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A combination of four effective components derived from Sheng-mai san attenuates hydrogen peroxide-induced injury in PC12 cells through inhibiting Akt and MAPK signaling pathways

CAO Guo-Sheng, LI Shao-Xia, WANG Yan, XU Ying-Qiong, LV Yan-Ni, KOU Jun-Ping *, YU Bo-Yang

Jiangsu Key Laboratory of TCM Evaluation and Translational Research, Department of Complex Prescription of TCM, China Pharmaceutical University, Nanjing, 211198, China

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[ABSTRACT] The present study was designed to investigate whether a combination of four effective components derived from Sheng-mai san **(**SMXZF; ginsenoside Rb1: ginsenoside Rg1: DT-13: Schizandrol A as 6 : 9 : 4 : 5) could attenuate hydrogen peroxide (H_2O_2)-induced injury in PC12 cells, focusing on the Akt and MAPK pathways . The PC12 cells were exposed to H_2O_2 (400 μmol·L^{-1}) for 1 h in the presence or absence of SMXZF pre-treatment for 24 h. Cell viability was measured by MTT assay. The efflux of lactate dehydrogenase (LDH), the intracellular content of malondialdehyde (MDA), the activities of superoxide dismutase (SOD), and caspase-3 were also determined. Cell apoptosis was measured by Hoechst 33342 staining and Annexin V-FITC/PI staining method. The expression of Bcl-2, Bax, cleaved caspase-3, Akt, and MAPKs were detected by Western blotting analyses. SMXZF pretreatment significantly increased the cell viability and SOD activity and improved the cell morphological changes, while reduced the levels of LDH and MDA at the concentrations of 0.1, 1 and 10 μg·mL^{-1}. SMXZF also inhibited H_2O_2-induced apoptosis in PC12 cells. Moreover, SMXZF reduced the activity of caspase-3, up-regulated the protein ratio of Bcl-2 and Bax and inhibited the expression of cleaved caspase-3, p-Akt, p-p38, p-JNK and p-ERK1/2 in H_2O_2-induced PC12 cells. Co-incubation of Akt inhibitor or p38 inhibitor partly attenuated the protection of SMXZF against H_2O_2-injured PC12 cells. In conclusion, our findings suggested that SMXZF attenuated H_2O_2-induced injury in PC12 cells by inhibiting Akt and MAPKs signaling pathways, which might shed insights on its neuroprotective mechanism.

[KEY WORDS] Combination of effective TCM components; Sheng-mai san**;** SMXZF; PC12 cells; Hydrogen peroxide; Akt; MAPKs

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Introduction

Most neurodegenerative diseases, such as Alzheimer's disease [1], Parkinson's disease (PD) [2-3], and stroke [4-5], are characterized by oxidative stress-induced cell damage, which can lead to a progressive loss of cognitive function, mitochondrial dysfunction, and apoptosis in neuronal cells [6-7]. Previous studies have indicated that increasing levels of reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2) through oxidative metabolism or decreasing clearance of H_2O_2 induced by oxidative stress and subsequent cell death is owing to anti-oxidant deficiency in the cell [8-9]. Enzymes and antioxidant nutrients, which have several natural defense mechanisms and capture the chain reaction of ROS initiation and production, cannot prevent the damage completely [10-11]. And the brain is vulnerable to oxidative stress damage due to its high energy use and metabolic demands, high cellular content of lipids and proteins, extensive axonal and dendritic networks, and low levels of endogenous scavengers [12-14]. A number of signaling pathways are involved in protecting cells against H_2O_2-induced neuronal

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[*Corresponding author] Tel/Fax: +86-25-86185158, E-mail: junpingkou@cpu.edu.cn or junpingkou@163.com
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damages, such as anti-inflammation, anti-oxidation, and anti-apoptosis pathways. Cell apoptosis is one of the major factors responsible for H_2O_2-induced damages [15-17]. In the process of cell apoptosis, caspase-3 is the most important terminal enzyme; Bax and its related protein Bcl-2 act as regulators of apoptosis at the mitochondrial level. Overexpression of Bax protein following oxidation stress plays a key role in DNA fragmentation and neuronal death and induces the release of apoptogenic factors by heterodimerized at the Bcl-2-interacting domain of the mitochondrial membrane. On the contrary, the anti-apoptotic protein Bcl-2 neutralizes Bax by interacting with it and inhibiting activation of the apoptosis signaling cascade [18]. In addition, two important signaling pathways, including Akt and MAPKs (p38, JNK, and ERK1/2) pathways, majorly regulate the caspase-3 activity and the ratio of Bcl-2/Bax proteins level in the process of cell apoptosis [19-21].

