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Original Article

BQ-869, a novel NMDA receptor antagonist, protects against excitotoxicity and attenuates cerebral ischemic injury in stroke

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Abstract: Stroke is one of the three diseases that cause human death in current world, and it is the common, frequently occurring disease in the middle-old ages. NMDA receptors mediate glutamate-induced cell death when intensely or chronically activated, which is an important cause of neuronal cell death after acute injuries. Here, we demonstrated that BQ-869, a potent NMDA receptor antagonist, blocked NMDA receptor in concentration-dependent and dose-dependent manner, attenuated NMDA-induced Ca^{2+} influx, inhabited NMDAR-mEPSC in hippocampal pyramidal neurons, improved athletic ability of rats with MACO, decreased infarction size in focal cerebral ischemia rats and reduced stroke mortality. Taken together, our data demonstrate the neuroprotective effect of BQ-869 might be through inhibiting NMDA-mediated excitotoxicity. These findings indicate that BQ-869 is the most potent antagonist of NMDA receptors, and provide new insights with potential therapeutic applications for the treatment of stroke.

Keywords: NMDA receptor, excitotoxicity, BQ-869, MACO, cerebral ischaemia

Introduction

Stroke, sometimes referred to as cerebrovascular accident (CVA), is the loss of brain function due to ischemia or hemorrhage [1]. The World Health Organisation (WHO) defined stroke as a neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours. As the second leading cause of death worldwide, stroke is a common and frequently occurring disease for middle-aged and old people. 95% of strokes occur in people age 45 and older, and two-thirds of strokes occur in those over the age of 65. In the past two decades globally, noticeable increases (a 68% increase) took place in the absolute numbers of people with incident stroke [2]. About 70% of strokes are cerebral ischemia stroke. Ischemia stroke is the result of a disturbance of the cerebral circulation, which causes ischemia, hypoxia, leading to necrosis of ischemic center and penumbra around the ischemic. Although the research of stroke neural protection drugs has lasted for decades, and more than 100 clinical trials have been finished, discouraging news accumulated as one by one the clinical trails were terminated. Ultra-early thrombolytic therapy is the key to salvage ischemic penumbra, and active cerebral protection is the core of reducing the reperfusion injury.

Glutamate is thought to serve as the major excitatory neurotransmitter in the central nervous system (CNS) and plays an essential role in neural development, excitatory synaptic transmission and plasticity [3-5]. Under ischemic brain damage, such as ischemia and hypoxia, excessive glutamate is released and accumulated, and then the abnormal surplus of glutamate activates three classes of ionophore-linked postsynaptic receptors: N-methy-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) and kainate (KA). The NMDA receptor distributes in cerebral cortex and hippocampal neuron, which not only is a receptor-gated ion channel, but also participates directly in spatial learning [6]. High-freq-

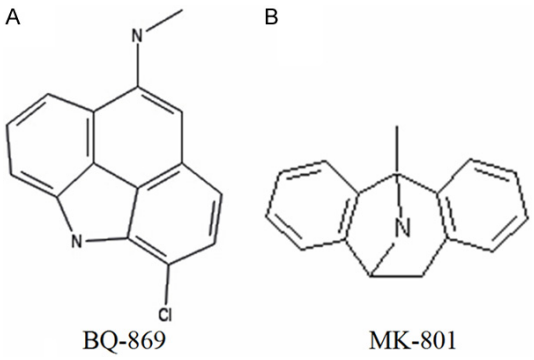

Figure 1. The chemical structure of BQ-869 (A) and MK-801 (B).

uency stimulation to the NMDA postsynaptic receptor induces a large amount of Ca^{2+} to influx directly through the receptor-gated ion channels [7]. This rapidly disorder of permeability and late-onset calcium overload is excitotoxicity. Ca^{2+} overload triggers several downstream lethal reactions including protease, phospholipase and protein kinases activation to lyse of intracellular elements and cell death finally [8-11]. Given this, over-stimulation of NMDA receptor can be considered as a primary intracellular event that induces neuronal death in stroke, and blocking NMDA receptor can inhibit the inward flow of calcium ions to alleviate neuron injury. As NMDA receptor can be an effective therapeutic target for stroke, clinical trials of glutamate NMDA receptor antagonists had been tested, but results are disappointing [12]. For instance, selfotel is a competitive glutamate antagonist that has been tested in double-blind, randomized, placebo-controlled phase III clinical trials. Studies indicated selfotel had a significant increase of serious adverse effects like neurotoxic and the phase III trial was prematurely terminated [13]. Sacco et al. reported gave-stinel, another glutamate antagonist, administered up to 6 hours after an acute ischemic stroke did not improve functional outcome at 3 months [14]. Thus, some drug with good efficacy need to be urgently exploited and utilized. As an uncompetitive antagonist MK-801, also named dizocilpine, have been extensively studied for use in treatment of diseases with excitotoxic components [15]. Although the initial clinical application has stalled, the effects of MK-801 at NMDA receptor are efficient and significant. The development of NMDA receptor antagonists is actively pursued, and we find a novel NMDA receptor antagonist with higher appetency, BQ-869, which was synthesized by Shanghai Institute of Materia Medica, Chinese Academy of Sciences (**Figure 1**).

In present study, we hypothesized that BQ-860 had good efficacy in the treatment of stroke; therefore we explored the effects of BQ-869 on NMDA receptor in vitro and on cerebral ischemia in vivo. We first examined weather and how BQ-869 affects NMDA receptor-mediated currents, and then the change of NMDA-induced Ca^{2+} influx and NMDAR sminiature excitatory post synaptic currents caused by BQ-869 was tested. We also investigated a potential role of BQ-869 on reducing infarction areas in MACO models. These results, altogether, present the first evidence that BQ-869 has neuroprotective effect via inhibiting glutamate excitotoxicity and reducing the infarction areas after cerebral ischemia, and provide the theoretical and experimental basis for clinical application and development of new stroke drugs.

