Effect of hippocampal L-NBP on BDNF and TrkB expression and neurological function of vascular dementia rats

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Received October 31, 2016; Accepted August 10, 2017

DOI: 10.3892/mmr.2017.7539

Abstract. The pathogenesis of vascular dementia (VD) is associated with neuronal degeneration, apoptosis or necrosis following ischemic brain injury. L-butylphthalide (L-NBP), has been demonstrated to exhibit potent anti-ischemic and anti-VD effects, however the associated specific mechanism remains to be elucidated. The present study generated a VD rat model, in which the effect of L-NBP on neurological function and expression levels of brain-derived neurotrophic factor (BDNF) and tyrosine kinase receptor B (TrkB) were observed. A total of 90 male Sprague Dawley rats were randomly divided into sham, model and L-NBP groups (n=30). The VD model was generated by ligation of bilateral common carotid artery. A Morris water maze was used to test learning and memory functions. Animals were then sacrificed and cortical and hippocampal tissues were extracted. Hematoxylin and Eosin staining was used to observe brain tissue injury, and reverse transcription-quantitative polymerase chain reaction was employed to measure BDNF and TrkB mRNA levels. Western blotting was employed to measure BDNF, TrkB and serine-threonine protein kinase (Akt) protein levels. Immunohistochemistry staining was used to detect the N-methyl-D-aspartate receptor (NMDAR) levels. VD rats exhibited elongated escape latency and lower crossing times, with significant neuronal damage. L-NBP treatment shortened escape latency, increased crossing times and improved cortical and hippocampal injury. BDNF, TrkB, Akt and NMDAR expressions in the treatment group were significantly increased compared with the model group ($P<0.05$). L-NBP may therefore enhance hippocampal expression of BDNF, TrkB, Akt and NMDAR, decrease ischemic injury of VD rats, and improve learning and memory.

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Key words: L-butylphthalide, vascular dementia, brain derived neurotrophic factor, tyrosine kinase receptor B

Introduction

Vascular dementia (VD) is a syndrome that frequently occurs following brain atherosclerosis or stroke, and is an intellectual disorder resulting from various cerebrovascular diseases (1,2). VD primarily manifests as dysfunction of memory and cognition, accompanied with motor, language, visual or personality disorders (3,4). The pathogenesis of VD is associated with neuronal degeneration, apoptosis or necrosis resulting from ischemic brain injury (5,6). Currently, no effective method has been developed to treat VD. Butylphthalide (NBP) is a lipid-soluble drug that has previously been demonstrated to target multiple areas of the brain to induce protective effects. Various pharmacological functions that NBP exhibits includes shrinking focal ischemic lesions, improving microcirculation, elevating blood flow at the ischemic region and decreasing effects of neurological damage (7,8). L- and D-type isomers of NBP have differing effects, as D-NBP may antagonize the L-NBP function in treating neuron degeneration, whereas L-NBP has potent anti-ischemic and anti-VD effects in the brain, however the underlying functional mechanism remains to be fully elucidated (9,10).

Brain-derived neurotrophic factor (BDNF)) is distributed in the central nervous system. During neural development, it improves the pathological status of neurons, facilitates their survival and differentiation, and protects against injury. It may additionally modulate neuronal functions via multiple signal transduction pathways. BDNF may affect learning and memory functions in a synergistic manner with the N-methyl-D-aspartate receptor (NMDAR) (11,12). Tyrosine kinase receptor B (TrkB) is important during neuronal differentiation, growth and development and may alleviate neuronal injury. Following brain ischemia, cell apoptosis is associated with signal pathways including phosphatidyl inositol-3/serine-threonine protein kinase (PI3K/Akt) and mitogen activated protein kinase, exerting a protective role (13,14). The present study established a rat VD model via bilateral ligation of common carotid arteries, and observed the effect of L-NBP on neurological functions and hippocampal BDNF and TrkB expression levels, in an attempt to investigate the underlying mechanism of the protective effects of L-NBP against VD.

