Research Progress on Neuroprotective Effects of Salvianolic Acid B in an Animal Model of Parkinson's Disease

Jie ZHOU*, Tong ZHU, Changdong LI, Yaqiang NAN, Li CAO

Department of Neurosurgery, 940th Hospital of Joint Logistics Support Force, Lanzhou 730050, China

Abstract As an active ingredient extracted from *Salvia miltiorrhiza*, the neuroprotective effects of salvianolic acid B in Parkinson's disease include antioxidation, improvement of mitochondrial function, modulation of neuroinflammation, inhibition of apoptosis, promotion of neuronal differentiation and proliferation, and influence on intestinal flora. As an adjuvant drug, salbutamol B can be used in combination with conventional therapeutic drugs to enhance the efficacy and minimize the side effects, which provides a method and basis for the early diagnosis and treatment of Parkinson's disease in clinical practice.

Key words Parkinson's disease, Salvianolic acid B, Neuroprotection, Antioxidant, Modulation of neuroinflammation, Inhibition of apoptosis, Improvement of mitochondrial function

1 Introduction

As a progressive neurodegenerative disease, Parkinson's disease (PD) has a high prevalence in the elderly population, with an average age of onset around 65 years[1]. The main pathologic change in PD is the degenerative death of dopamine (DA) neurons in the midbrain substantia nigra, resulting in a significant reduction in striatal dopamine content[2]. Although the exact etiology is unknown, genetic factors, environmental problems, ageing, oxidative stress, *etc.* may be involved[3]. The main symptoms of PD include slow movement, involuntary trembling of the limbs or body, and rigidity of the body, which makes it difficult for the patient to bend the body[4]. As the fourth most common neurodegenerative disease among the elderly, PD has a prevalence of 0.4% in people over 40 years of age. Although physiologic DA neurons degeneration decreases with age, only a small number of the elderly population suffers from this disease, suggesting that aging is only a precipitating factor in the onset of PD. Although genetic factors are also believed to be related to the occurrence of PD, less than 10% of patients have a family history[4]. Currently, PD is treated with drugs and surgery, but there is no cure[5]. As a common neurodegenerative disease, the etiology and pathogenesis of PD still need further research.

Salvianolic acid B (Sal B) is a phenolic acid compound extracted from the traditional Chinese medicine *Salvia miltiorrhiza*, which has a variety of pharmacological action. In the study of PD, Sal B has attracted much attention due to its potential neuroprotective effects[6]. In terms of antioxidant effects, Sal B has multiple phenolic hydroxyl groups, which gives Sal B an advantage in antioxidant activity. Besides, Sal B has a significant effective in scavenging free radicals and reducing cellular damage caused by oxidative stress[7]. In terms of anti-inflammatory effects, Sal B can alleviate inflammation by regulating the expression of inflammation-related factors, thus inhibiting the inflammatory response[8]. In terms of neuroprotection, Sal B has been proven to protect dopaminergic neurons and reduce neuronal death[9]. In terms of improving mitochondrial function, Sal B can improve mitochondrial function, increase cellular energy metabolism and antioxidant capacity[10]. In terms of inhibiting apoptosis, Sal B can inhibit apoptosis through multiple pathways and prolong cell survival time[11]. In PD, Sal B has been proven to reduce the death of nigrostriatal dopaminergic neurons, improve movement disorders, and enhance the quality of life, and its mechanism of action involves a variety of aspects including antioxidant, anti-inflammatory, and mitochondrial function improvement[12].

Although the potential therapeutic effect of Sal B in PD is encouraging, there is still a lack of sufficient human clinical trial data to support its widespread clinical application. More clinical studies are needed to validate its effectiveness and safety in the treatment of neurodegenerative diseases such as PD.

2 Pharmacologic effects of Sal B

As an active ingredient extracted from *Salvia miltiorrhiza*, Sal B is widely used in traditional Chinese medicine and modern medicine[6]. The main pharmacological effects of Sal B are shown below:

2.1 Anticoagulation Sal B inhibits platelet aggregation and thrombosis and exerts its anticoagulant effect by inhibiting thrombin and fibrin formation, thus improving blood flow[13].

