European Review for Medical and Pharmacological Sciences 2018; 22: 5377-5384

L-butyl phthalein improves neural function of vascular dementia mice by regulating the PI3K/AKT signaling pathway

D.-P. CHEN[1], S.-H. HOU[1], Y.-G. CHEN[1], M.-S. CHEN[1], Z.-Z. HU[1], Z.-J. Z[illegible]G[2]

[1]Department of Neurology, The Affiliated Longyan First Hospital of Fujian M[illegible]cal University, Longyan, Fujian, China
[2]Department of Neurology, The First Affiliated Hospital of Fujian Medic[illegible]versi[illegible]uzhou, Fujian, China

Dongping Chen and *Shuhong Hou* contributed equally to this w[illegible]

Abstract. – OBJECTIVE: L-3-n-butylphthalide (L-NBP) is a type of anti-ischemic cranial nerve protective drug that may act on vascular dementia (VD). Phosphatidylinositol-3 kinase (PI3K)/protein kinase B (AKT/PKB) signaling pathway can up-regulate B-cell lymphoma 2 (Bcl-2) expression, reduce reactive oxygen [illegible]cies (ROS) production, and alleviate cell a[illegible]sis. This study aimed at investigating the [illegible]of L-NBP on neurological function and cell a[illegible]tosis in VD mouse through regulating PI3K/A[illegible] signaling pathway.

MATERIALS AND METHODS: [illegible]mice wer[illegible] divided into four groups, in[illegible]am, VD, VD + solvent, and VD + L-[illegible] HT22 [illegible]ls were cultured in vitro and trea[illegible]by ischemia/reperfusion (I/R). HT22 cells [illegible]divi[illegible] groups, including I/R[illegible] + [illegible]VD + L-NBP, and VD + L-NBP [illegible]LY29400[illegible]ups. Phosphorylated AKT ([illegible]KT) and Bcl-[illegible]ressions were tested. R[illegible]nt in hippo[illegible]pus tissue was dete[illegible]d by [illegible]ytometry. Cell apoptosis was e[illegible]luated by t[illegible]rase-mediated deoxyuridi[illegible]phosphate-bi[illegible]ick end labeling (TUNE[illegible]ssay.

RE[illegible]LTS: R[illegible]content and cell apoptosis inc[illegible]d, w[illegible]p-AKT and Bcl-2 expressions reduc[illegible]pocam[illegible]tissue from VD group compar[illegible]h sha[illegible]oup. L-NBP significantly regu[illegible]p-AKT and Bcl-2 expressions an[illegible]creas[illegible]S content and cell apoptosis [illegible]ippocam[illegible]us tissue. I/R treatment marked[illegible] HT22 cell apoptosis and ROS induc[illegible]nd reduced p-AKT and Bcl-2 expressions. L-NBP treatment markedly up-regulated p-AKT and Bcl-2 levels, restrained cell apoptosis, and reduced ROS content in TH22 cells intervened by I/R. LY294002 apparently attenuated the protective effect of L-NBP on HT22 cells.

CONCLUSIONS: L-NBP protects VD by up-regulating PI3K/AKT signaling pathway, elevating Bcl-2 expression, reducing nerve cell apoptosis, and restraining ROS production.

Key[illegible]
Vasc[illegible]ntia, L-3-n-butylphthalide, PI3K/AKT, TH22 cells.

Introduction

Vascular dementia (VD) is a kind of brain circulation dysfunction and brain damage syndrome because of cerebrovascular factors, resulting in learning and memory impairment, accompanied by language, visual space, directional force, abstract thinking, and personality disorder. VD is the second largest dementia encephalopathy after Alzheimer's disease (AD)[1-3]. In recent years, VD shows rising and younger trend, leading to severe impact on quality of life, health, and huge burden to society and family[4,5].

L-3-n-butylphthalide (L-NBP) is a type of anti-ischemic cranial nerve protective drug commonly used in clinic[6,7]. It plays its effect through increasing the blood supply, improving microcirculation in ischemia area, alleviating cerebral edema, perfecting energy metabolism, protecting mitochondrial function, inhibiting glutamate release, preventing platelet accumulation and thrombosis[8,9]. Phosphatidylinositol-3 kinase (PI3K) is an important member of growth factor receptor superfamily that can be activated by the stimulus of cytokines and mitogen.

Corresponding Author: Dongping Chen, MD; e-mail: dui55361995@163.com

It participates in the regulation of cell proliferation, cell cycle, and apoptosis by activating downstream protein kinase B (PKB/AKT)[10,11]. PI3K/AKT signaling pathway is a classical pathway in antagonizing apoptosis and promoting survival that exists in various tissues and cells. Scholars[12-14] revealed that PI3K/AKT signaling pathway plays an important regulatory role in up-regulating B-cell lymphoma-2 (Bcl-2) expression, alleviating cell injury after ischemia and hypoxia, antagonizing apoptosis, and facilitating cell survival. However, it is still unclear about the role of L-NBP on VD mouse model and related mechanism. This study established VD mouse model and observed the impact of L-NBP on neurological function, cell apoptosis, and PI3K/AKT signaling pathway to evaluate the potential mechanism.