Materials and methods

Cell culture

Hippocampal cell cultures were prepared from embryonic day 18 SD rat embryos as previously described [16]. In brief, whole hippocampi were dissected from Sprague-Dawley rats at postnatal day 2. Tissue was digested by 0.125% trypsin for 20 min at 37°C, mechanically dissociated by pipetting. The dissociated cells were cultured in Neurobasal medium containing 2% B27, 0.5 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO_2. Culture medium was changed twice a week. All experiments were performed on cultures at day 10-14.

Patch clamp recordings

Currents activated by excitatory amino acids were measured in the whole-cell and outside-out patch-clamp configurations [17]. Pipettes contained an internal solution of 120 mM cesium methanesulfonate, 5 mM CsCl, 10 mM Cs_2EGTA, 5 mM Mg $(OH)_2$, 5 mM MgATP, 1 mM Na_2GTP, and 10 mM Hepes (pH 7.4). The external solution was 160 mM NaCl, 2 mM $CaC1_2$, and 10 mM Hepes (pH 7.4). In the experiments, 300 nM tetrodotoxin and 10 μM bicuculline methiodide were added to the external solution

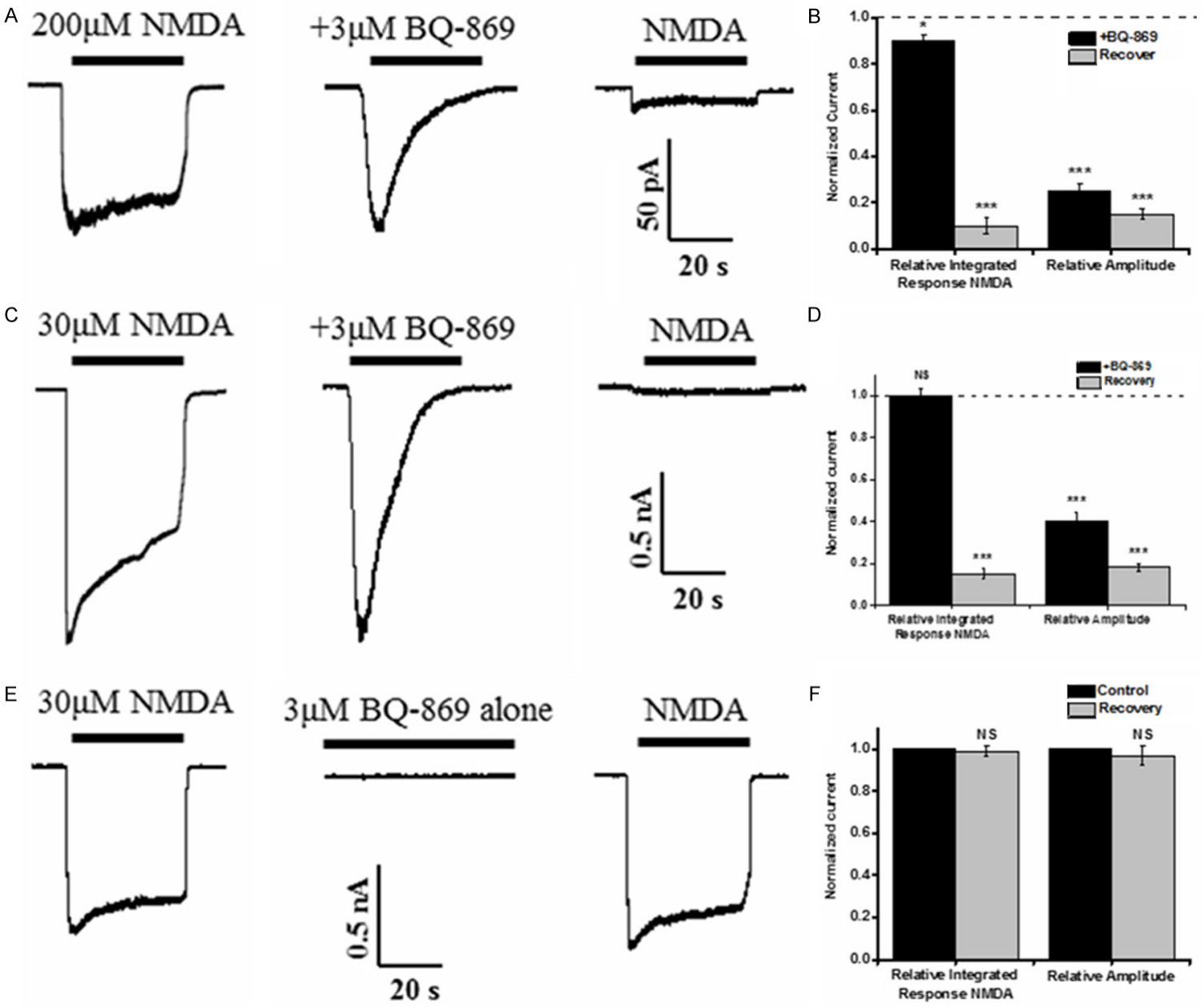
A
200μM NMDA
+3μM BQ-869
NMDA
50 pA
20 s
B
+BQ-869
Recover
Normalized Current
Relative Integrated Response NMDA
Relative Amplitude
C
30μM NMDA
+3μM BQ-869
NMDA
0.5 nA
20 s
D
+BQ-869
Recovery
NS
Normalized current
Relative Integrated Response NMDA
Relative Amplitude
E
30μM NMDA
3μM BQ-869 alone
NMDA
0.5 nA
20 s
F
Control
Recovery
NS
Normalized current
Relative Integrated Response NMDA
Relative Amplitude

