The *in vivo* effect of *Rhodiola quadrifida* extracts on the metabolic activity of blood granulocytes in mice

EWA SKOPIŃSKA-RÓŻEWSKA[1], MAŁGORZATA BYCHAWSKA[2], EWA SOMMER[1], ANDRZEJ K. SIWICKI[3]

[1]Department of Pathology, Biostructure Center, Medical University, Warsaw, Poland; [2]Department of Laboratory Diagnostics and Immunology, Institute of Tuberculosis and Lung Diseases, Warsaw, Poland; [3]Department of Microbiology and Clinical Immunology, Mazury and Warmia University, Olsztyn, Poland

Abstract

Rhodiola quadrifida (Rq) roots and rhizomes are traditional natural drugs used in Asia as adaptogens and antidepressants.

The aim of this work was to study the in vivo effect of aqueous and 50% hydro-alcoholic extracts of Rq rhizomes on the metabolic activity of blood granulocytes in mice. Mice were fed for 7 days Rq extracts in daily doses 50, 100, 200 or 400 μg.

The metabolic activity of blood phagocyting cells was determined based on the measurement of their chemiluminescent activity in scintillation counter, after stimulation by zymosan. Both extracts stimulated granulocytes activity in 400 μg doses, in lower dose (200 μg) only hydro-alcoholic extract was effective.

Key words: *Rhodiola quadrifida, mice, granulocytes, chemiluminescence.*

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Introduction

Species *Rhodiola quadrifida* (Pall.), Crassulaceae, grows in China, Tibet and Mongolia, and extracts prepared from *Rhodiola quadrifida* (Rq) are used as traditional drugs in these countries. Rq has anti-inflammatory, bacterio- and fungo- static properties, and was used also as a tonic, adaptogen, and antidepressant [1, 2]. Information about immunotropic activity of *Rhodiola* is very scarce. We previously reported that extracts of *R. rosea* and *R. quadrifida* influence tumor angiogenesis and some parameters of specific and non-specific cellular immunity [3-8].

The main group of chemical substances present in *Rhodiola* extracts are phenolic glycosides (rosavin characteristic for *R. rosea*, mongroside characteristic for *R. quadrifida* and salidroside characteristic for both species). Some medicinal products and dietary supplements containing *Rhodiola rosea* are present on the market, recommended as adaptogens and antidepressants. However, *Rhodiola quadrifida* is almost unknown and there are mostly our experimental data concerning its immunotropic activity [4-7]. The aim of the present work was to evaluate the effect of aqueous and 50% hydro-alcoholic extracts of Rq roots in the experimental model of *in vivo* non-specific granulocyte-mediated immunity in mice.

Materials and Methods

Preparation of extracts

Rhizomes of *R. quadrifida* were collected in Altai mountain in Mongolia, thanks to dr H. Wiedenfeld. The Mongolian plant material was identified; voucher specimen was deposited at the herbarium of the Institute of Botany of Mongolian Academy of Science in Ulaanbatar. Samples for the study were obtained by Prof. M. Furmanowa, head of the scientific project PBZ-KBN-092/PO5/2003. Sample extractions and their chemical analysis were performed by the scientists from the Research Institute of Medicinal Plants

Correspondence: Ewa Skopińska-Różewska, Department of Pathology, Biostructure Center, Medical University, Chałubińskiego 5, 02-004 Warsaw, Poland, Email: ewaskop@hotmail.com

(Dr A. Mścisz and dr S. Mielcarek) as described before [5]. Briefly: air-dried finely powdered rhizomes were extracted two times with water (aqueous extract) or 50% ethanol (hydro-alcoholic extract), evaporated in a rotary vacuum evaporator and lyophilized. Both extracts were dissolved in 10% ethyl alcohol before administration to the animals.

Animals

The study was performed on 8-10- weeks old female inbred Balb/c mice, 20-22 g of body mass, delivered from the Polish Academy of Sciences breeding colony.

For all experiments animals were handled according to the Polish law on the protection of animals and NIH (National Institutes of Health) standards. All experiments were accepted by the local Ethical Committee (nr 1/N/WDP-1/19.01.2006).

In vivo experiment

Rhodiola quadrifida extracts were administered to Balb/c mice *per os* in daily doses of 50, 100, 200 or 400 µg (each group consisted of 8 mice). These doses corresponded to 25, 50, 100 or 200 mg given to 70 kg person (applying the coefficient equal 7 for adjusting differences between mouse and human in relation of the surface to body mass). Mice received drugs by Eppendorff pipette, in 40 µl of 10% ethyl alcohol, for 7 days. Control mice (16 animals) were fed 40 µl of 10 % ethyl alcohol. On the day 8^{ght} mice were bled in anaesthesia from retro-orbital plexus and sacrificed with Morbital.