Materials and Methods

Main Reagents and Materials

Male C57BL/6 mice at 8 weeks old and weighted 22-25 g were purchased from ...tor River (Beijing, China). Dulbecco's ...ified Eagle Medium (DMEM), fetal b...e serum (FBS), and trypsin were purch...d from Gibco BRL. Co. Ltd. (Grand Isla... NY, USA). Dispase was deri...m Roch... Pharmaceutical (Basel, Sw... Rabbit anti-mouse Bcl-2 antibod...s got ...n Santa Cruz Biothechnology (S... Cruz ...SA). Rabbit anti-mouse A...T, p... b... (p-AKT), β-actin, ...horse... peroxidase (HRP) conjugate... condary a...dies were provided by ... Biotechnol... (Cambridge, MA, ...A). ...mmunoprecipitation assay (RI...), transfer...ediated deoxyuridine t...osphate-biotin... end labeling (TUN... apopt...is detection kit, and Annexin V... kit w... provided by Beyotime (Haimen, ...a). CFH-D... probe was purchased from S... Aldric... St. Louis, MO, USA). ...e hip... m... neuron HT22 cells were bo... from ... Chemical Reagent co., Ltd (Sh...hai, Chi...a). PI3K/AKT specific inhibito... was synthesized by MedChemExpress (Monmouth Junction, NJ, USA).

Mice were used for all the experiments, and ...cedures were approved by the Animal Ethics ...mmittee of The Affiliated Longyan First Hospital of Fujian Medical University (Fujian, China).

VD Mouse Model Establishment

The mice were raised at free eating and drinking, 12 h day/night cycle, temperature 22-25°C, and relative humidity 50%-60%. The e... began after 1 week's adaptive br...g. T... mouse was anesthetized by 10%... al hydrate abdominal injection and fixed on ...ting table. Then, the neck skin was disinfected ...e incision was made on neck mid... line. T... cle and connective tissue w... separated to bilateral common caro...rtery ...gation. ...e vagus nerve was pr...d to ...d dam...ge. A small incision wa...ad... comm...arotid artery to put t... occlusio... the ...ting of the middle c...al artery. T... occlusion was fixed ...in was close...fter 2 h, the occlusion ...s ren...upon diethyl ether rapid anesthesia. Antibiotic... used to prevent infec... mice in the ... group received the s... treatment without bilateral common carotid ...ry ligation. A ...tal of 10 mice were selected ...ntrol.

...was asse... by Zea Longa scoring. Score 0, ... without neurologic deficits; score 1, ...eft forepaw cannot be fully extended; score 2, turn left during walking; score ...aning during walking; score 4, loss of ...sness without spontaneous walking. The mice in score 1-3 were considered as successful modeling. The mice in score 0 and 4 were excluded from the experiment. A total of 30 mice were successfully established as VD model.

On the 21st day after modeling, the mice were killed and the hippocampus tissue was collected. The sample was embedded by paraffin, used for protein extraction, and digested for reactive oxygen species (ROS) content detection.

Experimental Animal Grouping and Intervention

The VD mice were randomly equally divided into three groups, including single VD group with normal feeding without other treatment after modeling, solvent group with corn oil gavage after modeling at 15 mg/kg/d for 20 days, and L-NBP group with L-NBP gavage after modeling at 15 mg/kg/d for 20 days.

Morris Water Maze Test

On the 21st day after modeling, the mice received Morris water maze test. Morris water maze device is a cylindrical tank at diameter 120 cm and height 50 cm. The tank was divided into four quadrants on the bottom. A transparent

organic glass cylindrical platform at diameter 12 cm and height 30 cm was put at the centre of a quadrant. The video camera was used to record the movement locus and search the incubation period of the platform. The water was added to the tank and stained by ink for non-transparent. The water surface was about 2 cm over the platform surface and the water temperature was maintained at 25°C. The mice swam for 2 min on the day before the experiment to adapt to the environment. The mouse was trained for 4 times each day from the first day. The roadmap and time needed for the mouse to search and climb up to the platform were recorded. The mouse was guided to the platform once it cannot find the platform within 120 s. The time interval between each time of training was 60 s. The experiment was repeated after 24 h to reflect the memory retention.

TUNEL Assay

The hippocampus tissue slice was dewaxed by xylene for 5-10 min, absolute ethyl alcohol for 5 min, 90% ethyl alcohol for 2 min, 70% ethyl alcohol for 2 min, and distilled water for 2 min. The slice was added with 20 μg/ml proteinase without DNase and incubated at 37°C for 20 min. Next, the slice was treated by 50 μl TUNEL detection liquid composed of 5 μl TdT enzyme and 45 μl fluorescence liquid at 37°C avoid of light for 60 min. After washed by phosphate buffered saline (PBS) three times and sealed, the slice was observed under the microscope to calculate the apoptotic cell ratio.

