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ORIGINAL ARTICLE

Regulation of Hematopoiesis in Cancer Patients: Placebo-Driven, Double-Blind Clinical Trials of β-Glucan

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ABSTRACT

AIM: The role of glucan in the stimulation of immunity is well established, including oncological models. In this report, we focused on effects of glucan supplementation of cancer patients in post-treatment periods.

MATERIALS AND METHODS: We measured the levels of C-reactive protein, ferritin, transferrin, soluble transferrin receptors, hemoglobin, total number of erythrocytes and index of sTfR/log ferritin.

RESULTS: We found significant improvements in the levels of hemoglobin, total number of erythrocytes and index of sTfR/log ferritin.

CONCLUSIONS: We concluded that the short 60 day supplementation with orally-administered glucan significantly improved some parameters of hematopoiesis.

Key words: Cancer; Glucan; Ferritin; Hematopoieses; CRP; Ferritin; Erythrocytes

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INTRODUCTION

Anemia occurs in more than 50% of cancer patients and significantly affects both the decreasing quality of life and the survival rate. Pathology of anemia in cancer patients varies. Most often it is a direct result of cancer effects, as cancer cells produce and release various cytokines[1,2]. In addition, anemia can be induced as a result of myelotoxic effects of chemotherapy and/or irradiation[3]. In cancer patients, anemia is a complex condition and is usually caused by a combination of effects. Immune and cancer cells secrete a wide range of cytokines able to inhibit hematopoiesis (IL-, IL-4, IL-6, IFN, and TNF-α), which might play a role in development of anemia either via suppression of erythropoietin production or by stimulation of heptin and ferritin production, which help to regulate input and output of Fe[3]. The treatment of anemia during cancer therapy is aimed at the inhibition of negative effects of anemia and on helping patients live a longer, better life[4].

Beta glucans stimulate hematopoiesis through binding to glucan receptors on hematopoietic progenitors. The most commonly used receptors are Dectin-1 and complement receptor type 3 (CR3, CD11b/CD18)[5,6]. Activation of hematopoietic progenitor cells and leukocytes by polymerized saccharides results in increased production and secretion of granulocyte colony stimulating factor (G-CSF). Recent studies demonstrated that orally administered glucan is well tolerated in cancer patients and supports hematopoiesis suppressed by chemotherapy and irradiation[3,6]. Similarly, glucan can help to reverse the suppression of hematopoiesis by cytotoxic effects of anti-cancer treatment[7]. Experimental studies clearly demonstrate the important effects of glucan in induction of hematopoiesis in clinical practice[6,8]. Both in vitro and in vivo experiments demonstrated that glucans induce production and secretion of various cytokines and hematopoietic growth factors[3,6-9]. Glucan application results in the increase of IL-1 levels in plasma, the increase of chemotaxis and neutrophils adhesion and mobilization of peripheral progenitor cells resulting in regeneration of hematopoiesis. Similarly, phagocytosis

of opsonized tumor cells and tumoricidal activity of NK cells and macrophages are increased by glucan treatment[3,9]. These palliative effects were found in patients with lymphoma, lymphoid leukemia and other malignant diseases[6].

Findings mentioned above led us to the current study. We studied the effects of orally-delivered glucan on hematological characteristics of cancer patients at the end of complex therapy. Most of these patients arrived at our department of medical immunology with a diagnosis of chronic fatigue syndrome or with a diagnosis of suspected immunosuppression. Our current study describes our findings in glucan-supplemented cancer patients treated three to five months after finishing cancer treatment.

MATERIALS AND METHODS

Protocol

A randomized, double-blind, placebo-controlled trial compared β-glucan #300 and a placebo in cancer patients after surgery and chemotherapeutic and radiotherapeutic treatment. We evaluated 27 patients (8 males and 19 females). Average age of the patients was 61.9 years, with an average age of 65.2 years in placebo group and 59.9 years in glucan-supplemented group. The most common diagnosis among women was breast cancer, in men colorectal cancer. All patients completed basic treatment. Glucan supplementation started three to five months after original treatment. At the beginning of the study, we evaluated complex immunological and hematological parameters. In all tested individuals, we found problems similar to chronic fatigue syndrome or immunodeficiency. In addition, reduced immune response with a higher risk of infectious diseases and breathing problems were found in most of the patients.

In this report, we focus on parameters of secondary induced markers of anemia resulting from original treatment. We measured levels of C-reactive protein (CRP), transferrin, ferritin, soluble transferrin receptor and ratio of ferritin with transferrin receptor, hemoglobin and number of erythrocytes.

