MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 10154-10163
DOI: 10.12659/MSM.918479

Received: 2019.07.03
Accepted: 2019.10.11
Published: 2019.12.30

Brain-Derived Neurotrophic Factor Alleviates Ropivacaine-Induced Neuronal Damage by Enhancing the Akt Signaling Pathway

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Source of support: Departmental sources

Background: Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors that modulate critical metabolic activities, including apoptosis, proliferation, and differentiation modulation. Although numerous studies have focused on the damaging effects of BDNF on neurons, the underlying relationship between these effects remains unclear. In the present study, we investigated the protective effect of BDNF on neuronal injury induced by ropivacaine and assessed whether it is related to the Akt signaling pathway.

Material/Methods: Human neuroblastoma cell line SH-SY5Y cells were stimulated with ropivacaine at different concentrations to induce neuronal injury. MTT analysis, flow cytometry, immunohistochemistry, qRT-PCR, and Western blot were used to investigate the proliferation activity, apoptotic level, and expression of Akt, PCNA, Bax, Bcl-2, and cleaved caspase-3, collectively demonstrating the underlying regulatory mechanisms.

Results: Compared with the control group, the morphological damage and proliferation inhibition of SH-SY5Y cells induced by ropivacaine were dose-dependent and time-dependent, accompanied by a significant decrease in Akt expression. We treated cells with BDNF or SC79, which is a selective cell-permeable small molecule Akt activator. The results showed that, compared to the ropivacaine group, the morphological damage of neurons was alleviated; cell proliferation activity was enhanced; apoptotic rate was reduced; PCNA, Bcl-2, and phosphorylated Akt expression levels were increased; and Bax and caspase-3 gene and protein expression were decreased. We were able to reverse these effects by administering API-2, an Akt inhibitor.

Conclusions: BDNF can alleviate ropivacaine-induced neuronal injury by activating Akt signaling pathway, consequently modulating the proliferation and apoptosis of neurons.

MeSH Keywords: Neuronal Migration Disorders • Oncogene Protein v-akt • Receptor, trkB

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/918479

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Background

In recent years, with the application of new drugs and the optimization of monitoring equipment, the safety of anesthesia has been gradually improved [1]. Meanwhile, the side effects of various anesthetics, including those used in local anesthesia, on organs and tissues of patients have gradually received increasing attention [2]. Although local anesthesia has less cardiopulmonary complications when compared to general anesthesia, its other adverse effects were reported to include respiratory dysfunction, epilepsy, palpitation, cardiac dysfunction and neuronal injury [3,4]. Nevertheless, the function of the awake brain as a real-time monitor was proved to be important, suggesting a complex of constantly improving and reviewing the role of local anesthesia in clinical medicine.

Ropivacaine (ROP) is one of the most commonly used local anesthetics in clinical medicine and stomatology [5]. Its main functional form is optical pure S (–) isomer, which has extraordinary biochemical and therapeutic properties, being widely used in clinical medicine [6]. Being an amide anesthetic with similar structure to bupivacaine but with slightly weaker overall effect, ropivacaine's motor blocking effect was 66% that of bupivacaine, while the analgesic effect was only 60% [7]. However, intrathecal administration of ROP was reported to induce neuronal injury and triggered cells apoptosis in a dose-dependent manner in rats [8]. It is important to find a method to improve ROP-caused neuronal injury.

Being one of the neurotrophic factors, brain-derived neurotrophic factor (BDNF) extensively exists in various procedures such as neuronal proliferation, survival regulation, and differentiation modulation, and consequently plays a crucial role in neurogenesis, synapse formation, and synaptic plasticity [9–11]. BDNF has also been proved to be associated with diverse neurological diseases such as schizophrenia, autism, mood disorders, and drug-induced brain injury [12]. It was previously delineated that upregulation of BDNF expression can promote activation of the Akt signaling pathway; it is used in pathophysiological procedures following brain injury and is involved in neurocyte morphology, dysfunction, and apoptosis [13]. Interestingly, there was no report about the neurological modulation effect of BDNF on the Akt pathway in ropivacaine-induced neuronal injury.

In view of the current situation, we innovatively and systematically elucidate BDNF's potential neuroprotective effects against ropivacaine and the mechanism underlying it.

Material and Methods

Cell culture and grouping

Human neuroblastoma cells SH-SY5Y (CRL-2266, ATCC, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) and 100 U/ml penicillin, 100 mg/ml streptomycin (Gibco, Rockville, MD, USA) in an incubator (Thermo Fisher, Scientific, USA) with 5% CO_2 at 37°C. In order to verify the effect of ropivacaine on cell metabolism, the cells were divided into a control group and a ropivacaine treatment group. The final concentration of ropivacaine was 1, 2, 3, 4, and 5 mM by phosphate-buffered saline (PBS) gradient dilution. In the control group, SH-SY5Y cells were treated with the same amount of PBS.

To investigate the protective effect of BDNF on neuronal injury and to explore its potential association with the Akt signaling pathway, the cells were divided into the following groups: Control group: SH-SY5Y cells were treated with PBS for 48 h; Rop group: 5 mM Ropivacaine treatment for 48 h; BDNF+Rop group: 20 ug/L BDNF treatment for 2 h and 5 mM ropivacaine treatment for 48 h; SC79+Rop group: 5 mg/L SC79 (HY-18749, MCE, USA) treatment for 2 h and 5 mM ropivacaine treatment for 48 h; BDNF+API-2+Rop group: 20 μg/L BDNF+10 μmol/L API-2 (HY-15457, MCE, USA) treatment for 2 h and 5 mM ropivacaine treatment for 48 h.

Cell morphology

After 48 h of ropivacaine administration, the cell morphology was observed under an inverted microscope and photographed.

3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) assay

After being digested by trypsinase, the cells were adjusted to the concentration of 1 *10^4/mL and incubated with 5% CO_2 at 37°C. After 24 h, 48 h, 72 h, and 96 h of incubation, the samples were treated with 20μL MTT (5 mg/mL). After 4 h, the supernatant was discarded and 200 μL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) was added. The absorbance optical density (OD) at 490 nm was read on a plate reader (Bio-Rad Laboratories).

