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ORIGINAL ARTICLE

Panax notoginsenoside Rb1 Restores the Neurotrophic Imbalance Following Photothrombotic Stroke in Rats

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Abstract

Mature brain-derived neurotrophic factor (mBDNF) has neuroprotection in cerebral ischemia. Conversely, the precursor of brain-derived neurotrophic factor (proBDNF) has the opposite function to its mature form, inducing apoptosis. However, whether the neuroprotection of *Panax notoginsenoside* Rb1 (PNS-Rb1) on ischemic stroke is due to, at least partially, its modulation of suppressing proBDNF/P75NTR/sortilin or upregulation of mBDNF is not clear. To test this hypothesis, rats induced by photothrombotic stroke were treated with PNS-Rb1 100 mg/kg or nimodipine 1 mg/kg twice a day until 3, 7, and 14 days. Our data indicate that PNS-Rb1 significantly reduced cerebral infarction rate, proBDNF/P75NTR/sortilin, and plasminogen activator inhibitor-1 (PAI-1) protein levels, and improved sensorimotor dysfunctions induced by ischemic stroke, upregulation of BDNF/TrkB levels, and its processing enzymes (tissue plasminogen activator, tPA) in a time-dependent manner. Taken together, our findings indicate that the improvement of sensorimotor dysfunctions by PNS-Rb1 following ischemic stroke is made, at least partially, by activating the BDNF/TrkB and inhibiting proBDNF/sortilin/P75NTR.

Keywords *Panax notoginsenoside* Rb1 · Photothrombosis · BDNF · TrkB · proBDNF

Introduction

Stroke, a very common neurological disorder in the aging population, harms motor and sensory functions as a consequence of neuronal and vascular damage (Donnan et al. 2008). Cerebral ischemia induces degenerative cellular reactions, including the release of excitatory amino acids, the formation of oxygen free radicals, Ca^{2+} overload, the activation of several cellular enzyme systems such as Ca^{2+}-dependent proteases, and the initiation or genomic responses that can reduce blood flow outside the area (Masumu and Hata 2003). Recovery from ischemic stroke mainly depends on the formation of new neurons and blood vessels, which includes the formation of new astrocytes and oligodendrocytes, along with synaptogenesis and axogenesis (Hermann and Chopp 2012).

Panax notoginsenoside Rb1 (PNS-Rb1) is believed to be an active compound of ginseng herbs and classified as the panaxadiol group (Tachikawa et al. 1999). At present, the research on PNS-Rb1 is mainly focused on the central nervous system (Ong et al. 2015) and cardiovascular system (Lee and Kim 2014). Previously, PNS-Rb1 was shown to protect neuron against oxidative damage induced by hydrogen peroxide (Ye et al. 2008) and oxygen-glucose deprivation in vitro control of cerebral ischemia (Ye et al. 2009).Our recent research showed PNS-Rb1 ameliorated cognitive and sensorimotor deficits after ischemic stroke, which were associated with the modulation of the Akt/mTOR/PTEN signaling pathway

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(Yan et al. 2018). However, although the function of PNS-Rb1 is evident in protecting the neurons from damage caused by ischemic stroke, the underlying mechanisms are not clear.

Mature brain-derived neurotrophic factor (mBDNF) is one of the neurotrophic factor family, which binds to its specific receptor tropomyosin-related kinase B (TrkB) and related to the survival, growth, and function of neurons. mBDNF has long been implicated in neuroprotection in certain pathological conditions, such as cerebral ischemia (Choi et al. 2015). Some enzymes such as matrix metalloproteinase enzyme (MMP-7) and tissue plasminogen activator(tPA)can be responsible for proBDNF cleavage to mBDNF (Seidah and Chretien 1999; Barker 2009).On the other hand, the precursor form of brain-derived neurotrophic factor (proBDNF) is released as a diffusible protein and has the opposite function to mBDNF via binding to P75 neurotrophin receptor(P75NTR) and sortilin receptors (Hashimoto 2013, 2016; Hempstead 2014), which plays an important role in neurite collapse, synaptic depression, and neuronal death (Yang et al. 2014; Sun et al. 2012). P75NTR is a 75 kDa transmembrane glycoprotein and its cytoplasmic juxtamembrane region, which is known as the chopper domain, has been proven to be essential in the induction of neuronal death (Coulson et al. 2000). The other half intracellular death domain is responsible to initiate apoptosis (Liepinsh et al. 1997; Coulson et al. 2004). Sortilin is a co-receptor, which is considered to be the key in initiating programmed cell death. The mature part of proBDNF is attached with the P75NTR, whereas the prodomain or precursor part is attached with the sortilin and thereby creates a high-affinity heterotrimeric complex (Nykjaer et al. 2004) that mediates programmed cell death process. Our recent study suggests that proBDNF and P75 NTR are significantly upregulated in the ischemic brain in a rat photothrombotic ischemic stroke model (Rahman et al. 2018).

Therefore, we propose that potential neuroprotection of PNS-Rb1 in photothrombotic stroke is induced, at least partially, by modulating the proBDNF/P75NTR/sortilin signaling pathway along with the upregulation of mBDNF/TrkB. Accordingly, in order to assist understanding the underlining mechanisms of PNS-Rb1 in photothrombotic ischemic model, in this study, photothrombotic stroke rats were treated with PNS-Rb1 to measure the behavioral phenotypes, proBDNF/P75NTR/sortilin, and mBDNF/TrkB proteins.

Materials and Methods

Animal Methods and Drug Administration

All procedures involving rats were approved by the Animal Research Ethics Committee of Kunming Medical University. Male adult Sprague-Dawley rats (250–320 g) were from the Animal Center of Kunming Medical University. The rats were exposed to a 12-h light/dark cycle, with constant supply of water and food. SD male rats were randomly divided into sham group, ischemic control group, PNS-Rb1 administration group (Division of Chinese Materia Medica and Natural Products, National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Public Health, China, dissolved in 0.9% isotonic saline/5% ethanol at 100 mg/kg), and the positive control group (nimodipine also called Nimotop 1 mg/kg). Both groups were induced by photothrombosis besides sham group.