Materials and methods

Experimental animals. A total of 90 healthy male Sprague-Dawley rats (6 months old, body weight 240-260 g) were purchased from Laboratory Animal Center, Chinese Medicine Academy (Beijing, China) (certificate no. SYXK-2013-0025), and were housed separately in a specific-pathogen-free grade facility at 23±1˚C with 50±20% relative humidity. All rats had free access to food and water ad libitum and kept on a 12:12 h light:dark cycle. Animals were randomly divided into sham, model and L-NBP groups (n=30 each). Following establishment of the VD model, the L-NBP group received drugs via gastric perfusion (10 mg/kd/day) for 3 weeks at 1 ml/100 g volume. An equal volume of vegetable oil was administered to sham and model groups. The present study was approved by the ethics committee of Jilin University (Changchun, China).

Drugs and reagents. L-NBP (99% purity; Shijiazhuang Pharmaceutical Group Co., Ltd., Hebei, China) was diluted in vegetable oil at 10% working concentration. Chloral hydrate and paraformaldehyde were purchased from Kemiou Chem (http://public.company.lookchem.cn/) (Tianjin, China). Rabbit anti-BDNF, rabbit anti-Akt1/2 and rabbit anti-NMDAR antibodies were purchased from Boster Biological Technology (Pleasanton, CA, USA). TRIzol® reagent and reverse transcription kit were provided by Invitrogen; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody was purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Primers were provided by Invitrogen; Thermo Fisher Scientific, Inc. Morris water maze apparatus (Model DMS-2) was provided by Pharmacological Institute, Chinese Medicine Academy (Beijing, China).

Animal model preparation. A Morris water maze test was firstly performed to screen out 110 rats with normal learning and memory functions. A total of 90 were randomly selected to generate a VD model using bilateral ligation of common carotid artery, as previously described (15). In brief, bilateral common carotid artery (2-VO) was permeant ligated in two surgeries with a 72 h time interval. Prior to each surgery, rats fasted for 8 h. Following anesthesia with 10% chloral hydrate, rats were laid in the supine position, with a middle incision made in the neck. The bilateral common carotid artery was separated carefully to leave the nerves intact. Double ligations were performed using surgical silks. A total of 30 rats in the sham group received only vessel separation and not ligation. During surgery, rectal temperature of rats was kept at 36.5-37.5˚C. Penicillin sodium was applied at the focal region to prevent infection, via intramuscular injection (200,000 units) daily for 3 days. A total of 6 weeks following surgery, the Morris water maze was used to select 60 rats for the VD model group. The judgement criteria were: (averaged escape latency-escape latency in control group)/escape latency of target group >20%. These animals were randomly divided into model and L-NBP groups (n=30), the latter of which received L-NBP (10 mg/kg) via gastric perfusion for three weeks (1 ml/100 g volume). Sham and model groups received equal volumes of vegetable oil.

Behavioral test. The water-maze test included navigation and exploration sessions. The water temperature was maintained at 20±2˚C and water depth was 30 cm. During the navigation test, rats were trained for 5 consecutive days and the escape latency from entering the pool to climbing onto the platform was recorded. In the navigation task, swimming path and times of crossing the original platform within 2 min were recorded.

Hematoxylin and eosin (H&E) staining for brain tissue pathology. Rats from all groups were anesthetized with 10% chloral hydrate and brain tissue was fixed in 4% paraformaldehyde at 4˚C overnight. Rats were then rapidly decapitated followed by removal of the cerebellum, olfactory bulb and lower brain stem. Brain sections from 4 mm posterior of the chiasm toward the cerebellum were embedded in paraffin by RM2126 microtome and were stained using the H&E method with staining in hematoxylin solution for 8 min and counterstaining in eosin solution for 1 min at 23˚C. A light field microscope was used to observe tissue morphology.

Semi-quantitative polymerase chain reaction (semi-qPCR) for BDNF and TrkB mRNA level in brain tissues. Total RNA was extracted from brain tissues, and reverse transcription used to obtain cDNA (High-Capacity cDNA Reverse Transcription kit; Thermo Fisher Scientific, Inc.). UV spectrometry was used to quantify DNA concentrations. Using specific primers (Table I), PCR was performed, amplified products were analyzed in 1.5% agarose gel electrophoresis in triplicate and visualized by staining with ethidium bromide. Gene expression level was expressed as relative level against β-actin.