Hippocampal Neuron Cell Ischemia-Reperfusion (I/R) Treatment *in vitro*

HT22 cells were cultured in low glucose DMEM without FBS to stimulate ischemia condition and maintained at 5% CO_2 and 95% N_2 to stimulate an anaerobic environment *in vivo*. After 12 h, cells were changed to routine medium containing FBS and cultured at 5% CO_2 and 95% air. After 12 h simulated reperfusion.

HT22 cells were divided into four groups, including single I/R group treated by I/R; L-NBP group treated by I/R after 12 h pre-treatment of 10 μg/ml L-NBP; solvent group treated by I/R after 12 h pre-treatment of corn oil; and combined treatment group treated by I/R after 12 h pre-treatment of 10 μg/ml L-NBP and 10 μM LY294002. The cells were collected for protein, apoptosis, and ROS content detection.

Cell Apoptosis Detection

The cells were re-suspended in 500 μl binding buffer and incubated in 5 μl Annexin V-FITC avoid of light for 15 min. Next, the cells were added with 5 μl propidium iodide (PI) and tested on Beckmann CytoFLEX flow cytometry to evaluate cell apoptosis.

ROS Content Detection

The cells were digested by trypsin and incubated in 0.1% DCFH-DA diluted by serum-free medium at 37°C for 20 min. After washed by serum-free medium for three times, the cells were detected on Beckmann CytoFLEX to assess ROS content.

Western Blot

Total protein was extracted by RIPA from cell tissues for 20 min and centrifuged at 12000 ×g for 10 min. A total of 40 μg protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane. Next, the membrane was blocked and incubated in primary antibody at 4°C overnight (AKT, p-AKT, Bcl-2, and β-actin at 1:1000, 1:2000, and 1:10000, respectively). Next, the membrane was incubated in HRP labeled secondary antibody (1:30000) for 60 min after washed by PBST for three times. At last, the protein expression was detected by enhanced chemiluminescence (ECL).

Statistical Analysis

All data analyses were performed on SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). The measurement data were depicted as mean ± standard deviation (SD). The Student's *t*-test was used to compare the differences between two groups. Tukey's post-hoc test was used to validate the ANOVA for comparing measurement data among the groups. $p < 0.05$ was considered statistically significant.

Results

Mouse Learning and Memory Functions Deteriorated in VD Model

The mice in sham group exhibited good mental state, normal activity, food, and drink, and rapid movement. The mice in VD model group presented lack of mental state, lethargy, drowsiness, decreased activity, feeding, and drinking, accompa-

Table I. Mouse learning and memory functions comparison.

Group	Cases	Learning ability		Memory ability	
		Escape latent time (s)	Mistake times	Platform stay time (s)	Swimming sp
Sham	10	46.3± 3.9	14.2 ± 1.3	34.6 ± 2.9	1 1.7
VD	10	87.6 ± 5.8*	37.2 ± 2.9*	18.2 ± 1.4*	+ 1.2*
VD + solvent	10	89.2 ± 6.1*	36.9 ± 2.7*	17.3 ± 1.5*	2*
VD + L-NBP	10	73.3 ± 5.5*#	22.2 ± 1.8*#	28.3 ± 2.3*#	14.8

*$p < 0.05$, compared with Sham group, #$p < 0.05$, compared with VD + solvent.

nied by weight loss, irritability, excitement, and ataxia symptoms. Compared with sham group, the escape latent time significantly prolonged and mistake times evidently increased, while platform stay time and swimming speed markedly reduced in VD model group ($p < 0.05$). L-NBP gavage apparently improved mouse learning and memory functions, resulting in the decrease of escape latent time and mistake times, and elevated platform stay time and swimming speed ($p < 0.05$). The corn oil gavage presented no statistical impact on mouse learning and memory fun s (Table I).

L-NBP Reduced Hippocampal Cell Apoptosis Through Up-Regulating PI3K/AKT Signaling Pathw tivity and Bcl-2 Expression

Flow cytometry detecti howed at ROS content significantly i ed in m- pus tissue from VD g up a with group, while L-NB vage dly declined ROS content. Sol gavage fail ffect ROS content compa with V up (re 1A). TUNEL ass ealed that c osis markedly enha pocampus ue from VD compared th sh up, whereas L-NBP gavage apparently allev ell apoptosis. Solvent ga ibited no sig nt influence on cell a osis compared with VD group (Figure 1B). tern blot det ion demonstrated that p-AKT Bcl-2 prote significanly down-regulated i pocampus ue in VD group than that in Sha e L-NBP gavage markedly enhanced p and Bcl-2 protein levels. Solvent avage did not impact p-AKT and Bcl-2 protein ons in hippocampus tissue (Figure 1C).