Animals were fasted for 12 h before surgery. The rats were anesthetized with 3% pentobarbital (10 mg/kg body weight) by intraperitoneal injection (i.p.). The body temperature was monitored and maintained at 37 °C by a thermostatically controlled heating pad. The rats are placed in a stereotactic frame. The scalp was longitudinally incised (2.0–2.5 cm) and retracted to expose the skull. The periost was gently removed, and coronal and sagittal sutures are identified. A laser beam of 8 mm in diameter (G Laser Technologies) and 560 nm wave length was stereotactically positioned onto the skull 0.5 mm anterior to the bregma and 3.5 mm right from the midline. The skull was illuminated for 20 min. Before illumination, rose bengal (0.133 mL/kg, 10 mg/mL saline) is slowly injected through the tail vein. Afterwards, the skin was sutured. After the surgical procedures, the rats were returned to their cages in a temperature-controlled room.

Administration groups (1% PNS-Rb1 100 mg/kg, nimodipine 1 mg/kg) were i.p. administrated twice a day until 3, 7, and 14 days after ischemic stroke. At the same time, sham and control groups were i.p. administered with an equal volume of solvent (0.9% isotonic saline/5% ethanol) twice a day until 3, 7, and 14 days after the surgery.

Behavioral Testing

In all rats, a battery of behavioral tests were trained 3 days before photothrombosis and performed at 3, 7, and 14 days after ischemic stroke by an investigator who was blinded to the experimental groups.

Corner Test

The animal entered a corner which was made by two vertical boards at an angle of 30° in front of the nose. Contact with the whiskers led to arear and the direction in which the rat turned was recorded. The non-ischemic rats turned non-selectively left or right, but the ischemic rats preferentially turned toward the non-impaired side. The scores toward the nonimpaired side were recorded from ten trials for each test (Roza et al. 1997).

Adhesive Tape Removal Test

An adhesive tape was attached to both fore paws and animals were placed in its own cage without changing the normal housing condition. The time taken for the rat to contact and remove the tape from its paws was recorded for a maximum of 60 s (Bouet et al. 2009).

mNSS

According to Chen's method (Chen et al. 2001), the modified neurological severity scores (mNSS) including motor, sensory, reflex, and balance tests were performed. Neurological function was recorded on a scale of 0 to 18 (normal score, at 0 point, mild injury at 1~6 points, moderate injury at 7~12 points, severe injury at 13~18 points, maximal deficit score at 18 points).

Histological Assessments of the Brain Damage Following Photothrombotic Stroke

At 3 days after photothrombotic stroke, the infarct areas were evaluated with 2% triphenyl-2, 3, 4-tetrazolium-chloride (TTC) stain. The brains were coronal cut into 2-mm-thick slices and stained with saline containing 2% TTC (Sigma, St. Louis, MO, USA) at 37 °C for 10 min. The infarct volumes were measured using ImageJ (NIH).

Immunohistochemistry for the Expressions of TrkB, P75NTR, and Sortilin

To investigate potential regulatory role of PNS-Rb1 on mBDNF and proBDNF, effects of PNS-Rb1 treatment on expressions of TrkB, P75NTR, and sortilin in cerebral cortex were examined by immunohistochemistry (IHC).

After behavioral tests, rats were decapitated at different time points after photothrombosis and the brain will be dissected. Via the center of infarcted area, the brain will be cut into two parts. One part will be frozen for WB, ELISA and the other part is fixed by 4% paraformaldehyde for IHC. Then, brains were embedded in paraffin and cut into serial histologic sections for 5 μm. Briefly, dewaxed brain sections (4 serial sections/brain) were processed for antigen recovery in a pressure cooker with 0.01 M of citric acid salt buffer (pH 6.0). Blocked with 5% normal goat serum in PBS, and incubated with primary anti-body (1:50 rabbit anti-P75NTR,Cell-Signaling Technology Co. Ltd., USA; 1:50 anti-sortilin and 1:100 anti-TrkB, Abcam Science & Technology Co. Ltd., UK; 1:1000 anti-TPA, Abcam Science & Technology Co. Ltd., UK; 1 μg/mL anti-PAI1, Abcam Science & Technology Co. Ltd., UK) at 37 °C for 1 h, and after washes with secondary antibodies (1:300, BOSTER, China) at 4 °C overnight, followed by DAB color reaction. Finally, the sections were counter-stained with hematoxylin before dehydration and being cover-slipped for image acquisition and analysis using the high-resolution color pathology report analysis system HPIAS (Wuhan Qianting Science & Technology Co. Ltd., Wuhan, China). The distributions of TrkB, P75NTR, and sortilin in cerebral cortex were observed, and optical density of positive cells was measured. The higher the absorbance was, the stronger the expression was.

