

# The effects of simvastatin on hippocampal caspase-3 and Bcl-2 expression following kainate-induced seizures in rats

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**Abstract.** Status epilepticus (SE) causes neuronal loss and apoptosis by inducing several apoptosis-regulatory genes. Two such genes, cysteinyl aspartate-specific protease-3 (caspase-3), an apoptosis activator, and B-cell leukemia-2 (Bcl-2), an apoptosis suppressor, are tightly regulated for their expression and activation. Statins, inhibitors of HMG-CoA reductase, have been recently recognized as neuroprotective drugs. However, their underlying mechanisms are still unclear. In this study, we examined the neuroprotective effects of simvastatin in a rat model of SE induced by kainic acid (KA). Feeding of simvastatin for 3 days after kainate injection rescued SE-induced neuronal apoptosis, as determined by histological examination of brain sections at the level of the dorsal hippocampus. Semi-quantitative RT-PCR showed that SE treatment markedly increased caspase-3 mRNA expression and reduced Bcl-2 mRNA expression in the hippocampus. Similarly, western blot analysis and immunohistochemical analysis of the rat hippocampus demonstrated that under SE treatment, caspase-3 protein levels significantly increased and peaked at 72 h, whereas Bcl-2 protein levels decreased from 6-24 h following SE. Interestingly, simvastatin could reverse the aforementioned SE-induced changes, suggesting that the neuroprotective effects of simvastatin against neuronal apoptosis may be achieved by inhibiting caspase-3 expression and increasing Bcl-2 expression.

## Introduction

Status epilepticus (SE) in adult rodents and humans can cause hippocampal neuronal loss and may result in temporal lobe

epilepsy (TLE) (1-3). Neuronal loss during chronic epilepsy mainly results from cell apoptosis or necrosis (4-6), which is triggered by the activation of a number of signal transduction factors (7). Kainic acid (KA), an analogue of the excitatory amino acid glutamate, has been widely used in inducing TLE in animal models (8,9). KA-induced SE in the amygdaloid complex activates several signal transduction factors, including B-cell leukemia-2 (Bcl-2), Bcl-2-associated X protein (Bax), and cysteinyl aspartate-specific protease-3 (caspase-3) (10,11). In the KA model, deletion or inhibition of pro-apoptotic genes protects the brain against seizure-induced neuronal death (12,13). Since currently available anti-epileptic drugs merely treat symptoms but do not cure the disease, it is imperative to develop neuroprotective drugs that prevent apoptosis after SE.

Statins, inhibitors of HMG-CoA reductase, inhibit cellular synthesis of cholesterol and isoprenoids and are commonly used to reduce cholesterol levels in humans. In addition to their lipid-lowering and thus beneficial cardiovascular effects, statins have also been suggested to exert neuroprotective actions in the central nervous system. For example, statins play anti-inflammatory and vasoprotective roles in cultured brain cells and endothelial cells (14). Statins inhibit a number of inflammatory processes important to brain damage and suppress the secretion of cytokines during spinal cord injury and ischemic stroke (15,16). The neuroprotective effects of statins have also been reported in various diseases, including traumatic brain injury, brain ischemia, and Alzheimer's disease, in both animal models and clinical studies (17-20). In KA-induced seizure, treatment with statins provides anti-apoptotic effects (21). However, the mechanisms underlying the anti-apoptotic role are not clear. In this study, we examined whether simvastatin regulates apoptosis and exerts its neuroprotective effects by modulating the expression of Bcl-2 and caspase-3.

## Materials and methods

**Ethics statement.** Animal care and handling was conducted in compliance with the Chinese Animal Welfare Act and was approved by the Medical Ethics Committee of the First Clinical College of Harbin Medical University (Approval ID, 201001).

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**Animals.** Adult male Wistar rats (n=120), weighing 180–200 g, were provided by the Animal Center of Jilin University, China. The rats were housed in individual cages in a controlled environment (constant 22–25°C; 50–60% humidity; 12/12 h light/dark cycle, lights on at 7 a.m.) for at least 1 week before being used in the experiment. The rats had free access to standard laboratory food and water. In addition, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data. All the experiments were conducted in the morning to avoid circadian variations.

**Experimental groups and drug administration.** The rats were randomly divided into 4 groups (n=30 per group): a saline group (sham), an epilepsy group, an epilepsy plus saline group, and an epilepsy plus simvastatin group. In the sham group, rats received saline intraperitoneally. In the epilepsy group, KA was dissolved in isotonic saline (pH 7.3) and administered intraperitoneally to rats at a dose of 10 mg/kg. In the epilepsy plus saline group, SE were induced in rats with KA injection followed by oral administration of saline starting at 0.5 h after SE once a day for 3 consecutive days. In the epilepsy plus simvastatin group, rats were subjected to KA lesions followed by oral administration of simvastatin (1 mg/kg/day) (Zocor®; MSD, USA), starting at 0.5 h after SE for 3 consecutive days. The dose was selected according to our previous study (22,23). The rats were sacrificed at indicated time points after SE or sham operation.