I/R Treatment Induced Hippocampal Neuron Apoptosis and Downregulated PI3K/AKT Signaling Pathway Activity and Bcl-2 Expression

Flow cytometry demonstrated that ROS content significantly increased and cell apoptosis enhanced in HT22 cells treated by I/R (Figure 2A and B). Western blot showed that p-AKT and Bcl-

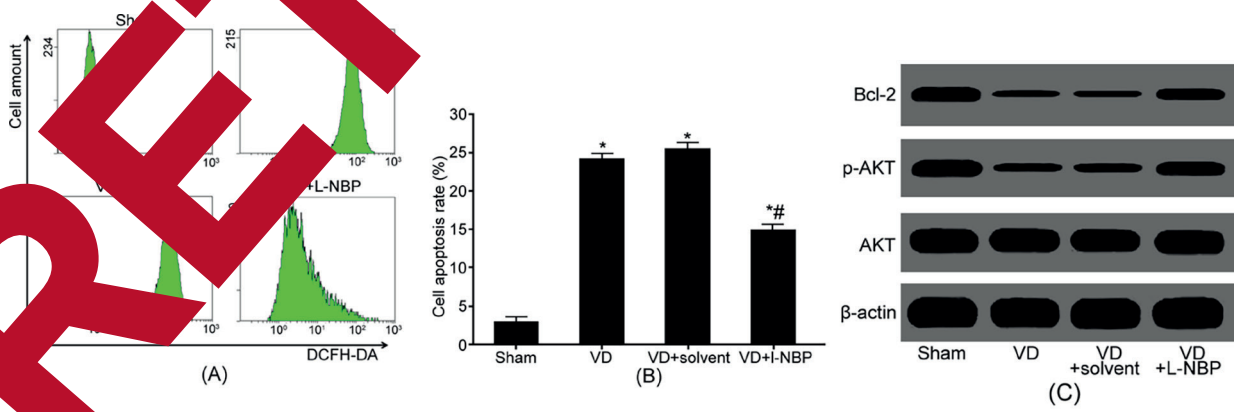

Figure 1. L-NBP reduced hippocampal cell apoptosis through upregulating PI3K/AKT signaling pathway activity and Bcl-2 expression. **A,** Flow cytometry detection of ROS content. **B,** TUNEL assay detection of cell apoptosis. **C,** Western blot detection of protein expression. *$p < 0.05$, compared with Sham group, #$p < 0.05$, compared with VD + solvent.

2 protein contents significantly reduced in HT22 cells treated by I/R (Figure 2C).

L-NBP Antagonized TH22 Cell Apoptosis Induced by I/R Through Enhancing PI3K/AKT Signaling Pathway

Flow cytometry presented that L-NBP pretreatment markedly declined ROS production and cell apoptosis in HT22 cells induced by I/R, while the solvent control failed to show the statistical impact (Figure 3A, B). The LY294002 intervention significantly increased ROS content and cell apoptosis in HT22 cells compared with L-NBP pretreatment group (Figure 3A, B). Western blot showed that L-NBP pretreatment significantly up-regulated p-AKT and Bcl-2 protein contents in HT22 cells induced by I/R, whereas LY294002 intervention markedly alleviated the influence of L-NBP on p-AKT and Bcl-2 expressions (Figure 3C).

Discussion

The major pathogenesis of VD is a cerebr... cular lesion, of which ischemic cerebrov... disease accounts for the leading morbidit... At present, the incidence of VD is about 2-7%, v... it is up to 6-12% in the population older t... 70. There are more than 18 mil... VD patien... around the world, which is e... over 30 million till 2020[4].

L-NBP is a yellow oil... id mo... pound extracted from ... the ... tute of Chinese Ac... y of ... al Sciences. Its optical isomer ... lude L-NB... tral butylphthalide (D-N... l despun bu... thalide (LD-NBP), o... ich ... P shows the strongest efficacy. T... existence o... BP exhibits an antagonis... ect on the phar... logical effect of L-NB... s an ... ti-ischemic cranial nerve protect... drug c... monly used in clinic, L-NBP is a ... of ... w drug ... ith independent intellectual ... ty righ... China[16]. L-NBP plays ... pact ... ng cranial nerve function aft... chem... increasing blood supply, improv... the mic... ocirculation in ischemic area, s... bral infarction area, alleviating ... ebral edema, improving energy metabolism, ... ducing cell apoptosis, promoting cell survival, ... asing intracellular calcium concentration, pro... ting mitochondrial function, inhibiting oxygen free radical generation, elevating antioxidant enzyme activity, suppressing glutamate release, and preventing platelet aggregation and thrombosis[8,9].