Figure 2. Patch clamp recordings from three different cultured hippocampal neurons. (A) Left panel: The inward current elicited by 200 μM NMDA; Middle panel: The inward current elicited by 3 μM BQ-869 simultaneously; Right panel: The blockade by BQ-869 persisted long when the drug had been washed out. (B) The statistics for peak currents and ions per 15 s of (A). (C) Left panel: The inward current elicited by 30 μM NMDA; Middle panel: The inward current elicited by 3 μM BQ-869 simultaneously; Right panel: The blockade by BQ-869 persisted long when the drug had been washed out. (D) The statistics for peak currents and ions per 15 s of (C). (E) Left panel: The inward current elicited by 30 μM NMDA; Middle panel: The inward current elicited by 3 μM BQ-869 alone; Right panel: The blockade by BQ-869 disappeared when the drug had been washed out. (F) The statistics for peak currents and ions per 15 s of (E) ($*P \leq 0.05$, $***P \leq 0.001$).

to suppress spontaneous activity. To record NMDAR miniature excitatory post synaptic currents (mEPSC), the $MgCl_2$ concentration was reduced to 0.1 mM and 10 μM 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (f) quinoxaline-2,3-dione (NBQX) and 50 μM picrotoxin (Sigma) were added to the bath solution to block AMPA-receptor-mediated excitatory currents and c-aminobutyric acid (GABA) receptor-mediated inhibitory currents, respectively.

Intracellular calcium measurements with Fluo-4

To assess intracellular calcium levels, 1.5 × 104 hippocampal neurons cells were seeded in a 96-well black plate, washed with Hank's buffered salt solution (HBSS) and loaded with the calcium indicator Fluo 4-AM (5 μM) for 30 minutes at 37°C. Then the dye was removed, cells were kept in HBSS and baseline calcium levels for each well were assigned. The fluorescence of each well was determined with a confocal laser scanning microscope, and scanned using iexcitation at 488 nm and emission at 525 nm. To measure the effect of BQ-869 on intracellular calcium levels, we treated cells with 200 and 30 μM NMDA and/or 3 μM BQ-869 and fluorescence was measured every 60 seconds for a time course of 200 minutes.

Establishment of focal cerebral ischemia reperfusion model

Pensiveness Sprague-Dawley rats weighing 250 to 300 g were used in the study. Briefly, the rats were anesthetized by 4% isoflurane with a mixture of 60% medical air and 40% oxygen and anesthesia was maintained with 2% isoflurane. Rats were subjected to intraluminal middle cerebral artery occlusion (MACO) as described previously [18] and then block by advancing a nylon suture in the internal carotid artery to the origin of the middle cerebral artery. After 30 min of middle cerebral occlusion, blood flow was restored by removing the suture, and the mice were allowed to recover for 24 h. The rectal temperature was controlled at 37 ± 0.5°C during surgery. Alternatively, rats were subjected to MCAO surgery without occlusion (sham).

Rota-rod test

After the administration of BQ-869 the Sprague-Dawley rats with MCAO surgery were placed one by one on the rotating rod. When the rat falls from the rotating rod, note down the fall of time. Compare the fall off time of control animals and drug treated animals.

TTC staining and evaluation of infarction volume

Infarct area was measured from the brain slices using 2,3,5-triphenyltetrazolium chloride staining. TTC staining was performed at 24 hours after reperfusion to determine the infarction volume. Brain tissues were sectioned into 2-mm-thick slices and immersed in 2% solution of TTC (Sigma) for 30 minutes at 37°C. Then, the stained slices were fixed by immersion in 4% formaldehyde solution for hours. The infarct area was traced and measured using image analysis system (Image-J analysis software).

Statistical analysis

The data were expressed as mean ± SEM. Two-tailed student's t test was used for comparison of two independent groups. A one-way ANOVA analysis of variance was used to compare multiple groups. The software SPSS 13.0 was used to analyze all these data, and statistical significance was defined as $P \leq 0.05$.

Results

BQ-869 blocked NMDA receptor

The patch clamp recordings illustrate the blocking action of BQ-869 on inward current activat-