2.2 Anti-inflammatory Sal B can inhibit inflammatory signaling pathways, such as blocking the release of inflammatory mediators and inhibiting the activation of inflammatory cells, reducing the inflammatory response[8].

2.3 Antioxidant Sal B has antioxidant activity, and it can neutralize free radicals and reduce cellular oxidative stress, thus protecting cells from oxidative damage[7].

Received: May 10, 2024 Accepted: July 5, 2024

Supported by Research on the Neuroprotective Mechanism of Salvianolic Acid B on Parkinson's Disease; Funded Project of Gansu Province Health Industry Scientific Research Program (GSWSKY2018-43); Mechanism Research on the Regulation of Antioxidant Dysregulation in Parkinson's Disease Model by Salvianolic Acid B through Nrf2-ARE Signaling Pathway; Hospital Graduate Student Supervisor Special Project (Hospital Health [2022] yxky011); Mechanism and Clinical Efficacy Study on Treatment of Parkinson's Disease by Exenatide Combined with Deep Brain Electrical Stimulation; Science and Technology Plan Project of Lanzhou Science and Technology Bureau (2023-ZD-167).

* Corresponding author. E-mail: zhoujie7089@163.com

2.4 Vasodilation Sal B can promote the release of nitric oxide (NO) from vascular endothelial cells, which in turn leads to vasodilation and improved blood flow[13].

2.5 Myocardial protection Sal B protects cardiomyocytes from ischemia-reperfusion injury, reduces myocardial infarct size, and improves myocardial function[13].

2.6 Anti-tumor Sal B shows potential anti-tumor activity against some tumor cells by inhibiting proliferation, inducing apoptosis and anti-angiogenesis[14].

3 Pathogenesis of PD

PD is a progressive neurological disorder with a multifaceted pathomechanism. The main pathological mechanisms of PD are shown below:

3.1 Loss of dopamine beurons The main feature of PD is the progressive degeneration of dopaminergic neurons in the substantia nigra. This degeneration leads to a decrease in the production of dopamine in the brain, which in turn affects motor control[15].

3.2 Oxidative stress and impaired mitochondrial function Oxidative stress is present in PD, whereby the intracellular production of harmful free radicals and oxidants exceeds antioxidant defenses. This can lead to impaired mitochondrial function, affecting nerve cell energy production and increasing the risk of cell death[16].

3.3 Inflammatory responses Inflammatory responses are present in the brain tissue of patients with PD, including activated neuroglia and the production of inflammatory mediators. These inflammatory responses may play a role in maintaining or accelerating the loss of dopamine neurons[17].

3.4 Abnormal protein metabolism PD is associated with abnormal protein metabolism in which one protein, called alpha-protein, plays a key role in the survival and function of dopamine neurons. Abnormal protein accumulation may lead to cell death and impaired nervous system function[18].

These pathologic mechanisms interact to cause the neurological problems of PD, such as symptoms of muscle rigidity, slow movement, tremor, and imbalance[4]. However, the exact cause of PD is still not fully understood, so research into the disease is still ongoing to further explore deeper pathological mechanisms and develop more effective treatments[5].

4 Mechanism of neuroprotective effect of Sal B in PD

4.1 Antioxidant In order to regulate intracellular redox signaling and drive key factors for tissue cell repair, the right amount of reactive oxygen species is necessary, however, excessive reactive oxygen species can lead to an imbalance in homeostasis between intracellular oxidation and antioxidant, resulting in the loss of function of important molecules of the tissues and cells[19]. The high energy and oxygen consuming metabolic properties of the brain, along with other intrinsic factors such as its weak antioxidant capacity, result in a higher probability of neurons and glial cells receiving oxidative stress damage[20]. In order to reduce the probability of neurons and glial cells receiving oxidative stress damage, we can start from the inhibition and elimination of free radicals, and Sal B as a natural antioxidant in the nervous system can inhibit and scavenge free radicals, inhibit the activity of oxidative enzymes that catalyze the generation of reactive oxygen species, and enhance the activity of antioxidant enzymes, and at the same time, promote the generation of endogenous antioxidants[21]. It was shown that pretreatment of SH-SY5Y cells with Sal B significantly reduced 6-hydroxy dopamine (6-OHDA)-induced reactive oxygen species production and blocked the increase in intracellular calcium ions induced by 6-OHDA. The data indicated that 6-OHDA-induced apoptosis was reversed by Sal B treatment; furthermore, Sal B reduced the activation of extracellular signal-regulated kinases and induced 6- OHDA-inhibited activation of protein kinase C. Thus, Sal B is one of the effective treatments for PD[22].

