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Original Article

Paeoniflorin Improves Regional Cerebral Blood Flow and Suppresses Inflammatory Factors in the Hippocampus of Rats with Vascular Dementia*

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ABSTRACT **Objective**: To explore the delayed neuroprotection induced by paeoniflorin (PF), the principal component of *Paeoniae radix* prescribed in Chinese medicine, and its underlying mechanisms in rats subjected to vascular dementia (VD). **Methods**: A rat model of VD was induced by bilateral common carotid arteries occlusion (BCCAO). Low-dose or high-dose PF (20 or 40 mg/kg once per day) was administrated for 28 days after VD. The behavioral analysis of rat was measured by water morris. Regional cerebral blood volume (rCBV), regional cerebral blood flow (rCBF) and mean transit time (MTT) were measured in the bilateral hippocampus by perfusion-weighted imaging (PWI). The levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) were measured by commercially available enzyme-linked immunosorbent assay kits. Protein levels were evaluated by western blot analysis. mRNA levels were evaluated by real time-polymerase chain reaction. Western blotting was used to estimate p65 translocation. **Results**: The behavioral analysis showed that PF could decrease the escape latency time ($P<0.05$), and increase the residence time of the original platform quadrant and the across platform frequency in water maze in VD rats ($P<0.05$). Likewise, PF remarkably promoted the rCBV ($P<0.05$), rCBF and decreased per minute MTT ($P<0.05$) in hippocampus of VD rats. Furthermore, PF decreased the release of IL-1β, IL-6 and TNF-α as well as inhibited the mRNA expression of IL-1β, IL-6 and TNF-α in the hippocampus of VD rats ($P<0.05$ or $P<0.01$). PF also could decrease the protein expressions of inducible nitric oxide synthase and cyclooxygenase-2 in the hippocampus of VD rats ($P<0.05$ or $P<0.01$). In addition, PF significantly inhibited the nuclear factor κB (NF-κB) pathway in the hippocampus of VD rats. **Conclusions**: PF significantly attenuates cognitive impairment, improves hippocampus perfusion and inhibits inflammatory response in VD rats. In addition, the anti-inflammatory effects of PF might be due to inhibiting the NF-κB pathway. PF may be a potential clinical application in improving VD.

KEYWORDS paeoniflorin, vascular dementia, hippocampus, cerebral blood flow

The prevalence of dementia dramatically increases in ageing populations, affecting 1% of 60–64 year olds, and up to 40% of those over age 85 years. While vascular dementia (VD) is one of the most frequent causes of dementia in the elderly, causing a major burden on health care systems in ageing societies. As the second most common form of dementia, VD is a decline in thinking skills and caused by reduced blood flow to the brain, usually induced by chronic cerebral ischemia or series of strokes. Clinically, cognitive dysfunction is the most frequently symptom occuring in the early stage of VD patients. The studies have all reported the frequent involvement of hippocampal damage in the physiopathology of VD in stroke patients.[1,2]

VD is triggered by various vascular disorder, with 80% being ischemic cerebrovascular disease. In patients with VD, intellectual decline is often associated with ischemic brain damage in the form

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of hippocampus lesions. Thus, it seems important to measure cerebral blood flow (CBF) of hippocampus to elucidate the pathophysiology of VD. It has been suggested that improving cerebrovascular lesions could mitigate the symptoms of VD. Future research involved in VD should focus on improving permanent cerebral ischemia.

Chronic inflammatory changes are a common feature of VD, this could result in neurodegenerative changes. Chronic inflammation is involved in a broad spectrum of neurodegenerative diseases. Elevated cytokines and chemokines as well as the accumulation of activated neuron, such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), are found in or near the pathologic lesions of VD. Furthermore, the cytokines could contribute to neuronal damage and cellular death in the initiation and progression of VD.[3]

Nuclear factor κB (NF-κB) is one of the best-characterized transcription factors. It is expressed ubiquitously and regulates the expression of many genes, most of which encode proteins that play an important and often determining role in the processes of inflammation. NF-κB activation results in the secretion of proinflammatory cytokines, which is involved in the degeneration of neurons in the central nervous system.[4] NF-κB could promote the inflammation process by targeting the downstream genes, such as TNF-α, IL-1β, iNOS, and COX-2. Inhibition of NF-κB signaling can ameliorate neurodegeneration and memory impairment.[5] These results indicate that suppression of neuroinflammation and NF-κB pathway may be a beneficial therapy for VD.

