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Neuroprotective Role of an N-acetyl Serotonin Derivative via Activation of Tropomyosin-related Kinase Receptor B after Subarachnoid Hemorrhage in a Rat Model

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Abstract

N-[2-(5-hydroxy-1H-indol-3-yl) ethyl]-2-oxopiperidine-3-carboxamide (HIOC), an N-acetyl serotonin's derivative, selectively activates tropomyosin-related kinase receptor B (TrkB). This study is to investigate a potential role of HIOC on ameliorating early brain injury after experimental subarachnoid hemorrhage (SAH). One hundred and fifty-six adult male Sprague-Dawley rats were used. SAH model was induced by endovascular perforation. TrkB small interfering RNA (siRNA) or scramble siRNA was injected intracerebroventricularly 24 hours before SAH. HIOC was administrated intracerebroventricularly 3 hours after SAH and compared with brain-derived neurotrophic factor (BDNF). SAH grade and neurologic scores were evaluated for the outcome study. For the mechanism study, the expression of TrkB, phosphorylated TrkB (p-TrkB), phosphorylated extracellular signal regulated kinase (p-ERK), B-cell lymphoma 2 (Bcl-2) and cleaved caspase 3 (CC3) were detected by Western blots, and neuronal injury was determined by double immunofluorescence staining of neuronal nuclei and terminal deoxynucleotidyl transferase-mediated uridine 5'-triphosphate-biotin nick end-labeling. Knocking down of TrkB decreased the expression of Bcl-2 and aggravated neurologic deficits 24 hours after SAH. HIOC activated TrkB/ERK pathway, decreased neuronal cell death, improved neurobehavioral outcome, and these effects were abolished by TrkB siRNA. HIOC was more potent than BDNF in reduction of apoptosis 24 hours post-SAH. Thus, we conclude that administration of HIOC activated TrkB/ERK signaling cascade and attenuated early brain injury after SAH. HIOC may be a promising agent for further treatment for SAH and other stroke events.

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Keywords

Subarachnoid Hemorrhage; Early Brain Injury; Tropomyosin-related Kinase Receptor B; Apoptosis; N-acetyl Serotonin Derivative; Brain-derived Neurotrophic Factor

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Introduction

Subarachnoid hemorrhage (SAH) accounts for only 5% of all stroke incidents. However, due to its high rate of death and complications, the management of SAH remains to be one of the toughest challenges faced by physicians (Suarez et al., 2006; van Gijn et al., 2007). The importance of early brain injury after SAH has been emphasized in the last decade as a key issue that may affect the prognosis of SAH (Caner et al., 2012; Chen et al., 2014; Kusaka et al., 2004; Sehba et al., 2012). Recent studies have shown that anti-apoptosis may attenuate early brain injury after experimental SAH (Hasegawa et al., 2011b).

Tropomyosin-related kinase receptor B (TrkB) belongs to the receptor tyrosine kinase (RTK) family (Boulle et al., 2012). Phosphorylation of TrkB ameliorated brain injury in ischemic stroke models (Cui et al., 2013; Van Kanegan et al., 2014). Our previous study revealed that the brain-derived neurotrophic factor (BDNF)/TrkB signaling pathway was involved in apoptosis after SAH (Hasegawa et al., 2011a). N-acetyl serotonin (NAS), which is an intermediate in the endogenous synthesis of melatonin from serotonin, has been demonstrated activated TrkB receptor and exerted anti-depressant effects in a TrkB-dependent manner (Jang et al., 2010). N-[2-(5-hydroxy-1H-indol-3-yl) ethyl]-2-oxopiperidine-3-carboxamide (HIOC) is one of the derivatives of NAS. It selectively activates TrkB receptor with greater potency than NAS (Shen et al., 2012). However, the role of HIOC in stroke models has not been evaluated.

In the present study, we hypothesized that administration of HIOC would activate TrkB and its downstream effector extracellular signal regulated kinase (ERK), thereby, attenuating neuronal injury after experimental SAH.

Materials and methods

Animals

One hundred and fifty-six adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighting 280–320g were used in this study. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Loma Linda University.

