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Original article

Protection of Salidroside on Primary Astrocytes from Cell Death by Attenuating Oxidative Stress

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ABSTRACT

Objective To investigate whether salidroside (SAL) has protective and anti-oxidative effects on astrocytes. **Methods** Firstly, SAL was extracted from the roots of *Rhodiola rosea* with 70% ethanol and butanol to obtain crude phenylethyl alcohol glycosides which have been known as bioactive part of *R. rosea*; Secondly, WST-1 assay was carried out to assess the cell viability of astrocytes and cortical neurons under the treatment of the purified (> 95%) SAL. Moreover, WST-1 assay was also used to evaluate the cytoprotective effects of SAL preventing astrocytes from staurosporine-induced cell death; Thirdly, we examined the spontaneous reactive oxygen species (ROS) and staurosporine-induced ROS generation in astrocytes in the absence or presence of SAL. **Results** SAL was observed to improve the astrocytes viability but not cortical neurons. In addition, SAL was able to ameliorate staurosporine-induced cell death. Moreover, SAL was able to attenuate the spontaneous ROS and staurosporine-induced ROS generation. **Conclusion** We here confirm that the anti-oxidative effect of SAL on primary astrocytes might be an important mechanism accounting for the cytoprotective effects from SAL.

Key words

astrocytes; cell death; oxidative stress; reactive oxygen species; salidroside

1. Introduction

Rhodiola rosea L. belongs to genus *Rhodiola* L., subfamily of Sedoideae, family of Crassulaceae. *R. rosea* is an important medicinal plant widely distributed at high altitude in the Arctic and mountainous regions in Europe and Asia, such as in Yunnan, Qinghai provinces and Tibet Autonomous Region, China (Alberdi et al, 2010; Cai et al, 2012). Species in *Rhodiola* L. were used as traditional Tibetan medicines with many medical functions, such as clearing lungs, eliminating toxins from the body, and treating various epidemic diseases, fatigue, traumatic injuries, and burns (Han et al, 2002a; Zuo et al, 2014). On the other hand, the roots of species in *Rhodiola* L. had a long application history in traditional Chinese medicine (TCM) for enhancing human physical and mental performance (Bayliak and Lushchak, 2011), improving cognitive functions (Spasov et al, 2000), reducing mental fatigue (Darbinyan et al, 2000; Shevtsov et al, 2003), promoting free radical mitigation (Wing et al, 2003), and strengthening learning and memory (Abidov et al, 2003; Chen

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et al, 2008; De Bock et al, 2004). Salidroside (SAL), rosavins, and *p*-tyrosol, isolated from the roots of *R. rosea*, were considered as the most important therapeutic components, and they were regarded as the standard to evaluate the quality of plant as well (Kelly, 2001).

Reactive oxygen species (ROS), including superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radical, are regarded as factors involved in fundamental mechanisms of a variety of diseases, such as atherosclerosis, diabetes, cancer, neuro-degeneration, and aging (Ray et al, 2012). Cellular ROS are generated endogenously as in the process of mitochondrial oxidative phosphorylation, or they may arise from the interactions with exogenous sources such as xenobiotic compounds. Oxidative stress occurs when ROS overpower the cellular anti-oxidant defense system, which was indicated by an induction of ROS levels, resulting in a decrease in the cellular anti-oxidant capacity. Oxidative stress led to ROS-mediated damage of nucleic acids, proteins, and lipids, which may occur directly or indirectly. These processes have been implicated in carcinogenesis (Trachootham et al, 2009). ROS regulated several signaling pathways through interaction with critical signaling molecules, affecting a variety of cellular processes, such as cell proliferation, metabolism, differentiation, and cell survival (Ray et al, 2012).

