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Altered expression of TRAIL on mouse T cells via ERK phosphorylation by *Rhodiola rosea* L. and its marker compounds

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ABSTRACT

Rhodiola rosea L. extracts have shown neuroprotective, anti-fatigue, anti-inflammatory and anti-tumor properties. However, the studies on their effect on T cell function are rather scarce. We examined the potential of *R. rosea* extract and its major constituents – salidroside, rosarin, rosavin and rosin to alter cell growth of human Jurkat T cells, apoptosis of splenic mouse CD3 T cells and expression of the surface markers and phosphorylation of extracellular signal-regulated kinase (ERK). The initial screening for cell viability in Jurkat T cells and for apoptosis of mouse T cells showed the strongest activity for rosavin and rosarin. Rosarin and rosavin did not alter significantly the dynamic of CD69 expression upon stimulation, but altered TNF-related apoptosis-inducing ligand (TRAIL) expression. Rosavin inhibited TRAIL up-regulation, while rosarin showed an opposite effect. Indeed, rosarin increased the frequencies of $CD3^{+}TRAIL^{+}$ T cells and the fold inhibition of ERK phosphorylation. Our data showed that different effects of rosarin and rosavin on TRAIL expression can involve distinct action on ERK signaling and hence highlighted their potential to manipulate TRAIL as a tool to rescue the resistance to apoptosis in auto-immune diseases and cancer.

1. Introduction

Rhodiola rosea L. (golden root) is a plant species of family Crassulaceae, well-known for its adaptogenic activities, able to increase the nonspecific resistance of the human body and relief the stress symptoms of emotional, mental or physical origin (Panossian et al., 2014). Numerous *in vitro* and *in vivo* studies have confirmed that *R. rosea* possesses anti-stress, anti-depressive, anti-inflammatory, anti-diabetic, anti-viral and anti-oxidant activities (Wang et al., 2016a; Zhang et al., 2016a; Zhao et al., 2015). The plant species exhibits neuro-, cardio- and hepato-protective effects, has benefits on learning and memory processes, and ameliorates lung and traumatic brain injuries (Chen et al., 2012; Vasileva et al., 2016; Tang et al., 2016). *R. rosea* inhibits cell proliferation and induces cell apoptosis in various cells and cell lines, *e.g.* human neuroblastoma (Zhang et al., 2007), glioma cells (Zhang et al., 2013), lung (Wang et al., 2014), breast (Zhao et al., 2015), sarcoma (Cai et al., 2012) and ovarian cells (Kormosh et al., 2006). The reported regulatory mechanisms, standing behind these activities, include decreased production of pro-inflammatory cytokines (Zhang et al., 2016a), inhibition of NF-κB (Zhang et al., 2016b; Tang et al., 2016) and ERK/MAPK (Guan et al., 2011a; Li et al., 2013), modulation of PI3K/Akt and mTOR (Chen et al., 2012, 2016), activation of Nrf2-antioxidant signaling pathways (Tang et al., 2016), increase in serum levels of glutathione and total superoxide dismutase anti-oxidant enzymes (Zhang et al., 2016a). The pharmacological properties of *R. rosea* extracts are supposed to be tightly associated with the phenylethanoids salidroside (Sal) and *p*-tyrosol content, as well as, the phenylpropanoids rosin, rosavin and rosarin, accumulated and further stored in the rhizomes and roots of the plant (Marchev

Abbreviations: Con A, concanavalin A; ERK/MAPK, extracellular signal-regulated kinase/mitogen-activated protein kinase; HSQC, Heteronuclear single quantum coherence spectroscopy; IFN-γ, interferon gamma; IL-1β, interleukin 1 beta; mTOR, mechanistic target of rapamycin signaling pathway; NF-κB, nuclear factor-kappa B; NMR, Nuclear magnetic resonance; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; PI3K/Akt, phosphatidylinositol-3-kinase; Sal, Salidroside; TCR, T cell receptor; Th1, T helper cell type 1; TGF-β1, transforming growth factor beta 1; TNF-α, tumor necrosis factor alpha; TRAIL, TNF-related apoptosis-inducing ligand.

