EFFECT OF FUCOIDAN, HABERLEA RHODOPENSIS AND PROPOLIS ON MOBILIZATION OF THE CD34+ STEM CELLS IN RATS

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Abstract

We evaluated the influence of fucoidan, Haberlea rhodopensis (H. rhodopensis) and propolis on the hematopoietic stem cells (CD34+) levels into the blood stream of rats. Four groups of male Wistar rats (n = 10) were treated intraperitoneally as follows: 1st group – 0.9% NaCl; 2nd group – fucoidan (100 mg/kg b.w.); 3rd group – H. rhodopensis (50 mg/kg b.w.) and 4th group – propolis (100 mg/kg b.w.). Two hours after a single administration of the substances, blood samples were collected and analysed. We found a significant increase in the number of CD34+ cells in the blood stream after the administration of the tested substances. Fucoidan also caused a significant increase in the number of leukocytes compared to the control group. These results show that the tested substances could mobilize stem cells in the peripheral blood of rats. Fucoidan may increase the count of circulating white blood cells (WBC), H. rhodopensis decreases this value, while propolis does not influence leukocytes level in peripheral blood.

Rezumat

A fost evaluată influența fucoidanului, Haberlea rhodopensis (H. rhodopensis) și a propolisului asupra nivelelor sanguine de celule stem hematopoietice (CD34+) la șobolani. Patru grupuri de șobolani masculi Wistar (n = 10) au fost tratați intraperitoneal după cum urmează: grupul I (control) – ser fiziologic; grupul II – fucoidan (100 mg/kg corp); grupul III - H. rhodopensis (50 mg/kg corp) și grupul IV - propolis (100 mg/kg corp). Animalele au fost supuse unei singure administrații a substanțelor, iar la 2 ore după aceasta, probele de sânge au fost colectate și analizate. S-a înregistrat o creștere semnificativă a numărului de celule CD34+ din sânge, după administrarea substanțelor testate. Fucoidanul a determinat, de asemenea, o creștere semnificativă a numărului de leucocite, comparativ cu grupul control. Aceste rezultate arată că substanțele testate ar putea mobiliza celule stem din sângele periferic al șobolanilor. Fucoidanul poate crește numărul de leucocite circulante, H. rhodopensis scade acest număr, în timp ce propolisul nu influențează nivelul leucocitelor din sângele periferic.

Keywords: CD34+ cells, fucoidan, Haberlea rhodopensis, propolis

Introduction

CD34+ cells are hematopoietic stem and progenitor cells (HPSC), derived from the bone marrow (BM). HPSC are capable of trafficking between BM and blood compartments. They also can differentiate into hematopoietic lineages including leukocytes (white blood cells, WBC), erythrocytes (red blood cells, RBC) and platelets (PLT), maintaining the normal haematopoiesis. The progenitor cells reside in specific niches in BM and their count in the peripheral blood is usually low [7, 11, 12].

Recently, the mechanisms of mobilization are studied intensively. Granulocyte colony-stimulating factor acts as a direct activator of granulocytes and leads to their expansion. One of the disadvantages of the therapy with this cytokine is the variations in the number of HPSC, mobilized by the patients. Moreover, some of them could not mobilize the amount of stem cells, required for autologous transplantation [17].

Over the past few years, the interest in natural substances which can mobilize HPSC in peripheral blood is increasing. Latest studies are focused on fucoidan, a composition of sulfated polysaccharides, derived from brown algae species [5]. This natural product not only shows important pharmacological activities (e.g. antioxidant, antitumor effect) but also induces rapid stem cells mobilization [3, 5]. H. rhodopensis, as a resurrection plant, was subjected to extensive research recently [14, 16]. Extracts of the plant are found to show antioxidant properties, increase skin elasticity and can be used as anti-aging cosmetic products. They also showed antibacterial and cytotoxic activities against human cancer cells [16].
Propolis, a substance produced by honeybees from plants has been found to exert antimicrobial, cytostatic and wound healing effect [2, 9]. Orsolic et al. reported that water-soluble derivatives of propolis can be used for the treatment of cytopenia due to their stimulating effect on the haematopoesis in mice [13]. These authors studied the influence of propolis on the formation of different types of spleen colonies. However, the influence of H. rhodopensis extracts and propolis on the CD34 positive cells and mature blood cells remained unclear.

The aim of this study was to evaluate the effect of fucoidan, H. rhodopensis and propolis on the number of circulating CD34+ cells in peripheral blood of rats. We performed complete blood count (CBC) in order to determine the eventual influence on blood cell maturation.

Materials and Methods

Animals

The experiment protocol was approved by the Animal Health and Welfare Directorate at Bulgarian Food Safety Agency (permit No 86/09.01.2014) and Medical University – Plovdiv Research Ethics Committee (No 3/26.06.2014).

Forty male Wistar rats (weight between 180 - 200 g) were divided into four groups (n = 10). The animals received intraperitoneal injection with the following solutions: 1st group – 0.9% NaCl; 2nd group – fucoidan (100 mg/kg b.w.); 3rd group – H. rhodopensis (50 mg/kg b.w.) and 4th group – propolis (100 mg/kg b.w.). Rats were kept under standard laboratory conditions (temperature 22 ± 1°C, humidity 45% and 12 h light cycle). The rodents received food and water ad libitum.