Western blotting for protein levels of BDNF, TrkB and Akt. Rat brain tissues were lysed in RIPA lysis buffer (Thermo Fisher Scientific, Inc.). Proteins were collected by centrifugation at 13,000 x g for 5 min at 4˚C, and were quantified using a BCA protein assay kit. Protein samples were separated by 12% SDS-PAGE with loading of 20 mg protein per lane, and were transferred to a polyvinylidene membrane. Following blocking with 5% bovine serum albumin (Thermo Fisher Scientific, Inc.). at room temperature for 1 h, the membrane was incubated with primary antibodies against BDNF (1:1,000; catalog no. ab108319; Abcam), TrkB (1:1,000; catalog no. ab33655; Abcam), Akt (1:2,000; catalog no. ab18785; Abcam) or β-actin (1:2,000; catalog no. ab8227; Abcam) at 4˚C overnight. Following washing 3 times with Tris buffered saline-Tween-20, a secondary antibody (1:1,000; catalog no. 65-6120; Thermo Fisher Scientific, Inc.) was added for a 1 h incubation at room temperature. A chromogenic substrate (1-Step Ultra TMB-Blotting solution; catalog no. 37574; Thermo Fisher Scientific, Inc.) was then added for development, followed by exposure to a dark room. Quantity One software version 4.6.5 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to analyze protein bands, which were expressed as a relative level against the internal reference.

Immunohistochemistry for NMDAR protein expression. Rats were sacrificed and brain tissue extracted. Following removal of the cerebellum and the olfactory bulb, the cerebral cortex and hippocampus were collected, fixed with 2% paraformaldehyde overnight at room temperature, dehydrated through 70, 80,

Table I. Primer sequences.

Target gene	Sequence (5'→3')	Fragment length (bp)
BDNF	Forward: GTGACAGTAGTAGCGAGTGGG Reverse: TATCCTTATGAACCGGCA	278
TrkB	Forward: CCAAGAGGCTAAATCCAGTCC Reverse: CCAGGTTACCAACATGCTAATA	248
β-actin	Forward: GAGACCTACAAGACCCCAGCC Reverse: TCGGCGCATCGGTACCGCTCA	445

BDNF, brain-derived neurotrophic factor; TrkB, tyrosine kinase receptor B.

