Danshensu protects against 6-hydroxydopamine-induced damage of PC12 cells *in vitro* and dopaminergic neurons in zebrafish

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Approved by _____

Supervisor

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碩士學位論文

丹參素在帕金森病實驗模型上的神經保護作用及其機理研究

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Abstract

Danshensu protects against 6-hydroxydopamine-induced damage of PC12 cells *in vitro* and dopaminergic neurons in zebrafish

By Zhong-Yan Zhou

Thesis Supervisor: Prof. Simon Ming-Yuen Lee

Chinese Medicinal Science

The overproduction of reactive oxygen species (ROS) has been implicated in the development of neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD). Previous studies have indicated that Danshensu (beta-3,4-dihydroxyphenyl-lactic acid), a main hydrophilic component of the Chinese materia medica *Salviae Miltiorrhizae Radix et Rhizoma* (Danshen, Pharmacopoeia of PR China), has ROS scavenging and antioxidant activities, however its mechanism of action was not clear. In this study, we investigated whether the protective effects of Danshensu against neurotoxin 6-hydroxydopamine (6-OHDA)-induced oxidative stress involved the Nrf2/HO-1 pathways. Pretreatment with Danshensu in PC12 cells significantly attenuated 6-OHDA-induced cytotoxicity and the production of ROS. Danshensu activated the nuclear translocation of Nrf2 to increase heme oxygenase-1 (HO-1), conferring protection against ROS. Danshensu induced the phosphorylation of Akt, and its cytoprotective effect was abolished by PI3K, Akt and HO-1 inhibitors. These results confirmed the crucial role of PI3K/Akt and HO-1 signaling pathways as the underlying mechanistic action of Danshensu. Taken together, the results suggest

that Danshensu enhances HO-1 expression to suppress 6-OHDA-induced oxidative damage via PI3K/Akt/Nrf2 signaling pathways. Moreover, 6-OHDA-induced dopaminergic neuronal loss in zebrafish could be reduced by Danshensu, further supporting the neuroprotective potential of Danshensu.

Key words

Danshensu, ROS, 6-OHDA, Parkinson's disease, PC12, Zebrafish

摘要

探討丹參的水溶性成份丹參素在帕金森病實驗模型上的神經保護作用及其機制。

活性氧自由基（reactive oxygen species, ROS）的過多產生與神經退化性疾病（如帕金森病和阿爾茲海默癡）的發生有著重要的關聯。過去的研究表明，丹參的主要水溶性成份丹參素（beta-3,4-dihydroxyphenyl-lactic acid）具有清除活性氧自由基和抗氧化的活性。然而，它的作用機理並不清晰。在本研究中，我們主要探討了丹參素抵抗六羥基多巴胺（6-hydroxydopamine, 6-OHDA）誘導的氧化壓力的作用是否通過Nrf2/HO-1信號通路。在PC12細胞模型上，前處理丹參素有效地減弱了六羥基多巴胺誘導的細胞毒性和活性氧自由基的產生。丹參素引起了轉錄因子Nrf2的轉核，進而引起了HO-1的表達來抵禦活性氧自由對細胞的損傷。丹參素能增加Akt的磷酸化，並且PI3K、Akt、HO-1的特異性抑製劑都能在不同程度上抑制丹參素的的保護效果。以上結果證實了PI3K/Akt/HO-1信號通路在丹參素神經保護作用機理上扮演著重要角色。綜上所述，本研究結果表明丹參素通過增強HO-1的表達來減弱六羥基多巴胺誘導的氧化損傷，這個過程是由PI3K/Akt/Nrf2信號通路介導的。此外，在斑馬魚模型上，丹參素減少了六羥基多巴胺誘導的斑馬魚多巴胺神經元丟失，這為丹參素的神經保護作用作了進一步的印證。

綜上，丹參素可能成為一個有潛力的預防帕金森藥物。

關鍵字:

丹參素，活性氧自由基，六羥基多巴胺，帕金森病，PC12細胞，斑馬魚

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List of Abbreviations

6-OHDA, 6-hydroxydopamine;

ALP, autophagy-lysosomal pathway;

CNS, central nervous system;

DMSO, dimethyl sulfoxide;

dpf, day post fertilization;

ETC, electron-transport chain

F-12K, Kaighn's modification of Ham's F12 medium;

FBS, Fetal bovine serum;

GSH, glutathione;

Nrf2, NF-E2 p45-related Factor 2

MDA, Malondialdehyde

MTT, 3-[4, 5-dimethyl- thiazol-2-yl]-2, 5-diphenyl tetrazolium bromide;

MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

MPP^+, 1-Methyl-4-phenylpyridin-1-ium

PBS, phosphate-buffered saline;

PD, Parkinson’s disease;

ROS, reactive species oxygen;

RNS, reactive nitrogen species;

SOD, superoxide dismutase;

SNpc, substantia nigra pars compacta;

UPS, ubiquitin proteasome system;