Flow cytometry

After 48 h of treatment with ropivacaine, the cells were treated with trypsin, centrifuged, and collected. After washing with PBS, the cells with a total number of about 1×10^6/mL were resuspended by 250 μL 1× binding buffer, then 195 μL of these resuspended cells were added with 5 μL FITC-labeled Annexin-V,

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followed by 3-min incubation, after which we added 20 μg/mL propidium iodide solution. After 10-min incubation, we added 1×binding buffer of 400 μL and performed flow cytometry (Gallios; Beckman Coulter, Inc., Brea, CA, USA). The results were analyzed by cell quest software (BD Biosciences, San Diego, CA, USA).

Cellular immunohistochemistry

The cells underwent 48-h treatment with ropivacaine and were further immobilized with polyformaldehyde, administrated by 0.5% Triton X-100 and treated with 3% H_2O_2 for 15–20 min before being blocked by serum albumin (BSA) for 30 min. Thereafter, they were treated with rabbit anti-Akt antibody (1: 100, ab8805, Abcam, UK), anti- proliferating cell nuclear antigen (PCNA) antibody (1: 100, ab15497, Abcam, UK), incubated for 30 min, and washed. Goat anti-rabbit IgG (Proteintech, USA) labeled with horseradish peroxidase were further added, followed by 30-min incubation and then were washed by PBS. Finally, after incubation with streptavidin-biotin complex (SABC) for 30 min, the samples underwent PBS washing, diaminobenzidine (DAB) staining, slight hematoxylin re-staining, dehydration, transparency, and sealing. They were placed under 400-power optical microscope (Olympus, Japan) with the positive cells being counted.

Real-time fluorescence quantitative polymerase chain reaction (RT-PCR)

Total RNA extraction kits (Invitrogen, Carlsbad, CA, USA) were used. After total RNA was extracted from each cell line, it underwent reverse transcription into cDNA for Real-time PCR reaction system. Mastercycler® nexus X2 (Eppendorf, Hamburg, Germany) was used for RT-PCR. Conditions were 95°C for 10 min, 95°C for 15 s, and 60°C for 60 s (40 cycles). The data were processed by $2^{-\Delta\Delta Ct}$ method with the GAPDH mRNA being used as internal reference.

The sequences of primers (Shanghai Biotechnology Service Co.) were as follows:
Caspase-3,
Forward: 5'-GTGCTATTGTGAGGCGGTT-3',
Reverse: 5'-CGAGATGTCATTCCAGTGCT-3';
Bcl-2-associated X protein (Bax),
Forward: 5'-TCGAGGACGACTTCAACTATGG-3',
Reverse: 5'-ACAGCAAAATTAAGGCAGGACTC-3';
B-cell lymphoma-2 (Bcl-2),
Forward: 5'-AACCCCAGCGACTCTTTTATG-3',
Reverse: 5'-GGCAATCTGTACCTCTGCTTG-3';
GAPDH,
Forward: 5'-TGTGGGCATCAATGGATTTGG-3',
Reverse: 5'-ACACCATGTATTCCGGGTCAAT-3'.

Western blot analysis

The total cell protein was extracted and quantified by BCA kit (Shanghai Biyuntian Biotechnology Co.). Protein was separated by electrophoresis with 4%–12% Novex Bis-Tris gradient gel (Invitrogen Company, USA). Furthermore, 40 μg of each sample underwent disodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (Mini-Protean-3, Bio-Rad, Hercules, CA, USA), followed by transfer to polyvinylidene fluoride (PVDF) membranes (Merck, Darmstadt, Germany) for 60 min. The membranes were washed 3 times in tris buffer solution tween (TBST) for 10 min each, followed by blocking in 5% non-fat milk for 1 h, and incubation with the following primary antibodies at 4°C overnight: Rabbit anti-Bax Antibody (1: 1000, ab53154, Abcam, UK), anti-Bcl-2 Antibody (1: 1000, ab59348, Abcam, UK), anti-cleaved caspase-3 Antibody (1: 500, ab49822, Abcam, UK), and beta-actin (1: 1000, ab8227, Abcam, UK) polyclonal antibodies. The membranes were washed 3 times in TBST (TBS with 1 ml/L Tween-20) 10 min each, and incubated with goat anti-rabbit IgG (1: 2000, Abcam ab6721) for 1 h, and the membranes were then washed 3 times in TBST. The Emitter Coupled Logic (ECL) chemiluminescence system was used for signal detection on the specimen membrane. The expression level of protein was standardized by β-actin, and the gray-scale scanning and quantitative analysis were carried out by Image J software (National Institutes of Health).

Western blot analysis was also used for determination of Akt signaling pathway activity. The experimental protocol was same as above mentioned, with the antibodies being: rabbit anti-Akt Antibody (1: 500, ab8805, Abcam, UK) and anti-p-Akt Antibody (1: 500, ab38449, Abcam, UK). The protein expression level was also standardized with β-actin.

Statistical methods

SPSS19.0 statistical software was used for data analysis, with the results being presented as mean±SD. One-way analysis of variance (ANOVA) was used for differences among multiple groups, and Fisher's Least Significant Difference (LSD) test for follow-up analysis. $P<0.05$ was considered as statistically significant.

Results

Effects of ropivacaine on morphology, cell proliferation, and apoptosis

As shown in Figure 1A, the cells in the control group had normal morphology, distinct neurites, and intact structure. With the increase of ropivacaine concentration, the number of cells decreased and their structure damage gradually increased,