Paeoniflorin (PF, Figure 1), the principal component of *Paeoniae radix* ("Shaoyao" in Chinese), has been widely investigated as antioxidant, cognitive enhancer and endothelium-dependent vasodilator.[6,7] Recently, the study found that PF could produce a dose-dependent decrease in both neurological impairment and infarct volume in acute transient cerebral ischemia in a manner different from its classic agonists.[8,9] Therefore, the present study focused on evaluating the neuroprotection of PF during VD development and its possible mechanisms on hippocampus with the goal of identifying therapeutic strategies against VD in the future.

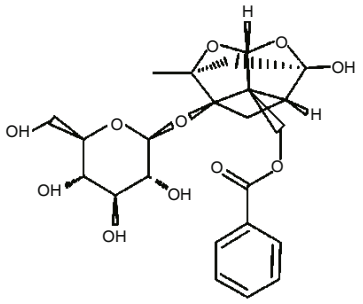

Figure 1. Chemical Structure of PF

METHODS

Animals

Male Sprague Dawley rats (16–18 months, 300–450 g body weight) were used. Animals were housed with free access to food and water at a constant temperature of 22±2 ℃, humidity of 55%±5%, and a 12-h light/dark cycle. This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Provincial Hospital Affiliated to Shandong University.

Materials

PF (purity>98%) was purchased from Guangrun Biotechnology (Nanjing, China). Following primary antibodies (Santa Cruz Biotechnology, Santa Cruz, USA) were used in western blot: anti-β-actin (sc-130656), anti-iNOS (sc-649), anti-COX2 (sc-23983), anti-p65 (sc-372), anti-histone H3 (sc-8654), anti-NF-κB inhibitor α (IκBα, sc-371) and anti-phospho-IκBα (P-IκBα, sc-101713). Enzyme-linked immunosorbent assay (ELISA) kits to measure TNF-α (H052), IL-1β (H002) and IL-6 (H007) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Primers were synthetized by Kangcheng Biotechnology (Guangzhou, China).

Study Design

PF and saline were administered by oral gavage daily for 28 days. Rats were divided into 4 groups (*n*=10 for each group): control group; model group: VD (60 min) + saline (2 mL/kg, once per day for 28 days); low-dose PF group (PFL): PF (20 mg/kg, once per day) was administrated for 28 days after VD; and high-dose PF group (PFH): PF (40 mg/kg, once per day) was administrated for 28 days after VD.

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Establishment of VD

Forty-five rats were deeply anesthetized with mebumal sodium (50 mg/kg) by intraperitoneal injection, and a midline incision was made to expose the bilateral common carotid arteries. Briefly, ischemia was induced by intraluminal filament (18.5±0.5 mm internal diameter) occlusion of the bilateral common carotid arteries (BCCA) for 60 min, while rats in the control group only underwent surgical exposure of the BCCA. Postoperative neurological function was scored on a 5-point scale, where 0 indicated no neurological deficit, 1 (failure to extend left forepaw fully) indicated mild focal neurological deficit, 2 (circling to the left) indicated moderate focal neurological deficit, 3 (falling to the left) indicated severe focal deficit, and 4 (did not walk spontaneously) indicated a depressed level of consciousness. Rats that scored ≥3 points were selected and used for experiments. At 28 days after operation, animals were sacrificed. Five rats were eliminated for death in the surgery process or not successfully modeled, and eventually 40 rats were randomized into four groups as described above.

Water Maze

Rats were trained in a 1.5-m diameter open-field water maze filled with water (26 ℃) and made opaque with latex liquid. Prominent extra-maze visual cues around the room remained in fixed positions throughout the experiment. During behavioral testing, animals were required to locate a hidden submerged platform 10 cm in diameter (1.5 cm below the surface), which remained in the same position across trials for individual animal but was counterbalanced across animals. Four equally spaced points (north, south, east, and west) around the edge of the pool were used as starting positions. The animals were given 4 trials per day for 6 days. Trials began as the rat placed in the pool facing the side wall at a start position and ended once the animal had found the platform; if the rat had not found the platform within 120 s, it was guided there by hand. After a period of 30 s on the platform, the rat was immediately re-placed in the pool at a different start position for the next trial. The latency time, distance and swimming speed of the rats were monitored. A video camera mounted to the ceiling directly above the center of the maze was used in conjunction with the EthoVision animal tracking system.

