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Salidroside promotes erythropoiesis and protects erythroblasts against oxidative stress by up-regulating glutathione peroxidase and thioredoxin

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ABSTRACT

Ethnopharmacological relevance: Rhodiola rosea is commonly used in China and Tibet folk medicine for the treatment of high altitude sickness, anoxia and mountain malhypoxia.
Aim of study: Salidroside (SDS) is an active ingredient of *Rhodiola rosea*. This study attempted to examine the potential erythropoiesis-stimulating and anti-oxidative effect of SDS in TF-1 erythroblasts.
Materials and methods: The erythropoiesis-promoting effect was determined by treating human TF-1 cells, one of the popular *in vitro* models for studying erythropoiesis, with SDS in the presence and absence of erythropoietin (EPO) through the measurement of the expression of a series of erythroid markers such as glycophorin A (GPA), transferrin receptor (CD71) and hemoglobin (Hb). The potential protective effect of SDS against H_2O_2-induced apoptosis and its underlying mechanism in TF-1 erythroblasts were examined by flow cytometry and Western blot analysis.
Results: SDS promotes erythropoiesis in the EPO-treated cells and it also reduces the number of apoptotic cells in TF-1 erythroblasts after H_2O_2 treatment probably through the up-regulation of protective proteins thioredoxin-1 (Trx1) and glutathione peroxidase-1 (GPx1).
Conclusion: Our study provides evidence to explain the ethnopharmacological role of SDS and *Rhodiola rosea* in Chinese medicine. Our findings also support the use of SDS as an erythropoiesis-adjuvant agent to correct anemia and malhypoxia.

1. Introduction

In the 19th century, medicinal plant *Rhodiola rosea* was used as a folk medicine in France, Germany and many European countries to fight fatigue. Results from recent studies support the use of *Rhodiola rosea* to enhance both the physical and mental performance (Spasov et al., 2000; Shevtsov et al., 2003). Consistent with this observation, results from a double-blind, placebo-controlled study with 56 young physicians on night duty indicate that participants received rhodiola extract retained a high level of mental acuity than those taking placebo (Darbinyan et al., 2000).

Salindroside (SDS) ($C_{14}H_{20}O_7$, structure shown in Fig. 1) is an active constituent of *Rhodiola rosea*. It has been reported that SDS possesses anti-aging, anti-cancer (Wang et al., 2009), anti-viral (Wang et al., 2009), anti-inflammatory (Skopinska-Rozewska et al., 2008), anti-hypoxia (Ye et al., 1993; Yu et al., 2008) and anti-oxidative (Kanupriya et al., 2005; Yu et al., 2007) properties. SDS is also used to protect neurons (Yu et al., 2008), liver cells (Ma et al., 2009) and to improve sleeping quality (Li et al., 2007). In China and Tibet, SDS has long been used as a blood tonic and adaptogen to prevent high altitude sickness, and to treat resisting anoxia and mountain malhypoxia (Wu et al., 2008). At high altitudes, where the partial pressure of oxygen in the lungs and blood are reduced, kidney releases hormone erythropoietin (EPO) to stimulate erythropoiesis to produce more erythrocytes to compensate for the low oxygen level (Savourey et al., 2004). During erythropoiesis, erythroblasts are exposed to a high level of oxidative stress conditions in the body. Not surprisingly, erythroid cells in healthy individuals contain an array of antioxidant enzymes that protect cells against oxygen radicals (Johnson et al., 2005). Therefore, regulation of oxidative stress is particularly important during the development of erythrocytes (Socolovsky, 2007). To date, little information is available in the literature about the effects of SDS on erythropoiesis. Also, very little is known about the mechanism by which SDS provides protection against oxidative stress in erythroblasts.

In this study, we employed a commonly used erythropoiesis model TF-1 cells to examine the effect of SDS on erythropoiesis and its possible protective effect against oxidative stress. We show here

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for the first time that SDS promotes erythropoiesis and up-regulates the level of anti-oxidative enzyme glutathione peroxidase-1 (GPx1) and thioredoxin-1 (Trx1) to counteract oxidative stress.

2. Materials and methods

2.1. Materials

SDS was purchased from International Laboratory (San Bruno, CA, USA). Human erythroleukemic cell line TF-1 was purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Recombinant human EPO and granulocyte-macrophage colony stimulating factor (GM-CSF) were obtained respectively from Calbiochem (Darmstadt, Germany) and Sigma (St. Louis, MO, USA). FITC-conjugated annexin-V was purchased from Roche (Basel, Switzerland) and propidium iodide (PI) was purchased from Sigma. PE-conjugated anti-GPA and mouse anti-CD71 monoclonal antibody were purchased from BD Pharmingen (San Diego, CA, USA). FITC-conjugated goat anti-mouse secondary antibody was from Calbiochem. Anti-GPx1, anti-catalase and anti-Trx1 antibodies were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA). CM-H_2DCFDA (5-(6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate) was obtained from Invitrogen.

