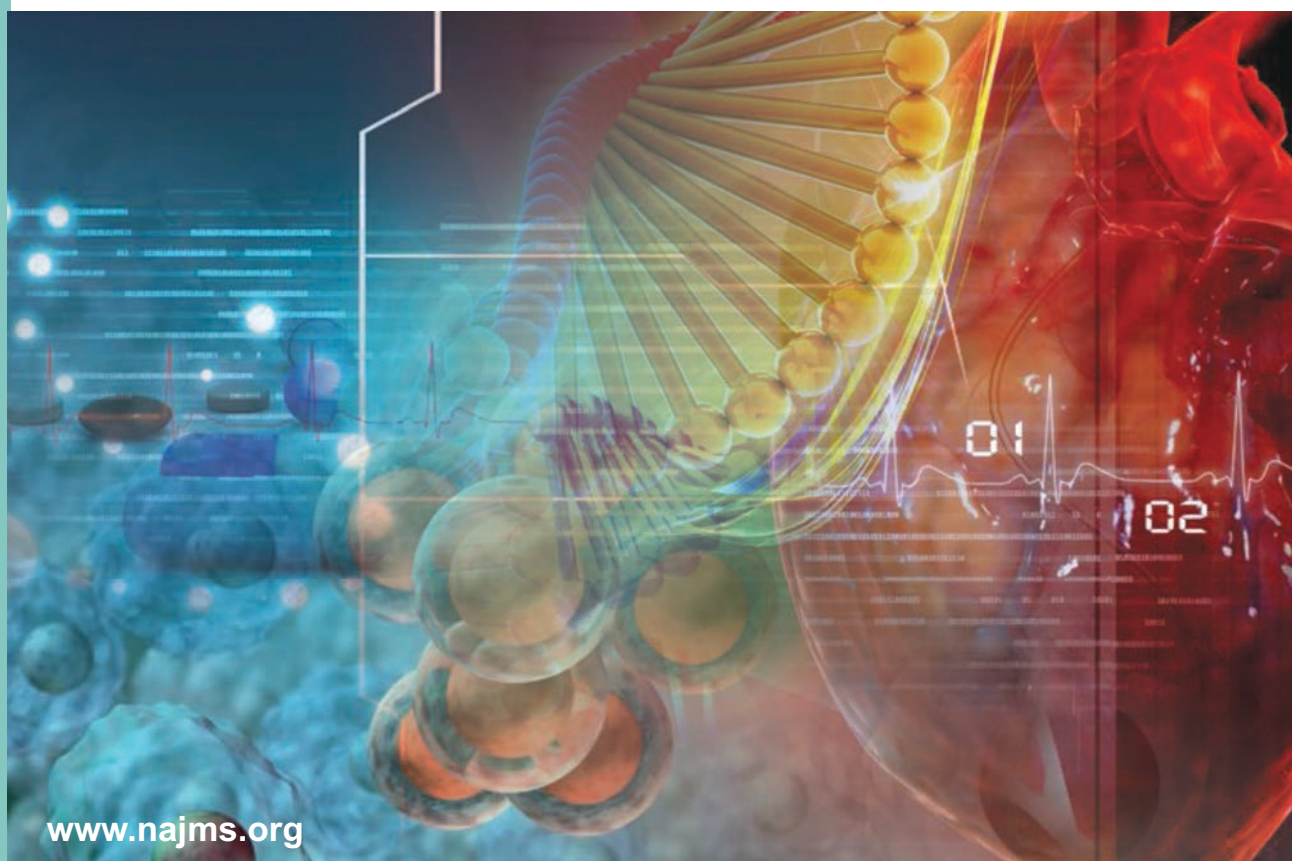


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Addition of Selenium Improves Immunomodulative Effects of Glucan

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Abstract

Background: Selenium (Se) is an established essential nutrient that plays a role in various biological processes including cancer development. Similarly, stimulation of immune reactions by β -glucans is well-documented. **Aims:** In the current study, we focused on the stimulation of phagocytosis and interleukin (IL)-2 production and on potentiation of anticancer immunity by a combination of glucan with two types of Se. **Materials and Methods:** Phagocytosis was evaluated using synthetic microspheres; cancer development was measured either using breast cancer cells or using lung cancer cells. **Results:** Using two different murine models of cancer, we showed that the Se/glucan combination strongly suppressed the growth of cancer, mostly probably *via* stimulation of immunity. **Conclusions:** A combination of glucan with Se offers superior stimulation of immunity and inhibition of cancer growth.