**KA-induced rat seizure model.** SE was induced in rats using KA administration. After KA was administered, the behavior of rats was observed for 3–4 h and documented to determine the duration and severity of seizure activity using a previously established seizure scoring scale (24). This method was widely used to study epilepsy in rodents. The behavior of rats was divided into 5 stages: stage 1, immobility; stage 2, forelimb and/or tail extension; stage 3, head bombing, as well as forelimb clonus with rearing and falling; stage 4, minor clonic seizures; stage 5, severe tonic-clonic seizures; and stage 6, death. Only those rats exhibiting at least 2 h of continuous stage 4/5 seizures were included in this study. Seizure parameters monitored included latency of convulsions and duration of severe (stage 4/5) seizure activity.

**Histological analysis.** Neuronal damage was assessed by histological examination of brain sections from the dorsal hippocampus of rats sacrificed at 6, 12, 24, 48, 72 or 96 h after SE or sham operation. The rats were deeply anesthetized with 4% halothane and then transcardially perfused with 200 ml of heparinized 0.9% saline followed by 500 ml of 4% paraformaldehyde (no. 158127; Sigma-Aldrich, Beijing, China) in 0.1 mol/l of phosphate-buffered saline (pH 7.4) (P5368; Sigma-Aldrich). After rats were decapitated, the brains were immersed in 4% paraformaldehyde for 3 days, embedded in paraffin, and sliced on a rotary microtome into 6-mm thick sections. Coronal sections consisting of the dorsal hippocampus were selected and processed for hematoxylin and eosin (H&E) staining. The paraffin-embedded sections were dewaxed by baking at 55–65°C for 45–60 min, cleaned with xylene three times for 5 min each, and rehydrated with 100%

ethanol twice for 5 min and 70% ethanol once for 5 min. After 5-min washes in distilled water, the sections were stained with hematoxylin (H9627; Sigma-Aldrich) for 2 min and then washed twice in running water for 1 min. The sections were dipped in ammonium hydroxide solution and then washed again using running water for 1 min. After being rinsed in graded ethanol (80% ethanol for 1 min and 95% ethanol for 1 min), each section was dipped 10 times in eosin-Y (E4009; Sigma-Aldrich) for 30–45 sec for counterstaining. Following two additional rinses in 100% ethanol for 1 min each, the sections were dehydrated in a graded series of alcohol, cleared in xylene, and coverslipped with Permount.

**Semi-quantitative RT-PCR.** The rats were anesthetized with 4% halothane and decapitated at 12 and 72 h after 2 h of SE. The hippocampus was then immediately isolated and put on an ice-cold glass stage. Total-RNA was extracted from the hippocampus using an RNA isolation reagent, TRIzol (TRIzol® Reagent; Invitrogen Life Technologies, Beijing, China), and reverse transcription was performed using oligo(dt) priming according to the manufacturer's instructions (ThermoScript™ RT-PCR System; Invitrogen Life Technologies). Primer sequences were as follows: rat caspase-3 (GenBank accession no. NM\_012922), F, 5'-CTGGACTGCGGTATTGAG-3' and R, 5'-GGAACATCGGATTTGATT-3'; rat Bcl-2 (GenBank accession no. NM\_021850), F, 5'-CTACCCAAGTTAGCATTCC-3' and R, 5'-CAAAGTCCCTATTTATCCCT-3'; and rat  $\beta$ -actin (GenBank accession no. NM\_031144), F, 5'-AGCCA TGTACGTAGCCATCC-3' and R, 5'-GCTGTGGTGGTG AAGCTGTA-3'. The PCR reactions were conducted as follows: 5 min at 94°C; 35 cycles (for caspase-3 and Bcl-2) or 30 cycles (for  $\beta$ -actin) of 30 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C; and final elongation for 10 min at 72°C. The amplified DNA fragments were 465 bp for caspase-3, 389 bp for Bcl-2, and 222 bp for  $\beta$ -actin. The PCR products were run on a 2% agarose gel and visualized by UV light. Band densities were quantified using the Scion Image software (Scion Corporation Frederick, MD, USA) and signals from caspase-3 and Bcl-2 were normalized to those from the housekeeping gene  $\beta$ -actin.

**Western blot analysis.** Rats were sacrificed at 6, 12, 24, 48, 72 or 96 h after 2 h of SE or sham operation. Immediately after decapitation, the hippocampus was quickly dissected and then homogenized using a Dounce homogenizer and lysed on ice in 400  $\mu$ l of RIPA buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS]. Proteins, 20  $\mu$ g per lane as determined using the BCA protein assay kit (no. 23227; Pierce, Rockford, IL, USA), were separated on 12% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes (RPN2020F; GE Healthcare, Beijing, China). The membranes were first probed with primary antibody against cleaved caspase-3 (1:1,000; no. 9664; Cell Signaling Technology, Inc., Danvers, MA, USA; recognizing the active form p17 of caspase-3), Bcl-2 (1:50; ab7973; Abcam Inc., Cambridge, MA, USA), or  $\beta$ -actin (1:200; sc-47778; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). The specificity of the immunoreactivity for each antibody was confirmed by preabsorption experiments. After washes, the membranes were then incubated with horseradish peroxidase-

conjugated secondary antibodies (1:5,000; for caspase-3 and Bcl-2: sc-2004; and for  $\beta$ -actin: sc-2005; were from Santa Cruz Biotechnology, Inc.). Subsequently, the protein bands were visualized with ECL (RPN2109; GE Healthcare) and quantified by densitometry.