Perfusion-Weighted Imaging

After completion of the Morris water maze test, magnetic resonance perfusion-weighted imaging (PWI) was performed using a 1.5-Tmagnetic resonance scanner (Intera®; Philips Healthcare, Best, The Netherlands). A 47-mm surface coil was placed on the head region and a bolus injection of gadolinium diethylenetriaminepenta-acetic acid (0.2 mmol/kg) was administered through a tail vein cannula. Regional cerebral blood volume (rCBV), regional cerebral blood flow (rCBF) and mean transit time (MTT) were measured in the bilateral hippocampus, and mean values were calculated.

Measurement of TNFα, IL-1β and IL-6 Levels

IL-1β, IL-6 and TNFα levels were measured by commercially available enzyme-linked immuno sorbent assay (ELISA) kits.

Real-Time Polymerase Chain Reaction

Total RNA of the hippocampus was isolated using Trizol (Invitrogen, USA) and reversed transcribed to cDNA using a reverse transcriptase kit (Takara, Dalian, China). Quantitative real-time polymerase chain reaction (RT-PCR) was performed using ABI 7500 system (Applied Biosystems, USA) by a SYBR green kit (Takara,Dalian, China). The primers are in Table 1.

Western Blot Analysis

Briefly, cytoplasmic and nuclear proteins of the hippocampus were collected using cytoplasmic and nuclear protein extraction kit (Thermo, USA) according to the manufacturer's instruction. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 5% skimmed milk for 1 h and incubated overnight at 4 ℃ with anti-iNOS, anti-COX2,

Table 1. Primers Used in Real-time PCR

Gene	Forward primers (5'-3')	Reverse primers (5'-3')
IL-1β	CACACTAGCAGGTCGTCATCATC	ATGAGAGCATCCAGCTTCAAATC
IL-6	GCCCTTCAGGAACAGCTATGA	TGTCAACAACATCAGTCCCAAGA
TNF-α	CAGAGCAATGACTCCAAAGTA	CAAGAGCCCTTGCCCTAA
β-actin	GTCAGGTCATCACTATCGGCAAT	AGAGGTCTTTACGGATGTCAACGT

anti-NF-κB, anti-IκBα and anti-p-IκBα. Actin was used as a loading control. Subsequently, the membranes were incubated with the corresponding secondary antibodies and the reaction was visualized with chemiluminescence reagents provided with an enhanced chemiluminescence kit (Bioworld, USA) and exposed to X-ray film. The intensity of the blots was quantified with densitometry.

Statistical Analyses

The water Morris was performed with repeated measures and two-way ANOVA followed by a Bonferroni multiple group comparison. For statistical analysis, a standard software package (SAS 10.0) was used. All data were presented as means ± standard error of the mean (SEM). Values of $P<0.05$ were considered statistically significant.

RESULTS

PF Improved Cognitive Impairment in VD Rats

As shown in Figure 2A, the mean escape latency time of the model group was significantly increased compared with the control group ($P<0.01$), while PF-treated VD rats showed significant improvements compared with the model rats after the training periods ($P<0.01$). As shown in Figure 2B, the residence time of the original platform quadrant of the model group was significantly decreased compared with the control group ($P<0.01$), while PF-treated VD rats showed significant ascension compared with the model rats. As shown in Figure 2C, the across platform frequency of the model group was significantly lower compared with the control group ($P<0.01$), and the across platform frequency by the PF-treated VD rats was significantly higher than that of VD rats ($P<0.01$, Figure 2C).

PF Increased rCBF in VD Rats

Data regarding rCBF, rCBV and MTT were presented in Table 2. The model group had lower rCBF and rCBV and higher MTT compared with the control group at 4 weeks after surgery ($P<0.05$). In addition, rCBF and rCBV were significantly higher and MTT was significantly lower in PF groups compared with the model group at 4 weeks after surgery ($P<0.05$ or $P<0.01$).