PI3K/AKT signaling pathway widely exists in multiple tissues and cells. Under the st... growth factor or mitogen, PI3K ca... activa... ed through conformation change... d translate phosphatidylinositol (4,5)-bispho... (PIP2) to phosphatidylinositol (3,4,5)-trisphos... (PIP3). PIP3 can phosphorylate A... protein ... 473 and Thr308 loci under th... istant of 3-ph...

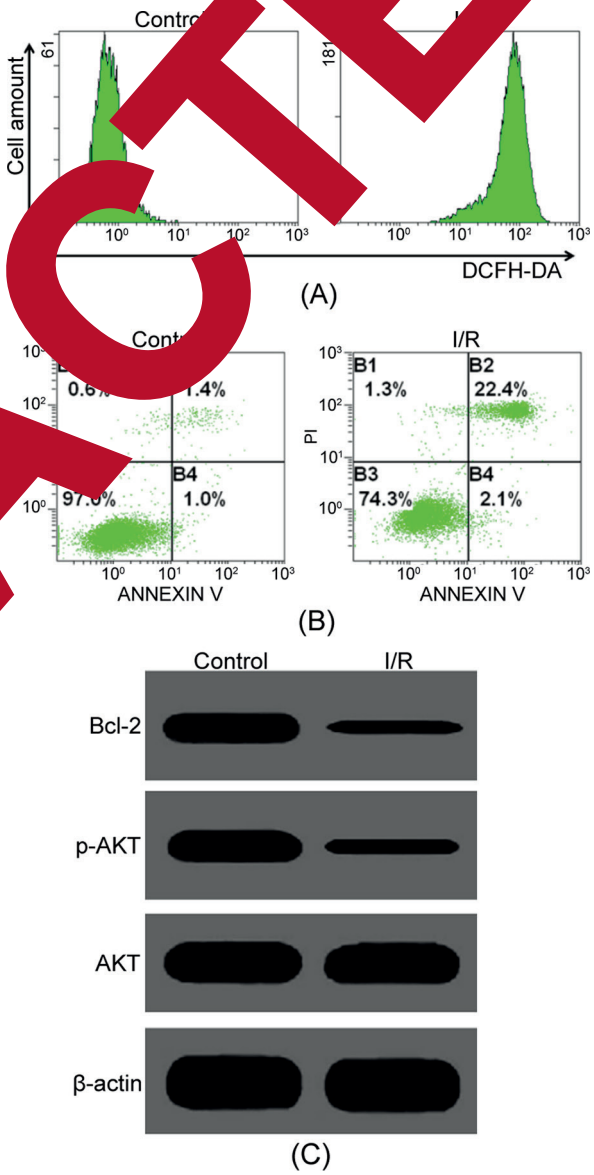

Figure 2. I/R treatment induced hippocampal neuron apoptosis and downregulated PI3K/AKT signaling pathway activity and Bcl-2 expression. **A,** Flow cytometry detection of ROS content. **B,** Flow cytometry detection of cell apoptosis. **C,** Western blot detection of protein expression.