Glucan

Yeast-derived insoluble glucan #300 was purchased from Transfer Point (Columbia, SC, USA). This glucan is over 85% pure. A single daily dose of 500 mg was used for 60 days. Placebo group was treated with pills of the same size and color.

Methods

Ferritin was evaluated using an Imulite 2000 analyzator employing a FER-IMULITE 2000 kit (Siemens Diagnostics) as recommended by the manufacturer. CRP, transferrin and soluble transferrin receptor (sTfR) were measured using nephelometer Siemens BN II (Siemens Health Care Diagnostic, Germany) as suggested by the manufacturer. Additional hematological parameters were measured using an automatic hematologic analyzer XS – 1000iXA-800i (Symex Corp., Kobe, Japan).

At the beginning and end of the study, we evaluated the score of problems using a modified form as described by others[10,11].

Statistical analysis

Statistical significance was evaluated by a Wilcoxon paired t-test using a GraphPad Prism 5.04 software (GraphPad Software, USA). Statistical significance between individual groups used a $p<0.05$ level.

RESULTS

Table 1 summarizes the levels of tested parameters in both groups of patients. In both groups, levels found at the beginning and the end of the study are given. Levels of CRP were not statistically significant in any group, similar to the levels of transferrin, ferritin and soluble transferrin receptor. Index of sTfR/log ferritin shows insignificant differences in the placebo group, with highly significant differences ($P < 0.002$ level) in glucan-supplemented group (Table 1, figure 1). Similar results were found in the case of hemoglobin levels, where the increase in the glucan-supplemented group was significant at the $P < 0.0373$ level (Table 1, figure 2). When we measured the total number of red blood cells, we found no changes in the placebo group, but significant increase ($P < 0.048$) in the glucan-supplemented group (Table 1, Figure 3). Parameters monitored following the signs of iron metabolism did not differ significantly by gender or diagnosis.

We also focused on index of chronic fatigue syndrome, as its evaluation is gaining more and more attention in cancer patients, in both postsurgical stage and during subsequent treatment. We found improvement of subjective problems in more than 25% of glucan-supplemented patients, whereas in placebo group, the improvement was reported in only 8% of patients.

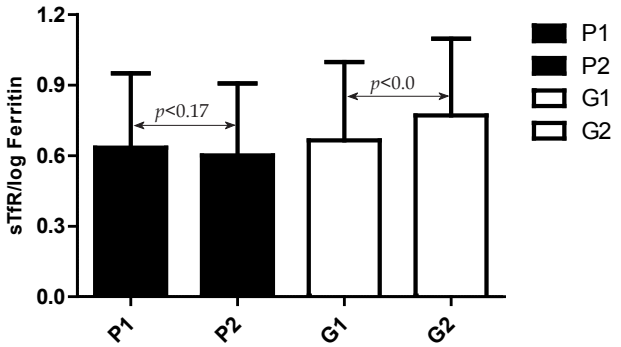

Figure 1 Transferin receptor/log ferritin index in placebo and glucan supplemented groups (P1 and G1 before, P2 and G2 after supplementation).

Table 1 Effects of glucan on individual parameters.

			Control group n=10				Glucan group n=17			
		Normal range	Mean before	SD	Mean after	SD	Mean before	SD	Mean after	SD
C-reactive protein	mg/mL	< 2.5	3.661	4.79	2.413	2.26	2.483	2.58	3.81	8.761
transferrin	mg/mL	2.0 - 3.8	2.568	0.4376	2.633	0.4342	2.762	0.4468	2.654	0.4421
ferritin	µg/L	20.0 - 300	167.55	114.4	150.71	102.12	100.21	78.189	116.8	122.5
sTfR *	mg/L	0.8 - 1.7	1.249	0.2872	1.262	0.255	1.323	0.132	1.292	0.1448
sTfR/log Ferritin		0.5 - 1.4	0.6333	0.3161	0.6	0.3069	0.6647	0.3332	0.7706	0.3264
hemoglobin	g/L	130 - 175	144.44	10.69	143.67	12	138.65	8.054	142.59	7.425
RBC **	$x10^{12}$/L	4.6 - 6.2	4.932	0.3011	4.836	0.4317	4.598	0.3542	4.951	0.8126

* sTfR: soluble transferrin receptor; ** RBC: Red Blood Cells.