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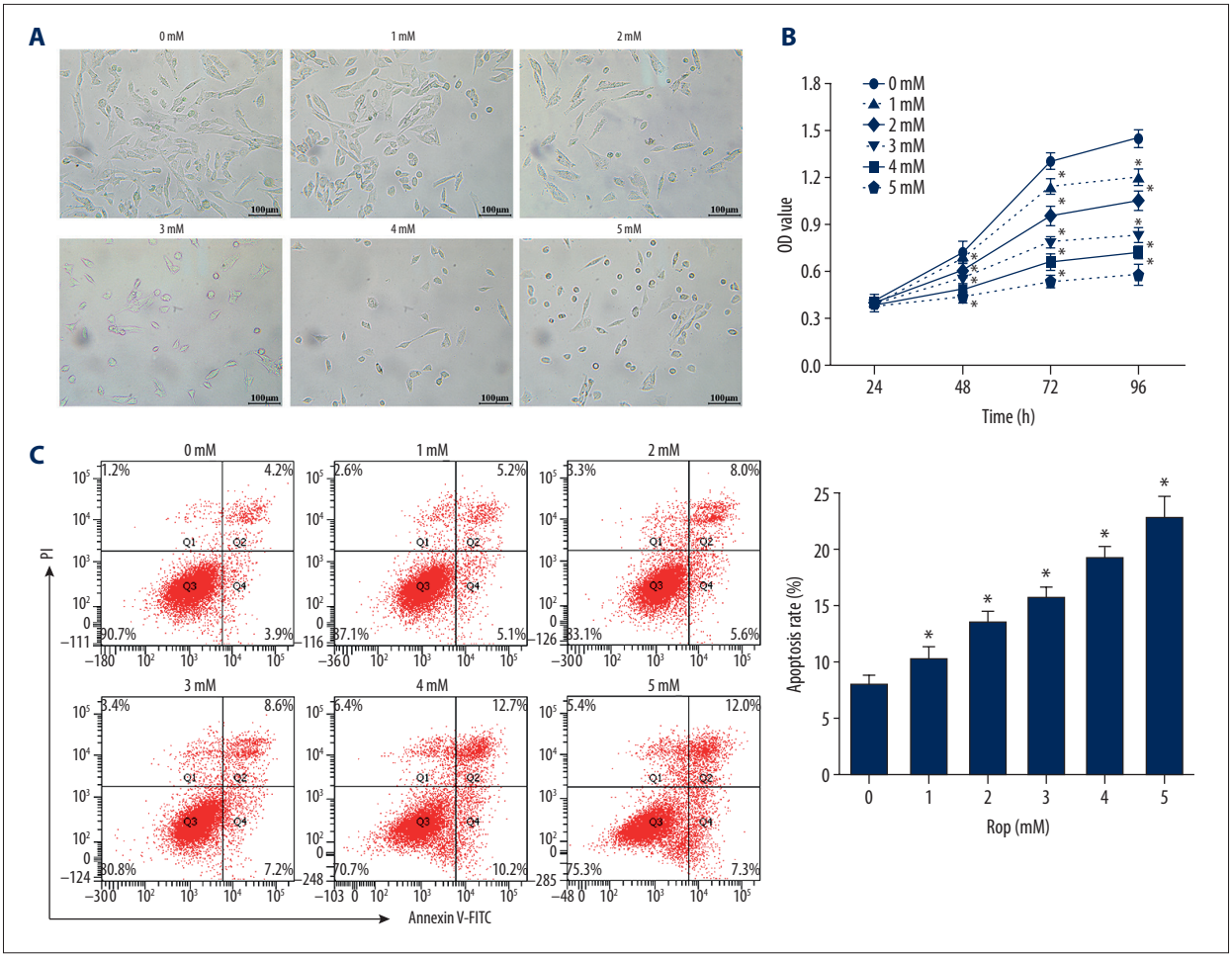

Figure 1. Ropivacaine affects SH-SY5Y cell morphology, cell proliferation, and apoptosis. (**A**) Morphological observation of SH-SY5Y cells (×200); (**B**) SH-SY5Y cell viability; (**C**) Flow cytometry and percentage of apoptotic cells. Compared with the control group, * P<0.05.

presented by round and atrophic cell body with incomplete structure, suggesting that ropivacaine could damage cells in a dose-dependent manner. The MTT evaluation (Figure 1B) showed that the cell proliferation ability decreased with the time and concentration increase of ropivacaine (p<0.05). The cell apoptosis experiment (Figure 1C) showed that when compared with the control group, the number of apoptotic cells increased significantly with the increase of ropivacaine concentration (p<0.05).

Effect of ropivacaine on the expression of Akt and PCNA

The immunohistochemistry (Figure 2) showed that after 48 h of ropivacaine intervention on SH-SY5Y cells, the expression of Akt and PCNA protein decreased with the increase of ropivacaine concentration (p<0.05).

Effect of ropivacaine on proliferation and apoptosis

In comparison with the control group, the neurons in the Rop group disappeared with their structure being round, severely atrophic, and incomplete and their number decreased (Figure 3A). BDNF alleviated the cell morphology atrophy and alleviated the damage with the effect of SC79 being revealed to be similar. Compared with the BDNF+Rop group, the damage of SH-SY5Y cells in the BDNF+API-2+Rop group was aggravated, with its final result being similar to that in the Rop group.

MTT assay (Figure 3B) showed that when compared to the control group, with the prolongation of ropivacaine treatment time, the proliferation activity of cells in each group decreased in varying degrees (p<0.05). In comparison with the Rop group, BDNF or SC79 could significantly increase the cell activity (p<0.05), which could be further reversed by API-2 (p<0.05).