Keywords: Cancer, glucan, immunity, phagocytosis, selenium (Se)

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Introduction

During decades of research, numerous types of glucan have been isolated and described (for review see^[1]). In scientific literature, one can find hundreds of different components, all under the name glucan. Unfortunately, not all glucans were created equal and glucans widely differ not only in physicochemical properties such as molecular weight, branching, or molecular weight but also in biological properties. Despite decades of research, the correlation between these characteristics and biological effects were not fully explained and various hypotheses exist in the literature.^[2] In addition, numerous recent publications suggested that small polysaccharides or even glucan-based oligosaccharides were more active than their high molecular weight counterparts.^[3-5] With stimulating effects on the immune system and cancer development clearly established (for review see^[6]),

the search is on for possible improvements of glucan action. So far, the most promising agents complementing glucan's effects are vitamin C,^[7] resveratrol,^[8] and humic acid.^[9]

One of the promising possibilities is selenium (Se), which is a potent micronutrient important for various facets of mammalian health including the optimal immune response. Se was found to have antioxidant and anti-inflammatory properties,^[10] increased phagocytosis,^[11,12] and improved activity of natural killer cells in elderly humans.^[13] The effects of Se on overall health are summarized in recent reviews.^[14,15] Most of the attention on the biological effects of Se is focused on cancer. Se was found to have suppressive effects on breast cancer manifested *via* epigenetic mechanisms^[16] and a role in the prevention of prostate cancer.^[17]

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Only two studies, so far, have evaluated possible synergy between Se and β -glucan. Se nanoparticles-glucan composites had strong anticancer effects.^[18] Glucan combined with Se-linked pseudodisaccharide was found to have significant effects in several mouse cancer models.^[19] These studies led us to further evaluate the possible synergistic effects of two different types of Se used together with probably the most intensively studied Glucan #300.^[20]

Materials and Methods

Animals

Eight-8 week old female BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). All animal work was done according to the University of Louisville Institutional Animal Care and Use Committee (IACUC) protocol. Animals were sacrificed by CO₂ asphyxiation followed by cervical dislocation.

Material

Yeast-derived insoluble Glucan #300 was purchased from Transfer Point (Columbia, SC, USA), sodium selenite Na₂SeO₃ (Se#1) from Spectrum (Gardena, CA, USA), Se aminomin (Se#2) containing 1.2% of Se from Monarch Nutraceuticals (Ogden, UT, USA). Roswell Park Memorial Institute (RPMI) 1640 medium, glutamine, antibiotics, and cyclophosphamide were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and fetal calf serum (FCS) was from Hyclone Laboratories (Logan, UT, USA).

Phagocytosis

Phagocytosis of synthetic polymeric microspheres was described earlier.^[21] Briefly, 0.1 mL of peripheral blood from mice injected with various doses of glucan or phosphate buffered saline (PBS) was incubated *in vitro* with 0.05 mL of 2-hydroxyethyl methacrylate particles (HEMA; 5×10^8 /mL). The tubes were incubated at 37° C for 60 min, with intermittent shaking. Smears were stained with Wright stain (Sigma). The cells with three or more HEMA particles were considered positive. Mice were injected with either glucan or PBS (control). All experiments were performed in triplicate. At least 300 cells were examined in each experiment.