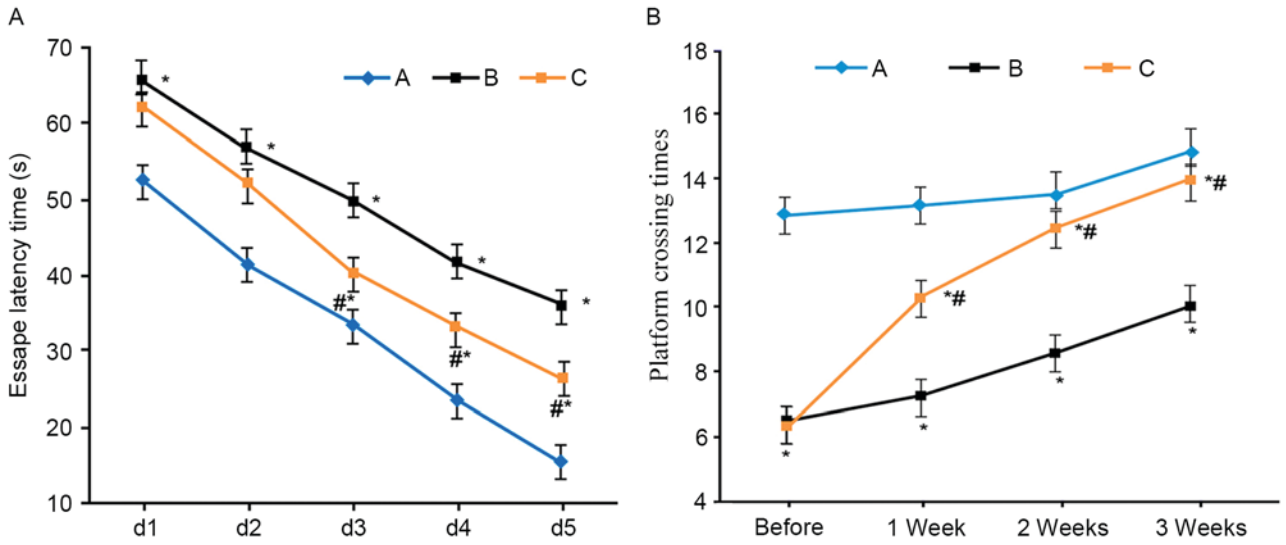

Figure 1. Effect of L-NPB on behavioural water-maze tests. (A) Escape latency and (B) platform crossing times in water-maze. *P<0.05 vs. sham group; #P<0.05 vs. model group. A, sham group; B, model group; C, L-NPE group. L-NBP, L-butylphthalide; d, days.

95% alcohol (5 min each) followed by 3 incubations in 100% alcohol (5 min each). Subsequently, the tissue was embedded in paraffin and sliced to 4 μm thickness using a RM2126 microtome. The slides were deparaffinized and transferred to 100% alcohol 2 times (3 min each), and then transfered once through 95, 70 and 50% alcohols respectively for 3 min each, followed by blocking of endogenous peroxidase activity by incubating sections in 3% hydrogen peroxide solution in methanol at room temperature for 10 min. Immunohistochemistry staining (Ultra Vision Detection System) was used to detect NMDAR protein levels using rabbit anti-mouse NMDAR polyclonal antibody (catalogue number PA1059) (1:1,500) (incubation at room temperature for 1 h) and biotinylated secondary antibody (1:200) (catalog no. ab64256, Abcam) (incubation for 10-15 min at room temperature). Following DAB development and counter-staining in Hematoxylin for 1-2 min at room temperature. The color of the antibody staining in the tissue sections was observed under a microscopy (CX31; Olympus, Corporation, Tokyo, Japan). Image-pro plus version 7.0 software was used to analyze the images.

Statistical analysis. SPSS software, version 20.0 (IBM Corp., Armonk, NY, USA) was used to analyze all data, in which measurement data were firstly tested for normal distribution. Those fitting the normal distribution were presented as the mean ± standard deviation. One-way analysis of variance was employed to compare means across multiple groups, followed by the least significant difference test in paired comparison. P<0.05 was considered to indicate a statistically significant difference.

Results

Learning and memory functions of VD rats. Compared with the sham group, VD model rats exhibited elongated escape latency and lower platform crossing times (P<0.05). L-NBP treatment shortened escape latency and increased platform crossing times (P<0.05 compared with model group; Fig. 1).

Pathology of VD rat brains. H&E staining results revealed a normal cortical structure and morphology of sham rats, which had regular arrangement of hippocampal tissues with intact morphology, high cell number, normal structure and clear nucleus. VD model rats had diffused injury in both cortical and hippocampal regions, the latter of which had irregular distribution of cells, with incomplete morphology, less cell number,

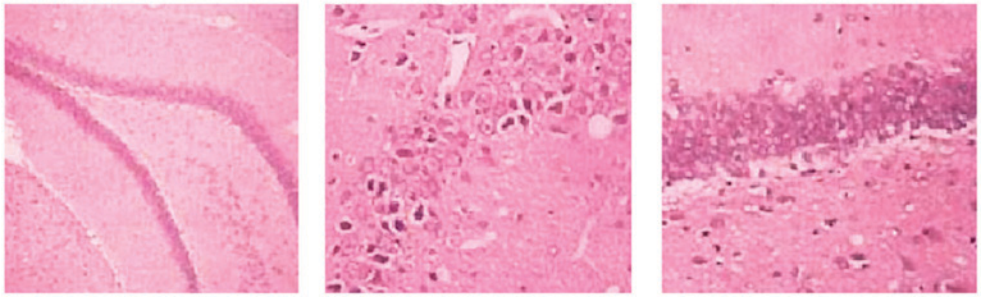

Figure 2. VD rat brain pathology. Pathology phenotype of hippocampal CA1 region in VD rats, viewed with haematoxylin and eosin staining. Magnification, x20. Left to right: Sham group, model group, and L-NBP group. VD, vascular dementia.