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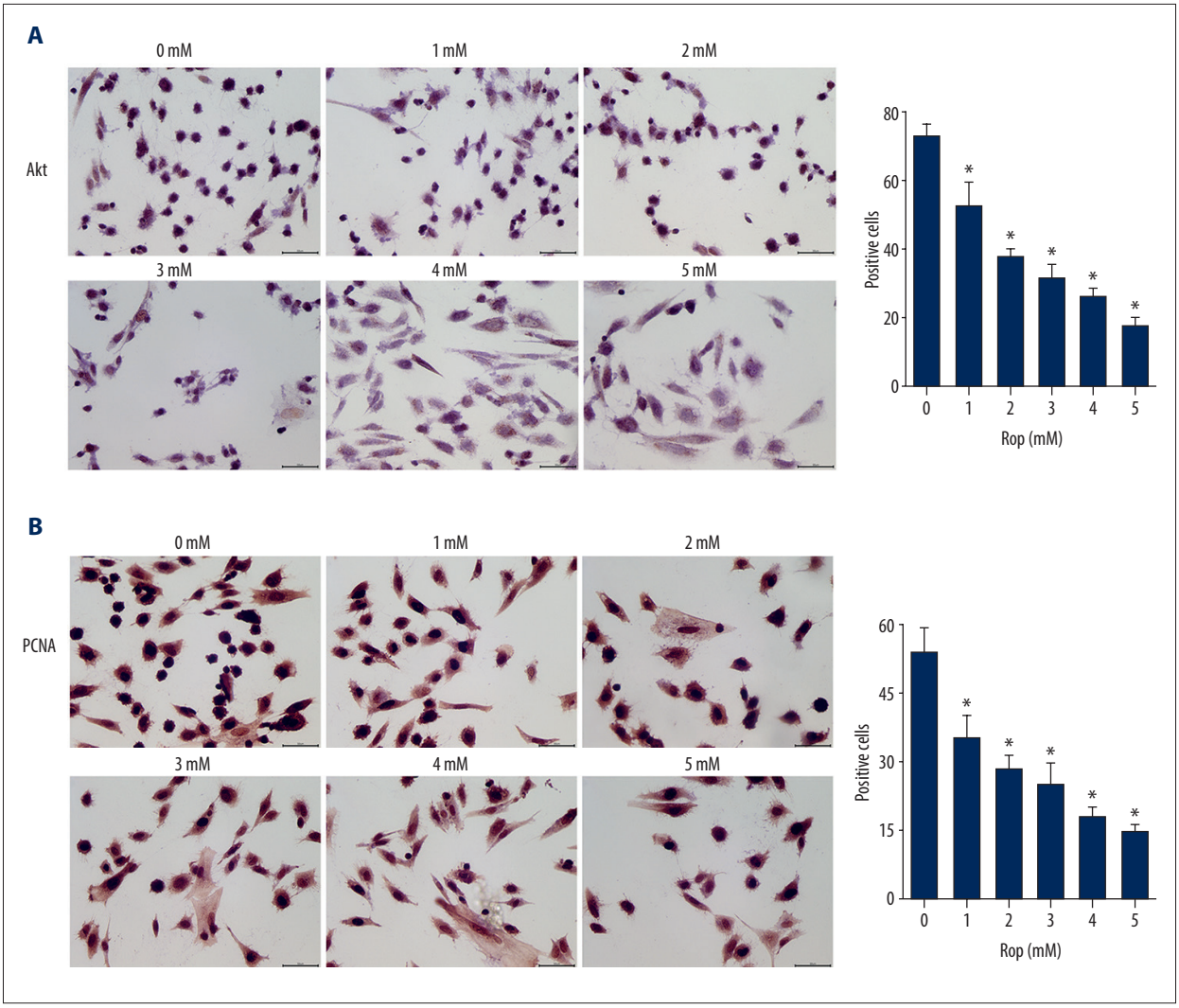

Figure 2. Ropivacaine reduces the expression of Akt and PCNA proteins. (**A**) Akt cell immunohistochemistry and the number of positive cells; (**B**) PCNA cell immunohistochemistry and the number of positive cells. Compared with the control group, * P<0.05.

The flow cytometry (Figure 3C) showed that the apoptotic rate of each group increased in varying degrees compared with the control group (p<0.05). BDNF or SC79 could significantly reduce the apoptotic level (p<0.05), with the above trends being further reversed by API-2 (p<0.05).

Effect of ropivacaine on Akt and PCNA expression via the Akt signaling pathway

Cellular immunohistochemistry (Figure 4) showed that the expression of Akt and PCNA protein decreased in different degrees in each group compared with the control group (p<0.05). Compared to the Rop group, the expression of Akt and PCNA protein was increased significantly by BDNF or SC79 (p<0.05), and the above trends could be reversed by API-2 (p<0.05).

Effect of ropivacaine on apoptosis-related proteins expression via the Akt signaling pathway

The expression of Bcl-2, Bax, and caspase-3 mRNA were analyzed (Figure 5A), and the results showed the expression of Bcl-2 decreased and the expression of Bax and caspase-3 increased when compared with the control group (p<0.05). The proteins were also investigated (Figure 5B). In contrast to the control group, the expression of Bcl-2 decreased and the expression of Bax and cleaved caspase-3 increased in different degrees (Figure 5) (p<0.05). Compared to the Rop group, BDNF or SC79 could increase the expression of Bcl-2 and decrease the expression of Bax and cleaved caspase-3 (p<0.05). Furthermore, API-2 could reverse the above trends (p<0.05).

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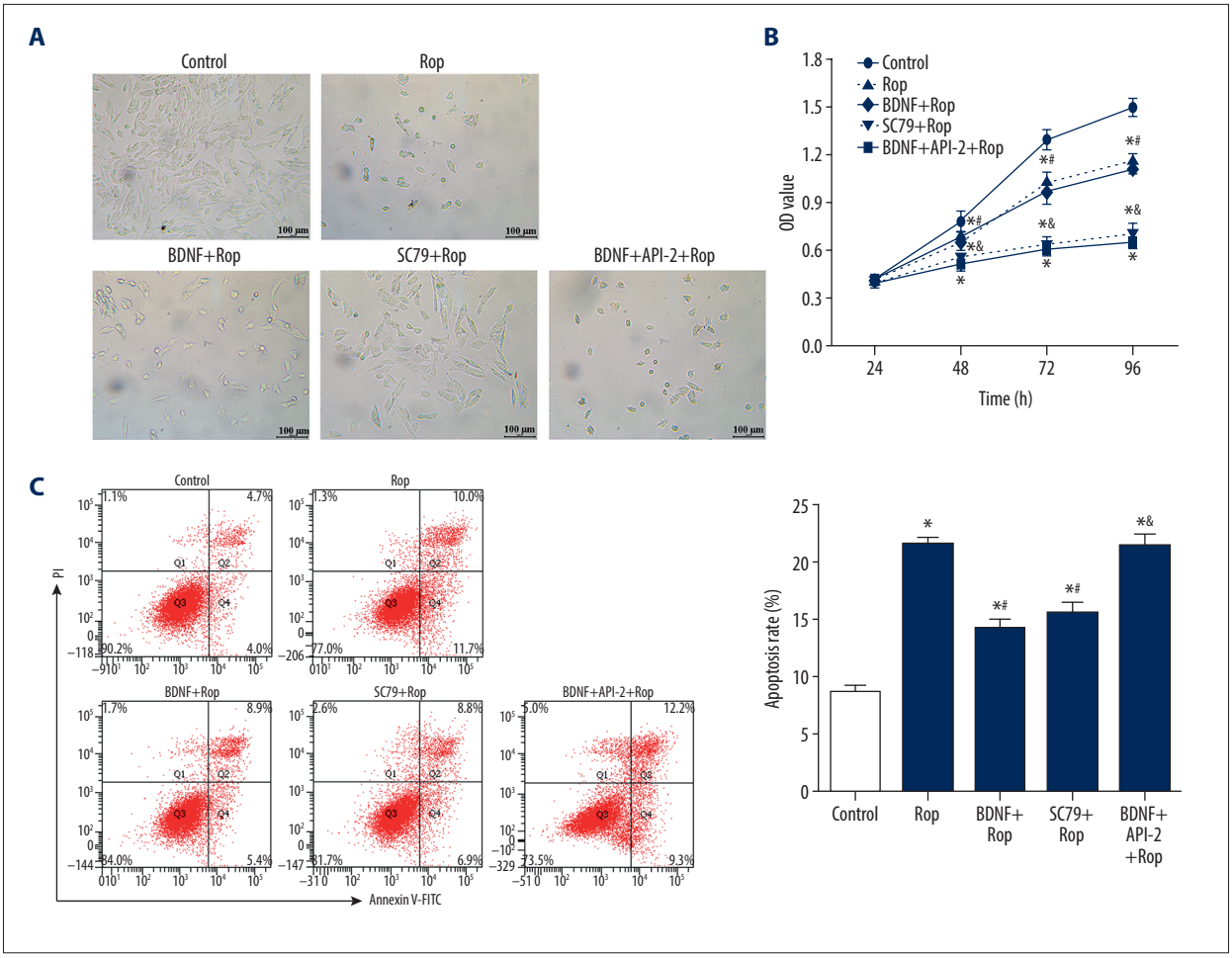