**Immunohistochemical staining.** Rats were anesthetized and perfused as described for the histological analysis. Coronal sections including the dorsal hippocampus were selected and processed for immunohistochemical staining. The paraffin sections were baked for 2 h in an oven, deparaffinized in xylene, and rehydrated in graded ethanol solutions. After three 5-min washes in 0.01 M PBS (pH 7.4), the sections were microwaved in 0.01 M sodium citrate buffer (pH 6.0) for 10 min, cooled to room temperature naturally, and then washed three times in PBS, 5 min each. The sections were incubated in 3% hydrogen peroxide for 10 min, washed using the same procedure as above, and then blocked in 10% normal goat serum for 1 h at room temperature. Rabbit monoclonal antibodies against cleaved caspase-3 or Bcl-2 (1:200; no. 2870; Cell Signaling Technology, Inc.) were diluted in the recommended antibody diluent (no. 8112; Cell Signaling Technology, Inc.) and incubated with sections overnight at 4°C. After three rinses in PBS, the sections were incubated with biotinylated goat anti-rabbit secondary antibody (1:2,000) for 1 h at room temperature and then with avidin-biotin-peroxidase solution (ABC reagent) for 30 min at room temperature. Following three additional washes in PBS, the sections were treated with 3,3'-diaminobenzidine tetrahydrochloride (DAB) for 2 min, counterstained in hematoxylin, dehydrated, and coverslipped. Normal goat serum, biotinylated goat anti-rabbit secondary antibody, ABC reagent, and DAB were all in the ABC staining kit (sc-2018; Santa Cruz Biotechnology, Inc.). To assess nonspecific immunostaining in our study, control samples were stained only with secondary antibody, and no labeling was detected. To count the number of positive neurons, 10 microscopic fields in the hippocampus, at a magnification of 50  $\mu$ m, were randomly chosen from each section, and the caspase-3- and Bcl-2-positive neurons were quantified using the Image-Pro Plus software (Media Cybernetics, Inc., Shanghai, China).

**Statistical analysis.** Data were expressed as mean  $\pm$  standard deviation and analyzed using one-way analysis of variance (ANOVA) and post hoc Fisher's PLSD after normality of the distribution was proved.  $P < 0.05$  was considered statistically significant.

## Results

**Simvastatin rescues SE-induced neuronal apoptosis in the hippocampal CA1 region.** To examine the effects of SE on the hippocampal CA1 neurons and the role of simvastatin in this process, we conducted H&E staining of the hippocampal CA1 region of rats. We demonstrated that 2-h SE induced selective neuronal death. Compared with hippocampal CA1 sections from the sham group rats (Fig. 1A and B), those from the epilepsy group showed partial cell death and neuronal loss following the 2-h SE. Typical morphological characteristics of apoptosis were observed in CA1 neurons, including cell shrinkage, nuclear condensation, and fragmentation