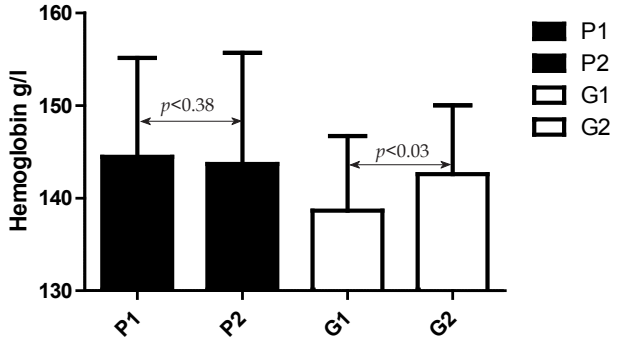

Figure 2 Hemoglobin levels in placebo and glucan supplemented groups (P1 and G1 before, P2 and G2 after supplementation).

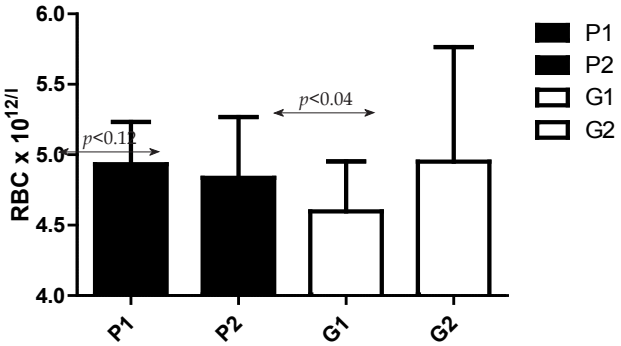

Figure 3 Red blood cells in placebo and glucan supplemented groups (P1 and G1 before, P2 and G2 after supplementation).

DISCUSSION

Oral administration of glucan did not influence the levels of transferrin, ferritin and soluble transferrin receptor. The lack of effects of glucan, in the case of ferritin, might be caused by the large individual variability of ferritin levels, together with its significant ability to react on inflammation. Together, the clinical significance of the ferritin level in iron deficit detection is questionable[1,7]. Due to the higher age of members of both groups, sex-based effects on ferritin levels were not found. From our data we can conclude that low levels of CRP and physiological levels of ferritin and transferrin found in both groups represent a positive marker of the overall health of these patients.

In most of the tested individuals, we found no significant inhibition of hemoglobin levels. The slightly lower levels found at the beginning of the study in the glucan-supplemented group were most probably caused by the fact that some members of this group showed low anemic syndrome with hemoglobin levels around 100-120 g/L. In these patients, glucan supplementation increased the hemoglobin levels to more than 130 g/L.

Reduction of soluble transferrin receptor levels is often described in bone marrow suppression[7]. The levels of sTfR increase based on the usage of iron inside the cell, and these levels reflect iron supply during the last three months, which is the time period when we started glucan supplementation. This fact might explain the small changes in this marker found in our study. Some authors believe that anemia, common in cancer disease, is caused by direct weakening of bone marrow response to erythropoietin stimulation. Our results are in agreement with Lee *et al*[7] who suggested that measurements of sTfR and ferritin have no real reflection of iron deficiencies.

On the other hand, evaluations of the transferrin receptor index are beneficial for the diagnosis of anemia in chronic inflammatory problems and post-infections stages[7,12]. In these cases, we have to remember that these levels can be significantly influenced by numerous causes. In order to eliminate possible effects of day-to-day or seasonal variations, we collected the samples at the same time period between 8 and 10 AM[13].

The addition of glucan to the food during cancer increased chemotaxis and adhesion of neutrophils, affected the production of myeloid growth factors resulting in increase of hematopoiesis and influenced peripheral progenitors cells in the blood[1,3,8,14]. In a model of irradiated mice, glucan caused regeneration of hematopoiesis and increased survival[15,16]. In addition, some studies showed a direct increase in the destruction of tumor cells and higher tumoricidal activity by glucan treatment[6]. Some authors reported higher levels of hemoglobin, leukocytes and platelets after glucan treatment of irradiated cancer patients[14]. Using a mouse model of chemotherapeutically induced immunosuppression, glucan positively affected monocytes in peripheral blood and affected maturation of hematopoietic progenitor cells and hematopoiesis[8]. Our previous experimental work on the mouse model supports these facts.

CONCLUSIONS

We followed-up the animal studies with this report showing positive effects of glucan in post-treatment period in cancer patients suffering from chronic fatigue syndrome. Our experience shows that subsequent treatment in post-surgical and/or post-chemotherapeutical and post-irradiation combined with monitoring of immune state is beneficial. Glucan supplementation improves an important part of hematopoiesis leading to the improvements of both physical and psychological conditions and reductions of possible subsequent complications resulting from subjective concerns about one's own health.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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