Interleukin-2 secretion

Purified spleen cells (2×10^6 /mL in RPMI 1640 medium with 5% FCS) obtained from mice injected with 100 μ g glucan or PBS were added into wells of a 24-well tissue culture plate. Cells were incubated for 48 h in a humidified incubator (37°C, 5% CO₂/95% air). The addition of 1 μ g of Concanavalin A (Sigma) was used as a positive control. At the endpoint of incubation,

supernatants were collected, filtered through 0.45 μ m filters (Merck Millipore, Tulagreen, Ireland), and tested for the presence of interleukin (IL)-2 using a Quantikine mouse IL-2 kit (R&D Systems, Minneapolis, MN, USA).

Blood sampling

The blood samplings were performed at day 0 and day 14 after the start of glucan/Se administration. Sampling was conducted with the tail vein. 0.2 mL of blood from each mouse was collected into a proper blood collecting tube containing anticoagulating agent. The total and differentiated white cell profiles were analyzed as previously described.^[22]

Breast tumor inhibition *in vivo*

Mice were injected directly into their mammary fat pads with 1×10^6 /mouse of Ptas64 cells in PBS. The experimental treatment was begun after palpable tumors were found (appropriately 14 days after injection of cells) and after the mice were assigned to experimental groups. Experimental treatment was achieved by intraperitoneal injections of tested samples diluted in PBS (once/day for 14 days). After treatment, the mice were sacrificed, and the tumors removed and weighed.^[23] These experiments were repeated three times with three mice per each group.

Cells

The Lewis lung carcinoma cells were obtained from Dr. G. Ross (University of Louisville, Louisville, KY, USA) and were cultivated as described in Kogan *et al.*, 2002.^[24] The BALB/c mouse-derived mammary tumor cell line Ptas64 was generously provided by Dr. Wei-Zen Wei of the Michigan Cancer Foundation, Wayne State University, Detroit, MI, USA. These cells were maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine and antibiotics.

Lewis lung carcinoma therapy

Mice were injected intramuscular (IM) with 5×10^6 of Lewis lung carcinoma cells. Cyclophosphamide (150 mg/kg) was used intraperitoneal (IP) at day 10 after tumor application. Glucan was used orally from day 0 to day 14 after tumor application. The control group of mice received daily IP PBS. Each group had a minimum of 5 mice. At the conclusion of the experiment, the mice were euthanized and the lungs were removed and fixed in 10% formalin, and the number of hematogenic metastases in lung tissue was estimated using a binocular lens at 8x magnification.

Statistics

Student's *t*-test was used to statistically analyze the data.

Results

As glucans are well-known stimulators of nonspecific immunity, we decided to first evaluate the possible effects of the glucan-Se combo on phagocytosis. Using 2-hydroxymethacrylate particles known for low nonspecific adherence to the cell membrane, we found that Se alone had no effects. Glucan alone showed dose-dependent stimulation of phagocytosis. The addition of Se caused a small increase in phagocytosis, which was significant in the case of 200 μg of glucan with Se#1 [Figure 1].

Next, we focused on the stimulation of cytokine production. Using IL-2 release as a model, we found strong synergistic effects of glucan combined with Se#1 at all glucan-Se ratios [Figure 2].

For subsequent experiments, we used only 100 μg of glucan, either alone or with Se. The amount of Se (10 μg) was the same as before. As mentioned in the Material and Methods section, blood samples were collected at the beginning and the end of supplementation in all the tested groups. No significant changes were observed in the levels of total white blood cell (WBC), lymphocytes, or eosinophils. All tested materials, either alone or together, caused a significant increase in the number of neutrophils (the highest effects were found in case of glucan/Se#1) and a decrease in the number of basophils. The number of monocytes increased only with application of glucan/Se#1 combination [Table 1].

In the next step, we evaluated the role of tested combinations in cancer development. First, we used mice inoculated with the Ptas64 mammary carcinoma cells. Our results found the Se alone had no effects on

the growth of mammary cancer but when combined with glucan, Se significantly suppressed cancer growth [Figure 3]. When we used a model of Lewis lung carcinoma cells, glucan alone caused a significant decrease of lung metastasis and the addition of Se caused an additional 34% suppression [Figure 4].