Figure 3. Observation of SH-SY5Y cell morphology, proliferation, and apoptosis level. (**A**) Morphological observation of SH-SY5Y cells; (**B**) Proliferative activity of SH-SY5Y cells; (**C**) Flow cytometry and percentage of apoptotic cells. Compared with the control group, * P<0.05, Compared with the Rop group, # P<0.05; Compared with BDNF+Rop group, & P<0.05.

Effect of ropivacaine on Akt signaling pathway activity

Western blot analysis (Figure 6) showed that the expression of p-Akt protein decreased in all groups compared with the control group, but the total expression of Akt did not change significantly. Compared with the Rop group, BDNF or SC79 could increase the expression of p-Akt/Akt ($p<0.05$), which could be further reversed by API-2 ($p<0.05$).

Discussion

According to the concept map (Figure 7), the SH-SY5Y cells were subjected to ropivacaine insult for reproducing its damage characteristics, showing that the number of SH-SY5Y cells decreased significantly and the surviving cells exhibited synaptic disappearance and cell body atrophy, which verified the rationality of ropivacaine administration to simulate neural injury under local anesthesia at the cellular level. Studies have also delineated that the dosage of anesthetics is more a determinant of the depth of anesthesia than the administration of anesthesia process, so the dose-dependent damage effect of ropivacaine should be paid more attention [14,15]. In this study, the cell morphological damage was observed gradually with the increase of ropivacaine concentration, while the cell proliferation ability also gradually decreased with the prolongation of ropivacaine treatment time, collectively suggesting that ropivacaine damages neurons in a dose-dependent and event-dependent manner. The apoptotic experiment further verified the adverse effect of ropivacaine on the survival of neurons from the point of view of programmed death.

The Akt pathway is closely related to cell metabolism and survival regulation [16]. Elaborate signaling pathway networks, including Akt, are the main mechanisms that regulate neuronal apoptosis after diverse pathophysiological procedures such as cerebral ischemia and drug injury [10,17]. PCNA plays a significant role in DNA synthesis and damage repair, closely

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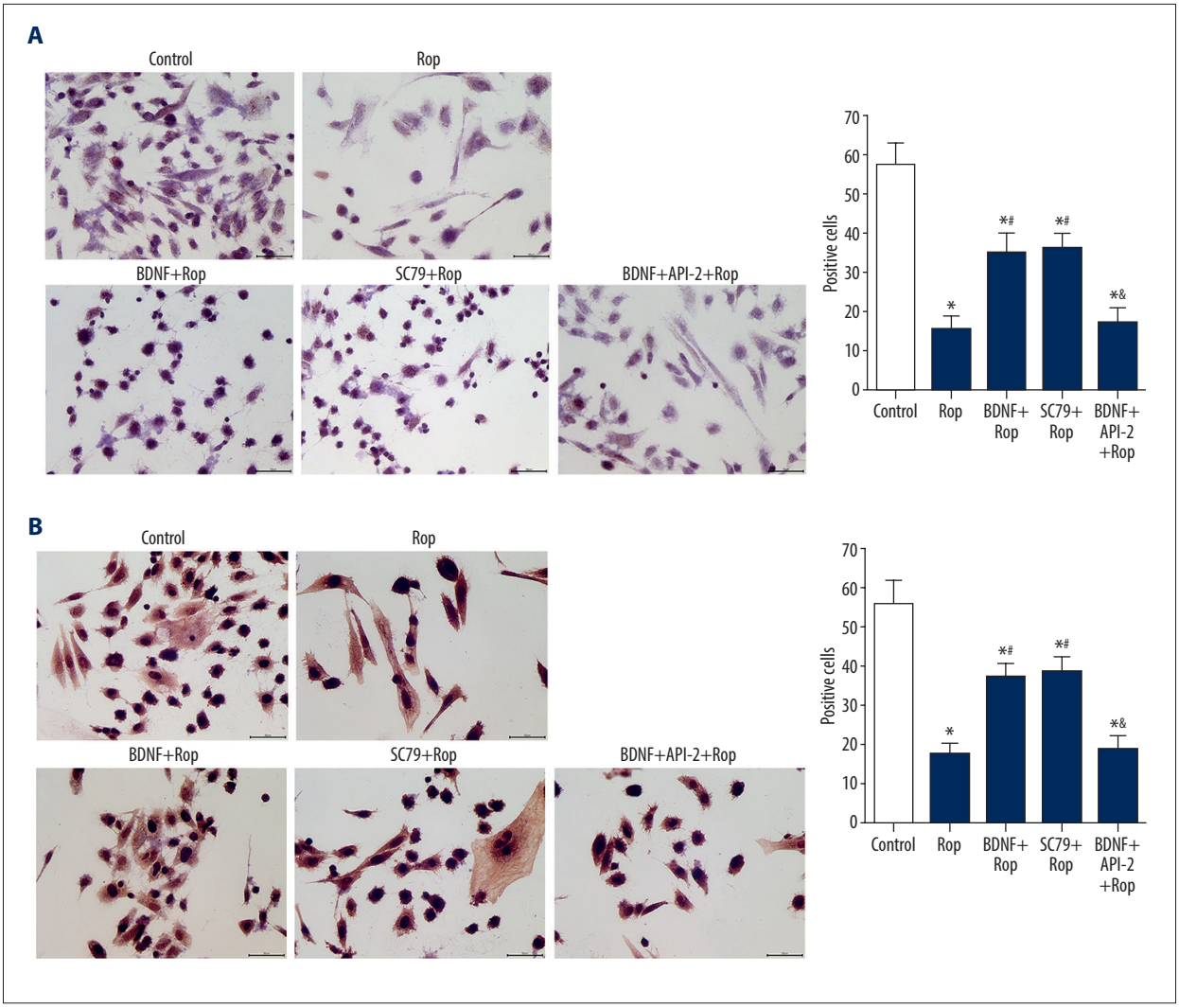