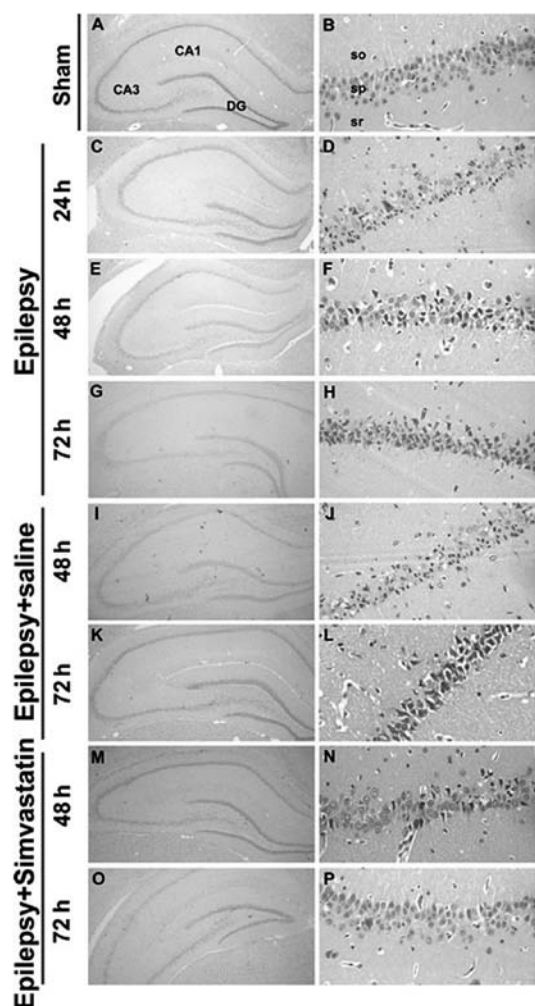


Figure 1. Simvastatin rescues SE-induced neuronal apoptosis in the hippocampal CA1 region. Neuronal damage and apoptosis were assessed by H&E staining and histological examination of brain sections from the hippocampal CA1 region. (A and B) In the sham group, brain sections revealed normal neurons in the hippocampus. (C-H) In the epilepsy group, sections from 24-72 h after a 2-h SE showed neuronal loss and apoptosis, displaying cell shrinkage, nuclear condensation, and fragmentation. At 72 h, a dramatic loss of neurons was observed, and the number of apoptotic neurons increased significantly. (I-L) The epilepsy plus saline group showed similar levels of neuronal loss and apoptosis as the epilepsy group. (M-P) Simvastatin treatment, as shown by 48- and 72-h post-SE sections, protected neurons from SE-induced neuronal loss and apoptosis.

(Fig. 1C-H). Twenty-four hours after SE, the CA1 cell layer showed a dramatic loss of neurons (Fig. 1C and D). The number of apoptotic neurons kept increasing at 48 and 72 h (Fig. 1E-H). Saline intake did not diminish the effect on neuronal death induced by SE (Fig. 1I-L). In contrast, simvastatin administration markedly rescued SE-induced neuronal apoptosis, especially at 48 and 72 h (Fig. 1M-P).

**Simvastatin reverses SE-induced changes in caspase-3 and Bcl-2 mRNA expression in the hippocampus.** To analyze the potential mechanisms underlying the neuroprotective role of simvastatin, we examined the mRNA expression of the pro-apoptotic gene, caspase-3, and the anti-apoptotic gene, Bcl-2, 72 and 12 h following SE, respectively. The semiquantitative RT-PCR results showed that the basal expression levels of caspase-3 and Bcl-2 mRNA were readily detectable in the

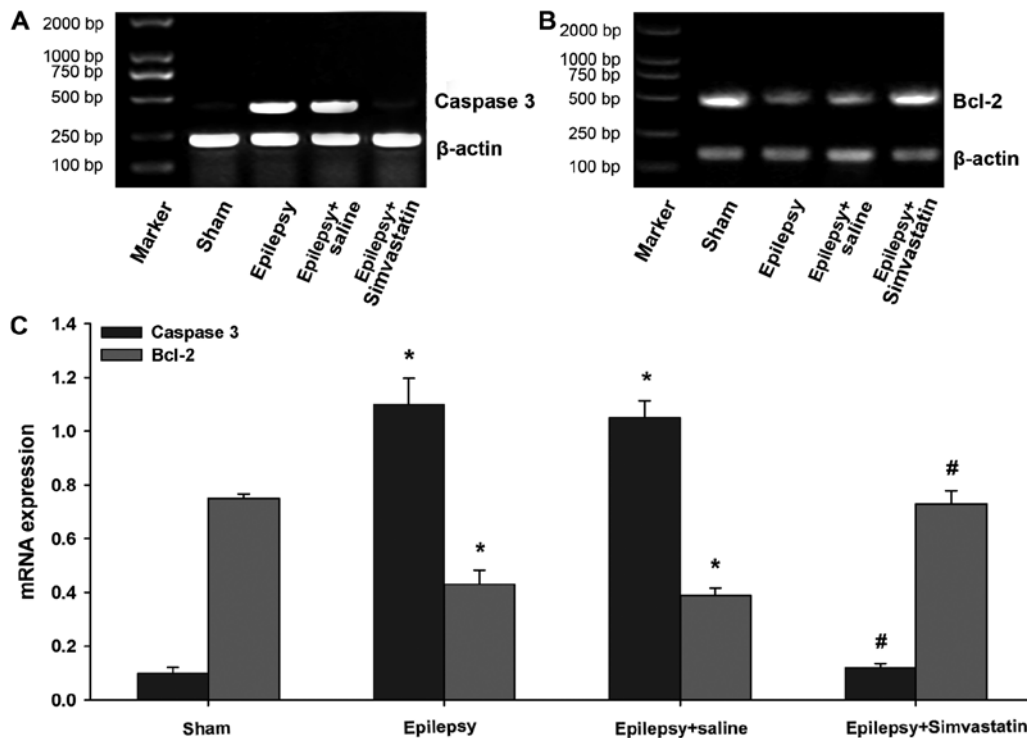


Figure 2. Simvastatin reverses SE-induced changes in caspase-3 and Bcl-2 mRNA expression in the hippocampus. The mRNA expression of the pro-apoptotic gene, caspase-3, and the anti-apoptotic gene, Bcl-2, in the hippocampus was examined 72 and 12 h after SE, respectively. The mRNA levels were measured using semiquantitative RT-PCR ( $n=6$  per group). (A) SE significantly increased caspase-3 mRNA expression, and this increase was abolished by intake of simvastatin (10 mg/kg), but not saline. (B) SE significantly decreased Bcl-2 mRNA expression, and this reduction was rescued by simvastatin intake (1 mg/kg), but not saline. (C) Quantification of caspase-3 and Bcl-2 mRNA levels. Expression of target genes was normalized to that of  $\beta$ -actin. \* $P<0.01$  vs. sham; # $P<0.01$  vs. epilepsy.