Experimental design

The experiment was designed as follows:

Experiment 1—To determine the role of TrkB receptor in SAH, twenty-nine rats were divided into four groups: sham with burr hole (n=6), vehicle (5µl sterile saline)+SAH (n=7), TrkB small interfering RNA (siRNA) (500pmol/5µl)+SAH (n=9) and scramble siRNA (500pmol/5µl)+SAH (n=7). Vehicle or siRNAs were injected intracerebroventricularly 24

hours before SAH was induced. Neurologic score, SAH grade, and the expression of TrkB, phosphorylated TrkB (p-TrkB), B-cell lymphoma 2 (Bcl-2) were tested 24 hours after SAH.

Experiment 2—For outcome evaluation, eighty-four rats were assigned into five groups: sham (n=16), SAH (n=14), SAH+vehicle (n=19), SAH+HIOC (6µg in 5µl vehicle) (n=17), and SAH+HIOC (30µg in 5µl vehicle) (n=18). Vehicle or HIOC were injected intracerebroventricularly 3 hours after SAH onset. The Neurologic score and SAH grade were assessed at 24 and 72 hours.

Experiment 3—Forty-three rats were divided into five groups for mechanism study: sham (n=10), SAH+vehicle (n=10), SAH+HIOC (n=10), scramble siRNA+SAH+HIOC (n=6), and TrkB siRNA+SAH+HIOC (n=7). The Scramble or TrkB siRNAs were injected 24 hours before SAH modeling. The expression of TrkB, p-TrkB, phosphorylated ERK (p-ERK) and cleaved caspase 3 (CC3) were measured by Western blot, and immunostaining and terminal deoxynucleotidyl transferase-mediated uridine 5'-triphosphate-biotin nick end-labeling (TUNEL) were conducted 24 hours after SAH.

Experiment 4—To compare the therapeutic effects between HIOC and BDNF in the SAH model, forty-eight rats were divided into four groups: sham (n=12), SAH+vehicle (n=12), SAH+HIOC (n=12), and SAH+BDNF (5µg in 5µl vehicle) (n=12). Vehicle, HIOC or BDNF were administered 3 hours after SAH modeling. Neurologic score, SAH grade and Western blots were detected at both 6 and 24 hours after SAH.

Brain samples from Experiments 3 and 4 in sham, vehicle and HIOC treatment groups at 24 hours after SAH were shared with the samples from Experiment 2.

SAH model

The SAH rat model was induced by endovascular perforation, as previously described (Chen et al., 2013). All animals were transorally intubated after induced anesthesia with 5% isoflurane in 70/30% medical air/oxygen, and a small rodent respirator (Harvard Apparatus, Holliston, MA) was used to maintain an adequate respiration. Anesthesia was then maintained with 3% isoflurane in 70/30% medical air/oxygen.

The external carotid artery (ECA) was identified and transected distally with a 3 mm stump. A 4-0 sharpened monofilament nylon suture was advanced into the internal carotid artery (ICA) through the ECA until resistance was felt (at 18–20 mm) and then was pushed 5 mm further to penetrate the bifurcation of the anterior and middle cerebral artery. The suture was then withdrawn and the ICA was reperfused to produce SAH. Sham-operated rats underwent the same procedure except the suture was withdrawn without perforation, after feeling resistance.

Intracerebroventricular (icv.) injection

An intracerebroventricular injection procedure was performed, as reported previously (Chen et al., 2013; Suzuki et al., 2010). A small burr hole was drilled into the skull according to the following coordinates relative to bregma: 1.5 mm posterior; 1.0 mm lateral. The needle of 10 µl Hamilton syringe (Microliter 701; Hamilton Company, Reno, NV) was stereotactically

inserted into the left lateral ventricle through the burr hole, 4.0 mm below the horizontal plane of bregma.

Five microliters of HIOC (Vitas-M Laboratory, Narva, Estonia) or BDNF (ProSpec-Tany Technogene, East Brunswick, NJ) in vehicle were infused at a rate of 0.5 μ l/min 3 hours after SAH induction, while 500pmol/5 μ l TrkB or scramble siRNAs (Santa Cruz Biotechnology, Santa Cruz, CA) were infused at the same rate 24 hours before SAH modeling. TrkB siRNA is a pool of three different siRNA duplexes in order to improve the knockdown efficiency. All TrkB siRNA sequences are provided in 5'→3' orientation: (a) sense, GGAUUCCGGCUUAAAGUUU, antisense, AAACUUUAAGCCGGAUCC; (b) sense, GGAUUUGUAUUGCCUCAAU, antisense, AUUGAGGCAAUACAAAUC; (c) sense, CCAUCACAUUUCUGAAUC, antisense, GAUUCGAGAAAUGUGAUGG. The syringe was left in situ for an additional 10 minutes before slowly removing it. In Experiment 1, the sham group rats were subjected to the same procedure, without inserting the needle.