It was also found that, Sal B could activate antioxidant-related pathways, exert neuroprotective effects, and preserve dopaminergic neuron function in PD models *in vivo*, improving motor dysfunction in Parkinson's animal models[23].

4.2 Modulation of neuroinflammation In PD, inflammation is the main driving factor for its occurrence, and the activation of microglia plays an irreplaceable role in its pathological response mechanism[24]. And Sal B can play a dual protective role against neuroinflammatory injury in vitro and in vivo by activating the antioxidant signaling pathway, and inhibit the release of pro-inflammatory cytokines in microglia to regulate neuroinflammation, and increase the expression and release of glial cell-derived neurotrophic factor in astrocytes, which can play a neuroprotective role[23].

4.3 Promote neuronal differentiation and proliferation In response to the application of the technology of converting the body's own mature cells into induced pluripotent stem cells (iPSCs) in PD, it has been shown that iPSC-induced neurons and DA differentiated from neuroglia with midbrain characteristics improve the behavior of a PD model after transplantation into the brain of the PD model[25]. However the neural differentiation of iPSC is not fast enough, limiting its use in PD. It was found that the incorporation of Sal B into the neural differentiation process of iPSCs could effectively increase the efficiency of iPSC differentiation into neural stem cells and promote the further differentiation and proliferation of neural stem cells because Sal B activated the AKT/GSK3β signaling pathway.[26]

4.4 Inhibition of apoptosis In the treatment of PD, inhibition of neuronal apoptosis is required because of the limited regenerative capacity of central nervous tissue cells. Studies have shown that Sal B prevents 6-OHDA-induced apoptosis in SH-SYSY cells, another cell model of PD, by decreasing 6-OHDA-induced caspase-3 activity and cytochrome c release, which inhibits neuronal apoptosis[22]. In addition to this, Sal B protected PC12 cells from hydrogen peroxide-induced cytotoxicity by reducing apoptosis and loss of superoxide dismutase, catalase and glutathione peroxidase activities, as well as inhibiting malondialdehyde production, lactate dehydrogenation release, and intracellular Ca^{2+} levels and caspase-3 activity, suggesting that Sal B is neuroprotective against some of these toxicities[27].

4.5 Improvement of mitochondrial function Mitochondria are the energy source of cells, and their abnormal oxidative phos-

phorylation function as well as abnormal or delayed activation of the mitochondrial unfolded protein response (UPRmt) after injury is an important reason for the loss of neuronal function[28], and mitochondrial dysfunction is one of the important causative factors of PD. Studies have shown that Sal B attenuates the decrease in mitochondrial membrane potential and the decrease in mitochondrial ATP synthesis caused by 1-methyl-4-phenylpyridinium ions, and increases the expression of mitochondrial DNA and PGC-1, NRF-1, TFAM genes as well as mitochondrial fusion-associated proteins after 1-methyl-4-phenylpyridinium ions treatment as well as inhibits mitochondrial fusion-associated proteins after the damage[29], therefore Sal B may regulate mitochondrial function by regulating the mitochondrial fusion-related proteins. Sal B may achieve benign biological effects on mitochondrial function repair by regulating mitochondrial biogenesis and mitochondrial fusion-related proteins, thus playing a neuroprotective role in PD.

4.6 Influence on intestinal flora There are multiple pathways of bidirectional communication of biological information between the intestinal flora and the brain, and the intestinal flora and its metabolites play a very important role in neurogenesis, neuroimmunity and neuroendocrinology.[30] Therefore, there exists a very close link between the intestinal flora and the occurrence of PD, and there exists a possibility that abnormalities in the immune function caused by abnormalities of the intestinal flora can trigger the degeneration of Parkinson's dopaminergic neurons. Degeneration of dopaminergic neurons in PD[31]. It has been shown that Sal B reduces serum MDA levels and increases the number and density of gut flora with anti-inflammatory and lipopolysaccharide production inhibition functions. In summary, Sal B can affect the species and density of gut microorganisms, but it is still questionable whether this effect is direct, and more in-depth studies are needed on the effects of Sal B on gut flora[32].

