In LPS mouse models, ONO-2506 could reduce the neuroinflammation and alleviate brain cell apoptosis in hippocampus. Moreover, the RAGE expression was inhibited after ONO-2506 treatment.

5. Materials And Methods

5.1 Experimental animals

Male C57BL/6 mice (supplied by animal Laboratory of Qingdao university, China), 10 weeks of age, 20-25 g of weight and specific pathogen free, were used in this study. The animals were housed with food and water available ad libitum, under a 12-h/12-h light–dark cycle. Experimental protocols were approved by the Institutional Animal Care and Use Committee at the Affiliated Hospital of Qingdao University.

Experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals (n=72) were randomly divided into 6 groups: Normal group, LPS group, LPS+ONO-2506 (10mg/kg) group, LPS+ONO-2506 (30mg/kg) group and LPS+ONO-2506 (90mg/kg) group, and Normal+ONO-2506 (30mg/kg) group. Animals in LPS group were given a dose of LPS (10mg/kg) intraperitoneally. LPS+ONO-2506 (10mg/kg) group, LPS+ONO-2506 (30mg/kg) group and LPS+ONO-2506 (90mg/kg) groups received treatment of LPS (10 mg/kg) and ONO-2506 (10 mg/kg or 30mg/kg or 90mg/kg, 60 min before LPS) intraperitoneally. After 24h, the mice were sacrificed by deeply anesthetizing with 1% sodium pentobarbital (60 mg/kg, intraperitoneal injection), and decapitated. Then brain hippocampus tissues in each group animals (n=12) were obtained for Western blotting (n=6) and TUNEL staining (n=6).

5.2 Experimental materials

ONO-2506 was supplied by Toronto Research Chemicals, Canada. In western blotting, primary antibodies used were RAGE (abcam ab3611), S100β (abcam ab52642), IL-1β (abcam ab9722), TNF-α (abcam ab6671) and β-actin (abmart P30002). Second antibody was HRP-conjugated Goat Anti-Rabbit IgG (CST 7004). Apoptosis Detection Kit (NO. MK1052) was supplied by Wuhan Boster Biological Technology, China.

5.3 Western blotting

For Western blot analyses, the hippocampus tissues isolated from the surrounding brain tissue were immediately snap-frozen in liquid nitrogen before stored at -80 ℃. For protein extraction, each 100mg samples were homogenized in 500μL RIPA lysis buffer (Beyotime Institute of Biotechnology, China) and then centrifuged (12000rpm, 4℃，10min). Equal amounts of protein (80μg) were loaded and separated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) through 10% gels. Then proteins were electrophoretically transferred from gel to a PVDF (polyvinylidene fluoride) membrane. Primary antibodies were diluted in blocking buffer and incubated with membranes at 4℃ for 12h. Then, the membranes were washed with TBST and then incubated with secondary antibodies for 2h. Immunoreactivity detection was accomplished using enhanced chemiluminescence reagents (Millipore, USA).

5.4 TUNEL staining

Estimation of apoptosis in the brain was determined using in Apoptosis Detection Kit (Boster, China). Mice brains were pre-fixed in 4% PFA and embedded with paraffin wax, following by sectioning to 5 μm thickness. Briefly, after deparaffinization and rehydration, serial sections were digested with proteinase K for 6 min at 37℃, followed by exposure to terminal deoxynucleotidyl transferase (TdT) in a reaction buffer containing digoxigenin-labeled nucleotides (DIG-d-UTP) at 37℃ for 2 hours. Slides were then placed in a stop/wash buffer for 30 min at room temperature, then exposed to anti-digoxigenin

peroxidase for 30 min at 37℃. The chromogen diaminobenzidine was used with hematoxylin counterstain to identify TUNEL-positive cells. Stained sections were then visualized using an Olympus microscope (Tokyo, Japan). The images were recorded and analyzed using Java-based image processing program Image J 1.52 software (NIH, Bethesda, MD, USA).

5.5 Statistical Analyses

The data were represented as mean values ± standard deviation. SPSS (IBM, USA, version 20.0) was used for all analyses. All calculations were 2-tailed. P□0.05 was considered to be statistically significant. Comparisons among multiple groups were determined by one-way analysis of variance.