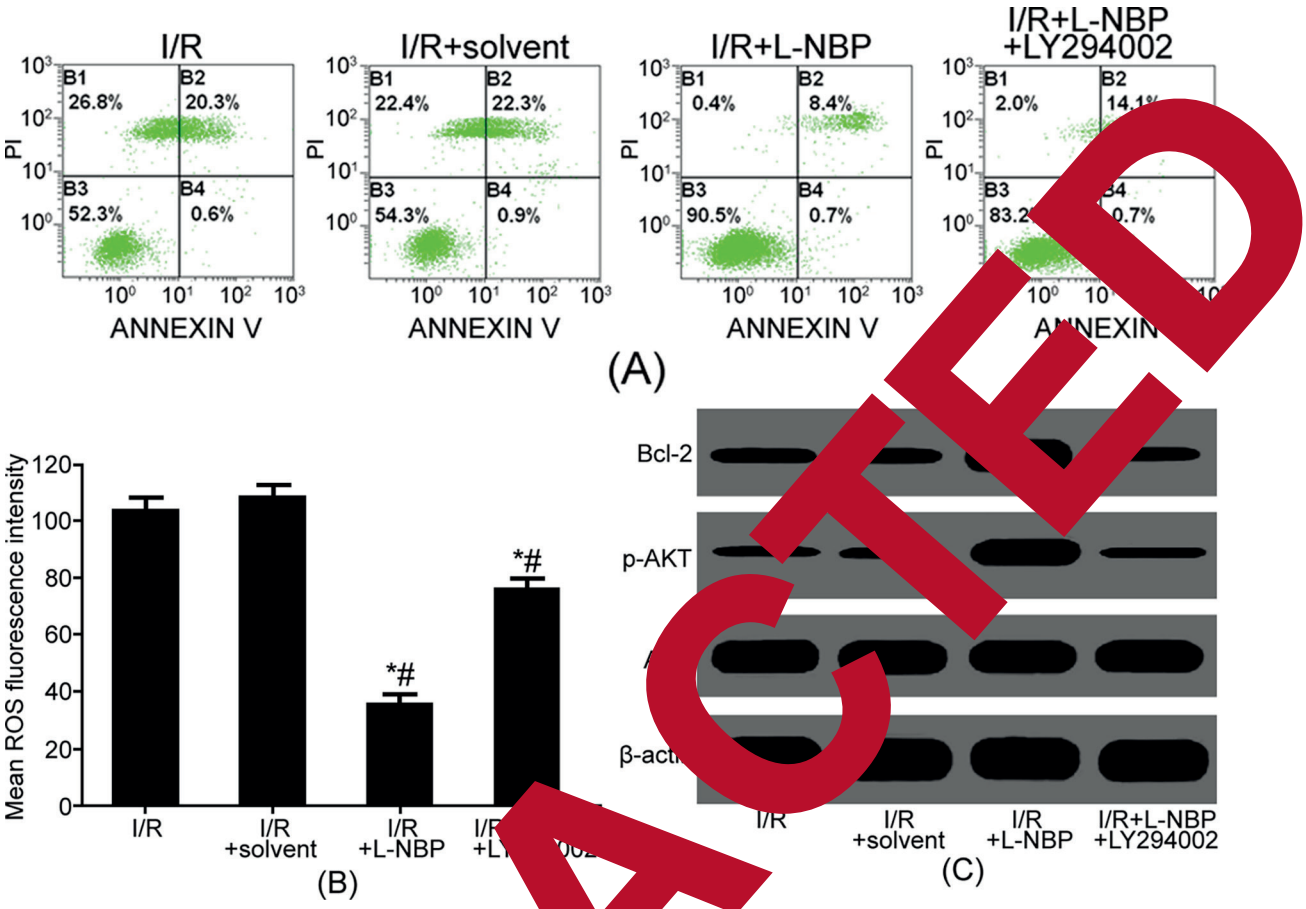

Figure 3. L-NBP antagonized TH22 ptosis ind by I/R through enhancing PI3K/AKT signaling pathway. **A,** Flow cytometry detection of cell ap w cytom tection of ROS content. **C,** Western blot detection of protein expression. *$p < 0.05$, compared w am gr $^{\#}p < 0.05$, pared with VD + solvent.

inositide-dependent ein ki (PDK1) and PDK2. Phosphory AKT con rious target genes trans nd translati us regulates cell su al, p ration, and apoptosis. PI3K/AKT naling pati a classic pathway in antag ng cell apoptos promoting cell survi PI3K/AKT signaling pathway plays a pro e rol inst tissue and cell ischemia and ol njury, luding heart[17], brain[18], lung[19], dney[20] l-2 is an important anptoti or cting mitochondrial functio strain C release, impacting calcium transme rane transport, and inhibiting a ase activating factor-1 (Apaf-1) vation . Bcl-2 also plays a crucial role in pressing ROS production and anti-oxidative [22]. Numerous studies[12-14] demonstrated that Bc s a key target gene of PI3K/AKT signaling pathway to play a role in anti-oxidation, antagonizing cell apoptosis, and promoting cell survival.

Currently, the role of L-NBP on VD mouse model and related mechanism is still unclear. This study established VD mouse model and observed the impact of L-NBP on neurological function, cell apoptosis, and PI3K/AKT signaling pathway to evaluate the potential mechanism.

This investigation showed that compared with sham group, the escape latent time significantly prolonged and mistake times markedly increased, while platform stay time and swimming speed markedly reduced in VD model group. L-NBP gavage apparently improved mouse learning and memory functions, resulting in the decrease of escape latent time and mistake times, and elevated platform stay time and swimming speed. It suggested that L-NBP markedly improved the neurologic function of VD mouse and enhanced learning and memory abilities. Xiang et al[16] reported that L-NBP treatment significantly improved the learning and cognitive function of