5 Clinical applications of Sal B in PD

5.1 Analysis of the efficacy of Sal B in the treatment of PD Currently, the main therapeutic drugs for PF are dopamine drugs, anticholinergic drugs, *etc.* However, the long-term use of these drugs may cause problems such as reduced efficacy and increased side effects[33]. As a herbal extract with neuroprotective effects, Sal B can be used as an adjuvant drug in combination with conventional therapeutic drugs to enhance the efficacy and reduce the side effects. Clinical studies have shown that Sal B combined with dopamine can significantly improve the motor function and life quality of patients with PD, and Sal B can also reduce the side effects caused by dopamine such as anisotropia.

Combining clinical practice and knowledge of the mechanism of PD, it can be suggested that the main cause of PD is located in the brain, and its pathological state is a deficiency of kidney essence, which cannot nourish the brain marrow, and there is stasis of blood in the body, which leads to poor blood circulation, so it is suggested that the root cause of PD lies in the deficiency of the kidneys[34]. According to existing research, *Salvia miltiorrhiza*, one of the most frequently used Chinese medicines, is found to play a role in activating blood circulation and removing blood stasis, as well as having a neuroprotective effect[34].

5.2 Efficacy and safety Sal B is often used as a pharmaceutical component in PD in clinical practice, and it has a variety of clinical efficacy and applications. With its vasoconstrictive and platelet aggregation increasing functions, Sal B can be used in the regulation of acute bleeding and hemostatic processes, and can promote the healing process of skin wounds[13]. Its astringent effect can narrow blood vessels and reduce exudation, thus accelerating the healing and repair of wounds[13]. Sal B inhibits the inflammatory response and reduces inflammatory cell-mediated injury and release of inflammatory factors[8]. It also possesses antibacterial and antiviral activity and reduces inflammatory symptoms by inhibiting the release of inflammatory mediators and the onset of inflammatory responses. Sal B has significant antioxidant activity that neutralizes free radicals and attenuates oxidative stress, and has potential in the prevention and treatment of oxidative damage associated with chronic diseases[7].

The clinical efficacy and recommended dosage of Sal B may vary depending on the specific disease, individual differences and drug compounding. In addition, Sal B, as an ingredient of traditional Chinese medicine, is often used as a component of Chinese medicinal preparations, and the specific efficacy of Sal B needs to be evaluated in combination with the drug combination and the specific formulation to comprehensively assess the therapeutic effect.

6 Summary and prospects

Sal B is an antioxidant among the most abundant active salvianolic acids in *Salvia miltiorrhiza*, which has clinical applications in PD as a herbal extract with neuroprotective effects. This article provides an overview of the pharmacological effects of Sal B and the pathological mechanisms of PD, and summarizes the mechanism of neuroprotective effects of Sal B in PD in six aspects: antioxidation, improvement of mitochondrial function, modulation of neuroinflammation, inhibition of apoptosis, promotion of neuronal differentiation and proliferation, and influence of intestinal flora. Meanwhile, efficacy analysis, curative efficacy and safety analysis were conducted on the clinical application of Sal B in PD.

Clinical studies have shown that Sal B combined with conventional drugs in the treatment of PD can improve the efficacy and reduce the side effects[34]; Sal B given preoperatively and postoperatively can reduce the incidence of surgical complications and shorten the postoperative recovery time. However, the current quantity and quality of clinical studies on the application of Sal B in PD are still limited, and further clinical trials with large samples and long-term follow-up are needed to verify its efficacy and safety. In addition, further in-depth studies on the mechanism of action of Sal B are also needed to provide a more reliable basis for its treatment in PD and other neurodegenerative diseases. Combination application of Sal B with other neuroprotective drugs or therapeutic methods is considered to enhance the therapeutic effect and improve the life quality of patients. Since specific diseases, individual differences and drug combinations can affect the dosage of Sal B, research on the improvement of dosage forms and admin-

istration routes of Sal B also needs to be further strengthened in order to better meet the clinical needs.