List Of Abbreviations

SAE: Sepsis associated encephalopathy; RAGE: Receptor for advanced glycation end products; LPS: lipopolysaccharides

Declarations

Ethics approval and consent to participate

Experimental protocols were approved by the Institutional Animal Care and Use Committee at the Affiliated Hospital of Qingdao University. Experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare no conflict of interest.

Funding

This work was supported by Youth Foundation of the affiliated hospital of Qingdao university (NO.2641).

Authors' contributions

YB conceived and designed the experiments. LJY, FW, CSX and DMM performed the experiments. YB and LJY analyzed the data. LJY and FW wrote the paper. All the authors agree to be accountable of all

aspects of the work. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Figures

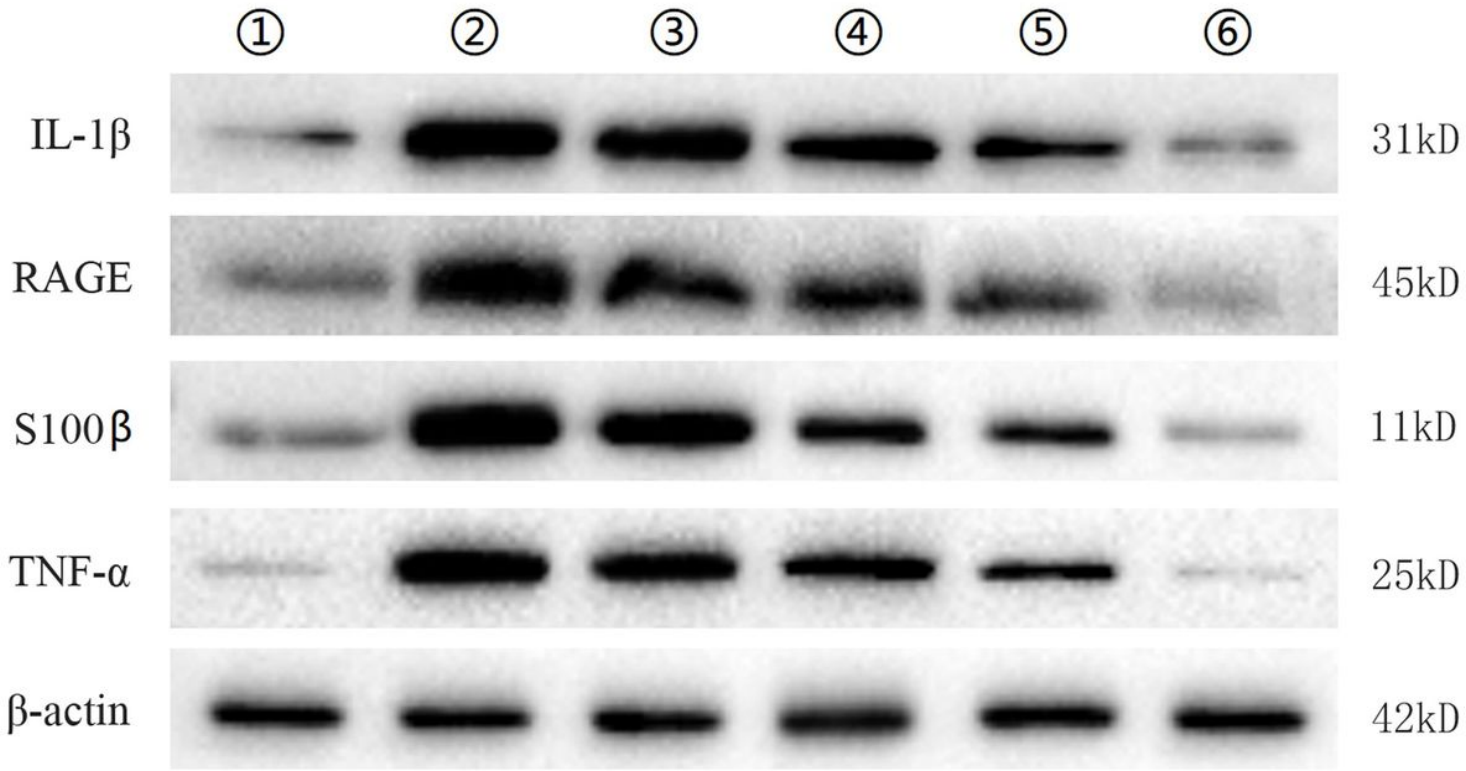

Figure 1

Representative Western blots of IL-1β, TNF-α, RAGE and S100β in groups ①Normal group; ②LPS group; ③LPS+ONO-2506 10mg/kg group; ④LPS+ONO-2506 30mg/kg group; ⑤LPS+ONO-2506 90mg/kg group; ⑥Normal+ONO-30mg/kg group