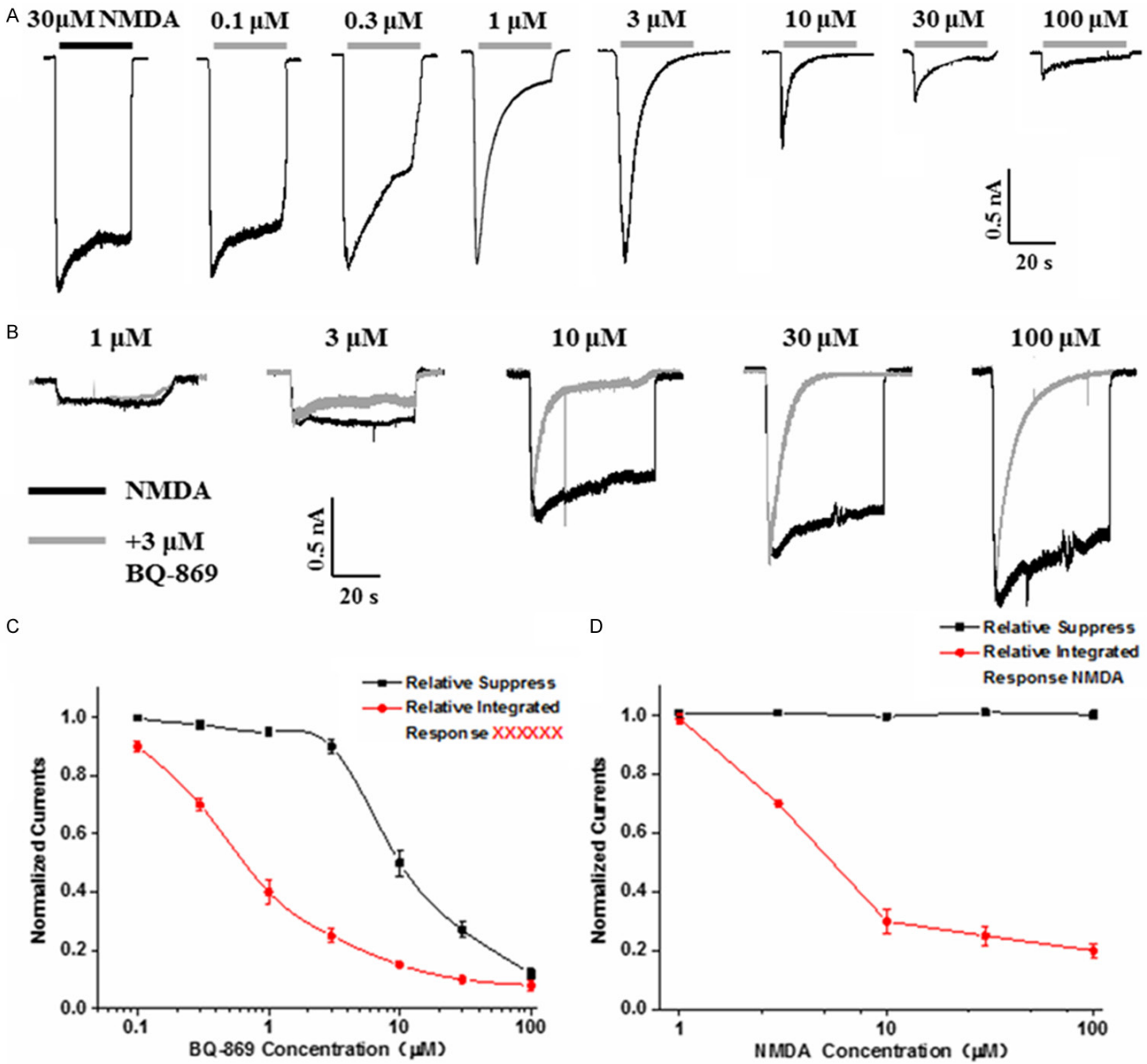
A
30μM NMDA
0.1 μM
0.3 μM
1 μM
3 μM
10 μM
30 μM
100 μM
0.5 nA
20 s
B
1 μM
3 μM
10 μM
30 μM
100 μM
NMDA
+3 μM
BQ-869
0.5 nA
20 s
C
Relative Suppress
Relative Integrated
Response XXXXXX
Normalized Currents
BQ-869 Concentration (μM)
D
Relative Suppress
Relative Integrated
Response NMDA
Normalized Currents
NMDA Concentration (μM)

Figure 3. BQ-869 blocked NMDA receptor in concentration-dependent and dose-dependent manner. (A) The inward current elicited by 30 μM NMDA alone and co-treating the increased concentration of BQ-869 (from 0.1 to 100 μM). (B) The inward current elicited by 3 μM BQ-869 and the increased concentration of NMDA (from 1 to 100 μM). (C) The statistics for peak currents and whole cell currents of (A). (D) The statistics for peak currents and whole cell currents of (B).

ed by NMDA in the hippocampal neurons. The results showed that application of 200 μM NMDA elicited an inward current that increased rapidly to a peak and then decayed to a steady current (**Figure 2A**). When applied 3 μM BQ-869 simultaneously, the current reached nearly the same peak but then was blocked progressively. The blockade by BQ-869 persisted long when the drug had been washed out with control solution and then tested with a subsequent application of NMDA. **Figure 2C** shows a similar experiment applied with low concentration of NMDA, and the result of 30 μM NMDA applying was similar to high concentration of NMDA. However, application of BQ-869 alone had no effect on the holding current and had little effect on the response of the cell to a subsequent application of NMDA (**Figure 2E**). Correspondingly analyzed with statistical methods shows that the peak current and total ions through cell per unit time (15 s) of BQ-869 applied together with NMDA were increased compared with recovery from BQ-869 blockade (**Figure 2B** and **2D**), but that of BQ-869 applied alone had no significant change compared with recovery (**Figure 2F**). These results suggests that BQ-869 can bind unless the channel has been opened by transmitter, that is, BQ-869 can exert its blocking action only when the receptor has been activated by NMDA.

BQ-869 blocked NMDA receptor in concentration-dependent and dose-dependent manner

To explore how did BQ-869 block NMDA receptor, we treated the hippocampal neurons with different concentrations of NMDA. Patch clamp recordings showed that NMDA-induced currents tended to be weaker after co-treating 30 μM NMDA and the increase of the concentration of BQ-86 (from 0.1 to 100 μM) compared to treating with 30 μM NMDA alone (**Figure 3A**). **Figure 3B** shows that the inhibition effect of BQ-869 to NMDA receptor enhanced with co-treating the increasing concentration of NMDA (from 1 to 100 μM) compared to treating with 3 μM BQ-869 alone. To verify the underlying mechanisms of electrophysiological traces, the currents data analyzed statistically. With co-treating of 30 μM NMDA and the increasing concentration of BQ-869, the suppression to peak currents slowly reduced then dropped rapidly when the concentration of BQ-869 was 3 μM (black) and to whole cell currents uniformly reduced (red) (**Figure 3C**). With co-application of 3 μM BQ-869 and the increasing concentration of NMDA, the suppression to peak currents almost unchanged (black) and to whole cell currents dropped rapidly then slowly reduced when the concentration of NMDA was 10 μM (red) (**Figure 3D**). All together, BQ-869 inhibited the current activated by NMDA in the hippocampal neurons by means of concentration-dependent and NMDA dose-dependent.