Western Blot for TrkB, P75NTR, and Sortilin Proteins

Western blot was also conducted to quantitate protein levels of TrkB, P75NTR, and sortilin in rat cerebral cortex. Briefly, total proteins were extracted from cerebral cortex with RIPA PMSF buffer (RIPA: PMSF = 100:1) followed by centrifugation to collect the supernatants. The protein concentration was assayed with BCA protein assay (Beyotime). Protein samples were separated by 8% or 10% SDS-PAGE electrophoresis and transferred to nitrocellulose membranes. After being blocked in a 10% milk solution in TBST (Tris-buffered saline with Tween 20, TBST) for 2 h, the membranes were incubated overnight at 4 °C with primary antibodies: rabbit anti-P75NTR (1:1000, Cell-Signaling Technology Co. Ltd., USA), anti-sortilin (1:1000, Abcam Science & Technology Co. Ltd., UK), anti-TrkB (1:1000, Abcam Science &

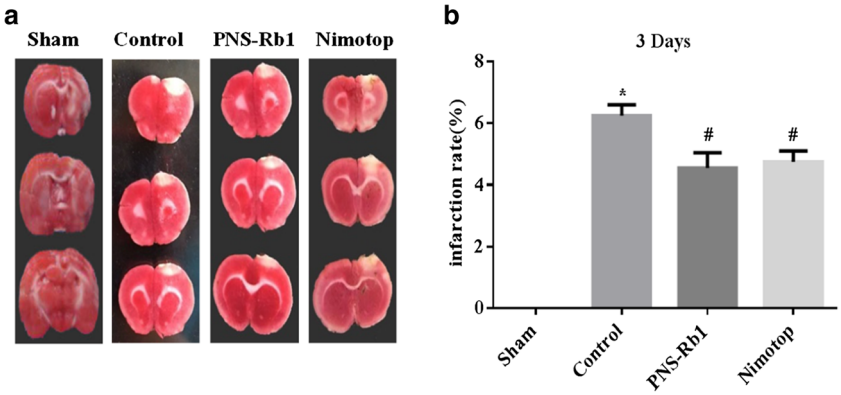

Fig. 1 Effects of PNS-Rb1 on brain infarct volume. **a** PNS-Rb1 reduced cerebral infarction volume as assessed by TTC staining at 3 days after photothrombotic stroke. **b** The comparison of infarct rate between different groups($^{\#}p < 0.05$ versus control group, $n = 6$)

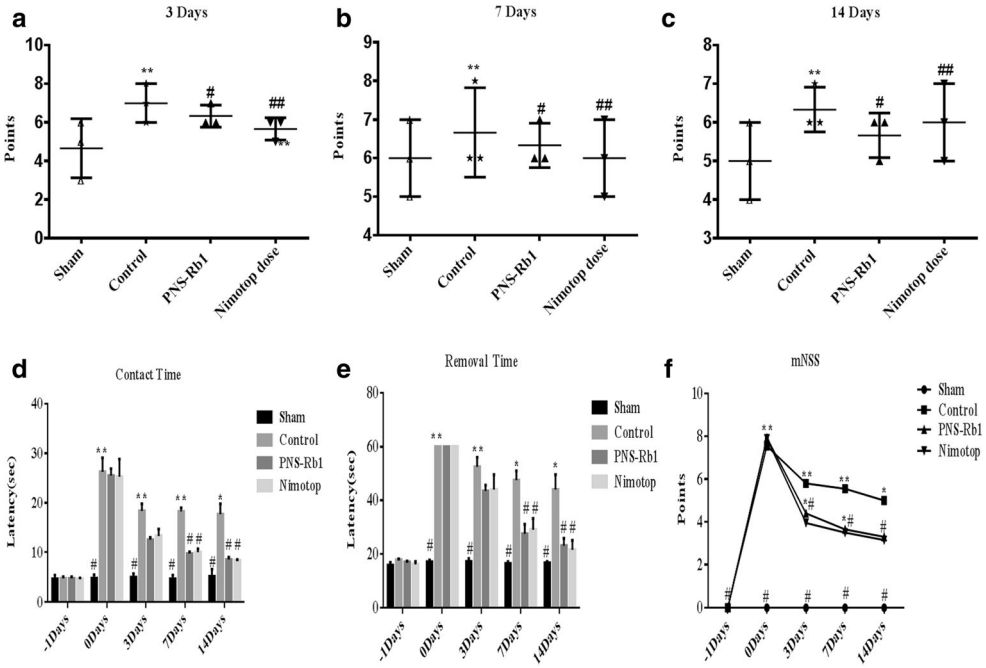

Fig. 2 PNS-Rb1 treatment improved sensorimotor deficits at 3, 7, and 14 days after ischemic stroke. **a**–**c** Corner test. **d**, **e** Adhesive tape removal tests. **f** Modified neurological severity score (mNSS) ($^{*}p < 0.05$; $^{**}p < 0.01$ versus sham group; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ versus model group, $n = 6$)

Technology Co. Ltd., UK), anti-TPA (1:1000, Abcam Science & Technology Co. Ltd., UK), anti-PAI1(1 μg/mL, Abcam Science & Technology Co. Ltd., UK), and anti-β-actin (1:500, Santa Cruz Science & Technology Co. Ltd., USA). After being washed, membranes were incubated with secondary antibodies for 1 h prior to being washed and developed with ECL reagents (Millipore Science & Technology Co. Ltd., Shanghai, China). The chemiluminescence signal was imaged using a ChemiDoc XRS system (Bio-Rad), and protein band signals were quantified by ImageJ 1.4.3.67 software (NIH, Bethesda, MD, USA). The signals of individual protein bands were normalized to the β-actin band intensity and represented in arbitrary units.

ELISA for mBDNF and proBDNF

In extracting the total protein, the protein concentration was assayed with BCA protein assay and dilution sample. mBDNF and proBDNF (Biosensis company) concentration are defined by the ELISA method. Briefly, standards and samples are pipetted into wells and mBDNF/proBDNF present in samples is tied with immobilization (Biosensis, USA) according to the manufacturer's instructions. Anti-mBDNF/proBDNF antibody is added. After the rinse, HRP-conjugated streptavidin is pipetted into the wells. TMB substratum solution is added into wells. Then, the intensity of the color is measured at 450 nm. Minimal concentration of mBDNF/proBDNF for detection is commonly lower than 2 pg/mL. The concentration of lysate mBDNF/proBDNF is expressed in pg/μg.