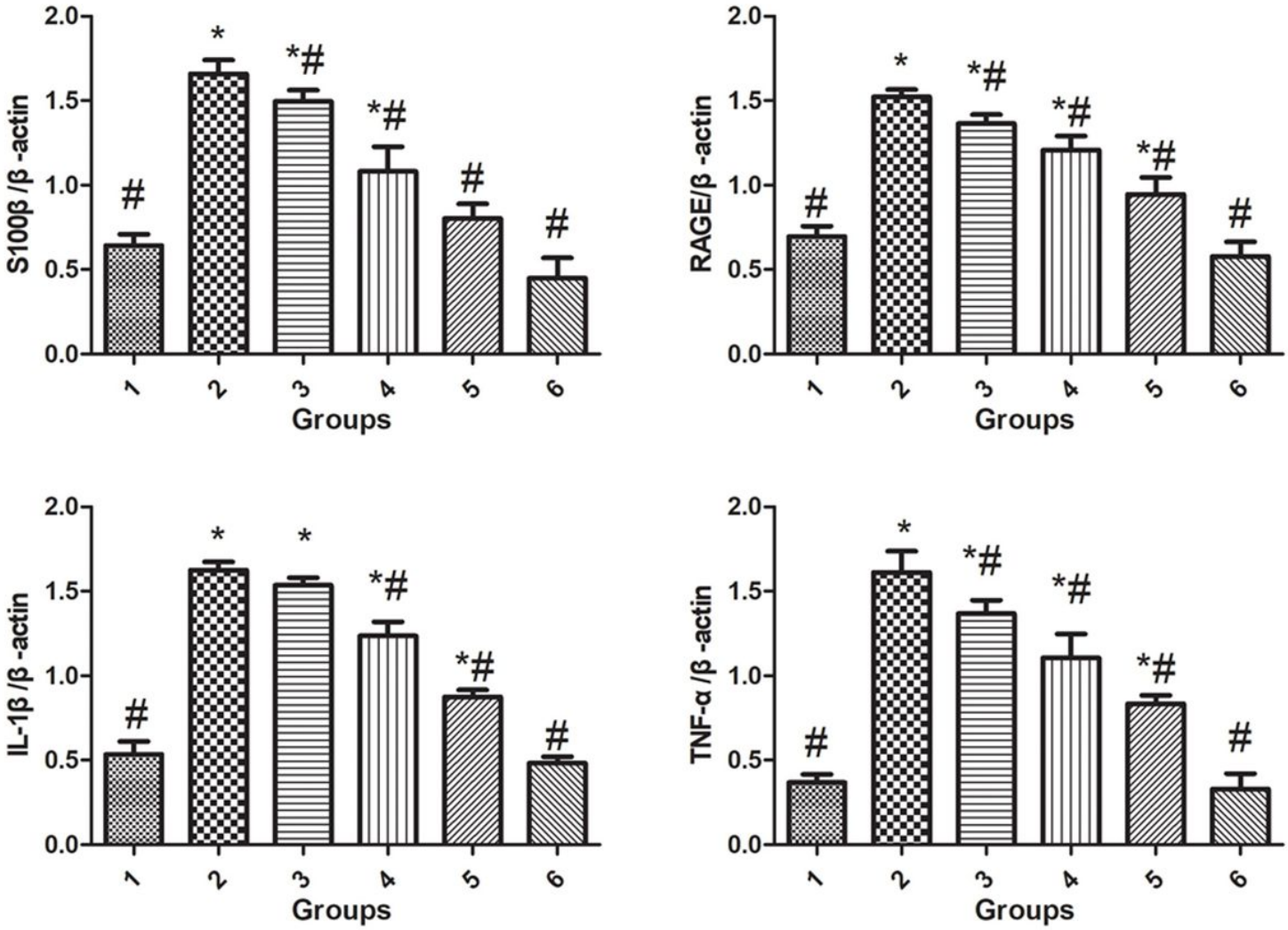

Figure 2

Quantitative densitometry analysis of Western blots for the ratio of S100β/β-actin, RAGE/β-actin, IL-1β/β-actin and TNF-α/β-actin 1 Normal group; 2 LPS group; 3 LPS+ONO-2506 10mg/kg group; 4 LPS+ONO-2506 30mg/kg group; 5 LPS+ONO-2506 90mg/kg group; 6 Normal+ONO-30mg/kg group *P□0.05, versus the Normal group; #P□0.05, versus the LPS group