Neurological scores

Neurological deficits were evaluated 24 and 72 hours after SAH using a 22-point scoring system including a Modified Garcia scale and beam balance test in the outcome study. The modified Garcia assessment consisted of six tests covering spontaneous activity, spontaneous movement of four limbs, forepaw outstretching, climbing, body proprioception, and response to whisker stimulation (3–18 points) (Garcia et al., 1995). For the beam balance test, the rats were placed on a beam to observe their walking distance within 1 minute (0–4 points). The mean neurologic scores were calculated from two blinded observers' grading.

SAH grade

An 18-point SAH severity grading system was used as previously described (Sugawara et al., 2008). The basal cistern was divided into six segments that can be scored from 0 to 3 according to the amount of subarachnoid blood clot. A total score was calculated by adding the scores from six segments (0–18 points). Animals receiving a score of less than 8 were excluded from the study.

Western blots

The protein extracted from the left hemisphere (perforation side) was used for the Western blot analysis. Equivalent total protein amounts (30 μ g) were loaded in each lane of SDS-PAGE gels. After gel electrophoresis, the protein was transferred onto a nitrocellulose membrane, which was then blocked by a blocking buffer for 2 hours at room temperature. The following primary antibodies were diluted to incubate with the membrane under gentle agitation at 4°C overnight: anti-TrkB, anti-phosphorylated TrkB (Abcam, Cambridge, MA), anti-phosphorylated ERK 1/2, anti-cleaved caspase 3 (Cell Signaling Technology, Danvers, MA), anti-Bcl-2 (Santa Cruz Biotechnology, Santa Cruz, CA). β -actin was used as an internal loading control (anti- β -actin) (Santa Cruz Biotechnology, Santa Cruz, CA). Appropriate secondary antibodies were incubated with the NC membrane for 2 hours at room temperature. Chemiluminescent detection was performed to identify the immune

bands with the kit (ECL Plus; Amersham Bioscience, Arlington Heights, IL). Data was analyzed by densitometry with ImageJ software.

Immunofluorescence

Immunofluorescence for brain slices was performed on fixed frozen sections as previously described (Hu et al., 2009). Twenty-four hours after SAH, rats were deeply anesthetized and transcardially perfused with phosphate-buffered solution (PBS) and 10% formalin. Rats' brains were rapidly isolated and postfixed in 10% formalin for 24 hours and then in 30% sucrose for 3 days. Coronal brain sections (10 μ m) were obtained with the help of cryostat (Leica CM3050S-3-1-1, Bannockburn, IL) and permeabilized with 0.3% Triton X-100 in PBS for 30 minutes. Sections were blocked with 5% donkey serum for 1 hour and incubated at 4 °C overnight with primary antibodies: anti-phosphorylated TrkB (Abcam, Cambridge, MA) and anti-neuronal nuclei (NeuN) (Millipore, Temecula, CA), followed by fluorescein isothiocyanate (FITC)- and Texas Red-conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) for 2 hours at room temperature. The colocalization of p-TrkB with the marker of neuron was examined by fluorescent microscope (Olympus OX51, Tokyo, Japan).

TUNEL staining

For double immunofluorescence staining of NeuN/TUNEL, a TUNEL kit (In situ Cell Death Detection Kit, Fluorescein, Roche, Mannheim, Germany) was used after the sections were probed with anti-NeuN primary antibody and Texas Red-conjugated secondary antibody. For quantitative analyses, the numbers of TUNEL-positive neurons in five microscope sections ($\times 20$), which were selected around the bleeding site in the subcortex, were averaged per animal.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used for different groups' comparison. Chi-square tests were used for mortality analyses. Data were shown as mean $\pm$ SEM. $P < 0.05$ was considered statistical difference.

Results

SAH severity score and mortality

SAH grading scores were similar among SAH groups in each time point of experiments (data not shown). Two animals were excluded from this study due to mild bleeding (one in TrkB siRNA + SAH group of Experiment 1, and one in SAH+HIOC low dosage group of Experiment 2). There is no significant difference on mortality among groups in each experiment (data not shown). No sham-operated animals died in this study.

Knocking-down of TrkB exacerbated neurologic deficits 24 hours after SAH

Gene silence of TrkB receptor slightly but significantly decreased the modified Garcia score 24 hours after SAH, when compared with the vehicle or scramble siRNA group (Fig. 1A).