PF Treatment Decreased the Levels of IL-1β, IL-6 and TNF-α, Down-Regulated the mRNA Expression Levels of IL-1β, IL-6 and TNFα in the Hippocampus

The IL-1β level was 0.79 ± 0.12 pg/mL in the control group, which were much lower than those subjected to model (2.23 ± 0.28 pg/mL). During the 4-week treatment period of PF at the doses of 20 and 40 mg/kg, the IL-1β level decreased to 1.35 ± 0.15 and 0.93 ± 0.12 pg/mL. The IL-6 level was 1.01 ± 0.12 pg/mL in the control group, which were much lower than those subjected to model

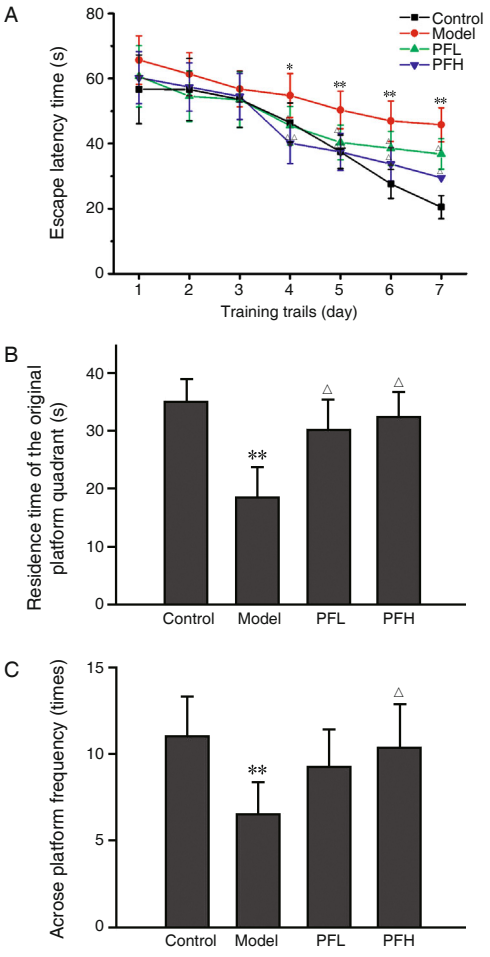

Figure 2. PF Attenuates Learning and Memory Impairment in VD Rats in the Morris Water Maze Test (n=7, $\bar{x} \pm$ SEM)

Notes: A: Escape latency time for escape to a submerged platform in the training trails; B: Residence time of the original platform quadrant in the probe trail; C: Across platform frequency in the probe trail; $^{*}P<0.05$, $^{**}P<0.01$, compared with the control group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, compared with the model group.

Table 2. Results of rCBV, rCBF and MTT in Rats Hippocampus of Four Groups ($\bar{x} \pm$ SEM)

Group	n	rCBV (mL/100 g)	rCBF (mL/100 g)	per min MTT (s)
Control	5	13.68 ± 2.67	3.29 ± 0.58	4.48 ± 0.63
Model	5	7.13 ± 1.06*	1.36 ± 0.29*	6.95 ± 1.27*
PFL	5	10.35 ± 1.74$^{\triangle}$	2.15 ± 0.28$^{\triangle}$	5.85 ± 0.84$^{\triangle}$
PFH	5	11.54 ± 1.68$^{\triangle}$	2.85 ± 0.43$^{\triangle\triangle}$	5.34 ± 0.68$^{\triangle}$

Notes: $^{*}P<0.01$, compared with the control group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, compared with the model group

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(2.40 ± 0.38 pg/mL). After PF treatment, the IL-6 level decreased to 1.55 ± 0.23 and 1.3 ± 0.15 pg/mL. The TNF-α level was 1.86 ± 0.16 ng/mL in the control group, which were much lower than those subjected to the model (1.8 ± 0.28 ng/mL). After PF treatment, the TNF-α level decreased to 1.35 ± 0.15 and 1.27 ± 0.15 ng/mL (Figure 3).