APP/PS1 dual-transgenic dementia mouse. Yang et al[23] revealed that L-NBP gavage remarkably improved the neurological function of cerebral ischemia mouse. This study also observed the improvement effect of L-NBP on the neurological function of dementia mouse, which was similar with Xiang et al[16] and Yang et al[23]. Cerebral ischemia can induce cerebral infarction or selectively cerebral neuron death. It may cause VD when the ischemia appeared in the region related to learning and memory. Hippocampus is the key for learning and memory, and is also sensitive to ischemia and anoxia. Therefore, this study investigated the impact of L-NBP on the hippocampus of the mouse model. It exhibited that L-NBP gavage significantly alleviated ROS production, reduced hippocampal cell apoptosis, and up-regulated p-AKT and Bcl-2 expressions in VD mouse. Yang et al[23] presented that L-NBP gavage significantly down-regulated cleaved caspase-3 and Bcl-2 associated X protein (Bax) expressions, promoted neuron regeneration, and improved neurological function in the cerebral ischemic rat. Xiang et al[16] found that L-NBP treatment markedly enhanced PI3K/AKT [illegible] way activity in cerebral tissue and im[illegible] cognitive and memory functions in AP[illegible]S1 dual-transgenic dementia mouse. Huai et [illegible] demonstrated that L-NBP apparently impro[illegible] learning and memory functio[illegible]d elevate[illegible] p-AKT expression in hippoc[illegible]us tissue from VD mouse. We observed [illegible]PI3K/[illegible]T pathway attenuation and Bcl[illegible]wn-reg[illegible]lay a regulatory role in hi[illegible]poc[illegible]l apop[illegible], ROS production, a[illegible]e pa[illegible]esis of VD. L-NBP interventi[illegible]leviated ce[illegible]ptosis and ROS productio[illegible]h elevating [illegible]K/AKT signaling pa[illegible]ay a[illegible] and Bcl-2 expression, which was in acco[illegible]e with the reports of Yang [illegible][23], Xiang et a[illegible]d Huai et al[24]. We al[illegible]ound [illegible]at I/R treatment significantly ind[illegible] HT22 [illegible] apoptosis and ROS production, [illegible]e[illegible]d p-AK[illegible]nd Bcl-2 expressions. L-NBP [illegible]atment[illegible]rkedly enhanced PI3K/[illegible] pati[illegible]ac[illegible]y and Bcl-2 expression, alle[illegible]ed ce[illegible]tosis, and antagonized ROS pro[illegible]tion. L-NBP combined PI3K/AKT specific [illegible]294002 significantly down-regulated p-AKT and Bcl-2 expressions and attenuated the apoptosis protective and anti-oxidative effects on hippocampal neuron cells. It suggested that L-NBP plays apoptosis protective and anti-oxidative effects on hippocampal neuron cells through affecting PI3K/AKT pathway activity and Bcl-2 expression. Lei et al[25] demonstrated that L-NBP protected nerve cell apoptosis and injury induced by Alzheimer's disease pathogenic factor Abeta25-35 through upregulat[illegible] expression, which was in accordan[illegible]ith o[illegible] results. Peng et al[9] also reported t[illegible]L-NBP has a protective effect on nerve cell ap[illegible]is induced by Abeta25-35. Both the reports of [illegible]et al[16] and Huai et al[24] showed t[illegible]L-NBP [illegible]ved neurological function an[illegible]egulated PI3[illegible] signaling pathway in d[illegible]entia m[illegible]. This w[illegible] showed that L-NBP [illegible]lates [illegible]K/AKT path-way activity and [illegible]tro[illegible]optosis [illegible]T22 cells induced b[illegible], while [illegible]2400[illegible]ectively attenuated t[illegible]otective im[illegible]L-NBP on hippocamp[illegible] cells, thus d[illegible]tly confirming the r[illegible] of L-[illegible]n VD. Except for PI3K/AKT signaling pathw[illegible]her pathways, such as ER[illegible]K, also play [illegible]al regulatory roles i[illegible] proliferation, survival, and apoptosis, and related to Al[illegible]imer's disease and VD. However, whether L-[illegible]P may protect neuron through a[illegible]ng ERK/[illegible]PK signaling pathway still nee[illegible]ther e[illegible]oration.

Conclusions

We found that PI3K/AKT signaling pathway down-regulation plays a role in neuron cell apoptosis and VD pathogenesis. L-NBP protects VD by up-regulating PI3K/AKT signaling pathway, elevating Bcl-2 expression, reducing nerve cell apoptosis, and restraining ROS production.

Acknowledgements

This work was supported by Youth Research Foundation of Fujian Health and Family Planning Commission (No. 2015-1-104) and Science and Technology Plan Project of Longyan City (No. 2017LY47).

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Quinn TJ, McCleery J. Diagnosis in vascular dementia, applying 'Cochrane diagnosis rules' to 'dementia diagnostic tools'. Clin Sci (Lond) 2017; 131: 729-732.

2) Chi CL, Zhang SA, Liu Z, Chang MX, Wang H, Huang Y. Research on the role of GLP-2 in the

central nervous system EPK signal transduction pathway of mice with vascular dementia. Eur Rev Med Pharmacol Sci 2017; 21: 131-137.

3) Vijayan M, Kumar S, Bhatti JS, Reddy PH. Molecular links and biomarkers of stroke, vascular dementia, and Alzheimer's disease. Prog Mol Biol Transl Sci 2017; 146: 95-126.

4) Rizzi L, Rosset I, Roriz-Cruz M. Global epidemiology of dementia: Alzheimer's and vascular types. Biomed Res Int 2014; 2014: 908915.

5) Barnes DE, Haight TJ, Mehta KM, Carlson MC, Kuller LH, Tager IB. Secondhand smoke, vascular disease, and dementia incidence: findings from the cardiovascular health cognition study. Am J Epidemiol 2010; 171: 292-302.