References

[1] SONG LP, WANG Q, ZHANG T, *et al.* Rehabilitation for Parkinson's disease:Chinese expert consensus[J]. Chinese Journal of Rehabilitation Theory and Practice, 2018, 24(7): 745 – 752. (in Chinese).

[2] PUSPITA L, CHUNG SY, SHIM JW. Oxidative stress and cellular pathologies in Parkinson's disease[J]. Molecular Brain, 2017, 10(1): 1 – 12.

[3] TOLLESON CM, FANG JY. Advances in the mechanisms of Parkinson's disease[J]. Discovery Medicine, 2013, 15(80): 61 – 66.

[4] HURBEN AK, TRETYAKOVA NY. Role of protein damage inflicted by dopa-mine metabolites in Parkinson's disease: Evidence, tools, and outlook[J]. Chemical Research in Toxicology, 2022, 35(10): 1789 – 1804.

[5] LUO XD, ZHENG CH, SU QZ. Parkinson's disease Chinese medicine understanding and diagnosis and treatment strategy[M]. Beijing:China Press of Traditional Chinese Medicine Co., Ltd., 2018. (in Chinese).

[6] TANG MK, REN DC, ZHANG JT, *et al.* Effect of salvianolic acids from radix salviae miltiorrhizae on regional cerebral blood flow and platelet aggregation in rats[J]. Phytomedicine, 2002(9): 405 – 409.

[7] TAO S, DUAN R, XU T, *et al.* Salvianolic acid B inhibits the progression of liver fibrosis in rats via modulation of the hedgehog signaling pathway[J]. Experimental and Therapeutic Medicine, 2022, 23(2): 116.

[8] CHEN T, LIU W, CHAO X, *et al.* Salvianolic acid B attenuates brain damage and inflammation after traumatic brain injury in mice[J]. Brain Research Bulletin, 2011, 84(2): 163 – 168.

[9] CAO W, GUO XW, ZHENG HZ, *et al.* Current progress of research on pharmacologic actions of salvianolic acid B[J]. Chinese Journal of Integrative Medicine, 2012, 18(4): 316 – 320.

[10] HE Y, JIA K, LI L, *et al.* Salvianolic acid B attenuates mitochondrial stress against Aβ toxicity in primary cultured mouse neurons[J]. Biochemical and Biophysical Research Communications, 2018, 498(4): 1066 – 1072.

[11] FU J, FAN HB, GUO Z, *et al.* Salvianolic acid B attenuates spinal cord ischemia-reperfusion-induced neuronal injury and oxidative stress by activating the extracellular signal-regulated kinase pathway in rats[J]. Journal of Surgical Research, 2014, 188(1): 222 – 230.

[12] NAN YQ, TAO JW, ZHOU J. Progress of the protective effect of salvianolic acid B against neurodegenerative diseases[J]. Medical & Pharmaceutical Journal of Chinese People's Liberation Army, 2021, 33(1): 112 – 116. (in Chinese).

[13] ZHANG AT, WANG CG, YAO T, *et al.* Protective mechanism and apoptosis related protein of salvianolic acid B induced angiogenesis on myocardial ischemia-reperfusion injury in rats[J]. Guiding Journal of Traditional Chinese Medicine and Pharmacy, 2021, 27(6): 20 – 24. (in Chinese).

[14] WANG GH, YANG XT, YANG GS. Effect and possible mechanism of salvianolic acid B on radiosensitivity of non-small cell lung cancer[J]. Chinese Journal of Radiation Oncology, 2021, 30(12): 1304 – 1308. (in Chinese).

[15] LI FJ, XU RY. Progress in the study of possible mechanisms of dopaminergic neuronal degeneration and clinical manifestations in Parkinson's disease[J]. Practical Clinical Medicine, 2012, 13(1): 135 – 138. (in Chinese).

[16] LIANG Y, YANG H. Effects of mitochondria and alpha-synuclein on pathogenesis of Parkinson disease[J]. Chinese Journal of Clinical Rehabilitation, 2005, 9(9): 148 – 150. (in Chinese).

[17] SUN SG, LI G, CAO XB, *et al.* Research on relation between Parkinson's disease and inflammatory reaction[J]. Chinese Journal of Neurology, 2003, 36(6): 401 – 403. (in Chinese).