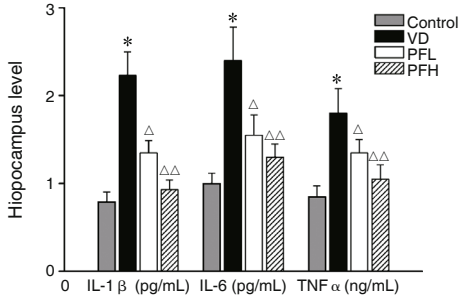

Figure 3. Effects of PF on the Levels of IL-1β, IL-6 and TNF-α in Hippocampus of VD Rat (*n*=7, $\bar{x}$ ± SEM)

Notes: *P<0.01, compared with the control group; △P<0.05, △△P<0.01, compared with the model group

The mRNA levels of IL-1β, IL-6 and TNFα in the model group were respectively 2.5, 4.1 and 7.1 times higher than those in the control group. However, after PF treatment for 28 days, the mRNA levels of IL-1β, IL-6 and TNFα were significantly decreased than those in the model group (P<0.05 or P<0.01, Figure 4).

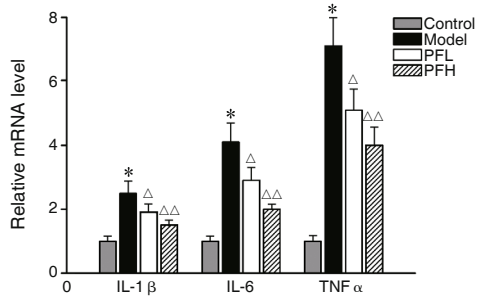

Figure 4. Effects of PF on the mRNA Levels of IL-1β, IL-6 and TNFα in Hippocampus of VD Rat by RT-PCR (*n*=4, $\bar{x}$ ± SEM)

Notes: *P<0.01, compared with the control group; △P<0.05, △△P<0.01, compared with the model group

PF Treatment Decreased the Relative Protein Levels of iNOS and COX-2 in the Hippocampus

The iNOS mRNA level in the model group was 4.8 times higher than that in control group. The COX-2 mRNA level in the model group was 2.3 times higher than that in the control group. After PF treatment for 28 days, the protein levels of iNOS and COX-2 were significantly decreased than those in the model group (P<0.05 or P<0.01, Figure 5).

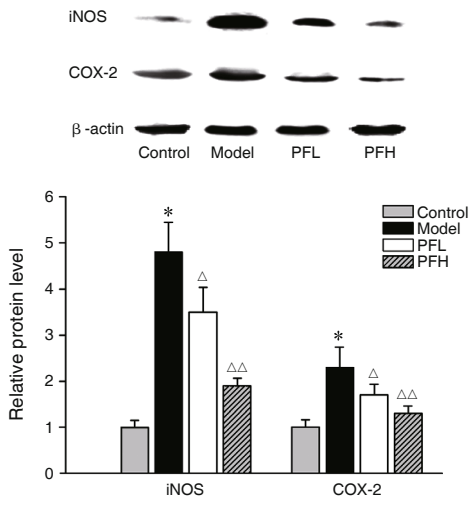

Figure 5. Effects of PF on the Protein Levels of iNOS and COX-2 in Hippocampus of VD Rat by Western Blotting (*n*=4, $\bar{x}$ ± SEM)

Notes: *P<0.01 compared with the control group, △P<0.05, △△P<0.01, compared with the model group

PF Inhibited VD-Induced Activation of NF-κB p65 Signaling Pathway in the Hippocampus

As shown in Figure 6, in the cytoplasm, the expression of p65 and IκBα were decreased in the model group than those of in the control group. The expression of P-IκBα were increased in the model group than those in the control group. After PF treatment for 28 days, the protein levels of p65 and IκBα were increased and P-IκBα were decreased than those in the model group. In the nucleus, the expression of p65 was increased in the model group than that in the control group. After PF treatment, the protein level of p65 was decreased than that in the model group. These studies revealed that PF inhibited the VD-induced degradation of IκBα and translocation of NF-κB p65. Moreover, the phosphorylation of IκBα was significantly increased in hippocampus of VD rats. PF treatment could decrease the phosphorylation of IκBα in the hippocampus of VD rats.