hippocampus of the sham rats (Fig. 2). SE significantly increased the caspase-3 mRNA expression 72 h post-SE (Fig. 2A and C) ( $P<0.01$ ) and significantly decreased Bcl-2 mRNA expression 12 h post-SE (Fig. 2B and C) ( $P<0.01$ ). Saline intake did not affect the mRNA expression of caspase-3 and Bcl-2 following SE (Fig. 2) ( $P<0.01$  vs. the sham group). Interestingly, simvastatin treatment completely reversed SE-induced changes in both caspase-3 and Bcl-2 mRNA expression, to levels not significantly different from that of the sham group (Fig. 2) ( $P<0.01$  vs. the epilepsy group;  $P>0.05$  vs. the sham group).

*Simvastatin reverses SE-induced changes in caspase-3 and Bcl-2 protein expression in the hippocampus (western blot analysis).* We further confirmed the influence on caspase-3 and Bcl-2 expression by examining protein levels. After SE-induced activation, full length caspase-3 (32 kDa) is cleaved into 2 mature subunits, p17 (17 kDa) and p12 (12 kDa). In this study, using an antibody specific to p17, we compared the levels of activated caspase-3 in the 4 groups. As expected, p17 staining was not detected in the sham hippocampus, whereas SE markedly increased the expression of activated caspase-3. This increase began at 24 h and peaked at 72 h after SE (Fig. 3A and C) ( $P<0.05$  vs. the sham group). Treatment with simvastatin, but not saline, significantly reduced the SE-induced increase of activated caspase-3 levels (Fig. 3A and C) ( $P<0.05$  vs. the epilepsy group).

Despite comparable Bcl-2 protein expression in the sham and epilepsy groups immediately after the 2-h SE (i.e., 0 h), Bcl-2 levels were significantly decreased from 6 to 24 h (Fig. 3B and D) ( $P<0.05$  vs. the sham group). Intake of simv-

astatin, but not saline, significantly reversed the reduction in Bcl-2 protein levels following SE (Fig. 3B and D) ( $P<0.05$  vs. the epilepsy group).

*Simvastatin reverses SE-induced changes in caspase-3 and Bcl-2 protein expression in the hippocampus (immunohistochemical analysis).* To characterize the cellular distribution of caspase-3 and Bcl-2 activation after SE, we performed immunohistochemical staining of caspase-3 in brain sections 72 h after SE and of Bcl-2 in sections 12 h after SE. Neurons in the hippocampal CA1 region prepared from sham animals showed some distribution of caspase-3 (Fig. 4A) and Bcl-2 (Fig. 5A) staining in the nucleus or cytoplasm. Under SE treatment, the caspase-3 expression in the hippocampal CA1 pyramidal cell layer increased significantly at 72 h (Fig. 4B). In contrast, SE treatment significantly decreased the expression of Bcl-2, which was predominantly localized in the cytosol (Fig. 5B). Saline intake did not affect SE-induced expression patterns of caspase-3 (Fig. 4C) or Bcl-2 (Fig. 5C). In contrast, simvastatin treatment significantly reduced SE-induced caspase-3 activation (Fig. 4D) and, rescued SE-induced reduction in Bcl-2 expression (Fig. 5D) in the hippocampal CA1 region.

To quantitatively analyze caspase-3 and Bcl-2 expression in the hippocampus, the mean intensity was analyzed by counting the number of positive neurons. Under SE treatment, the number of caspase-3-positive neurons increased significantly at 72 h (Fig. 4E) ( $P<0.01$  vs. the sham group), whereas the number of Bcl-2-positive neurons decreased significantly at 12 h (Fig. 5E) ( $P<0.05$  vs. the sham group). Treatment with simvastatin, but not saline, could reverse SE-induced changes