Discussion

Immunomodulators are not always known for clearly defined mechanisms of action. On the contrary, we usually know nothing about how the particular immunomodulatory works. Glucan, with at least 10,000 published studies, is an exception. However, despite well-established and well-defined action, the search on improving its effects continues. One way is to directly improve the effects of individual glucan by micromanipulating its properties.^[25]

More promising is the way of synergy. There are studies showing that some bioactive molecules have synergistic effects when combined with glucan. Numerous scientific studies have shown some beneficial effects when glucan was given in combination with vitamin C. The main reason why vitamin C shows synergistic effects is the fact that this vitamin has been shown to stimulate the exact same immune responses as glucan, i.e., macrophage activities, natural killer cell activity, and specific antibody formation.^[26] Similarly, glucan supplemented with resveratrol and vitamin C showed strong biological effects.^[27] Another possibility is the strong synergy between glucan and humic acid.^[28] In our study, we decided to evaluate the possibility of glucan-Se synergy, which was previously suggested.^[19]

The doses used in this study, 100 μg of glucan and 10 μg of Se were partly based on the results shown in Figure 1,

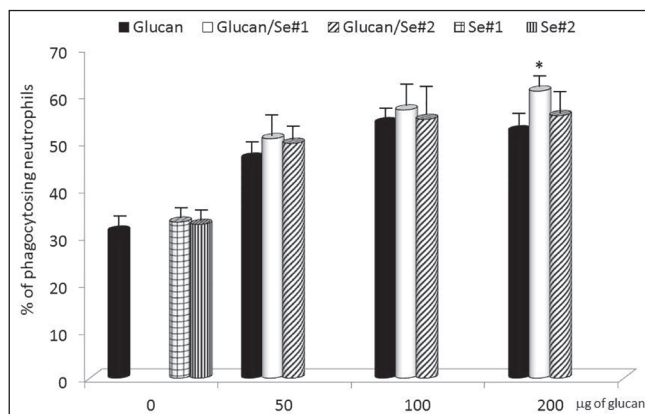


Figure 1: Effects of Se, glucan, or glucan/Se combination on phagocytosis of synthetic microspheres by peripheral blood granulocytes. Each value represents the mean \pm SD. *Represents significant differences between glucan alone and glucan/Se combination at $P \leq 0.05$ level

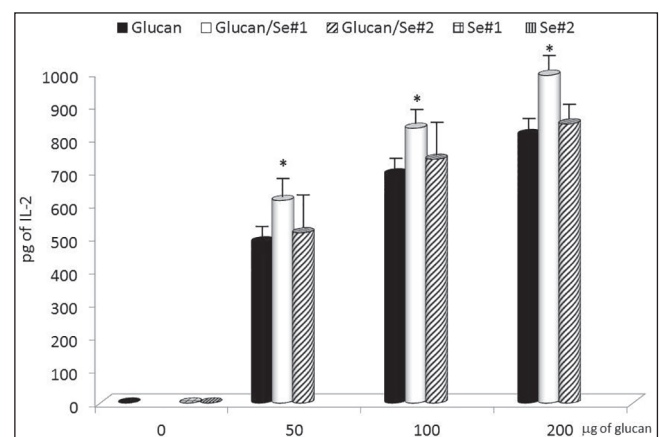


Figure 2: Effects of Se, glucan, or glucan/Se combination on IL-2 production. Each value represents the mean \pm SD. *Represents significant differences between glucan alone and glucan/Se combination at $P \leq 0.05$ level

Table 1: Total and differential white cell counts

Cells/mL	PBS [†]	Glucan	Se#1	Se#2	Glucan/Se#1	Glucan/Se#2
Total WBC [‡]						
0	5920±102	5920±102	5920±102	5920±102	5920±102	5920±102
14 days	5873±111	6103±106	6122±162	6283±171*	8200±117	6388±166
Neutrophils						
0	75±9	75±9	75±9	75±9	75±9	75±9
14 days	73±7	101±11*	255±17*	271±18*	555±62*	243±23*
Lymphocytes						
0	5182±155	5182±155	5182±155	5182±155	5182±155	5182±155
14 days	5207±161	5218±125	5027±172	5225±301	4996±212	5239±177
Monocytes						
0	271±18	271±18	271±18	271±18	271±18	271±18
14 days	281±14	305±15	281±12	299±19	333±17*	311±19
Eosinophils						
0	0	0	0	0	0	0
14 days	0	0	1	2	2	3
Basophils						
0	253±21	253±21	253±21	253±21	253±21	253±21
14 days	260±22	215±11*	201±12*	222±23*	201±12*	219±27*