Reagents

Fucoidan from Fucus vesiculosus (Sigma-Aldrich Co, St Louis, MO) was purchased and stored according to the instructions.

The total leaf extract of H. rhodopensis was prepared as follows: 48 hour maceration in 70% ethanol, followed by percolation. The obtained liquid extract was concentrated in a vacuum rotation evaporator (BUCHI, Rotavapor R II). The herb-to-extract ratio at the end of the procedure was 3:1.

Propolin® (Peych-LP, Bulgaria) (16% ethanolic propolis extract from the Rhodopi Mountains, Bulgaria) was purchased one day before its application.

Fucoidan, propolis, and H. rhodopensis extract were dissolved or diluted in 0.9% NaCl for intraperitoneal administration (0.1 mL/kg b.w.) on the day of the experiment.

Experimental

Two hours after single dose administration of the tested substances, venous blood samples were collected in tubes containing EDTA from the tail vein of the animals using a previously described method [1]. The samples were coded and send for analysis immediately. CD34+ cells level was measured by flow-cytometry (FC500-BECKMAN Coulter). CBC was performed using haematology analyser Advia 2120 (Siemens Diagnostica).

Statistical analysis

All results are shown as percentage ± SEM. The mean value of each parameter in the control groups was taken as 100% and values in the experimental groups were calculated and compared.

The statistical package SPSS 19.0 was employed for the evaluation. The normal distribution was tested with one sample Kolmogorov-Smirnov test. In a case of normal distribution, one way ANOVA with Tuckey post hoc test was used; non-parametric Wilcoxon signed rank test and Mann Whitney test were conducted in the other case. Results were considered significant at p < 0.05.

Results and Discussion

According to Mikirova et al. [10] cells associated with hematopoietic activity express CD34 biomarker. Elevated CD34+ levels in peripheral blood may be related to elevated levels of HPSCs in blood stream.
control group [8]. Irhimeh et al. reported a slight decrease in the lymphocytes and leucocytes after 12-day oral treatment with fucoidan (a clinical trial). Nevertheless, these authors found a small increase in the CD34+ cells [6]. Regarding the effect of propolis, it is possible that the increased levels of CD34+ cells are related to pinocembrin. Treatment of mice with pinocembrin (a flavonoid found in propolis) leads to elevated CD34+ cells levels in peripheral blood [18]. Circulating WBC count reached levels of 145.02% ± 17.60 after treatment with fucoidan as presented in Figure 2 left. We registered a significant decrease in WBCs levels in rats treated with H. rhodopensis (78.66% ± 7.80) when compared to the group treated with fucoidan (145.02% ± 17.60). No significant difference in RBC count was evaluated in the tested groups when compared to controls, as shown in Figure 2 right. A tendency of increase was observed in rats treated with fucoidan. H. rhodopensis (92.29% ± 7.78) and propolis (92.83% ± 8.18) groups revealed lower erythrocytes levels in comparison to rats treated with fucoidan (102.09% ± 17.60; p < 0.05).

![Figure 2](image_url)

**Figure 2.**
Effect of fucoidan, H. rhodopensis and propolis on WBC (left) and RBC (right) levels in peripheral blood of rats (*p < 0.05 versus controls; †p < 0.05 versus fucoidan)

Our findings are consistent with Sweeney et al. [15] who reported an increase in the WBC levels after treatment with fucoidan. The elevated count may be due to the mobilization of leucocytes into the peripheral blood from the bone marrow. Another possibility is the differentiation of the mobilized CD34+ cells into leucocytes.

The elevated levels of WBCs in the peripheral blood may be related to the antibacterial and antiviral properties of fucoidan, as mentioned by Coneac et al. [2].

Based on the slight decrease of WBC and RBC and the increase of PLT after treatment with H. rhodopensis, we can conclude that the total plant extract leads the differentiation of CD34+ cells to platelets. Our results are consistent with Georgieva et al. [4] who also reported lack of positive effect on immune function in rabbits.

We found no significant difference in the number of circulating leucocytes in contrast to Orsolic et al. [12] who registered an increase after treating mice with a water soluble derivative of propolis. The percent of circulating platelets showed no significant difference after treatment with the tested substances when compared to controls taken as 100% (data not shown). A tendency of increase was registered in the group treated with H. rhodopensis. Eventhough the platelets play a major role in the thrombosis, the process of thrombocyte aggregation is more complex and can be influenced by many factors (e.g. the presence of cations) [13]. Hence, further studies may enlighten the role of the herbal extract on the blood coagulation.

**Conclusions**

Fucoidan, H. rhodopensis and propolis elevated the CD34+ cells levels in the peripheral blood of rats. Fucoidan may increase the count of circulating WBC; H. rhodopensis decreases this value, while propolis does not influence leukocytes level in peripheral blood.

In addition, these results provided the first evidence that H. rhodopensis and Bulgarian propolis acts directly on CD34+ cells, augmenting their mobilization in the peripheral blood.

**Conflict of interest**

All authors declare no conflict of interest.

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References