Statistical Analysis

All data are expressed as the mean ± standard deviation (SD). Statistical analyses were conducted with SPSS 17.0 statistical software (IBM, Chicago, IL). The experimental data were analyzed by one-way ANOVA. Student-Newman-Keuls q-test was

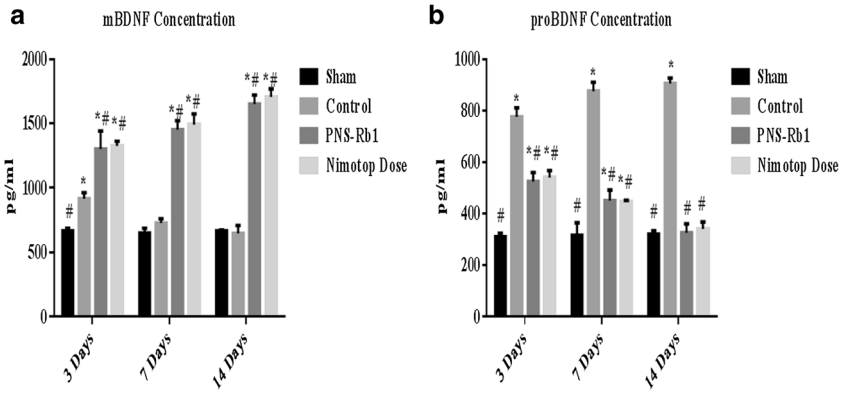

Fig. 3 Effects of PNS-Rb1 on mBDNF and proBDNF levels after cerebral ischemia in rats. **a** PNS-Rb1 treatment increased the concentration of mBDNF at 3, 7, and 14 days after ischemic stroke. **b** PNS-Rb1 treatment reduced the concentration of proBDNF at 3, 7, and 14 days after ischemic stroke ($^{*}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus model group, $n = 6$–8)

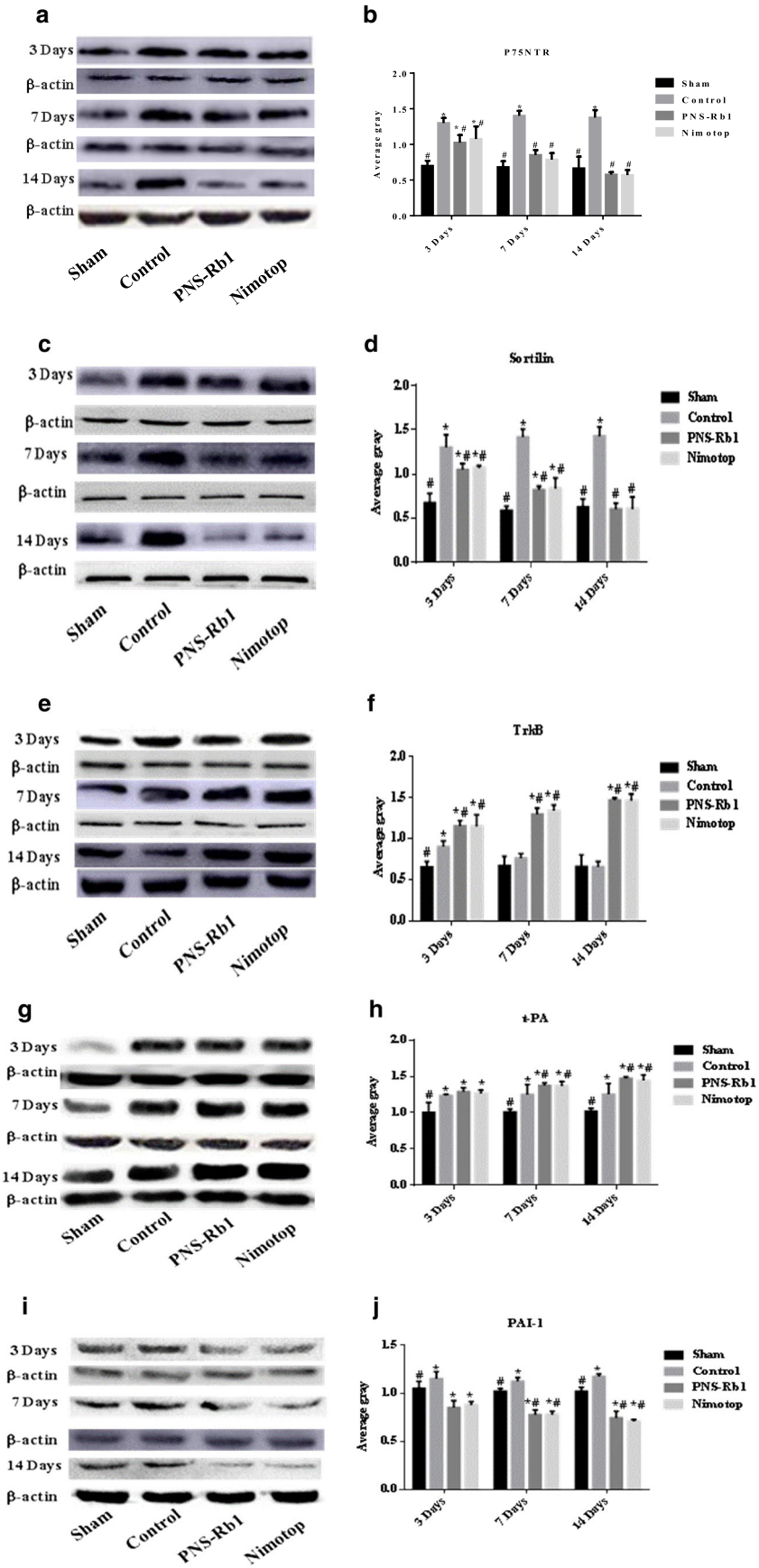

Fig. 4 PNS-Rb1 decreased the levels of P75NTR, sortilin, and PAI-1,while increased the expression of TrkB and tPA on days 3, 7, and 14 between different groups after cerebral ischemia in rats. **a** The expression of P75NTR by western blotting. **b** The average gray value comparison of P75NTR. **c** The expression of sortilin by western blotting. **d** The average gray value comparison of sortilin. **e** The expression of TrkB by western blotting. **f** The average gray value comparison of TrkB. **g** The expression of tPA by western blotting. **h** The average gray value comparison of tPA. **i** The expression of PAI-1 by western blotting. **j** The average gray value comparison of PAI-1 ($\bar{x} \pm s$) ($^{*}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus model group, $n = 6–8$)