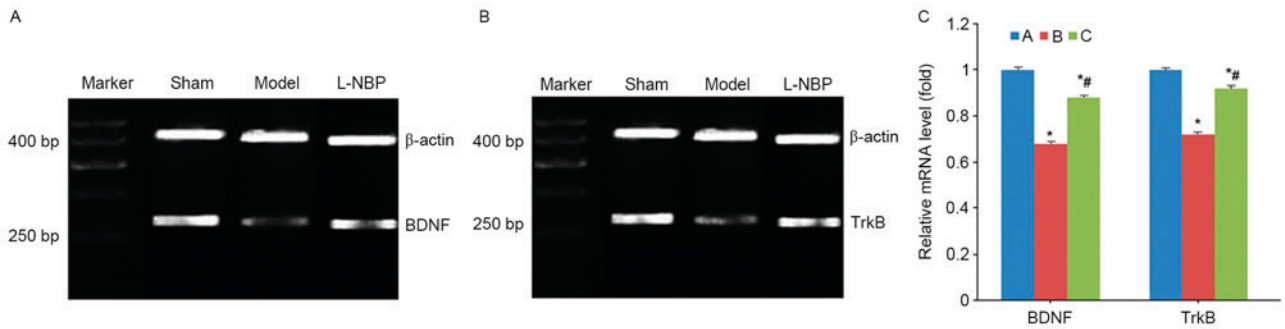

Figure 3. BDNF and TrkB mRNA levels in rat brain tissues. Polymerase chain reaction product bands (A) BDNF and (B) TrkB. (C) Quantitative analysis of fold increase of mRNA expression levels. *P<0.05 vs. sham group; #P<0.05 vs. model group. A, sham group; B, model group; C, L-NPB group. BDNF, brain-derived neurotrophic factor; TrkB, tyrosine kinase receptor B; L-NBP, L-butylphthalide; bp, base pairs.

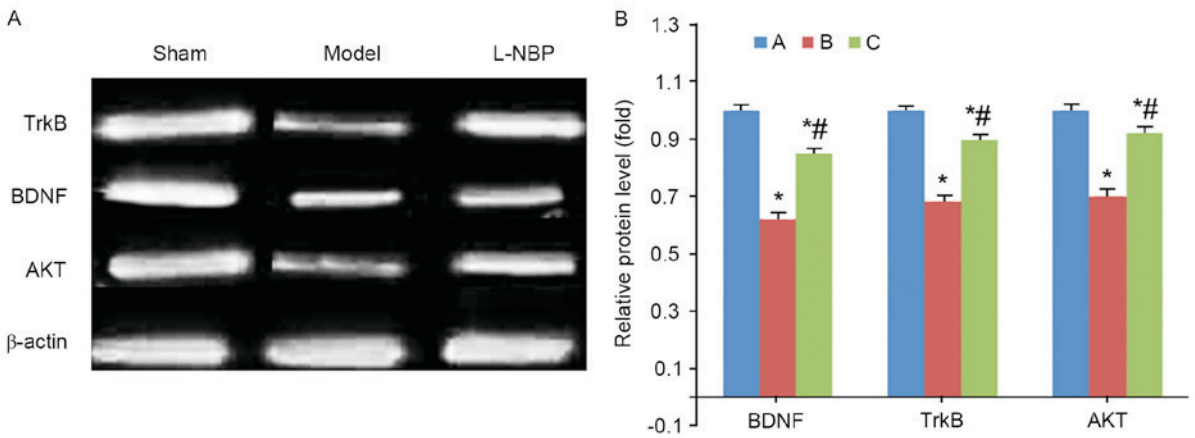

Figure 4. BDNF, TrkB and Akt protein expression in rat brain tissues. (A) Representative image and (B) quantitative analysis revealing fold-increase of BDNF, TrkB and Akt protein expression levels, detected by western blotting. *P<0.05 vs. sham group; #P<0.05 vs. model group. A, sham group; B, model group; C, L-NPB group. BDNF, brain-derived neurotrophic factor; TrkB, tyrosine kinase receptor B; Akt, serine-threonine protein kinase; L-NBP, L-butylphthalide.