DISCUSSION

In the current study, we have demonstrated that PF, when administered to rats for 28 days after VD, evokes significant improvement in functional neurologic recovery. In the present study, we found that PF attenuated memory impairment in VD rats. Furthermore, PF could improve cerebral blood flow and suppress the inflammatory response, and the underlying mechanism might be associated with the inhibition of NF-κB pathway.

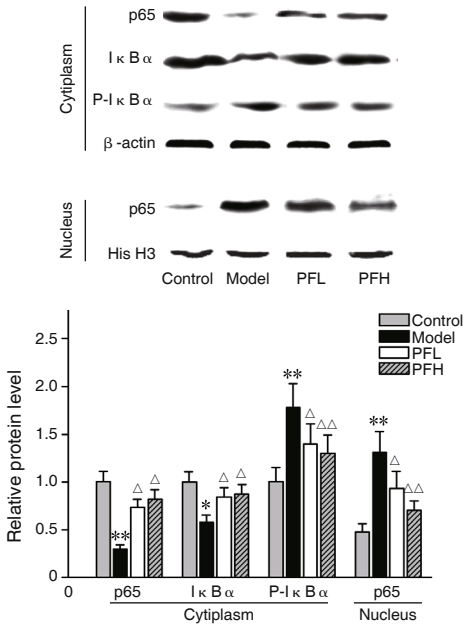

Figure 6. PF Inhibits NF-κB Activation in Hippocampus of VD Rat by Western Blotting (*n*=4, $\bar{x} \pm$ SEM)

Notes: $^{*}P<0.05$, $^{**}P<0.01$, compared with the control group; $^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$, compared with the model group

Cognitive impairment has been demonstrated in animals with chronic cerebral hypoperfusion and modeling patients with VD.[10,11] The reduction in CBF occurs in the early stages of vascular disease and it is possible for a variable period of chronic ischemia to precede a major vascular event.[12] This can result in the brain's cellular malfunction and degeneration, consequent significant hippocampal dysfunction and neuronal loss. In our study, treatment with PF increased rCBF and rCBV and improved regional tissue perfusion in the hippocampus of rats with VD.

Growing data from basic and clinical studies indicate that neuro-inflammation is involved in neuronal degeneration. It has been reported that the levels of inflammatory cytokines are significantly elevated in brains of VD patients, which suggests that inflammation might contribute to the pathogenesis of VD.[13] VD-induced overproduction of cytokines could lead to neuronal dysfunction. Therefore, inhibiting the production of proinflammatory cytokines may contribute to neuroprotection. In the present study, the levels of IL-1β, IL-6 and TNF-α were significantly decreased in the PF-treated rats compared with VD rats. Consistently, PF also could inhibit the mRNA levels of IL-1β, IL-6 and TNF-α, and the protein levels of iNOS and COX-2 induced by VD. Collectively, the current findings suggested that PF modulated inflammatory response induced by VD.

The NF-κB family of transcription factors is a central player in the regulation of inflammation and immune responses. Consequently, NF-κB dysregulation has been implicated in diverse human pathologies. NF-κB has been regarded as the key regulator of inflammatory processes, and many studies have showed that suppression of NF-κB pathway ameliorates the neuroinflammation.[14,15] Activation or inhibition of the NF-κB signaling pathway prevented IκB degradation, a requirement for p65 translocation to the nucleus.[16,17] The activity of NF-κB is primarily regulated by interaction with inhibitory IκB proteins. In most unactivated cells, IκBα-containing complexes constantly shuttle between the nucleus and the cytoplasm, whereas IκBβ- and e-containing complexes are predominantly cytoplasmic. Upon stimuli, IκB is phosphorylated by IκB kinase (IKK), which then is ubiquitinated and subsequent degraded, leading to translocation of NF-κB to the nucleus and binding to specific target genes, and increased the expression of proinflammatory factors.[18] This study demonstrated that PF significantly inhibited NF-κB p65 nuclear translocation by attenuating IκBα phosphorylation and degradation *in vivo*.

In conclusion, we demonstrated that PF significantly attenuates cognitive impairment, improves hippocampus perfusion and inhibits inflammatory response in VD rats. In addition, the anti-inflammatory effects of PF might be due to inhibiting the NF-κB pathway. These results suggest that PF may be a potential clinical application in improving VD.

Conflict of Interest

All the authors do not have any possible conficts of interest.