BQ-869 attenuated NMDA-induced Ca^{2+} influx

For intracelluar Ca^{2+} overload in physiological and pathological conditions plays a vital role in the process of NMDA-mediated excitotoxicity, we explored whether BQ-869 affected Ca^{2+} induced by NMDA. We directly treated the hippocampal neurons with NMDA, and observed the effects of BQ-869 on NMDA-triggered Ca^{2+} influx. Fluorescence analysis shows that 200 μM NMDA significantly increases intracellular Fluo-4 fluorescence intensity, but co-treating with 200 μM NMDA adding 3 μM BQ-869 decreased NMDA-induced fluorescence intensity by about 2-folds (**Figure 4A**). Similar results were observed when 30 μM NMDA was added, and co-treating with 30 μM NMDA and 3 μM BQ-869 decreased NMDA-induced fluorescence intensity by about 3-folds (**Figure 4C**). However, the intracellular Fluo-4 fluorescence intensity was unchanged when added 3 μM BQ-869 alone compared to 30 μM NMDA disposal (**Figure 4E**). Statistically, the total increased concentrations and the peak of increasing concentration of Ca^{2+} reduced after co-treating with NMDA adding BQ-869 in the hippocampal neurons, and the concentrations decreased with the declining concentration of NMDA (**Figure 4B** and **4D**). Accordingly, no significant change about the total increased concentration and the peak of increasing concentration of Ca^{2+} was found after adding BQ-869 alone (**Figure 4F**). These results indicated that

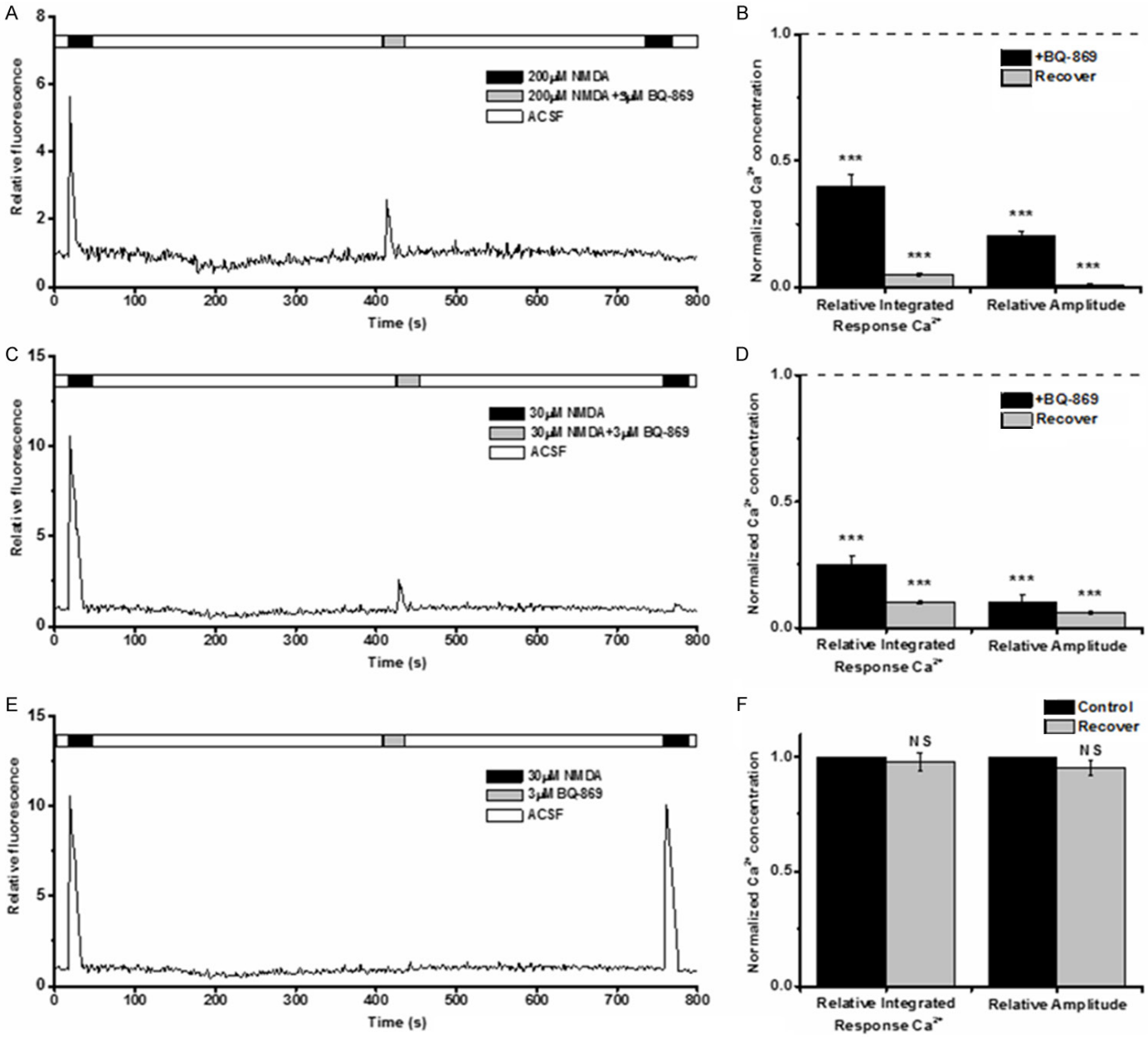
A
Relative fluorescence
Time (s)
200μM NMDA
200μM NMDA+3μM BQ-869
ACSF
B
Normalized Ca2+ concentration
+BQ-869
Recover
***
Relative Integrated Response Ca2+
Relative Amplitude
C
Relative fluorescence
Time (s)
30μM NMDA
30μM NMDA+3μM BQ-869
ACSF
D
Normalized Ca2+ concentration
+BQ-869
Recover
***
Relative Integrated Response Ca2+
Relative Amplitude
E
Relative fluorescence
Time (s)
30μM NMDA
3μM BQ-869
ACSF
F
Normalized Ca2+ concentration
Control
Recover
NS
Relative Integrated Response Ca2+
Relative Amplitude

Figure 4. Effects of BQ-869 on NMDA-induced calcium influx in cultured hippocampal neurons. (A) Fluorescence intensity increased by 200 μM NMDA, then decreased by co-treating with 200 μM NMDA and 3 μM BQ-869 about 2-folds. (B) The statistics for total increased concentrations and the peak of increasing Ca^{2+} concentration of (A). (C) Fluorescence intensity increased by 30 μM NMDA, then decreased by co-treating with 30 μM NMDA and 3 μM BQ-869 about 3-folds. (D) The statistics for total increased concentrations and the peak of increased Ca^{2+} concentration of (C). (E) Fluorescence intensity increased by 30 μM NMDA, then did not change after treating with 3 μM BQ-869 alone. (F) The statistics for total increased concentrations and the peak of increased Ca^{2+} concentration of (E) ($***P \leq 0.001$).