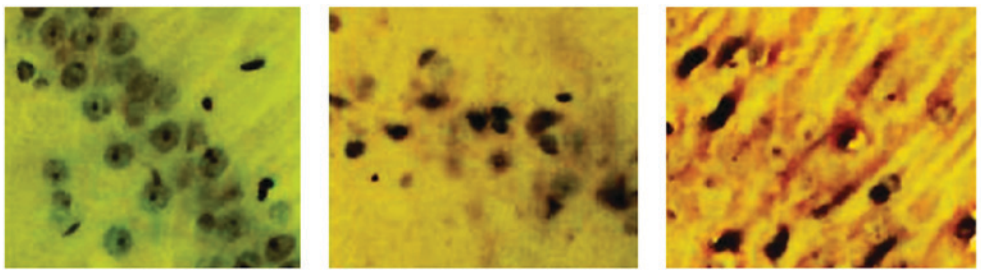

Figure 5. NMDAR protein analysis. Hippocampal CA1 regions underwent immunohistochemical analysis for detection of NMDAR protein expression. Magnification, x400. Left to right: Sham group, model group, and L-butylphthalide group. NMDAR, N-methyl-D-aspartate receptor.

abnormal structure, condensed nucleus and glial hypertrophy. The L-NBP treatment group had alleviated injury compared with the model group, as indicated by less neuron denaturing or necrosis (Fig. 2).

L-NBP increases BDNF and TrkB mRNA levels in VD rats. VD model rats demonstrated decreased mRNA levels of BDNF and TrkB ($P<0.05$ compared with sham group), whereas the L-NBP group exhibited increased mRNA expression levels of BDNF and TrkB ($P<0.05$ compared to model group, Fig. 3).

L-NBP increases BDNF, TrkB and Akt protein levels in VD rats. Compared with the sham group, model rats demonstrated decreased protein levels of BDNF, TrkB and Akt proteins ($P<0.05$). However, the L-NBP treated group had elevated expression levels of these proteins in brain tissues ($P<0.05$ compared with model group, Fig. 4).

NMDAR protein expression analysis. Model group exhibited a lighter staining of NMDAR in the hippocampal CA1 regions, compared with the sham group, whereas the L-NBP group revealed a darker staining compared with the model group (Fig. 5).

Discussion

The pathological mechanism of VD remains to be fully elucidated. BDNF and NMDAR are closely associated with the learning and memory process. Synaptic excitation may lead to BDNF release from axons via N-type calcium ion channels and intracellular calcium release (16). Voltage-gated ion channels and NMDAR may regulate BDNF release; a neurotrophic factor with a biological function dependant on specific binding to TrkB. Various studies have demonstrated that the co-activation of BDNF and its receptor TrkB elevates synaptic plasticity, facilitates axonal and dendritic growth and increases synaptic terminal density (17,18). Exogenous BDNF may additionally decrease brain infarction volume. BDNF-like immune reactive products are widely distributed in ipsilateral neocortex neurons. Following brain ischemia, BDNF expression is largely varied, with a significant suppression of expression levels in the hippocampus detectable during the first few days. A total of one-week following the ischemia, BDNF and TrkB expression levels are elevated, thus protecting neurons, although such enhanced self-protection does not have a long-term effect (19,20). It has previously been demonstrated that L-NBP may facilitate an increase of BDNF mRNA/protein expression levels in hippocampal regions of VD rats. BDNF mRNA levels are associated with the severity of ischemia-induced injury. Under conditions of severe ischemia, BDNF mRNA transcription is suppressed. Endogenous BDNF may significantly improve ischemia injury of brain tissues. However, BDNF is unable to easily penetrate the blood-brain barrier. L-NBP has multiple target sites, thus elevating its endogenous protective effects (21,22). L-NBP exhibits an increased potency in its therapeutic effect in treating acute ischemia brain stroke, compared with D-LBP, which may antagonize the anti-apoptotic effect of L-NBP. In the present study, VD model rats demonstrated an elongated escape latency and lower platform crossing times. L-NBP treatment shortened escape latency and increased platform crossing times. Pathological examination of rat brain tissues revealed diffused injury in cortical and hippocampal regions of model rats, which had abnormal structure of cortical and hippocampal neural tissues, with condensed nucleus and glial hypertrophy. L-NBP rats had alleviated neuron injury, with less necrosis of neurons, indicating that L-NBP alleviated neurological functions and improved memory/learning functions. L-NBP therefore resulted in certain neuroprotective effects on VD, which was consistent with previous studies that demonstrated that L-NBP may improve the cognitive function in diabetic rats (23), as well as the cognitive deficits and neuronal loss in the hippocampus of cerebral repetitive ischemia/reperfusion mice (24).