[18] YAN JN, ZHANG HY, JIANG YC, *et al.* Correlation of serum alpha-synuclein and autophagy-related proteins with Parkinson's disease[J]. Medical Journal of Communications, 2022, 36(3): 240 – 242. (in Chinese).

[19] SIES H, BERNDT C, JINES D P. Oxidative stress[J]. Annual Review of Biochemistry, 2017(86): 715 – 748.

[20] YU JT, ZHANG AR. Research progress of near-infrared spectroscopy cerebral oxygen saturation monitoring in surgery[J]. Chinese Science and Technology Journal Database (Citation Edition) Medicine and Health, 2022(3): 67 – 70. (in Chinese).

[21] FAN Y, LUO Q, WEI J, *et al.* Mechanism of salvianolic acid B neuroprotection against ischemia/reperfusion induced cerebral injury[J]. Brain Research, 2018(1679): 125 – 133.

[22] TIAN LL, WANG XJ, SUN YN, *et al.* Salvianolic acid B, an antioxidant from *Salvia miltiorrhiza*, prevents 6-hydroxydopamine induced apoptosis in SH-SY5Y Cells[J]. International Journal of Biochemistry and Cell Biology, 2008, 40(3): 409 – 422.

[23] ZHOU J, QU XD, LI ZY, *et al.* Salvianolic acid B attenuates toxin-induced neuronal damage via Nrf2-dependent glial cells-mediated protective activity in Parkinson's disease models[J]. PLoS One, 2014, 9(7): e101668.

[24] ZHU RF, ZHANG Y, LU YM. The role of microglia and microglia-mediated neuroinflammation in the pathogenesis of Parkinson's disease[J]. Journal of Nanjing Medical University (Natural Sciences), 2023, 43(4): 569 – 576. (in Chinese).

[25] WERNIG M, ZHAO JP, PRUSZAK J, *et al.* Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease[J]. Proceedings of the National Academy of Sciences of the United States of America, 2008, 105(15): 5856 – 5861.

[26] SHU T, LIU C, PANG M, *et al.* Salvianolic acid B promotes neural differentiation of induced pluripotent stem cells VIA PI3K/AKT/GSK3β/β-catenin pathway[J]. Neuroscience Letters, 2018(671): 154 – 160.

[27] ZENG G, TANG T, WU HJ, *et al.* Salvianolic acid B protects SH-SY5Y neuroblastoma cells from 1-methyl-4-phenylpyridinium-induced apoptosis[J]. Biological &Pharmaceutical Bulletin, 2010, 33(8): 1337 – 1342.

[28] SHPILKA T, HAYNES CM. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing[J]. Nature Reviews in Molecular Cell Biology, 2018, 19(2): 109 – 120.

[29] YANG JL, YANG X. Protective effects of salvianolic acid B on MPP^+-induced mitochondrial injury in PC12 cells[J]. Academic Journal of Second Military Medical University, 2015, 36(6): 685 – 689. (in Chinese).

[30] CRYAN JF, ORIORDAN KJ, COWAN CSM, *et al.* The Mi-crobiota-Gut-Brain axis[J]. Physiological Reviews, 2019, 99(4): 1877 – 2013.

[31] MATHEOUD D, CANNON T, VOISIN A, *et al.* Itestinal infection triggers Parkinson's disease-like symptoms in pink1-/-mice[J]. Nature, 2019, 571(7766): 565 – 569.

[32] ZHAO MH, FENG SN, YIN YW, *et al.* Effect of salvianolic acid B on antioxidant capacity and intestinal microflora in mice[J]. Food Science, 2020, 41(9): 105 – 112. (in Chinese).

[33] LUO XD, ZHENG CH, SU QZ. Chinese medicine understanding and treatment strategy of Parkinson's disease[M]. Beijing: China Press of Traditional Chinese Medicine Co., Ltd., 2018. (in Chinese).

[34] LUAN ZX, PAN Y, CHEN YF, *et al.* Medication rules of Bushen Huoxue in treatment of Parkinson's disease[J]. Chinese Journal of Geriatric Heart, Brain and Vessel Diseases, 2023, 25(2): 130 – 133. (in Chinese).