SAL is the major phenylpropanoid glycoside and pharmacologically active ingredient of *R. rosea*. In recent years, it was demonstrated that SAL exhibited many activities, such as anti-aging, anti-cancer, anti-inflammatory, anti-hypoxia, and anti-oxidative properties (Mao et al, 2010; Skopinska-Rozewska et al, 2008; Yu et al, 2007; 2008). Moreover, several studies showed that SAL had the protective effects on neurons (Zhang et al, 2011) and cardiomyocytes (Zhang et al, 2009). However, there were no reports of SAL on assaying its activities in astrocytes, and it remained unclear whether SAL could provide protection against cell death of astrocytes induced by oxidative stress. Astrocytes are demonstrated as the numerous cell types in the central nervous system, playing an important role in providing structural, trophic, and metabolic support to neurons and modulate synaptic activity. Without close communication with astrocytes, neurons can not survive in the brain. As a result, the survival of astrocytes modulates normal brain function. In this study, we are aiming at clarifying whether SAL was able to promote cell viability of astrocytes in the absence and presence of the cell-toxic compound staurosporine (STS). Moreover, we investigated whether SAL was capable of blocking ROS production, either spontaneous or in STS-treated cells.

2. Materials and methods

2.1 Preparation of SAL

The roots of *Rhodiola rosea* L. (8 kg, from Chengdu Herbal Medicine Market, China) were extracted for 4 h with boiling aqueous ethanol (70%) for three times. The solvent was filtered and evaporated in vacuum, and then the concentrated extract was successively partitioned with petroleum ether, ethyl acetate, and *n*-butanol. The *n*-butanol fraction (containing crude phenylethyl alcohol glycosides) was chromatographed repeatedly on silica gel and eluted with $CHCl_3$-CH_3OH (50:0→0:1). The eluent was combined by monitoring with thin-layer chromatography to obtain six fractions (Frs. 1−6). The Fr. 3 was subjected to silica gel and eluted with $CHCl_3$-CH_3OH (8:1) to yield SAL. The chemical structure of SAL was confimed by nuclear magnetic resonance (NMR) spectroscopy, mass spectrometry (MS) (Han et al, 2002b; Kasai et al, 1989; Nicholson and Wilson, 1989), and compared with the reference.

2.2 Astrocytes culture

Primary astrocytes-enriched cell cultures were obtained from newborn rats, according to the method described before (Zhu and Reiser, 2014). All procedures using tissue from animals were approved by corresponding regulations from Sachsen-Anhalt, Germany. In brief, newborn rats were decapitated; Total brains were removed and collected in ice-cold Puck's-D1 solution (NaCl, 137 mmol/L; KCl, 5.4 mmol/L; KH_2PO_4, 0.2 mmol/L; Na_2HPO_4, 0.17 mmol/L; glucose, 5.0 mmol/L; sucrose, 58.4 mmol/L, pH 7.4). The brains were gently passed through meshes (256 and 136 μm pore diameter) and centrifuged at 400 *g* for 5 min. The cells were re-suspended in Dulbecco's minimum essential medium (DMEM, Biochrom, Germany) containing 10% heat-inactivated fetal calf serum (FCS, Biochrom, Germany), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were plated in the flasks and kept in culture for 10−13 d, and then astrocytes were used for experiments.

As for the experiments, astrocytes were detached by trypsin and re-seeded on 96-well plates at a density of 3×10^4 cells per well. Astrocytes were kept in culture for 24 h before carrying out WST-1 assay or DCF-DA assay. All the cell cultures and sub-cultures were cultured in the humidified incubator with 10% CO_2 at 37 °C. The medium was changed for the first time after 5 d and thereafter every 2 d. For the induction of cell death, astrocytes were treated with 0.2 μmol/L STS in FCS-free DMEM for 24 h.

2.3 Cortical neuronal culture

Primary cortical neurons were obtained from brains of 1−3 day-old rats. The cell suspension was prepared according to the protocol described (Gorbacheva et al, 2013) and cells were plated on dishes coated with ethyleneimine polymer solution (1 mg/mL). The cells were allowed to sediment for 1 h at 37 °C in 5% CO_2, then the floating cells were removed and 1.5 mL culture medium (neurobasal medium A containing 2% supplement B-27 and 0.5 mmol/L *L*-glutamine) was added. Two days later, cytosine arabinoside (AraC, 10^{-5} mol/L) was added to suppress the growth of glial cells. For experiments, neurons were used at days 9−10 in culture. All animal procedures were approved by Ethics Committee of the German Federal State of Sachsen-Anhalt.