Figure 4. Immunohistochemistry of Akt and PCNA protein expression. (**A**) Akt cell immunohistochemistry and the number of positive cells; (**B**) PCNA cell immunohistochemistry and the number of positive cells. Compared with the control group, * P<0.05, Compared with the Rop group, # P<0.05; Compared with BDNF+Rop group, & P<0.05.

participating in a variety of physiological procedures, such as trans-lesion modulation, error-free injury bypass, and break-induced replication [18]. In our study, immunohistochemical results showed that the SH-SY5Y cells with positive expressions of Akt and PCNA protein significantly decreased after ropivacaine administration, and this trend was correlated with the increase of ropivacaine concentration. This revealed the involvement of the Akt signaling pathway in ropivacaine-induced nerve injury in a dose-dependent manner and further suggests its potential as a therapeutic target for alleviating local anesthesia-induced neuronal injury.

As a member of neurotrophic factor family, which can support the growth, survival, and differentiation of neurons, BDNF has attracted wide attention for its multipotency in diverse brain diseases, whereas its potential regulatory role in ropivacaine-induced neuronal injury was not explored and consequently became the focus of our research [19,20].To further verify the regulatory role of BDNF in this pathological procedure, the ropivacaine-damaged cells were treated with BDNF, showing that under the protection of BDNF, the morphological damages presented by synapse disappearance and cell atrophy were significantly reversed, which preliminarily indicates its protective role in ropivacaine-induced neuronal injury at the cytological level. Similar phenomena have also been observed in various related studies [21]. Additionally, similar protective effects were reproduced by the Akt pathway agonist SC79, while API-2, an antagonist of the same pathway, can significantly reverse these trends. Similar phenomena were further verified by MTT assay and flow cytometry analysis from perspectives of cell proliferative activity and apoptosis, together

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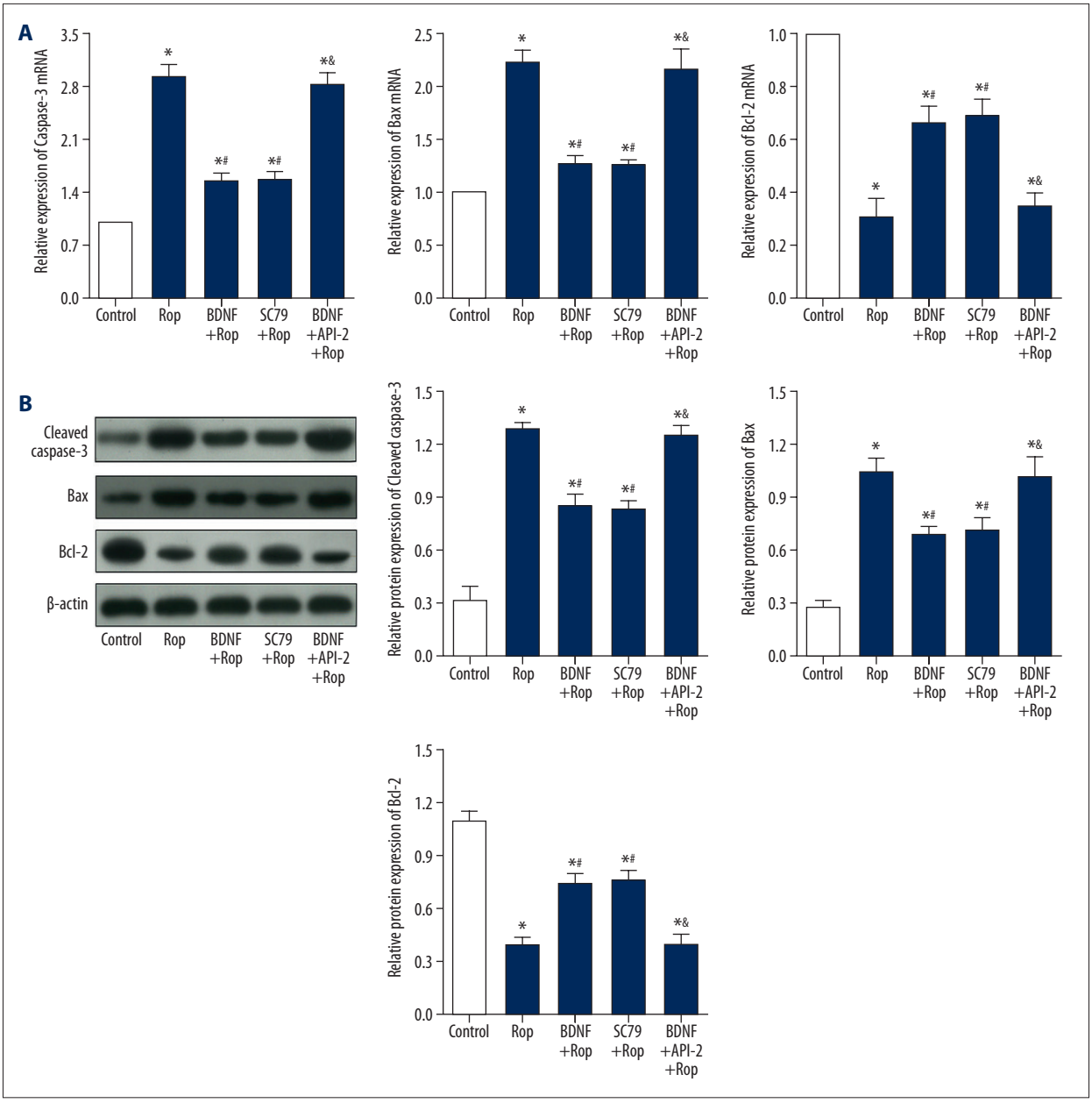