BQ-869 may inhibit NMDA-triggered Ca^{2+} influx and attenuate excitotoxicity in the hippocampal neurons.

BQ-869 inhibited NMDA-mEPSC in hippocampal pyramidal neurons

To determine whether BQ-869 inhibits NMDA-receptor mediated spontaneous mEPSC (NMDAR-mEPSCs), we tested its impact on hippocampal pyramidal neurons. After 10 μM BQ-869 perfusion, we recorded NMDAR-mEPSCs and detected a significant decrease within minutes, and BQ-869 mediated block of mEPSCs persisted following washout (**Figure 5A**). Moreover, the amplitude and frequency of NMDAR-mEPSC reduced drastically after application of BQ-869 (**Figure 5B** and **5C**). **Figure 5D** shows that 10 μM BQ-869 decreased the peak currents of action potential, and **Figure 5E** shows that the current was rapidly falling as BQ-869 worked. These results suggested that BQ-869 may inhibit NMDA-mEPSC in the hippocampal pyramidal neurons.

BQ-869 decreased infarction size in focal cerebral ischemia rats

As the therapeutic effects of BQ-869 in focal cerebral ischemia remained unknown, we establishment of MACO model to examine the impact of BQ-869 on cerebral infarction. In TTC staining, normal tissue stained deep red and infarct tissue with loss of mitochondrial enzyme activity did not stain, but appeared white. The border between stained and unstained tissues was well demarcated and could be identified easily by visual inspection [19]. **Figure 6A** shows that there was a existence of cerebral infarction in rats after ischemic injury (middle) compared with sham-group (left), but this existence of cerebral infarction disappeared when BQ-869 was made to act on rats with MACO (right). Quantitative analysis of infarction size in rats with MACO revealed that BQ-869 had remarkable function on decrease brain infarction (**Figure 6B**). In Rota rod, the latency time of the drug group was 160 s while control was 120 s (**Figure 6C**). This result suggested that BQ-869 administrated exhibited highly significant increase of athletic ability compared with control group of rats, which reflected the decrease of brain damage. Therefore, BQ-869 may decrease infarction size and has a neuroprotective role.

BQ-869 reduced stroke mortality

To assess the efficacy of BQ-869 in rats with focal cerebral ischemia, we investigated the date of infarction volume and mortality in rats treated normal saline (NS) and BQ-869 at the day 1, 3, 7, and 17. The infarct volume was significantly decreased in rats with MCAO treated with BQ-869 compared with the NS group (**Figure 7A**). Almost 61.5% (8/13) of BQ-869 treated rats were survival whereas in the NS group the experiment rats were totally dead, and the number of sham group rats was unaltered (**Figure 7B**). Thus, these results indicated that BQ-869 may reduce stroke mortality by decreasing infarction size after infarction size.

Discussion

Stroke is a deadly disease that continues to disrupt the lives of millions of patients and their families worldwide, and it is the third leading cause of death in the west, behind heart disease and cancer [20]. Death rates of stroke are accounts for about 10% of the global total [21]. These statistics are frightening despite decades of innovative research that led to the continuous development of newer drug. During a stroke, abnormal surplus of glutamate stimulation to the NMDA postsynaptic receptor induce a large amount of Ca^{2+} to influx directly through the receptor-gated ion channels. Calcium influx not only activates enzymes that digest the cells' proteins, lipids and nuclear material, but also leads to the failure of mitochondria [22], which can lead further toward energy depletion and may trigger cell death due to apoptosis [23]. Owing to the excitatory