No obvious neurologic deficits were observed 24 hours after siRNA injection, before perforation (data not shown).

Knocking-down of TrkB decreased the expression of p-TrkB and Bcl-2, 24 hours after SAH

The expression of TrkB did not change 24 hours after SAH, while it was down regulated in the TrkB siRNA group (Fig. 1C). Phosphorylation of TrkB and the expression of Bcl-2 were reduced 24 hours after SAH, and knocking down of TrkB led to further decrease (Figs. 1D, E).

Administration of HIOC after SAH improved neurologic scores, and TrkB siRNA abolished the effects

Impaired neurologic outcomes were observed after SAH, and HIOC (icv. injection) attenuated neurobehavioral deficits 24 hours after SAH in both low and high dosage groups (Fig. 2A). High dosage of HIOC administration significantly improved the neurologic function 48 and 72 hours after SAH, while the low dosage group showed no effects on increasing neurobehavioral scores compared with the SAH or SAH+vehicle groups (Fig. 2B). Therefore, the high dosage was selected for the following studies (experiments 3 and 4).

TrkB siRNA abolished the therapeutic effects of HIOC treatment, while scramble siRNA had no impact on modified Garcia scores 24 hours after SAH (Fig. 2C).

HIOC exerts neuroprotection via activation of TrkB/ERK pathway

Western blots analyses showed that TrkB siRNA significantly decreased TrkB expression (Fig. 3B). The phosphorylation of TrkB and its downstream effector ERK reduced 24 hours after SAH. HIOC treatment increased the level of p-TrkB and p-ERK (Figs. 3C, D). The expression of CC3 increased 24 hours after SAH, while HIOC treatment decreased this pro-apoptosis protein level (Fig. 3E). These effects of HIOC were prevented by TrkB siRNA, but not affected by scramble siRNA (Figs. 3C–E).

HIOC treatment attenuated neuronal injury in subcortex 24 hours following SAH

The expression of p-TrkB was detected in neurons in both sham-operated and SAH rats (Fig. 4A). The numbers of TUNEL positive neurons were strikingly increased in subcortex 24 hours after SAH, and HIOC treatment remarkably reduced neuronal apoptosis (Fig. 4B, C).

HIOC is equally potent as BDNF at 6 hours, but more potent 24 hours following SAH

There is no notable difference on modified Garcia scores among vehicle, HIOC and BDNF treatment groups 6 hours after SAH (Fig. 5A). The expression of p-TrkB and p-ERK significantly decreased 6 hours after SAH compared with sham group, while treatment with HIOC or BDNF remarkably increased the expression of p-TrkB and p-ERK (Fig. 5C, D). No statistical difference was found upon the phosphorylation level between the two treatment groups (Fig. 5C, D).

Twenty-four hours after SAH, the HIOC group showed better outcomes than the BDNF group (Fig. 6A). There is a trend ($P>0.05$) that the level of p-TrkB was higher in the HIOC

group than that of the BDNF group 24 hours after SAH (Fig. 6B), and the level of p-ERK was significantly lower in BDNF group compared with that in the HIOC group at 24 hours' time point (Fig. 6C). The expression of CC3 notably increased 24 hours after SAH was induced. HIOC significantly decreased the CC3 level compared with the vehicle group, while BDNF showed no marked effect on reducing the CC3 expression (Fig. 6D).

Discussion

The present study demonstrated a crucial role of TrkB in early brain injury after SAH that knocking down TrkB decreased Bcl-2 and aggravated neurological deficits. HIOC activated the TrkB/ERK pathway and decreased brain injury especially neuronal apoptosis after SAH. The therapeutic effect of HIOC was abolished by TrkB siRNA. Furthermore, the neuroprotective effect of HIOC was comparable to BDNF at 6 hours but more pronounced 24 hours after SAH.

TrkB is one of a major class of neurotrophin receptors, as they promote cell survival, differentiation and synaptic plasticity (Boulle et al., 2012; Obianyo and Ye, 2013). Several studies have demonstrated that TrkB signaling plays a pivotal role in central nervous system diseases, such as neurodegeneration, psychiatric disorders and ischemia stroke (Castello et al., 2014; Pandya et al., 2014; Van Kanegan et al., 2014). Our previous study suggested that inhibition of protein tyrosine phosphatases could preserve TrkB activation, thus preventing early brain injury after SAH in rat model (Hasegawa et al., 2011a). As anticipated, gene silencing of TrkB worsened the outcomes of SAH in this study (Fig. 1).