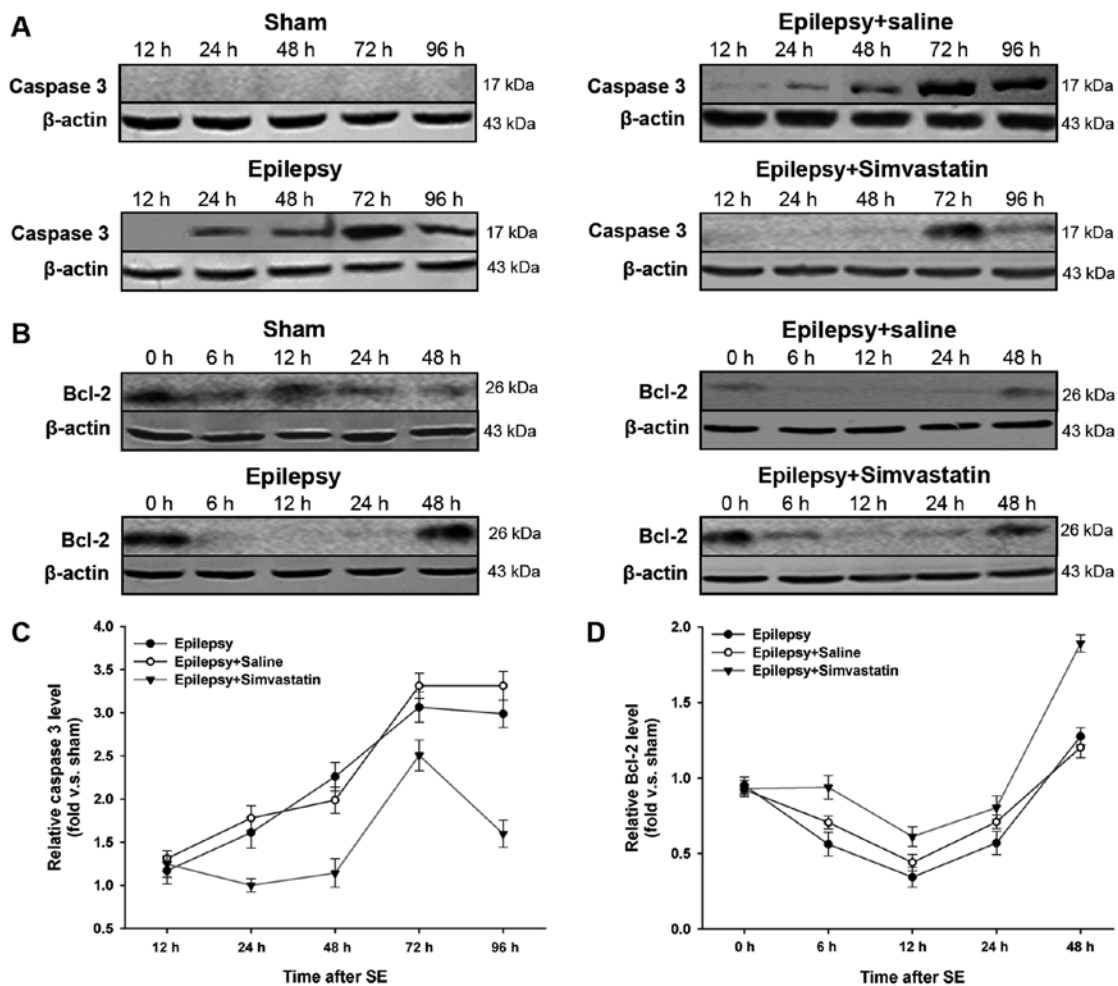


Figure 3. Simvastatin reverses SE-induced changes in caspase-3 and Bcl-2 protein expression in the hippocampus (western blot analysis). Western blot analyses showing representative images of caspase-3 and Bcl-2 protein expression in the hippocampus from the sham, epilepsy, epilepsy plus saline, and epilepsy plus simvastatin groups (the sham group,  $n=3$ ; each time point in the other three groups,  $n=3$ ). Band signals of target genes were normalized to those of  $\beta$ -actin. (A) The expression of the large cleavage form (17 kDa) of caspase-3 increased from 24 h after SE and peaked at 72 h. Under simvastatin (1 mg/kg) treatment, the level of caspase-3 after SE decreased and returned to the basal level at 96 h. (B) Bcl-2 protein levels decreased after SE from 6-24 h. Treatment with simvastatin (1 mg/kg) after SE upregulated the level of Bcl-2 expression. (C and D) Quantification of caspase-3 and Bcl-2 protein levels, showing fold changes vs. the sham controls. \* $P<0.05$  vs. sham; # $P<0.05$  vs. epilepsy.

in the number of caspase-3- and Bcl-2-positive neurons (Fig. 4E) ( $P<0.01$  vs. the epilepsy group) (Fig. 5E) ( $P<0.05$  vs. the epilepsy group).

## Discussion

SE as well as brief and/or repetitive seizures associated with epilepsy cause neuronal loss in the brain (25). In the KA-induced SE rat model, pyramidal cells in the CA1 and CA3 regions of the hippocampus undergo apoptosis and death (26,27). This apoptotic process in degenerating neurons is characterized by morphological changes, altered expression of Bcl-2-family proteins, and activation of the caspase family of cell-death proteases (28-30). Consistent with previous reports, in this study, we observed that under SE treatment, from 12 to 48 h, a small number of neurons demonstrate the typical characteristics of apoptosis, including cell shrinkage, nuclear condensation, and fragmentation, and the number of apoptotic neurons increases significantly at 72 h.

Apoptosis is triggered by a series of caspase cascades (31,32). Caspase-3, a crucial apoptotic regulator that is acti-

vated by caspases-9 or -8, is activated during seizure-induced neuronal death (32,33). Controversially, other studies have suggested that caspase-3 does not contribute significantly to SE-induced neuronal necrosis, because caspase-3 activation was not detected 6 and 24 h after 2-h SE (34,35). In our experiment, caspase-3 mRNA and protein expression increased in hippocampal neurons 24 h after the 2-h SE and peaked at 72 h. Our results strongly support the theory that caspase-3 plays an important role in neuronal cell death during SE. The sham control rats express only low basal levels of caspase-3 mRNA and do not exhibit activated caspase-3 protein, and this low level of expression may be interpreted as mediating normal brain development.