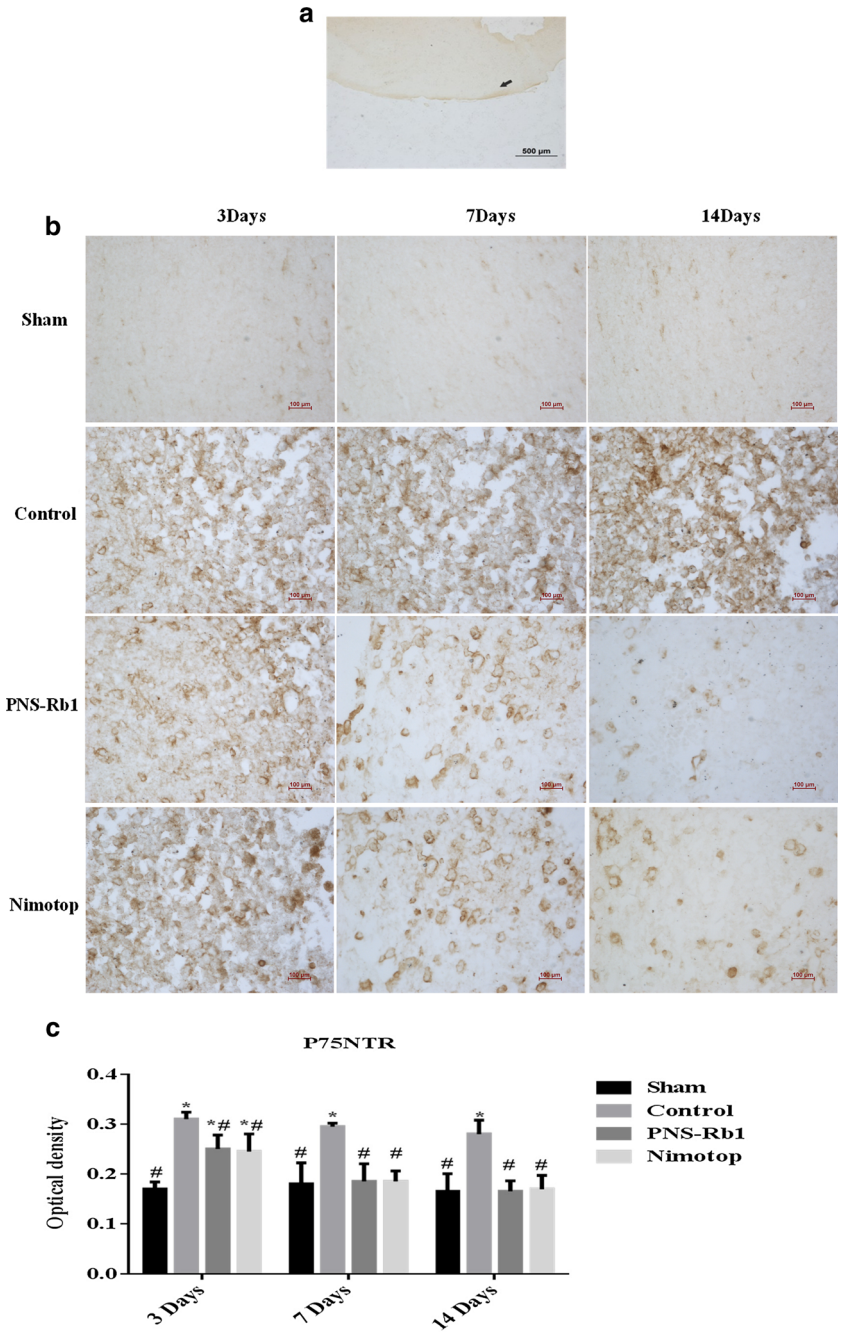

Fig. 5 The immunolocalization of P75NTR at 3, 7, and 14 days after ischemia in rats (immunohistochemical staining*200). **a** Area of detection. **b** The expression of P75NTR between different groups at different time. **c** The optical density of P75NTR-positive neurons in rats ($\overline{x} \pm s$) ($^{*}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus model group)

used to determine statistically significant differences with homogeneity test of variance. The significance level was set at $p < 0.05$.

Results

PNS-Rb1 Alleviated Brain Infarct Volume and Improved Sensorimotor Dysfunction After Ischemic Stroke

At first, we observed the effect of PNS-Rb1 on the brain infarct size. TTC staining showed that the positive control nimodipine group and the PNS-Rb1 group at 3 days had smaller infarct volumes than the control group (Fig. 1a, b).