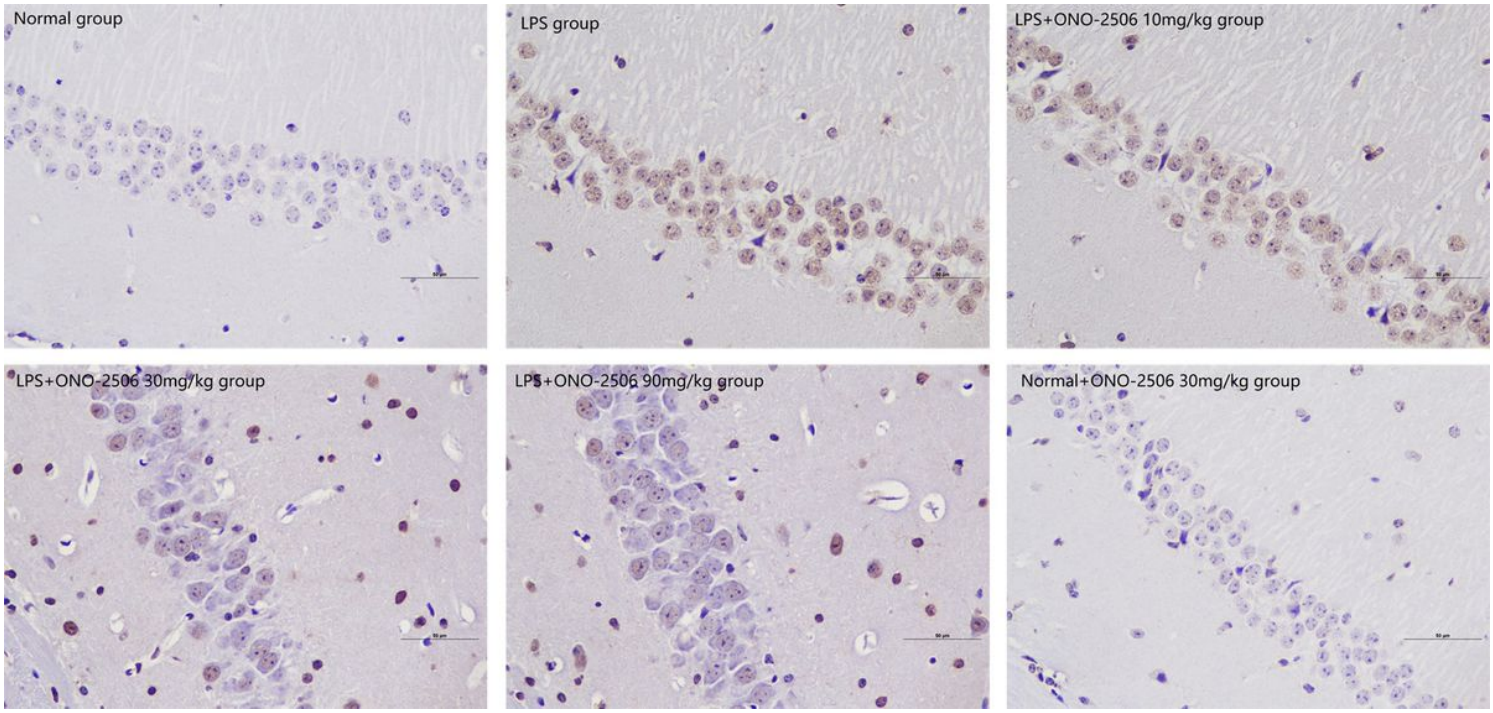

Figure 3

Representative TUNEL staining images in CA1 regions of hippocampus. Scale bar=50 μm