NMDAR primarily consists of NR1 and NR2, which are associated with synaptic plasticity. BDNF participates in long-term memory formation, and is important in maintaining learning/memory and regulating synaptic plasticity. The present study revealed significantly elevated BDNF, TrkB, Akt and NMDAR expression levels in L-NBP-treated VD rats, compared with model rats, suggesting that L-NBP facilitated regeneration of neurons and prevented their late onset apoptosis, via the BDNF-induced phosphatidyl inositol-3/Akt signaling pathway, thus protecting cognitive functions and synaptic transmission and protects against neuronal apoptosis. Endogenous neuroprotective effects have important roles in ischemic brain tissues. Their initiation, however, is relatively slow without exogenous stimuli. Administration of exogenous L-NBP may protect the integrity of neurons, facilitate synaptic plasticity and improve rat memory and learning. However, the main limitation of the present study was that a BDNF inhibitor was not administered under the treatment of L-NBP to confirm whether L-NBP exerts a protective role in cognitive function in VD rats. This will be the focus of future investigation.

In conclusion, L-NBP upregulated hippocampal expression of BDNF, TrkB, Akt and NMDAR in VD rats, thus alleviating ischemia-induced brain injury and improving learning and memory functions.

References

1. Lantz M, Sjöstrand C and Kostulas K: Ischemic stroke and patent foramen ovale: Risk factors and genetic profile. J Stroke Cerebrovasc Dis 22: 841-845, 2013.
2. O'Brien JT and Thomas A: Vascular dementia. Lancet 386: 1698-706, 2015.
3. Yakushiji Y: Cerebral microbleeds: Detection, associations and clinical implications. Front Neurol Neurosci 37: 78-92, 2015.
4. Ueno M, Chiba Y, Matsumoto K, Murakami R, Fujihara R, Kawauchi M, Miyanaka H and Nakagawa T: Blood-brain barrier damage in vascular dementia. Neuropathology 36: 115-124, 2016.
5. Li Y, Hai S, Zhou Y and Dong BR: Cholinesterase inhibitors for rarer dementias associated with neurological conditions. Cochrane Database Syst Rev: CD009444, 2015.
6. Kwon KJ, Kim MK, Lee EJ, Kim JN, Choi BR, Kim SY, Cho KS, Han JS, Kim HY, Shin CY and Han SH: Effects of donepezil, an acetylcholinesterase inhibitor, on neurogenesis in a rat model of vascular dementia. J Neurol Sci 347: 66-77, 2014.
7. Yin W, Lan L, Huang Z, Ji J, Fang J, Wang X, Ji H, Peng S, Xu J and Zhang Y: Discovery of a ring-opened derivative of 3-n-butylphthalide bearing NO/H2S-donating moieties as a potential anti-ischemic stroke agent. Eur J Med Chem 115: 369-380, 2016.
8. Wen XR, Tang M, Qi DS, Huang XJ, Liu HZ, Zhang F, Wu J, Wang YW, Zhang XB, Guo JQ, *et al*: Butylphthalide suppresses neuronal cells apoptosis and inhibits JNK-caspase3 signaling pathway after brain ischemia/reperfusion in rats. Cell Mol Neurobiol 36: 1087-1095, 2016.