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et al., 2016a, 2016b).

Recent studies have revealed that *R. rosea* can enhance cellular immunity by inhibiting the expression of apoptotic genes and reducing spleen and thymus lymphocyte apoptosis (Liu et al., 2015a, 2015b). Further, *R. rosea* extracts can influence the number and function of T lymphocytes. These extracts are shown to increase total $CD3^+$ and memory $CD4^+$ T cell pools, to elevate production of Th1 cytokines (IFN-γ, IL-2 and IL-12) and to decrease the number and function of cytotoxic $CD8^+$ T cells (Liu et al., 2015a). In pathological conditions of sepsis, however, the extracts reduce TNF-α and IL-6 expression, hence attenuating the disease (Liu et al., 2015b). *R. rosea* extract and its bioactive constituent Sal significantly elicit the proliferation of concavalin A (ConA)-stimulated T cells (Guan et al., 2011b; Skopińska-Różewska et al., 2011), revealing the vaccine adjuvant effect of Sal in ovalbumin-immunized mice (Guan et al., 2011b). Moreover, Sal intake promotes the production of immunoglobulins (total IgG, IgG1 and IgG2α) and improves the response of elderly rats to vaccine enhancing humoral and cell-mediated immune responses (Lu et al., 2013). In a model of Con A-induced liver damage, a pathology involving T cells, Sal decreases the number of liver-infiltrating T lymphocytes and inhibits the secretion of inflammatory cytokines and NF-κB activation, thus preventing hepatitis (Hu et al., 2014). Despite that the biological activity of *R. rosea* extracts and its constituent Sal on T cells have been reported, limited studies have been focused on the biological activity of other major constituents, such as rosin, rosavin and rosarin. Thus, the present study was undertaken to identify the constituents from *R. rosea* extract rhizomes and to investigate their effects on T cell activation and apoptosis.

2. Material and methods

2.1. Standards and chemicals

CD_3OD (99.8%) and D_2O (99.9%) were purchased from Deutero GmbH (Kastellaun, Germany). Trimethyl silylpropionic acid sodium salt-d_4 (TSPA-d_4), salidroside (≥98%), rosarin (≥98%), rosavin (≥98%), rosin (≥95%) were supplied from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant material and NMR analysis

For the NMR analysis *R. rosea* rhizomes, collected from its natural habitat in Bulgaria, were used (Marchev et al., 2016a). The sample preparation was performed as previously described by Georgiev et al. (2015). Briefly, freeze-dried *R. rosea* samples (50 mg) were homogenized in 2 mL Eppendorf tubes with equivalent amounts (0.75 mL) of CD_3OD and D_2O (KH_2PO_4 buffer, pH 6.0). TSPA-d_4 (0.005%; w/v) was added as an internal standard. After homogenization at room temperature the samples were sonicated for 20 min (35 kHz; UCI-50 Raypa® R. Espinar S.L., Barcelona, Spain). After 20 min of centrifugation (12 000 rpm) ca. 0.8 mL of the supernatant was transferred into 5 mm NMR tube. The 1H and 2D NMR spectra (COSY and HSQC) were recorded at 25 °C on an AVII + 600 spectrometer (Bruker, Karlsruhe, Germany), at a proton frequency of 600.13 MHz with 4.07 s relaxation time and CD_3OD as an internal lock.