Bcl-2, the founding member of the Bcl-2 family, suppresses apoptosis primarily via effects on mitochondria and one of its mechanisms is preventing cytochrome c release (36,37). Under KA-induced SE, Bcl-2 family members, including Bax and Bcl-2, are activated in the amygdaloid complex (36,37). In the present study, Bcl-2 mRNA and protein expression decreased 6 h after the 2-h SE and reached the trough level at 12 h, suggesting that SE may accelerate



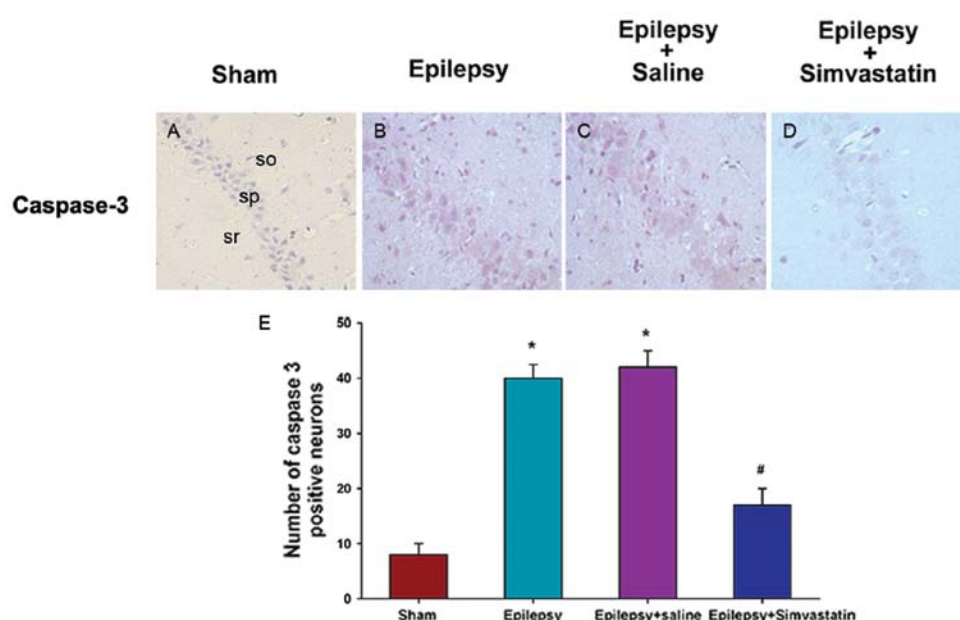


Figure 4. Simvastatin abolishes SE-induced increase in caspase-3 protein expression in the hippocampus at 72 h after SE (immunohistochemical analysis). Immunohistochemical images of caspase-3 in hippocampal sections (the sham group,  $n=5$ ; the other three groups,  $n=5$ ). (A) Caspase-3 was weakly expressed in the hippocampus of sham control rats. (B) Caspase-3 expression of the epilepsy group increased significantly in the hippocampal CA1 pyramidal cell layer at 72 h. (C) Caspase-3 expression of the epilepsy plus saline group showed a similar pattern as that of the epilepsy group. (D) Simvastatin (1 mg/kg) abolished the increase in caspase-3 expression under SE treatment. (E) Number of caspase-3-positive neurons. \* $P<0.01$  vs. sham; # $P<0.01$  vs. epilepsy. Scale bars: lower magnification, 50  $\mu\text{m}$ ; higher magnification, 500  $\mu\text{m}$ . so, stratum oriens; sp, stratum pyramidal; sr, stratum radiatum.