6) Bhatt PC, Pandey P, Panda BP, Anwar F, Kumar V. Commentary: L-3-n-butylphthalide rescues hippocampal synaptic failure and attenuates neuropathology in aged APP/PS1 mouse model of Alzheimer's disease. Front Aging Neurosci 2017; 9: 4.

7) Ma S, Xu S, Liu B, Li J, Feng N, Wang L, Wang X. Long-term treatment of l-3-n-butylphthalide attenuated neurodegenerative changes in aged rats. Naunyn Schmiedebergs Arch Pharmacol 2009; 379: 565-574.

8) Zhang Y, Huang LJ, Shi S, Xu SF, Wang XL, Peng Y. L-3-n-butylphthalide Rescues hippocampal synaptic failure and attenuates neuropath aged APP/PS1 mouse model of Alzheim ease. CNS Neurosci Ther 2016; 22: 979-9

9) Peng Y, Xing C, Lemere CA, Chen G, Wang L, Y, Wang X. l-3-n-Butylphthalide ameliora beta-amyloid-induced neuron icity in cu tured neuronal cells. Neur 08; 434 224-229.

10) Su W, Li S, Chen X, Yin P, Ma GABARAPL1 suppresses m is b PI3K/Akt pathway i ost Oncotarget 2017; 8: 449-445

11) Zhu L, Shen W. Paraoxo promotes cell prolifer metastasis b K/Akt in oral squa ce noma. Biomed Pharmacother 2017; 85: 712-

12) Wan ang L, Wang Y, Chen Z, Liu L. Exen protects HUVECs n t-BHP-induced osis vi I3K/Akt-Bcl-2-caspase-3 signal- Endoc s 2016; 41: 229-235.

13) Cao X, C, Zhang X, Lin W, Wang X, Gab1 r tes SDF-1-induced progression vi ition apoptosis pathway induced by K/AK X pathway in human chondrocoma. Tu Biol 2016; 37: 1141-1149.

14) Hu L, Sun Y, Hu J. Catalpol inhibits apoptosis in hydrogen peroxide-induced endothelium by activating the PI3K/Akt signaling pathway and modulating expression of Bcl-2 and Bax. Eu macol 2010; 628: 155-163.

15) Roman GC. The Epidemiology of va ar demen tia. Handb Clin Neurol 2008; 89 658.

16) Xiang J, Pan J, Chen F, Zheng Y, Zhang S, Feng W. L-3-n-butylphth ide in cogni tive impairment of APP/P ice by rkB/ PI3K/AKT pathway. Int in Exp Med 1706-1713.

17) Pei YH, Chen J, X ai XM RH, Wang X, Gong JB. Hydroxy ts agai myocardial ische epe injury ugh a PI3K/Akt-de ent me m. ators Inflamm 20 16: 1232103.

18) Jiao S P, Teng J. B mic acid protects nst c l ischemia/reperfusion injury by activating t K/Akt signaling pathway. Pharmacoth 6; 84: 1533-1537.

19 ang W, Zhang JQ, Me FM, Xue FS. Dexmedetomidine protects against lung ischemia-reperfusion injury b e PI3K/Akt/HIF-1alpha signaling pathway. J A n 2016; 30: 826-833.

20 o HY, Han The role of PTEN up-regulation pro glomerular mesangial cells proliferation ephritis pathogenesis. Eur Rev Med Pharmacol Sci 2017; 21: 3634-3641.

Li Y, Zhang S, Geng JX, Hu XY. Curcumin inhibits human non-small cell lung cancer A549 cell proliferation through regulation of Bcl-2/Bax and cytochrome C. Asian Pac J Cancer Prev 2013; 14: 4599-4602.

22) Kong CZ, Zhang Z. Bcl-2 overexpression inhibits generation of intracellular reactive oxygen species and blocks adriamycin-induced apoptosis in bladder cancer cells. Asian Pac J Cancer Prev 2013; 14: 895-901.

23) Yang LC, Li J, Xu SF, Cai J, Lei H, Liu DM, Zhang M, Rong XF, Cui DD, Wang L, Peng Y, Wang XL. L-3-n-butylphthalide promotes neurogenesis and neuroplasticity in cerebral ischemic rats. CNS Neurosci Ther 2015; 21: 733-741.

24) Huai Y, Dong Y, Xu J, Meng N, Song C, Li W, Lv P. L-3-n-butylphthalide protects against vascular dementia via activation of the Akt kinase pathway. Neural Regen Res 2013; 8: 1733-1742.

25) Lei H, Zhao CY, Liu DM, Zhang Y, Li L, Wang XL, Peng Y. l-3-n-Butylphthalide attenuates beta-amyloid-induced toxicity in neuroblastoma SH-SY5Y cells through regulating mitochondrion-mediated apoptosis and MAPK signaling. J Asian Nat Prod Res 2014; 16: 854-864.