Author Contributions

Zhang LG, Wang LJ preformed the data analysis and wrote the manuscript; Zhang Y, Zhang SC, Shi CG helped conceive and design the experiments; Shen QQ and Wang HF performed the animal model preparation, behavioral test and daily drug intervening; Zhang MY helped perform the analysis with constructive discussions.

REFERENCES

1. Viswanathan A, Rocca WA, Tzourio C. Vascular risk factors and dementia: how to move forward? Neurology

2009;72:368-374.
2. Shi CG, Yang YS, Li H, Zhang Y, Wang N, Wang SM, et al. J Asian Nat Prod Res 2014;16:667-676.
3. Ernst A, Buerger K, Hartmann O, Dodel R, Noelker C, Sommer N, et al. J Neuroimmunol 2010; 221(1-2):62-67.
4. Gasparini C, Feldmann M. NF-κB as a target for modulating inflammatory responses. Curr Pharm Des 2012;18:5735-5745.
5. Kim HG, Moon M, Choi JG, Park G, Kim AJ, Hur J, et al. Donepezil inhibits the amyloid-beta oligomer-induced microglial activation *in vitro* and *in vivo*. Neurotoxicology 2014;40:23-32.
6. Tabata K, Matsumoto K, Watanabe H. Paeoniflorin, a major constituent of peony root, reverses muscarinic M1-receptor antagonist-induced suppression of long-term potentiation in the rat hippocampal slice. Jpn J Pharmacol 2000;83:25-30.
7. Xiao L, Wang YZ, Liu J, Luo XT, Ye Y, Zhu XZ. Effects of paeoniflorin on the cerebral infarction, behavioral and cognitive impairments at the chronic stage of transient middle cerebral artery occlusion in rats. Life Sci 2005;78:413-420.
8. Chen DM, Xiao L, Cai X, Zeng R, Zhu XZ. Involvement of multitargets in paeoniflorin-induced preconditioning. J Pharmacol Exp Ther 2006;319:165-180.
9. Guo RB, Wang GF, Zhao AP, Gu J, Sun XL, Hu G. Paeoniflorin protects against ischemia-induced brain damages in rats via inhibiting MAPKs/NF-κB-mediated inflammatory responses. PLoS One 2012;7:e49701.
10. Al-Qazzaz NK, Ali SH, Ahmad SA, Islam S. Cognitive assessments for the early diagnosis of dementia after stroke. Neuropsychiatr Dis Treat 2014;10:1743-1751.
11. Choi BR, Lee SR, Han JS, Woo SK, Kim KM, Choi DH, et al. Synergistic memory impairment through the interaction of chronic cerebral hypoperfusion and amlyloid toxicity in a rat model. Stroke 2011;42:2595-2604.
12. D'Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. Brain Res Rev 2001;36:60-90.
13. Rosenberg GA, Bjerke M, Wallin A. Multimodal markers of inflammation in the subcortical ischemic vascular disease type of vascular cognitive impairment. Stroke 2014;45:1531-1538.
14. Sharma B, Singh N. Salutary effect of NFκB inhibitor and folacin in hyperhomocysteinemia-hyperlipidemia induced vascular dementia. Prog Neuropsychopharmacol Biol Psychiatry 2012;38:207-215.
15. Zhang J, Zhen YF, Pu-Bu CR, Song LG, Kong WN, Shao TM, et al. Salidroside attenuates beta amyloid-induced cognitive deficits via modulating oxidative stress and inflammatory mediators in rat hippocampus. Behav Brain Res 2013;244:70-81.
16. Min KJ, Cho KH, Kwon TK. The effect of oxidized low density lipoprotein (oxLDL)-induced heme oxygenase-1 on LPS-induced inflammation in RAW 264.7 macrophage cells. Cell Signal 2012;24:1215-1221.
17. Xie C, Kang J, Ferguson ME, Nagarajan S, Badger TM, Wu X. Blueberries reduce pro-inflammatory cytokine TNF-α and IL-6 production in mouse macrophages by inhibiting NF-κB activation and the MAPK pathway. Mol Nutr Food Res 2011;55:1587-1591.
18. Hayden MS, Ghosh S. Signaling to NF-κB. Genes Dev 2004;18:2195-2224.

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