As ischemic stroke triggered the sensorimotor deficits, we asked whether PNS-Rb1 improved the abnormal sensorimotor behavior. In order to answer this question, we examined the behavioral phenotype of PNS-Rb1 treatment in photothrombotic rats by mNSS, adhesive tape removal test, and corner test. Poor motor functions were observed by corner test and removal time in adhesive tape removal test at 3, 7, and 14 days in control rats (Fig. 2a–c and e, $p < 0.01$). Compared with the control group, both PNS-Rb1 and nimodipine groups

alleviated motor dysfunctions in corner test and removal time in adhesive tape removal test (Fig. 2a–c and e, $p < 0.01$, $p < 0.05$), especially at 7 and 14 days. At 3, 7, and 14 days after ischemic stroke, sensory deficits of ischemic rats were obtained by contact time in comparison with the sham group (Fig. 2d, $p < 0.01$). The contact time in PNS-Rb1 and nimodipine groups were significantly shortened, compared to the control group (Fig. 2d, $p < 0.01$). The contact and removal time in adhesive tape removal tests in both PNS-Rb1 and nimodipine groups was reduced (Fig. 2d, e $p < 0.05$), especially at 7 and 14 days. The mNSS points of PNS-Rb1 and nimodipine groups were also significantly reduced at 3, 7, and 14 days in comparison with the control group (Fig. 2f, $p < 0.05$). The results showed that PNS-Rb1 reduced brain infarct size and promoted the functional recovery after ischemic stroke.

Effect of PNS-Rb1 on mBDNF and proBDNF After Cerebral Ischemia in Rats

The mBDNF and proBDNF levels in brain were measured by ELISA. Compared with the sham group, the levels of mBDNF and proBDNF in control group were upregulated (Fig. 3a and b, $p < 0.05$). In comparison with the control group, the protein level of mBDNF in the PNS-Rb1 group or nimodipine group was increased at 3, 7, and 14 days after ischemia (Fig. 3a, $p < 0.05$), whereas the level of proBDNF

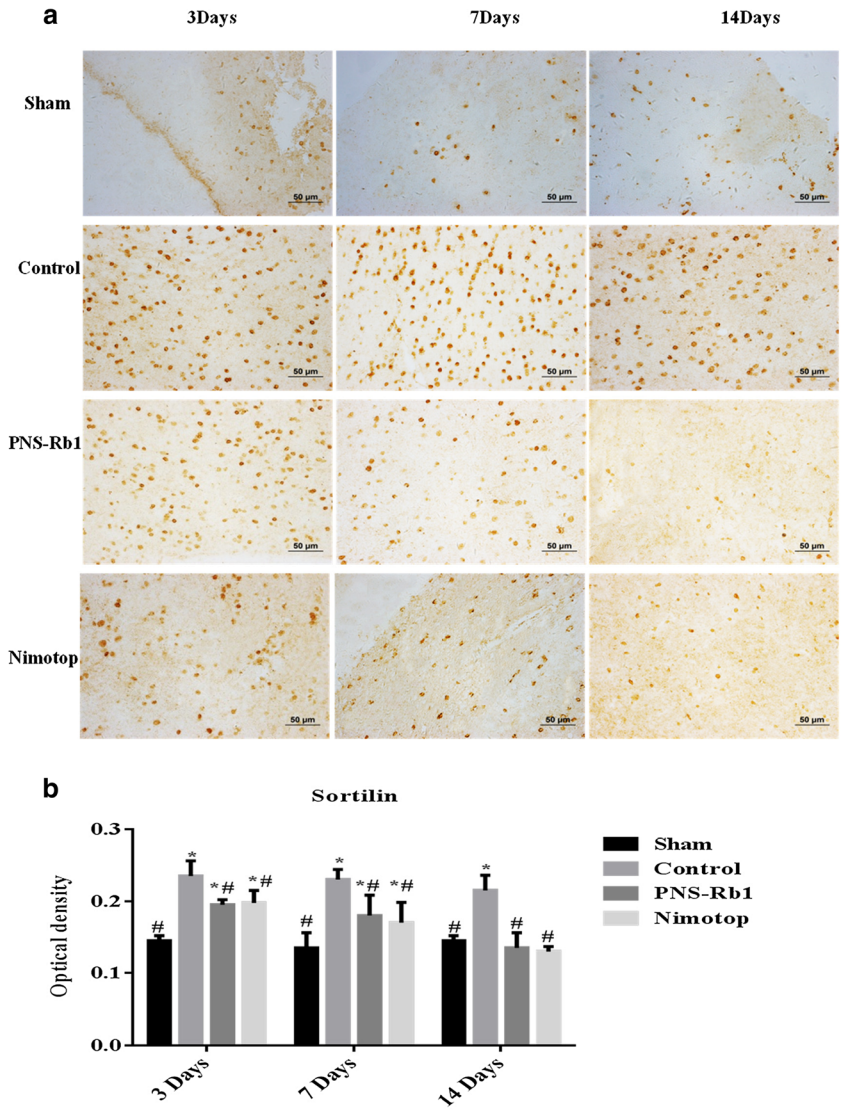

Fig. 6 Immunolocalization of sortilin at 3, 7, and 14 days after ischemia by immunohistochemical staining (immunohistochemical staining*200). **a** The expression of sortilin between different groups at different time. **b** The optical density of sortilin-positive neurons in rats ($\bar{x} \pm s$) ($^{*}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus model group, $n = 6$)