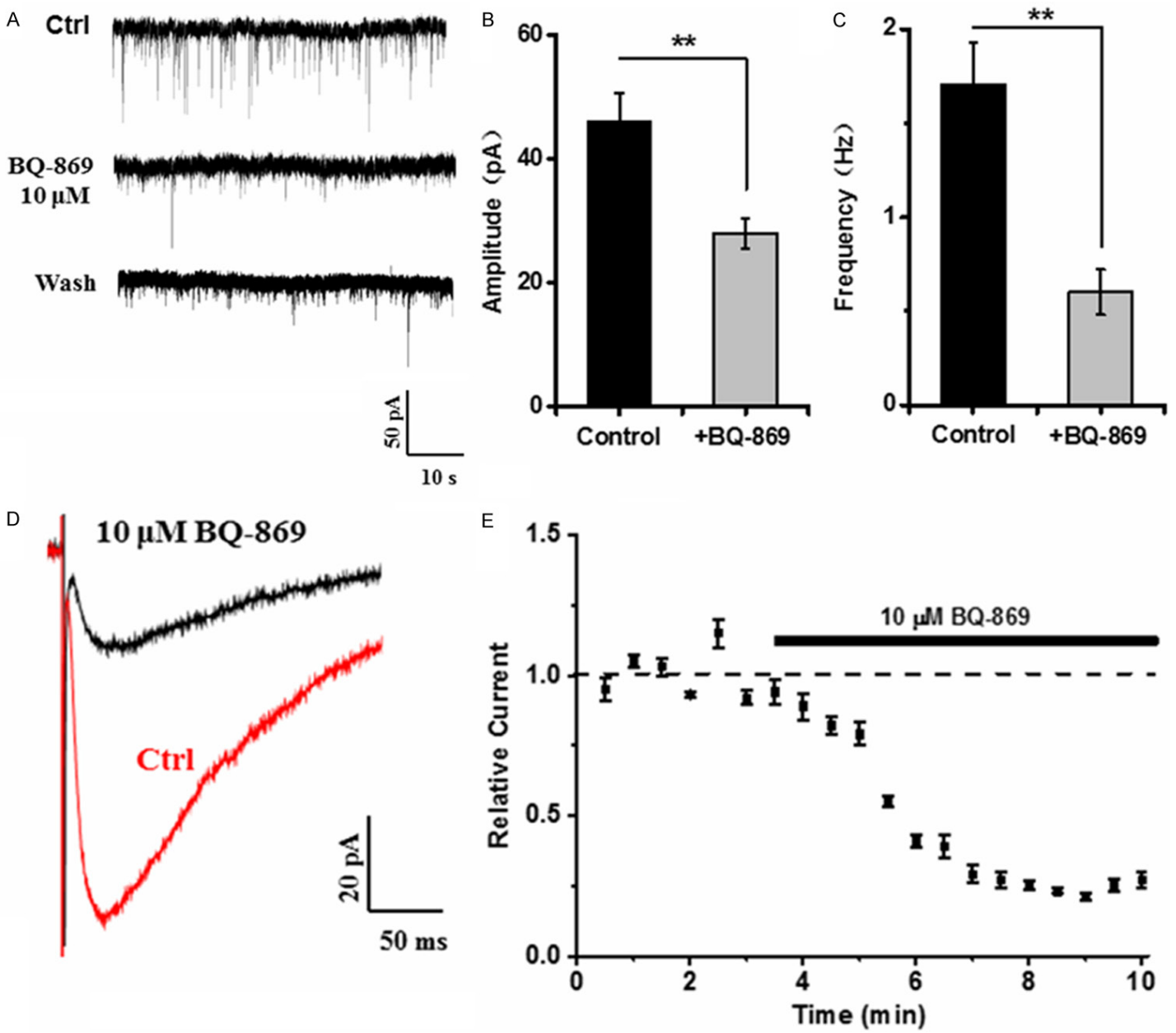
A
Ctrl
BQ-869
10 μM
Wash
50 pA
10 s
B
Amplitude (pA)
60
40
20
0
**
Control
+BQ-869
C
Frequency (Hz)
2
1
0
**
Control
+BQ-869
D
10 μM BQ-869
Ctrl
20 pA
50 ms
E
Relative Current
1.5
1.0
0.5
0.0
10 μM BQ-869
0
2
4
6
8
10
Time (min)

Figure 5. Effects of BQ-869 on NMDA-mEPSC in hippocampal pyramidal neurons. (A) Top panel: NMDAR-mEPSCs of predose; Middle panel: NMDAR-mEPSCs of 10 μM BQ-869; Bottom panel: NMDAR-mEPSCs of drug washout. (B, C) Quantification of amplitude and frequency of (A). (D) 10 μM BQ-869 decreased the peak currents of action potential. E. The statistics for the changed current of (D) (**$P \leq 0.01$).

neural toxicity induced by NMDA receptor in stroke, NMDA receptor antagonists have been suggested as potential therapeutic agents [13-15, 24]. Until fairly recently, ischemic stroke is treated as an important international problem at the forefront of science, for it occurs suddenly, develops quickly, lacks effective therapy, and with a poor prognosis [25]. The research and development of drugs should base on cognition to the occurrence and development of diseases. It is worth mentioning that the detailed clarification of brain tissue and ischemic stroke in the pathophysiological processes will give a big push for the selection of new target point and strategy.

In present study, the compound BQ-869 is a derivative of NMDA receptor antagonist MK-801, and the present study shows that it as an extremely potent neuroprotectant. BQ-869 blocked NMDA receptor in the hippocampal neurons, and the blocking action persisted long when the drug had been washed out with control solution and then tested with a subsequent application of NMDA. However, a previous study have reported that the inward current at 100 mM NMDA was markedly reduced in the presence of 5mM MK-801, and after washout of MK-801, the application of NMDA alone again induced a rapid inward current in clonal rat pheochromocytoma (PC12) cells [26]. The different suppression capabilities suggests BQ-869 shows higher NMDA receptor inhibitory effects than MK-801, a commonly used antagonist of NMDA receptors, which suggested BQ-869 is a new NMDA receptor antagonist with higher appetency. In addition, BQ-869 inhibited the NMDA-activated current by means of concentration-dependent and NMDA dose-dependent. It is important to master the dependent manner, for it has a guiding significance for the rational use of drug in clinical tests. Then fluorescence analysis showed that BQ-869 inhibited NMDA-triggered Ca^{2+} influx, and this proved BQ-869 can attenuate Ca^{2+} overload induced excitotoxicity in the hippocampal neurons. Recent studies suggest synaptic NMDA receptor activation mediated by spontaneous mEPSCs at rest can trigger signaling leading to synaptic plasticity within a time window of hours to several days, which plays an important role on ischemic stroke [27]. Here we found BQ-869 inhibits NMDAR-mEPSCs in the hippocampal pyramidal neurons, and it means that the NMDA receptor sensitivity to synaptically released glutamate is decreased. Buchan et al. Have reported that the neuroprotective activity of MK801 against transient global ischemia appears to be largely a consequence of postischemic hypothermia rather than a direct action on NMDA receptor channels [28]. However, we established intraluminal MCAO models to demonstrate the efficient effects of BQ-869 on stroke, and quantitative analysis of infarction size in rats with MACO revealed that BQ-869 had remarkable function on decrease brain infarction. As the results from our study show, BQ-869 may reduce stroke mortality by decreasing infarction size after cerebral ischemic injury. These results indirectly demonstrate the effect of BQ-869 on cerebral infarction is better than MK-801. In physiological terms, NMDA receptor participates in excitatory synaptic transmission, mediates signal transduction constituting the basis of memory and cognition, and is connected with physiological processes of synaptic plasticity, neuron growth and exists as well as long-termpotentiation (LTP) [29]. The Rota-rod test is an established motor task to evaluate balance and coordination aspects of motor performance in rats [30]. Present test showed that BQ-869 administrated exhibited highly significant increase of athletic ability compared with control group, which reflected the decrease of brain damage.