Figure 5. Bax, Bcl-2, caspase-3 mRNA, and protein expression. (**A**) Gene content; (**B**) Protein band and protein expression level. Compared with the control group, * P<0.05, Compared with the Rop group, # P<0.05; Compared with BDNF+Rop group, & P<0.05

implying the critical role of the Akt signaling pathway in the neuroprotective mechanism of BDNF.

Studies have shown that Thr308 and Ser473 is phosphorylated during activation of the Akt pathway. Thereafter, P-Akt will play a biological regulatory role through its kinase activity [22–24]. PCNA is a homotrimer with ring-shaped molecular structure that encircles the DNA of eukaryotic genomes [25]. It is closely involved in cell replication, consequently serving as an essential marker in the monitoring of proliferation activity [26].

In the present study, the expression and activation of Akt and PCNA in SH-SY5Y cells under different treatments were investigated. The results showed that after being treated with BDNF or SC79, the concentration of Akt and PCNA both increased significantly, with this upregulation of expression being further antagonized by API-2, revealing that Akt might be the key link in the neuroprotective effect of BDNF. The increase of p-Akt/Akt ratio following BDNF treatment also confirms the involvement of the Akt signaling pathway in the above procedures on the signaling level.

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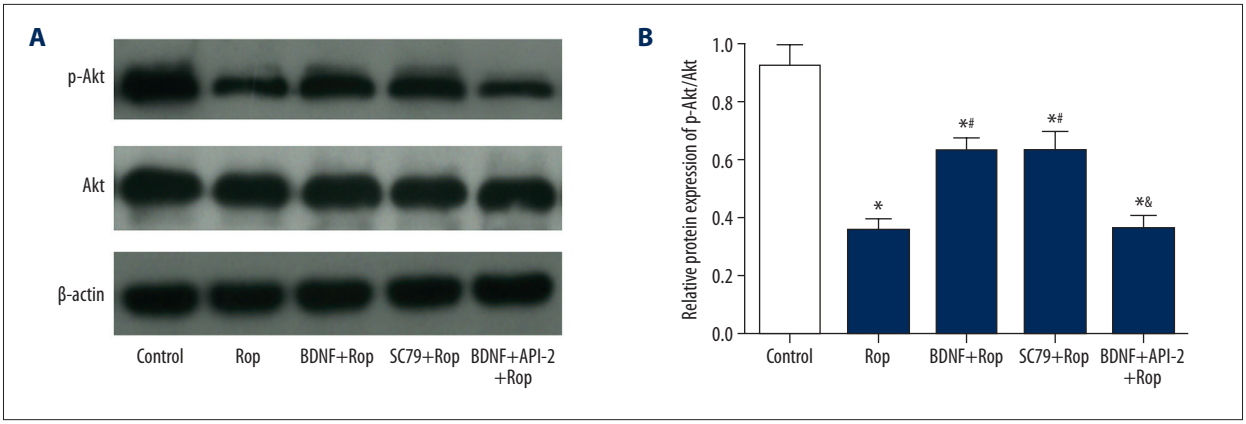

Figure 6. Akt signaling pathway activity. (**A**) Protein band; (**B**) p-Akt/Akt protein expression level. Compared with the control group, * P<0.05, Compared with the Rop group, # P<0.05; Compared with BDNF+Rop group, & P<0.05.

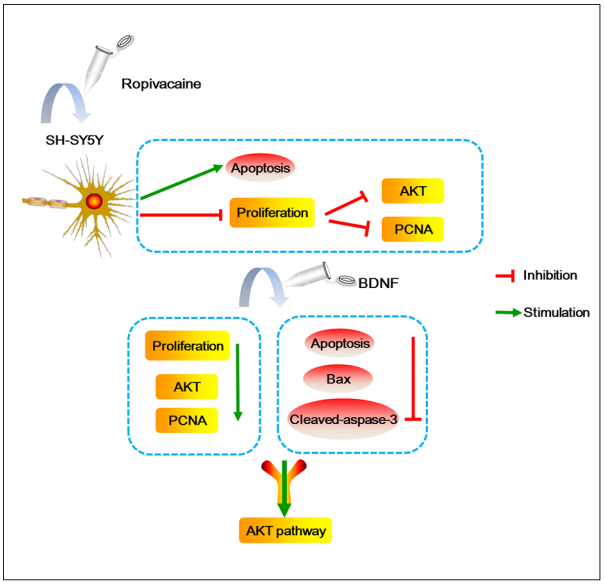

Figure 7. Concept map.

Previous studies have reported that Bcl-2 can be modulated by BDNF, serving as a potential therapeutic target, and is further upregulated by bone marrow stem cells in multiple sclerosis and ischemic stroke, resulting in inhibition of cell apoptosis [27]. Meanwhile, Bax and cleaved caspase-3 have been revealed to be apoptotic promoters and are widely involved in the regulation of neuronal survival [27]. We found that Bcl-2 was significantly increased by BDNF, while Bax and cleaved caspase-3 decreased evidently. These trends were significantly reversed by Akt signaling pathway antagonists, which demonstrates the mechanism of BDNF's neuroprotective effects from the downstream molecular point of view.

Conclusions

This study suggests that BDNF can alleviate ropivacaine-induced neuronal damage, the mechanism of which involves the Akt signaling pathway.

Conflicts of interest

None.