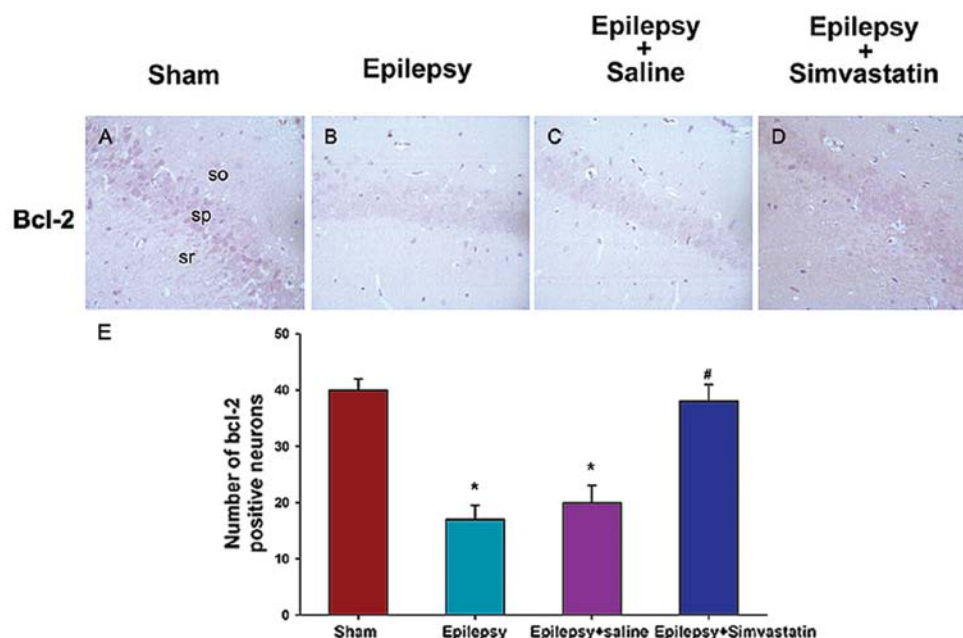


Figure 5. Simvastatin reverses SE-induced reduction in Bcl-2 protein expression in the hippocampus at 12 h after SE (immunohistochemical analysis). Immunohistochemical images of Bcl-2 in hippocampal sections (the sham group,  $n=5$ ; the other three groups,  $n=5$ ). (A) Bcl-2 staining in the hippocampus of sham control rats. (B) SE treatment significantly reduced Bcl-2 expression of the epilepsy group in the hippocampal CA1 pyramidal cell layer. (C) Bcl-2 expression of the epilepsy plus saline group showed a similar pattern as that of the epilepsy group. (D) Simvastatin (1 mg/kg) reversed SE-induced reduction in Bcl-2 expression. (E) Number of Bcl-2-positive neurons. \* $P<0.05$  vs. sham; # $P<0.05$  vs. epilepsy. Scale bars: lower magnification, 50  $\mu\text{m}$ ; higher magnification, 500  $\mu\text{m}$ . so, stratum oriens; sp, stratum pyramidal; sr, stratum radiatum.

apoptosis by inhibiting Bcl-2 expression. Indeed, Bcl-2 overexpression provides modest protection against hippocampal seizure damage (38), whereas the level of SE-induced hippocampal neuronal death in Bcl-2-deficient mice is twice as severe as that in wild-type mice (39).

Statins are important drugs to ameliorate neurodegenerative diseases due to its significant neuroprotective roles as well as the fact that they are well tolerated with relatively few side effects. Recent clinical trials have reported the neuroprotective function of statins in Alzheimer's disease (40), Parkinson's

disease (41,42), and multiple sclerosis (43). Although the exact mechanisms still remain unclear, several important discoveries have been made. First, statins reduce the circulating cholesterol levels and thus deplete lipid rafts, which contain many proteins involved in neurotransmitter systems (44) and have been linked to the processing of prion protein and the impairment of dopamine function in Parkinson's disease (45). Second, statins also exert cholesterol-independent effects by blocking the production of the non-sterol isoprenoid products of cholesterol synthesis, such as farnesylpyrophosphate and geranylgeranylpyrophosphate, which are active players in protein modification (46). Third, statins can influence the outcome of neurological insults through peripheral actions, including reducing oxidative damage, improving vascular function, and modulating peripheral inflammatory responses. Fourth, statins activate several neuroprotective and anti-apoptotic signaling pathways, including the brain-derived neurotrophic factor pathway, the protein kinase B (PKB/Akt) pathway, and the Ras-ERK signaling cascade (47-49). In this study, we demonstrated that treatment with simvastatin significantly attenuates SE-induced neuronal loss and apoptosis in the hippocampus. Simvastatin abolishes SE-induced increase in caspase-3 mRNA expression 24 h after the 2-h SE. A persistent reduction in caspase-3 protein expression was also observed. In contrast, simvastatin could significantly reverse SE-induced reduction in Bcl-2 mRNA and protein expression levels. In conclusion, our results suggest that the mitochondrial apoptotic pathways participate in the protective effect of simvastatin against KA-evoked prolonged seizures.

Controversies over the clinical use of statins include several *in vitro* studies reporting that statins are actually neurotoxic and induce cell death in neurons and glia (50,51). However, these experiments were usually carried out with very high statin concentrations of 0.1, 1 or even 10 mM. Thus, their physiological significance is unclear considering that the maximum concentration of pravastatin is only 0.1 mM in the serum of healthy subjects (52). Moreover, statins probably do not reach this concentration in the central nervous system (53). Different types of statins differ in their ability to attenuate KA-induced seizures. Simvastatin is one of the most effective statins against kainate-induced excitotoxicity (54). Further studies are required to examine whether simvastatin can influence apoptotic regulators other than caspase-3 and Bcl-2 and whether the anti-apoptotic properties of simvastatin depend on the dosages administered.

In conclusion, our study demonstrated that SE induces apoptosis in the rat hippocampus by increasing the expression of the pro-apoptotic caspase-3 and reducing the expression of the anti-apoptotic Bcl-2, in a time-dependent manner. Treatment with simvastatin rescues SE-induced apoptosis by reversing SE-produced changes in the expression of caspase-3 and Bcl-2. This study helps to further our understanding of the mechanisms of neuronal loss and apoptosis as well as the protective effects of simvastatin against SE-caused brain injury.

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