Sheng-mai san, which is composed of Panax ginseng, Ophiopogon japonicas, and Schisandra chinensis, is one of the famous complex prescriptions in traditional Chinese medicine with the effects of nourishing the Qi, tonifying the Yin, restoring pulse, and treating collapse [22-23]. It has been mostly used for cardiovascular and cerebrovascular diseases in clinic with significant therapeutic effects [24-25], which is partly ascribed to its anti-oxidative activities [26-27]. The proper proportion of effective constituents of SMXZF (the proportion of ginsenoside Rb1 : ginsenoside Rg1 : DT-13 : Schizandrol A as 6 : 9 : 4 : 5, their chemical structures were shown in Fig. 1) is derived from Sheng-mai san, a complex prescription for prevention and treatment of cardiovascular and cerebrovascular diseases. Recent studies have shown that SMXZF exerts significant protection against cerebral ischemia-reperfusion injury in a mouse model of stroke *in vivo* [28] and inhibits H_2O_2-induced PC12 cell apoptosis linked with caspase-3/ROCK1/MLC pathway *in vitro* [29]. However, the other possible underlying mechanisms of SMXZF on PC12 cells induced by H_2O_2 remain unclear. To provide further evidence for its potential use for most neurodegenerative diseases, we evaluated its neuroprotective activities and potential cellular mechanisms in the present study.

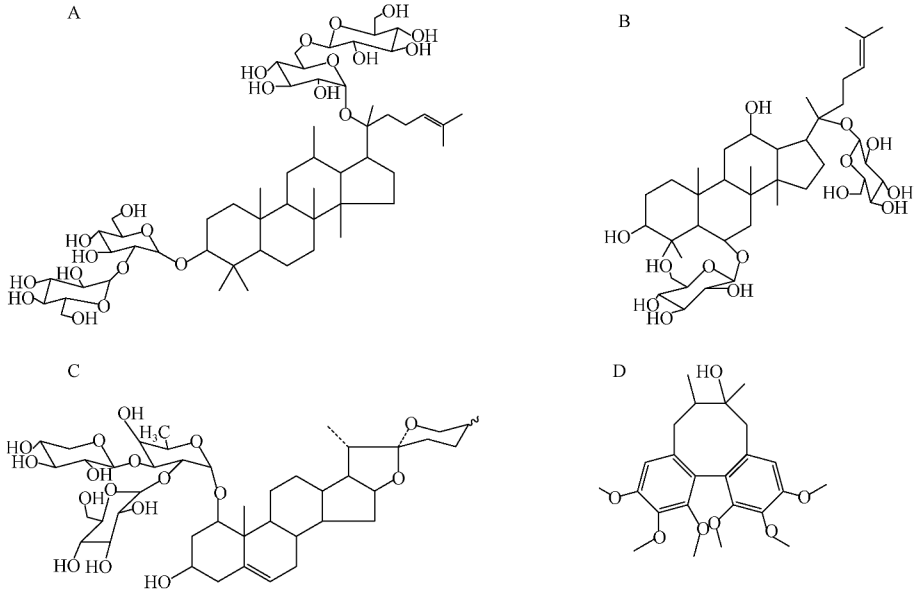

Fig. 1 Chemical structures of four components in SMXZF. A: Ginsenoside Rb1. B: Ginsenoside Rg1. C: DT-13. D: Schizandrin A

Materials and Methods

Test compounds, chemicals, and reagents

The effective constituents of SMXZF (ginsenoside Rb1, ginsenoside Rg1, and schizandrin) were purchased from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China), and DT-13 with the purity greater than 95% was kindly provided by Dr. QI Jin at the Department of Complex Prescription of TCM, China Pharmaceutical University (Nanjing, China). They were dissolved in Dulbecco's modified Eagle's medium (DMEM), which was purchased from GIBCO (New York, USA). Assay kits for lactate dehydrogenase (LDH), malondialdehyde (MDA), and superoxide dismutase (SOD) were purchased from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China). *N*-acetylcysteine (NAC, as an anti-oxidant) was purchased from Sigma (St. Louis, MO, USA). Hoechst 33342 (bisbenzimide), enhanced chemiluminescence (ECL) reagent, wortmannin (Akt inhibitor) and SB203580 (p38MAPK inhibitor) were obtained from Beyotime (Haimen, Jiangsu, China). Polyvinylidene fluoride (PVDF) membranes were purchased from Millipore (Bedford, MA, USA). 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Ameresco (Ameresco, OH, USA).