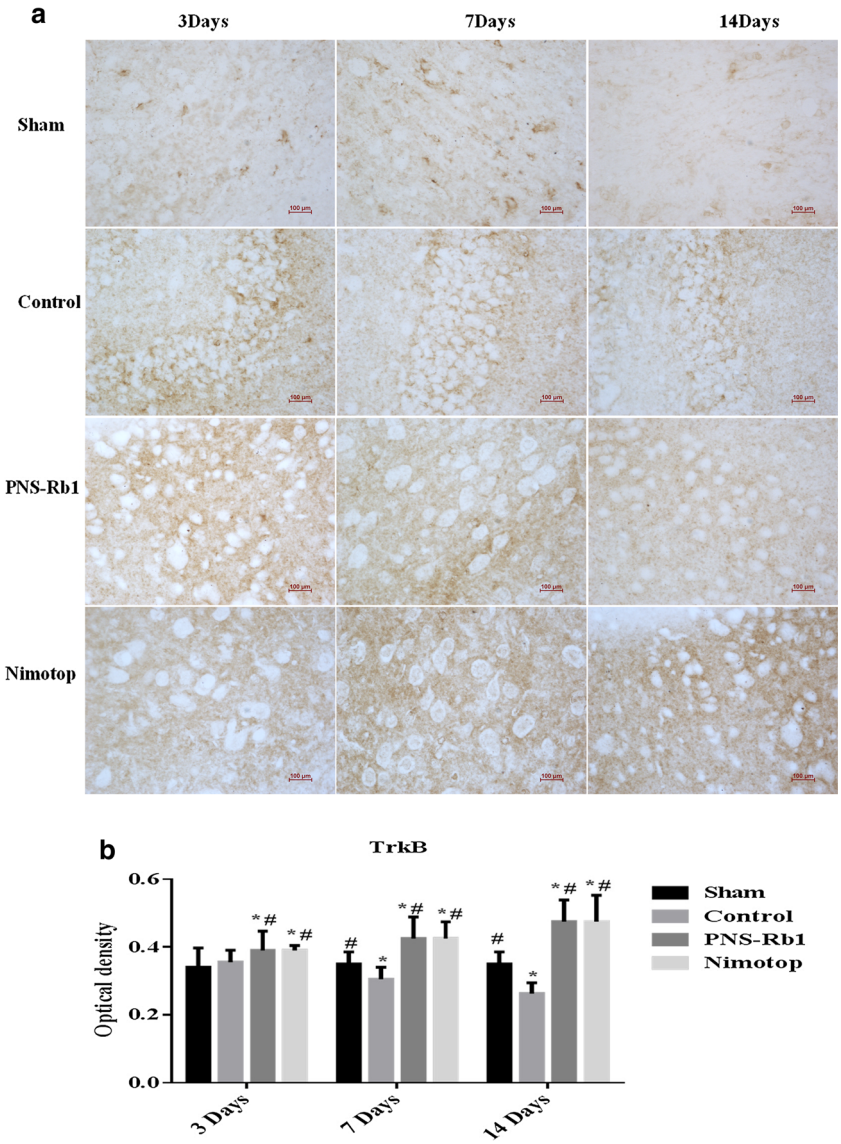

Fig. 7 Immunolocalization of TrkB at 3, 7, and 14 days after ischemia by immunohistochemical staining (immunohistochemical staining*200). **a** The expression of sortilin between different groups at different time. **b** The optical density of TrkB-positive neurons in rats ($\bar{x} \pm s$) ($^{*}p < 0.05$ versus sham group; $^{\#}p < 0.05$ versus model group, $n = 6$)

time-dependently decreased (Fig. 3b, $p < 0.05$). These data taken together indicate that PNS-Rb1 time-dependently inhibited proBDNF protein, whereas it increased the level of mBDNF after ischemic stroke.

The Expressions of TrkB, Sortilin, P75NTR, PAI-1, and tPA in Response to PNS-Rb1 Treatment

The expressions of TrkB, sortilin, P75NTR, tPA, and PAI-1were tested by western blot. Compared with the sham group, the expressions of sortilin, P75NTR, tPA, and PAI-1 significantly increased in the control group (Fig. 4 a–d, g–j; $p < 0.05$). PNS-Rb1 or nimodipine treatment significantly decreased the expressions of sortilin, P75NTR, and PAI-1 (Fig. 4a–d, i and j; $p < 0.05$), while increased TrkB and tPA on 3, 7, and 14 days, especially at 14 days, in comparison with the control group (Fig. 4e–h, $p < 0.01$). It is clear that PNS-Rb1 treatment significantly increased TrkB and tPA proteins, whereas it reduced the expressions of sortilin, P75NTR, and PAI-1 in a time-dependent manner.

Effect of PNS-Rb1 Treatment on TrkB, Sortilin, and P75NTR Levels by Immunohistochemistry

Immunohistochemistry was further performed to detect the positive expressions of TrkB, co-receptor sortilin, and P75NTR in the ischemic cortex (Figs. 5, 6, and 7). The expressions of P75NTR and sortilin in control group were higher than those of sham group at 3, 7, and 14 days (Fig. 5 a and b and 6 a and b, $p < 0.05$). After PNS-Rb1 or nimodipine administration, P75NTR and sortilin proteins were significantly reduced in a time-dependent manner after ischemia compared with the control group (Fig. 5a, b and 6a, b; $p < 0.05$). The expression of TrkB in the ischemic control group was lower than that of sham

group (Fig. 7a, b; $p < 0.05$), and the expression of TrkB in PNS-Rb1 or nimodipine administration group was time-dependently reduced after ischemia (Fig. 7a, b; $p < 0.05$). There were no significant changes of P75NTR, sortilin, and TrkB levels between PNS-Rb1 and nimodipine intervention (Figs. 5, 6, and 7). It is also suggested that TrkB level is upregulated while sortilin and P75NTR proteins are lower by PNS-Rb1 administration in a time-dependent manner.

Discussion

As our previous study (Yan et al. 2018) showed that PNS-Rb1 protected neuron from cerebral ischemia in a dose-dependent manner, especially at high dose (100 mg/kg), the present study investigated effect of PNS-Rb1 treatment at high dose on the expressions of mBDNF, proBDNF, and their receptors in cerebral cortex at different time points following ischemic stroke. Our results showed that PNS-Rb1 could reduce infarction size and alleviate sensorimotor deficits after photothrombotic ischemic possibly via activation of the mBDNF/TrkB signaling pathway and tPA but suppression of the proBDNF/P75NTR/sortilin signaling pathway and PAI-1.