In summary, our current study demonstrated that BQ-869, a potent NMDA receptor antagonist with higher appetency, blocked NMDA receptor in concentration-dependent and dose-dependent manner, attenuated NMDA-induced Ca^{2+} influx, inhabited NMDAR-mEPSC in hippocampal pyramidal neurons, improved athletic ability of rats with MACO, decreased infarction size in focal cerebral ischemia rats and reduced stroke mortality. Based on our findings, we suggest that the neuroprotective effect of BQ-869 might be through inhibiting NMDA-mediated mediated excitotoxicity. Therefore, we conclude that its beneficial effect on cerebral ischemic

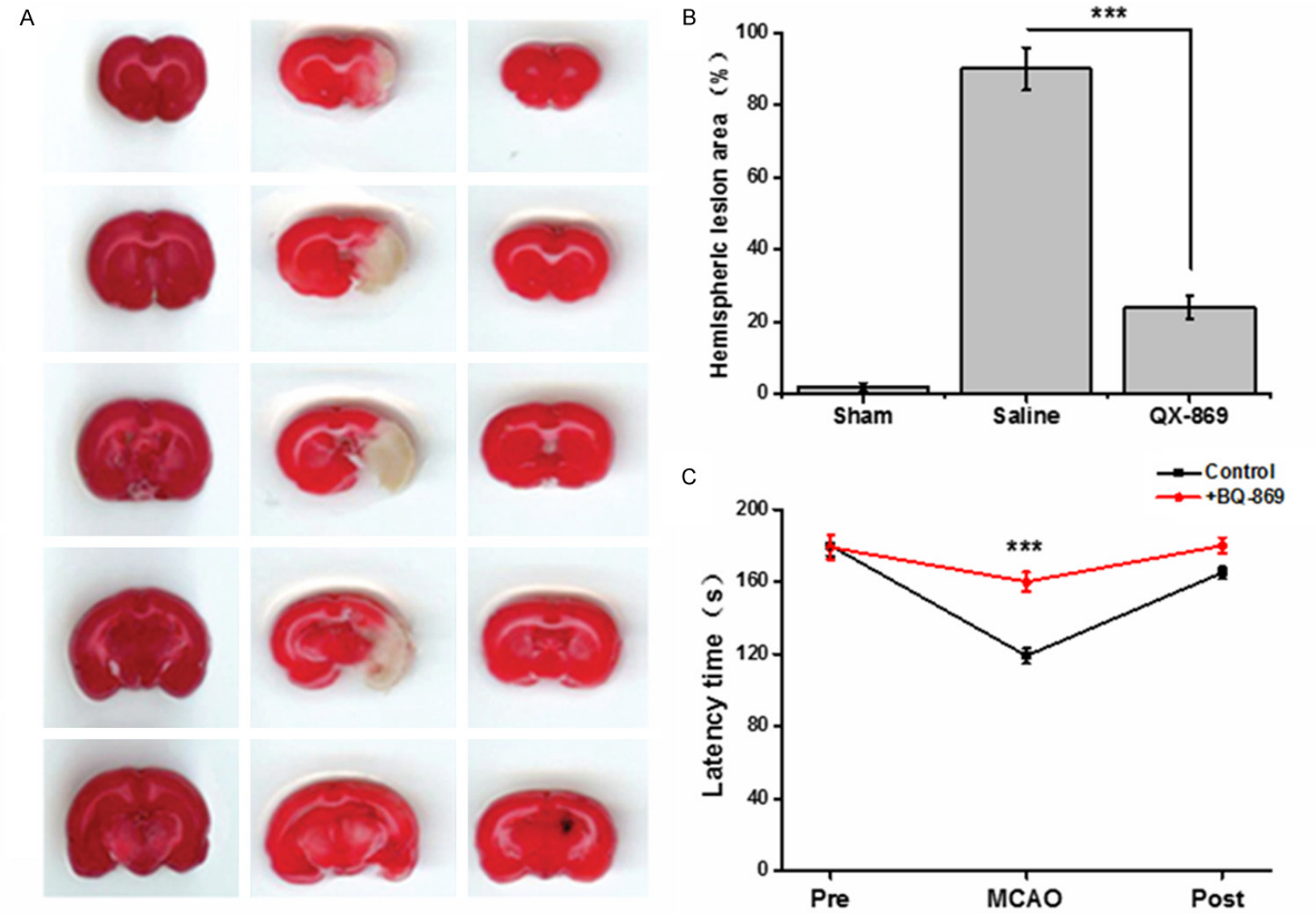

Figure 6. BQ-869 decreased infarction size in focal cerebral ischemia rats. (A) TTC staining of cerebral infarction. Left panel: sham; Middle panel: NS; Right panel: BQ-869. (B) The statistics for ischaemia-induced brain infarction area of (A). (C) Latency time of Rota-Rod test (***$P \leq 0.001$).

A

B

	Sham	Control	BQ-869
Total	10	13	13
Day3	10	10	12
Day7	10	3	10
Day14	10	0	8

Figure 7. BQ-869 reduced stroke mortality. A. TTC staining of cerebral infarction. Top panel: BQ-869; Bottom panel: NS. B. The mortality of MACO injury rats treated with NS or BQ-869 in 14 days.

injury has a therapeutic potential, giving a new drug of controlling stroke. BQ-869 will be a valuable new tool for elucidating the pathophysiological roles of stroke, and could be further explored in more vivo models even in clinical trials to justify the effectivity for prevention and treatment of stroke.

Disclosure of conflict of interest

None.

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