2.2. Cell culture

TF-1 cells were maintained at 37 °C, 5% CO_2 in RPMI 1640 medium (Gibco) supplemented with 10% FBS, 1% penicillin–streptomycin, 2.5 g/l glucose, 2 g/l sodium bicarbonate, 1 mM sodium pyruvate and 2 ng/ml GM-CSF as described (Lui and Kong, 2006, 2007). Prior to induction of differentiation, cells were kept in GM-CSF free medium for 16 h. Subsequently, EPO (10 ng/ml) were added and the time was considered as day 0 of the experiments. On alternative days, medium with EPO (10 ng/ml) in the presence or absence of SDS (100 μM) were renewed and the cell density was maintained at 4×10^5 cells/ml.

2.3. MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay was used to measure cell viability. Briefly, 20 μl MTT (0.5 mg/ml) solution was added to TF-1 cells (1×10^5/ml) after treatments and incubation was continued for 3 h at 37 °C, 5% CO_2. Subsequently, 200 μl DMSO was added to lyse the cells and the absorbance was measured at 540 nm.

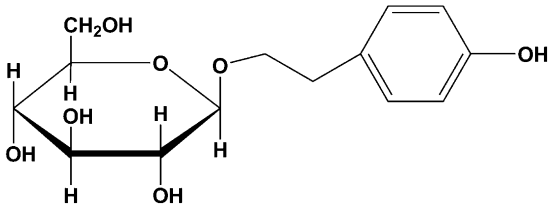

Fig. 1. Chemical structure of salidroside (SDS).

2.4. Phenotypic assays

The level of specific markers of erythroid cells such as glycophorin A (GPA), transferrin receptor (CD71) and hemoglobin (Hb) were evaluated by flow cytometry. Briefly, for GPA staining, cells (5×10^5) incubated with ice-cold PBS containing 0.5% BSA (w/v), 0.2 mg/ml mouse IgG for 30 min were labeled with PE-conjugated anti-GPA monoclonal antibody (1:500) for 30 min on ice in dark. For CD71 staining, cells (5×10^5) incubated with ice-cold PBS containing 0.5% BSA (w/v), mouse IgG and goat IgG (0.2 mg/ml each) for 30 min were labeled with mouse anti-CD71 monoclonal antibody (1:1000) for 30 min on ice in dark. After washing, cells were labeled with FITC-conjugated goat anti-mouse secondary antibody (1:2000) for another 30 min in dark. For the staining of Hb, cells ($\sim 1 \times 10^6$) after washing were fixed with 1% (v/v) paraformaldehyde at 4 °C for 30 min and permeabilized with 0.06% (v/v) Triton X-100 in PBS containing 0.1% (w/v) BSA at 4 °C for 10 min. Cells were then incubated with 0.5% (w/v) BSA, 0.2 mg/ml goat IgG and 0.2 mg/ml rat IgG in 0.5 ml PBS on ice for 15 min to block non-specific binding. Thereafter, cells were labeled with mouse anti-Hb monoclonal antibody (Abcam) (1:500) on ice for 30 min. After washing 3 times with PBS, cells were labeled with FITC-conjugated goat anti-mouse secondary antibody (Calbiochem) (1:2000) for another 30 min. After labeling, cells were washed with ice-cold PBS for three times and subjected to flow cytometric analysis (FACSCanto, BD Biosciences).

2.5. Study of cell death by annexin-V and PI assay

Degree of cell death after treatment was determined by using annexin-V/PI assay. Cells (5×10^5) were labeled with annexin-V-FITC (2%, v/v) for 20 min and then with PI (2.5 μg/ml) for 5 min at room temperature before flow cytometric analysis. In this assay, annexin-V-FITC is used to label the phosphatidylserine (PS) on the outer leaflet of plasma membrane to determine PS externalization (an indicator for cell death) while PI is used to label the cell nucleus of the late apoptotic cells in which

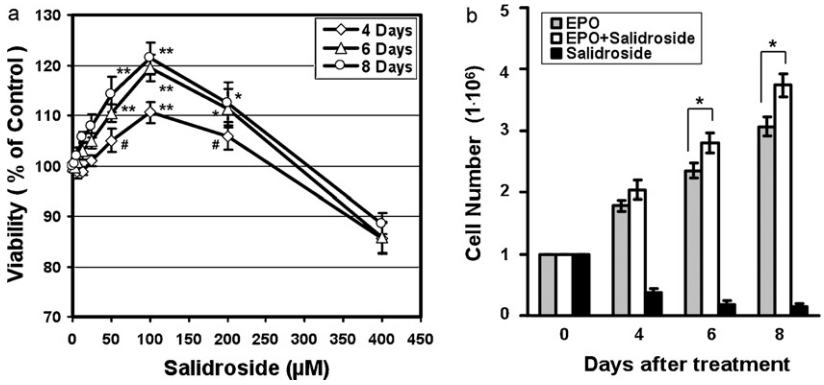

Fig. 2. Effect of salidroside on the viability and cell growth in TF-1 cells. TF-1 cells were cultured with EPO (10 ng/ml) with various concentrations of salidroside at 37 °C, 5% CO_2. Viability (% of control) was measured by MTT assay. Mean ± SD, $n = 3$, $^*P < 0.05$, $^{**}P < 0.005$, $^{\#}P > 0.05$ (a). TF-1 cells (1×10^5/ml) were treated with EPO (10 ng/ml), salidroside (100 μM) or in combination for 2–8 days. Total cell number was determined by flow cytometry. Results are mean ± SD, $n = 5$ (bar chart), $^*P < 0.05$ (b).