NAS was once considered only as the precursor of melatonin from serotonin. However, recent studies revealed that NAS could act as an agonist of TrkB and display neuroprotective properties *in vitro* and *in vivo* (Jang et al., 2010; Sompol et al., 2011). Meanwhile, Zhou et al. demonstrated that NAS could also inhibit mitochondrial cell death pathways and autophagy activation following ischemic insults (Zhou et al., 2014). As a new derivative of NAS, HIOC has been proven to selectively phosphorylate TrkB with higher efficiency and is more stable than NAS (Shen et al., 2012). However, we are not aware of a study on the effects of HIOC in stroke models. In the present study, we showed that HIOC had a pro-survival role through initiating TrkB downstream signaling cascade following SAH in a rat model. Treatment with HIOC 3 hours after SAH attenuated early brain injury, showing a relatively wide therapeutic time window of HIOC, which made it more clinically relevant.

TrkB is the primary high affinity receptor for BDNF and BDNF-TrkB axis has been revealed to be neuroprotective involving in various central nervous system disorders, including ischemic injury (Han and Holtzman, 2000; Han et al., 2011; Muller et al., 2008; Van Kanegan et al., 2014), which made BDNF a promising agent for stroke treatment. Therefore, in the current study, we compared the therapeutic effects of HIOC with BDNF after SAH. Our data showed that these two treatment groups exhibited no significant difference on stimulating TrkB phosphorylation 6 hours following SAH, while HIOC group showed improved neurobehavioral outcomes and exerted a tendency of activating TrkB more potently than the BDNF group 24 hours post-SAH (Fig. 5, 6). It has been reported that

BDNF has poor blood brain barrier penetrability, short serum half-life, and induces TrkB polyubiquitination and degradation, and these features made BDNF's clinical application problematic (Nagahara and Tuszynski, 2011; Shen et al., 2012). From this observation, it seems that HIOC, as a small compound, is more efficient and has prolonged effects on activating TrkB and its downstream effector than BDNF, thus a good candidate for future consideration.

Apoptosis is considered to be one of the most crucial factors that can cause early brain injury after SAH. Moreover, injured neurons following SAH may be associated with delayed neurological deterioration and poor long-term outcome (Sabri et al., 2008). TrkB activation leads to ERK1/2 phosphorylation, which is critical for numerous cellular activities such as survival, proliferation, apoptosis, motility, transcription, metabolism and differentiation (Ramos, 2008; Roux and Blenis, 2004). A previous study reported that p-ERK was significantly decreased in the dentate gyrus in SAH model and elevating its expression prevented SAH induced apoptosis (Lin et al., 2009). In agreement with this study, in our study, the level of p-ERK in the left hemisphere (perforation side) decreased after SAH compared with the sham, and treatment with HIOC prevented its decrease (Fig. 3). Based on these findings, we speculate that phosphorylation of ERK1/2 via activation of TrkB may result in the subsequent pro-survival signaling cascade, which is the activation of the 90 kDa ribosomal S6 kinase and its downstream pathway. The transcriptional factor cAMP-response element binding protein (CREB) phosphorylation is ribosomal S6 kinase-mediated and facilitates the neurons survival through increased transcription of pro-survival genes, such as Bcl2 family members (Anjum and Blenis, 2008). Indeed, the expression of Bcl-2 was reduced when TrkB was knocked down in this study (Fig. 1).

However, there are several limitations of this study need to be mentioned. First, we did HIOC intracerebroventricular injection only in our experiments. Intraperitoneal or intravenous injection route should be compared with intracerebroventricular injection route to assess blood brain barrier penetrability of HIOC, which might be important for further clinical application. Second, we cannot exclude the therapeutic effects of HIOC are partially from the antidepressant actions of NAS derivatives. So long-term effects of HIOC on SAH rat need to be evaluated to see if this treatment is related to antidepressant actions. Third, serotonin is a well-known platelet agonist with which an N-acetyl serotonin derivative might compete. The effects of HIOC on coagulation cascade and platelet activation might affect the results. Future studies of HIOC on clotting mechanism *in vivo* should help us to answer this question.

In conclusion, the present study demonstrated for the first time that knocking down of TrkB aggravated early brain injury and activation of TrkB with HIOC attenuated neuronal injury following SAH in a rodent model. HIOC has the potential to reduce SAH or other stroke injuries.