PNS-Rb1 Improves Sensory Motor Dysfunctions in Photothrombotic Ischemic Rats

Our results suggested that PNS-Rb1 remarkably improved the sensory and motor function in a time-dependent manner, as reflected by corner test and adhesive tape removal test. Moreover, PNS-Rb1 treatment attenuated brain infarct volume and reduced the mNSS scores. These data are consistent with our previous finding showing PNS-Rb1 significantly alleviates cognitive and sensorimotor deficits induced by ischemic stroke (Yan et al. 2018), and are also consistent with another study reporting that PNS-Rb1 has protective effects on cerebral ischemia injury (Huang et al. 2015). Overall, PNS-Rb1 ameliorated sensorimotor behavioral dysfunctions induced by ischemic stroke.

PNS-Rb1 Upregulates mBDNF/TrkB Levels in Cortex Following Ischemic Stroke

Mature BDNF and TrkB help with the cell survival, growth, and function of the neurons. The expression of the BDNF gene was upregulated by traumatic stress, such as cerebral ischemia (Tao et al. 2014). However, with the time extension, the expression of TrkB decreased, which suggested that the stress-induced expression of TrkB in pathological condition is difficult to maintain for a long time, which may be one of the mechanisms of CNS axon regeneration difficulty. Our immunohistochemical data indicated that the activation of these pathways is consistent with those shown by western blots. Our data showed that the expression of TrkB persists at a high level in cerebral ischemia after ischemia by PNS-Rb1 intervention. This data was consistent with the study that PNS-Rb1 promoted neurogenesis via increasing VEGF and BDNF expressions and activating the PI3K/Akt and ERK1/2 pathways, then attenuated ischemia/reperfusion injury in rat brain (Liu et al. 2015). Similarly, the recovery of sensorimotor behavioral function was evident in our control in response to the treatment of PNS-Rb1, which was in line with the role of PNS-Rb1 in promoting cell survival in the mouse hippocampus by upregulating the expression of BDNF (Hou et al. 2014).

Regulation of proBDNF/P75NTR/Sortilin Signaling Pathway in Ischemic Stroke by PNS-Rb1 Administration

The balance between mBDNF and proBDNF and their associated proteins may play an important role in the pathogenesis and recovery from ischemia (Rahman et al. 2018). It is well known that the proBDNF is found to act as a distinct ligand by binding to P75NTR and co-receptor sortilin to initiate cell death (Teng et al. 2005; Fan et al. 2008) and neurite collapse by activating RhoA (Sun et al. 2012). Recent study showed upregulations of proBDNF and P75NTR concomitant with sensorimotor deficits induced by ischemic stroke (Rahman et al. 2018). Inhibition of proBDNF/P75NTR/sortilin pathway can promote proliferation of neural precursors and increase the migration of cerebellar granule cells and neural stem cells during development (Xu et al. 2011; Li et al. 2017). However, there is no detailed investigation of a possible relationship between PNS-Rb1 and proBDNF/P75NTR/sortilin signaling pathway after ischemic stroke. Our data showed that proBDNF and P75NTR levels were significantly increased in cortex after stroke, which was consistent with the published results (Rahman et al. 2018). Our data also showed that PNS-Rb1 reduced expressions of proBDNF/P75NTR/sortilin after cerebral ischemia in a time-dependent manner. This data is consistent with the improved sensory-motor functions of PNS-Rb1 shown in this study and previous studies (Yan et al. 2018; Huang et al. 2015). The functions of proBDNF/P75NTR/sortilin in ischemia of our data were consistent with research that found that P75NTR receptor protein was significantly upregulated in the injured spinal cord, and anti-proBDNF treatment had a neuroprotective effect after spinal cord injury (Wong et al. 2010). Our data clearly demonstrated the imbalance was reflected by the decreased mature BDNF/TrkB signaling but the increased proBDNF/P75/sortilin signaling pathway in the control group of ischemic stroke in a time-dependent manner.

proBDNF-converting enzymes such as MMP and tPA facilitate conversion of proBDNF into mBDNF (Pang et al. 2004; Cao et al. 2014), thereby upregulation of proBDNF-converting enzyme tPA level following ischemia may have a protective function. Plasminogen activator inhibitor-1 (PAI-1) is the major inhibitor of tPA (Lawrence et al. 1990). PAI-1

inhibits the activity of tPA and thereby disrupts proBDNF cleavage to mBDNF. The gene of PAI-1 was also increased at earlier ischemia (Rahman et al. 2018). Our results also suggest that the upregulation of tPA and downregulation of PAI-1 in response to PNS-Rb1 during ischemia play a positive role by prompting proBDNF cleavage to mBDNF, as they help to generate more mBDNF. The increased level of mBDNF and decreased level of proBDNF in response to PNS-Rb1 treatment after ischemia of our data were consistent with research that found that the increment of mBDNF may have facilitated the recovery of sensorimotor function (Je et al. 2013), which we observed in our research. Overall, tPA in PNS-Rb1-administrated ischemic rats displays hyperactivity and the suppression of PAI-1 may mediate the function of PNS-Rb1.

Taken together, our study revealed that PNS-Rb1 eased neuronal damage after cerebral ischemia, at least partially, possibly via activating the mBDNF/TrkB and inhibiting proBDNF/sortilin/P75NTR signal pathway. This may be another possible mechanism by which PNS-Rb1 protects neurons by restoring the neurotrophic imbalance triggered by the cerebral ischemia. The present findings offer a theoretical basis for better understanding of the mechanism of PNS-Rb1 on cerebral ischemia in rats.

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Compliance with Ethical Standards

All experimental procedures involving animals were approved by the Animal Ethics Committee of Kunming Medical University and conducted between 7 a.m. and 7 p.m. in accordance with the guidelines of the National Health and Medical Research Council of China. Rats were acclimatized for a week before any procedures were initiated.

Conflict of Interest The authors declare that they have no conflict of interest.

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