References:

1. Pollock H, Forman S, Pollock T, Raccasi M: Conscious sedation/local anesthesia in the office-based surgical and procedural facility. Clin Plast Surg, 2013; 40(3): 383–88
2. Locke MC, Davis JC, Brothers RJ, Love WE: Assessing the outcomes, risks, and costs of local versus general anesthesia: A review with implications for cutaneous surgery. J Am Acad Dermatol, 2018; 78(5): 983–88
3. Dickerson DM, Apfelbaum JL: Local anesthetic systemic toxicity. Aesthet Surg J, 2014; 34(7): 1111–19
4. Quick-Weller J, Konczalla J, Duetzmann S et al: General anesthesia versus local anesthesia in stereotactic biopsies of brain lesions: A prospective randomized study. World Neurosurg, 2017; 97: 16–20
5. Niu Z, Tang J, Ren Y, Feng W: Ropivacaine impairs mitochondrial biogenesis by reducing PGC-1alpha. Biochem Biophys Res Commun, 2018; 504(2): 513–18
6. Da Silva CMG, Franz-Montan M, Limia CEG et al: Encapsulation of ropivacaine in a combined (donor-acceptor, ionic-gradient) liposomal system promotes extended anesthesia time. PLoS One, 2017; 12(10): e0185828
7. Mellick LB, Mellick GA, McCollum D: Paraspinous injections with ropivacaine for pediatric headache treatment. Ann Emerg Med, 2017; 70(6): 925–27
8. Zhong Z, Qulian G, Yuan Z et al: Repeated intrathecal administration of ropivacaine causes neurotoxicity in rats. Anaesth Intensive Care, 2009; 37(6): 929–36
9. Chang J, Yao X, Zou H et al: BDNF/PI3K/Akt and Nogo-A/RhoA/ROCK signaling pathways contribute to neurorestorative effect of Houshiheisan against cerebral ischemia injury in rats. J Ethnopharmacol, 2016; 194: 1032–42

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

10. Ding ML, Ma H, Man YG, Lv HY: Protective effects of a green tea polyphenol, epigallocatechin-3-gallate, against sevoflurane-induced neuronal apoptosis involve regulation of CREB/BDNF/TrkB and PI3K/Akt/mTOR signaling pathways in neonatal mice. Can J Physiol Pharmacol, 2017; 95(12): 1396–405
11. Shang W, Yang Y, Zhang J, Wu Q: Long noncoding RNA BDNF-AS is a potential biomarker and regulates cancer development in human retinoblastoma. Biochem Biophys Res Commun, 2018; 497(4): 1142–48
12. Bjorkholm C, Monteggia LM: BDNF – a key transducer of antidepressant effects. Neuropharmacology, 2016; 102: 72–79
13. Zhang G, Zhang T, Li N et al: Tetramethylpyrazine nitrone activates the BDNF/Akt/CREB pathway to promote post-ischaemic neuroregeneration and recovery of neurological functions in rats. Br J Pharmacol, 2018; 175(3): 517–31
14. Zheng T, Zheng CY, Zheng XC et al: Effect of parthanatos on ropivacaine-induced damage in SH-SY5Y cells. Clin Exp Pharmacol Physiol, 2017; 44(5): 586–94
15. Zhang AZ, Ficklscherer A, Gulecyuz MF et al: Cell toxicity in fibroblasts, tenocytes, and human mesenchymal stem cells-a comparison of necrosis and apoptosis-inducing ability in ropivacaine, bupivacaine, and triamcinolone. Arthroscopy, 2017; 33(4): 840–48
16. Zhang K, Wu S, Li Z, Zhou J: MicroRNA-211/BDNF axis regulates LPS-induced proliferation of normal human astrocyte through PI3K/AKT pathway. Biosci Rep, 2017; 37(4): pii: BSR20170755
17. Zhong JB, Li X, Zhong SM et al: Knockdown of long noncoding antisense RNA brain-derived neurotrophic factor attenuates hypoxia/reoxygenation-induced nerve cell apoptosis through the BDNF-TrkB-PI3K/Akt signaling pathway. Neuroreport, 2017; 28(14): 910–16
18. Boehm EM, Gildenberg MS, Washington MT: The many roles of PCNA in eukaryotic DNA replication. Enzymes, 2016; 39: 231–54
19. Song M, Martinowich K, Lee FS: BDNF at the synapse: Why location matters. Mol Psychiatry, 2017; 22(10): 1370–75
20. Kim JH: Brain-derived neurotrophic factor exerts neuroprotective actions against amyloid beta-induced apoptosis in neuroblastoma cells. Exp Ther Med, 2014; 8(6): 1891–95
21. Nikoletopoulou V, Sidiropoulou K, Kallergi E et al: Modulation of autophagy by BDNF underlies synaptic plasticity. Cell Metab, 2017; 26(1): 230–42
22. Su WS, Wu CH, Chen SF, Yang FY: Transcranial ultrasound stimulation promotes brain-derived neurotrophic factor and reduces apoptosis in a mouse model of traumatic brain injury. Brain Stimul, 2017; 10(6): 1032–41
23. Qi G, Mi Y, Wang Y et al: Neuroprotective action of tea polyphenols on oxidative stress-induced apoptosis through the activation of the TrkB/CREB/BDNF pathway and Keap1/Nrf2 signaling pathway in SH-SY5Y cells and mice brain. Food Funct, 2017; 8(12): 4421–32
24. Nguyen T, Kim CK, Cho JH et al: Neuroprotection signaling pathway of nerve growth factor and brain-derived neurotrophic factor against staurosporine induced apoptosis in hippocampal H19-7/IGF-IR [corrected]. Exp Mol Med, 2010; 42(8): 583–95
25. Choe KN, Moldovan GL: Forging ahead through darkness: PCNA, still the principal conductor at the replication fork. Mol Cell, 2017; 65(3): 380–92
26. Wang SC: PCNA: A silent housekeeper or a potential therapeutic target? Trends Pharmacol Sci, 2014; 35(4): 178–86
27. Xu D, Lian D, Wu J et al: Brain-derived neurotrophic factor reduces inflammation and hippocampal apoptosis in experimental *Streptococcus pneumoniae* meningitis. J Neuroinflammation, 2017; 14(1): 156

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