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Highlights

Knocking down of TrkB aggravated early brain injury after SAH.

Administration of HIOC improved neurologic deficits after SAH.

HIOC initiated TrkB/ERK pathway and decreased neuronal apoptosis after SAH.

HIOC exerted better therapeutic effects than BDNF following SAH.

Fig. 1. Tropomyosin-related Kinase Receptor B (TrkB) small interfering RNA (siRNA) exacerbated early brain injury at 24 hours after subarachnoid hemorrhage (SAH). (A) Modified Garcia score in each group. Representative Western blots (B) and quantitative analyses of TrkB (C), phosphorylated-TrkB (p-TrkB) (D) and B-cell lymphoma 2 (Bcl-2) (E) at left hemisphere 24 hours after SAH. $n = 6$ for each group. * $P < 0.05$ vs. sham, and # $P < 0.05$ vs. TrkB siRNA+SAH.

Fig. 2.

Administration of HIOC improved neurologic scores (modified Garcia score + beam balance score) at (A) 24 hours, (B) 48 hours and 72 hours after subarachnoid hemorrhage (SAH). (C) Tropomyosin-related Kinase Receptor B (TrkB) small interfering RNA (siRNA) injection reversed the therapeutic effects of HIOC (30 μ g) at 24 hours post-SAH. For (A) and (C), $n = 10$ for sham, vehicle and HIOC high dose groups; $n = 6$ for the other groups. For (B), $n = 6$ for each group. * $P < 0.05$ vs. sham, @ $P < 0.05$ vs. SAH, & $P < 0.05$ vs. vehicle, # $P < 0.05$ vs. HIOC (6 μ g), % $P < 0.05$ vs. HIOC (30 μ g), \$ $P < 0.05$ vs. scramble siRNA + HIOC.

Fig. 3. HIOC initiated tropomyosin-related kinase receptor B (TrkB)/extracellular signal regulated kinase (ERK) pathway at 24 hours following subarachnoid hemorrhage (SAH). Representative Western blots (A) quantitative analyses of TrkB (B), phosphorylated-TrkB (p-TrkB) (C), phosphorylated-ERK (p-ERK) (D) and cleaved caspase 3 (CC3) (E) at left hemisphere 24 hours after SAH. $n = 6$ for each group. $*P < 0.05$ vs. sham, $@P < 0.05$ vs. vehicle, $\#P < 0.05$ vs. HIOC, $\&P < 0.05$ vs. scramble small interfering RNA (siRNA) + HIOC.

Fig. 4.

Administration of HIOC attenuated neuronal apoptosis at 24 hours after subarachnoid hemorrhage (SAH). (A) Double immunofluorescence showed colocalization of phosphorylated tropomyosin-related kinase receptor B (p-TrkB) (green) with neuronal nuclei (NeuN) (red) in both sham and vehicle groups. (B) Terminal deoxynucleotidyl transferase-mediated uridine 5'-triphosphate-biotin nick end-labeling (TUNEL) staining (green) at subcortex in sham, vehicle and HIOC groups. (C) Cell counts of TUNEL-positive neurons ($n = 4$ for each group). * $P < 0.05$ vs. sham, # $P < 0.05$ vs. vehicle.

Fig. 5.

Therapeutic effects of HIOC and brain-derived neurotrophic factor (BDNF) at 6 hours after subarachnoid hemorrhage (SAH). (A) Modified Garcia scores in each group. Representative Western blots (B) quantitative analyses of phosphorylated tropomyosin-related kinase receptor B (p-TrkB) (C) and phosphorylated extracellular signal regulated kinase (p-ERK) (D) expression at left hemisphere in each group. $n = 6$ for each group. * $P < 0.05$ vs. sham, @ $P < 0.05$ vs. vehicle.

Fig. 6.

Therapeutic effects of HIOC and brain-derived neurotrophic factor (BDNF) at 24 hours after subarachnoid hemorrhage (SAH). (A) Modified Garcia scores in each group. Representative Western blots quantitative analyses of phosphorylated tropomyosin-related kinase receptor B (p-TrkB) (B), phosphorylated extracellular signal regulated kinase (p-ERK) (C) and cleaved caspase 3 (CC3) (D) at left hemisphere in each group. $n = 6$ for each group. * $P < 0.05$ vs. sham, @ $P < 0.05$ vs. vehicle, # $P < 0.05$ vs. HIOC.