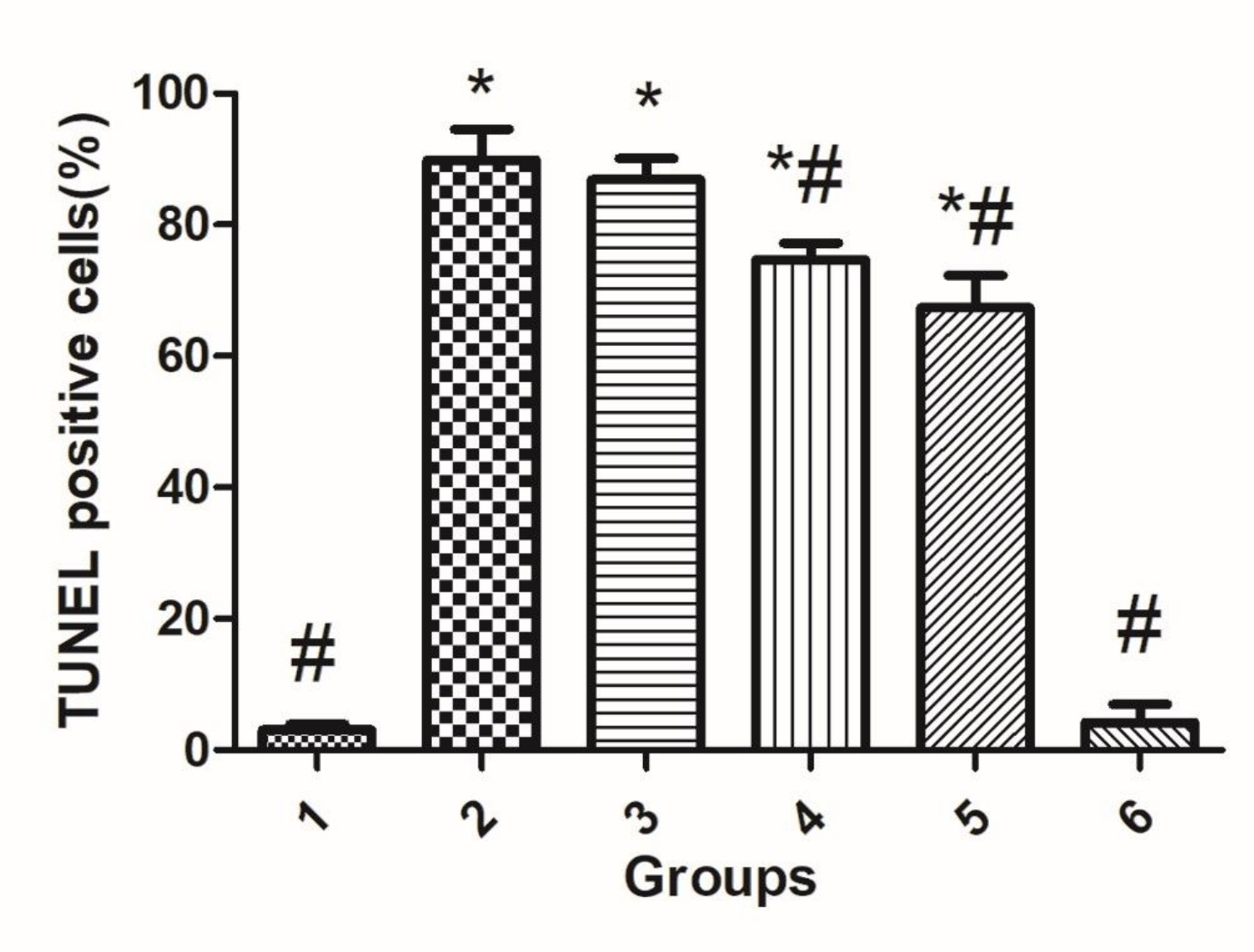

Figure 4

The mean percentages of TUNEL positive cells in CA1 regions of hippocampus. 1 Normal group; 2 LPS group; 3 LPS+ONO-2506 10mg/kg group; 4 LPS+ONO-2506 30mg/kg group; 5 LPS+ONO-2506 90mg/kg group; 6 Normal+ONO-30mg/kg group *P□0.05, versus the Normal group; #P□0.05, versus the LPS group

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