9. Niu H, Zhang Z, Wang H, Wang H, Zhang J, Li C and Zhao L: The impact of butylphthalide on the hypothalamus-pituitary-adrenal axis of patients suffering from cerebral infarction in the basal ganglia. Electron Physician 8: 1759-1763, 2016.
10. Jiang Y, Sun L, Xuan X and Wang J: Impacts of N-Butylphthalide on expression of growth factors in rats with focal cerebral ischemia. Bosn J Basic Med Sci 16: 102-107, 2016.
11. Lee SH, Ko IG, Kim SE, Hwang L, Jin JJ, Choi HH and Kim CJ: Aqueous extract of Cordyceps alleviates cerebral ischemia-induced short-term memory impairment in gerbils. J Exerc Rehabil 12: 69-78, 2016.
12. Sawamoto A, Okuyama S, Yamamoto K, Amakura Y, Yoshimura M, Nakajima M and Furukawa Y: 3,5,6,7,8,3',4'-Heptamethoxyflavone, a citrus flavonoid, ameliorates corticosterone-induced depression-like behavior and restores brain-derived neurotrophic factor expression, neurogenesis, and neuroplasticity in the Hippocampus. Molecules 21: 541, 2016.
13. Fang M, Yuan Y, Lu J, Li HE, Zhao M, Ling EA and Wu CY: Scutellarin promotes microglia-mediated astrogliosis coupled with improved behavioral function in cerebral ischemia. Neurochem Int 97: 154-171, 2016.
14. Xing M, Sun Q, Wang Y, Cheng Y and Zhang N: Hydroxysafflor yellow A increases BDNF and NMDARs in the hippocampus in a vascular dementia rat model. Brain Res 1642: 419-425, 2016.
15. Kato M, Iwata H, Katayama T, Asai H, Narita H and Endo T: Possible therapeutic effect of T-794, a novel reversible inhibitor of monoamine oxidase-A, on post-stroke emotional disturbances, assessed in animal models of depression. Biol Pharm Bull 20: 349-353, 1997.
16. Du R, Teng JF, Wang Y, Zhao XY and Shi ZB: Clinical study of Butylphthalide combined with Xue Shuan Tong on serum inflammatory factors and prognosis effect of patients with cerebral infarction. Pak J Pharm Sci 28 (5 Suppl): S1823-S1827, 2015.
17. Yang LC, Li J, Xu SF, Cai J, Lei H, Liu DM, Zhang M, Rong XF, Cui DD, Wang L, *et al*: L-3-n-butylphthalide promotes neurogenesis and neuroplasticity in cerebral ischemic rats. CNS Neurosci Ther 21: 733-741, 2015.
18. Zhao W, Luo C, Wang J, Gong J, Li B, Gong Y, Wang J and Wang H: 3-N-butylphthalide improves neuronal morphology after chronic cerebral ischemia. Neural Regen Res 9: 719-726, 2014.
19. Zhao Y, Li J, Zhang P, Chen C and Li S: Protective effects of dl-3n-butylphthalide against diffuse brain injury. Neural Regen Res 8: 2615-2624, 2013.
20. Hu J, Wen Q, Wu Y, Li B and Gao P: The effect of butylphthalide on the brain edema, blood-brain barrier of rats after focal cerebral infarction and the expression of Rho A. Cell Biochem Biophys 69: 363-368, 2014.
21. Diao X, Pang X, Xie C, Guo Z, Zhong D and Chen X: Bioactivation of 3-n-butylphthalide via sulfation of its major metabolite 3-hydroxy-NBP: Mediated mainly by sulfotransferase 1A1. Drug Metab Dispos 42: 774-781, 2014.
22. Ghadernezhad N, Khalaj L, Pazoki-Toroudi H, Mirmasoumi M and Ashabi G: Metformin pretreatment enhanced learning and memory in cerebral forebrain ischaemia: The role of the AMPK/BDNF/P70SK signalling pathway. Pharm Biol 54: 2211-2219, 2016.
23. Li J, Zhang S, Zhang L, Wang R and Wang M: Effects of L-3-n-butylphthalide on cognitive dysfunction and NR2B expression in hippocampus of streptozotocin (STZ)-induced diabetic rats. Cell Biochem Biophys 71: 315-322, 2015.
24. Huai Y, Dong Y, Xu J, Meng N, Song C, Li W and Lv P: L-3-n-butylphthalide protects against vascular dementia via activation of the Akt kinase pathway. Neural Regen Res 8: 1733-1742, 2013.