2.3. Extraction procedure and pure compounds

R. rosea extract (RRH) was obtained after triplicate extraction of lyophilized rhizomes with 70% methanol (1:20 w/v) under sonication, at 35 °C, for 15 min each. The combined extracts were filtrated through filter paper, evaporated at 50 °C, freeze-dried to complete dryness and stored at −20 °C prior to be used. RRH, Sal, rosin, rosavin and rosarin, used in the study, were dissolved at concentration of 2 mg/mL in 2% DMSO (dimethyl sulfoxide, endotoxin free; Sigma-Aldrich, Munich, Germany)/dH_20 and were then diluted in endotoxin free phosphate buffer (PBS, pH 7.4, Lonza, Basel, Switzerland). Controls contain 0.05% DMSO/PBS or PBS only.

2.4. Cell line and primary cell cultures

Human Jurkat cell line (clone E6-1, ATCC® TIB-152™) was used to study the effect of extract, and pure compounds on cell viability. Cells (1×10^5/mL) were seeded into 50 mL flaks (TPP, Trasadingen, Switzerland) and cultured in RPMI-1640 medium (Sigma-Aldrich, Munich, Germany) supplemented with 2 mM L-glutamine, 10 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, HEPES), 1 mM sodium pyruvate, 4500 mg/L glucose, 1500 mg/L sodium bicarbonate and 10% fetal bovine serum (FBS), all from Sigma-Aldrich (Sigma-Aldrich, Munich, Germany), at 37 °C in humidified 5% CO_2 incubator. Cells from exponentially growing cultures were used in the experiments.

Primary T cells were isolated from ICR mice and used to evaluate the effect of the extract, and pure compounds on the expression of activation and apoptotic markers. The mouse strain was purchased from the Charles River Laboratories (Wilmington, MA, USA) and then bred in the experimental animals facility at the Bulgarian Academy of Sciences (Slivnitsa, Sofia-region, Bulgaria). All procedures with animals were done under anesthesia and control of veterinarian and were approved by the Animal Care Committee at the Stephan Angeloff Institute of Microbiology (Sofia), according to the National and European Guidelines. Spleens from 7 week-old male mice were harvested and the splenocyte suspension was prepared as previously described (Dimitrova et al., 2012). CD3 T cells were enriched by magnet negative selection using cocktail of biotinylated antibodies against CD11b, CD19, CD45R/B220 and CD49b, all from Biolegend (London, UK) and streptavidin coupled Dynabeads® (Thermo Fisher Scientific, Waltham, MA, USA). Cell population was 85–90% positive for CD3 and over 96% viable (Trypan blue staining). CD3 T cells were cultured in RPMI-1640 medium supplemented with 1 mM L-glutamine, 1% penicillin/streptomycin solution, 50 μM 2-mercaptoethanol, 10% FBS (all reagents from Sigma-Aldrich, Munich, Germany), non-mitogen concentration of recombinant mouse IL-2 (50 U/mL; Biolegend, London, UK).

2.5. Cell growth and viability

Cells from Jurkat cell line (2×10^5/mL) were seeded at 96-well plates (TPP, Trasadingen, Switzerland) and stimulated with 25 μg/mL ConA (Sigma-Aldrich, Munich, Germany) or with plate-bound antibodies against CD3 (clone HIT3a, 1 μg/mL) and CD28 (clone 28.2, 2 μg/mL) (BD Pharmingen, San Jose, CA, USA) in the presence of increasing concentrations of the extract or pure compounds (0.0001–1000 μg/mL). Cell growth was measured by Quick Cell Proliferation Assay Kit II (Abcam, Cambridge, UK) based on the cleavage of the tetrazolium salt WST to formazan by cellular mitochondrial dehydrogenases. Briefly, 48 h after stimulation, WST dissolved in electro-coupling solution (ECS) was added to cell cultures for 3 h, the formed formazan crystals were solubilized and quantified spectrophotometrically (ELx800, BioTek, Winooski, VT, USA) measuring the absorbance at 450 nm with a reference wavelength of 630 nm. Controls contain unstimulated cells cultured in the presence of 0.05% DMSO/PBS or PBS. Cell viability was expressed as a percentage of control. WST reduction index was calculated for concentrations of the compound inhibiting cell growth by 50% (IC_{50}).