Chemiluminescence test (CL)

CL was measured using the method of Easmon and Cole with some modifications [9, 10] at room temperature, in scintillation counter (RackBeta 1218, LKB, Sweden). Briefly: samples of 0.05 ml heparinised blood were diluted 1:4 with PBS (Biomed Lublin, Poland) supplemented with 0.1% BSA (Sigma-Aldrich, USA) and 0.1% glucose (Polfa, Poland). Next, 0.05 ml of this diluted blood was mixed with 0.2 ml of luminol (Sigma-Aldrich, USA) solution (10^{-5} M) in PBS and placed in a scintillation counter in the "out of coincidence" mode for background chemiluminescence measurement. Then, the cells were activated by addition of 0.02 ml solution of opsonised zymosan (10 mg/ml) and chemiluminescence activity was measured for the next 15 min. Counting of leukocytes and blood smears examination were performed by routine methods and the results were shown as the maximum value of chemiluminescence (cpm) obtained for 10^3 granulocytes.

Statistical analysis

The results were verified statistically by a one-way ANOVA analysis of variance (GraphPad Prism software package), and the significance of differences between the groups was verified with a Tukey-Kramer Multiple Comparisons Test.

Results

Performed analysis of variance (ANOVA) revealed, that variation among columns means is significantly greater than expected by chance. The p value is <0.0001, considered extremely significant. Tukey-Kramer Multiple Comparisons Test indicates differences between control group and group of mice fed 0.2 mg of hydro-alcoholic extract ($p<0.01$), and between control mice and mice fed 0.4 mg of both types of extracts ($p<0.001$). The results are presented on Fig. 1.

Discussion

Our experiments showed *in vivo* immunostimulatory effect exerted by *Rhodiola quadrifida* hydro-alcoholic and aqueous extracts on mice granulocytes activity evaluated by chemiluminescence test.

Granulocytes provide the first line of defence against microbial pathogens and they have been shown to have the capacity to kill various of them. The most important event in the killing process is the generation of reactive oxygen species during the oxidative burst. The production of free oxygen radicals is a critical component of the killing mechanisms of phagocytic cells and is of great importance in protection against infectious diseases. This process leads to the emission of light proportional to free radical quantity-che-

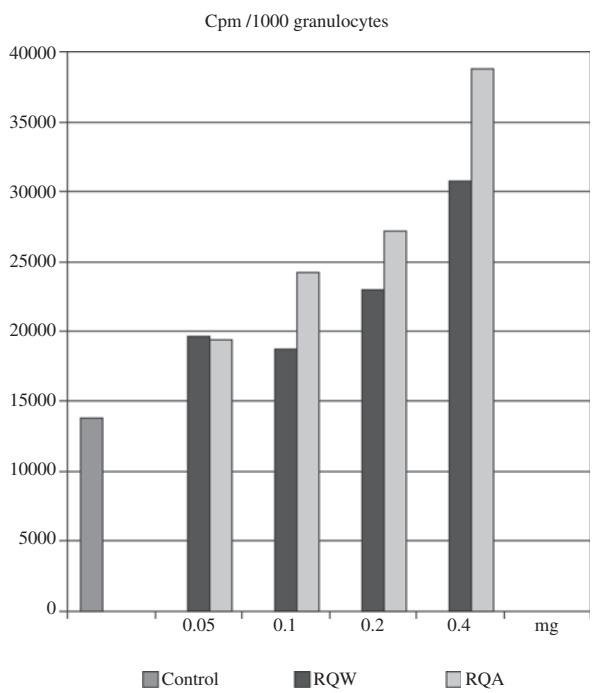

Fig. 1. The effect of feeding mice *Rhodiola quadrifida* aqueous (RQW) and 50% hydro-alcoholic (RQA) extracts on granulocyte chemiluminescence

miluminescence (CL) and is widely accepted as a method of measuring overall granulocytes metabolic activity [11].

Present findings obtained *in vivo* in mice confirm our earlier results obtained *in vitro* in rats, where we observed stimulatory influence of *Rhodiola rosea* and *Rhodiola quadrifida* extracts on non-specific cell-mediated immunity, evaluated by respiratory burst activity (RBA) and potential killing activity (PKA) tests [4-6].

At present, we cannot formulate one hypothesis explaining stimulatory effect of *Rhodiola quadrifida* on granulocyte metabolic activity.

There is a possibility that *Rhodiola's* activity might be secondary to the induction of opioid peptide biosynthesis by this herb as well as to the activation of both central and peripheral opioid receptors, what was discussed elsewhere [4].

It was also reported, that aqueous extract of other *Rhodiola* species, *Rhodiola sachalinensis* enhanced the expression of inducible nitric oxide synthase gene in RAW264. 7 macrophages [12]. As nitric oxide plays an important role in immune function, *Rhodiola* treatment could modulate several aspects of host defense mechanisms due to stimulation of the inducible nitric oxide synthase.

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