Results represent mean from three experiments ± SD, *Significant differences between the control and the treated group at $P \leq 0.05$ level, [‡]WBC = White blood cell, [†]PBS = Phosphate buffered saline

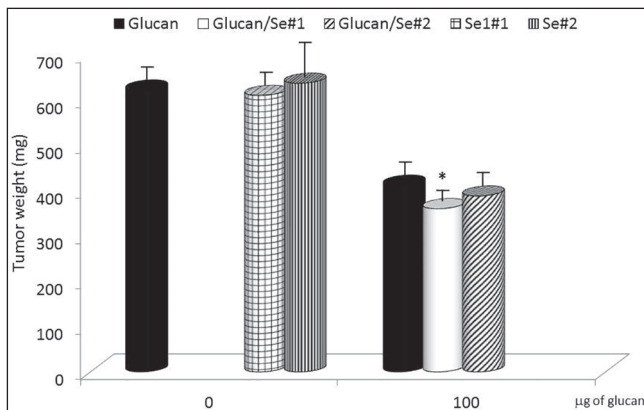


Figure 3: Therapy of BALB/c mice with Ptas64 mammary carcinoma. Data from three independent experiments are shown. For each experiment, groups of mice were tested for a response to a therapy as indicated by the weight of tumors after 2 weeks of therapy. For each experiment, individual groups were treated orally. Each value represents the mean ± SD. *Represents significant differences between glucan alone and glucan/Se combination at $P \leq 0.05$ level

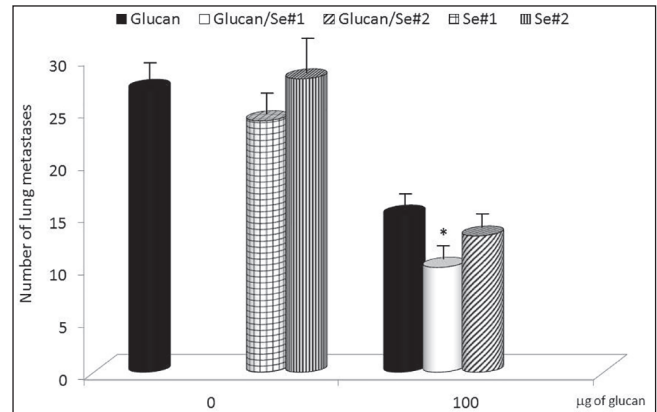


Figure 4: Effect of tested substances on lung cancer growth in cyclophosphamide treated mice. Cyclophosphamide was injected into mice on day 8 of the inoculation of 1×10^5 tumor cells followed by the daily oral doses of individual substances starting 48 h after injection of cyclophosphamide. Each value represents the mean ± SD. *Represents significant differences between glucan alone and glucan/Se combination at $P \leq 0.05$ level

and partly on older studies establishing the optimal dose of Glucan #300.^[21] Our results showing that sodium selenite Na_2SeO_3 was more active than Se aminomin support the previous findings demonstrating that not all Se components are biologically active.^[12]

Biological effects of Se were mostly studied on cancer models. Se was found to have suppressive effects on breast cancer manifested *via* epigenetic mechanisms^[16] and on prostate cancer *via* increase of antitumor immunity.^[29] Some studies even suggested a role in

prostate cancer prevention.^[17] In this study, we used two different mouse cancer models. The first one employed breast cancer cells and showed that the combination of glucan with sodium selenite offered stronger inhibition than individual components. The second model used lung cancer cells and showed identical results. Based on our previous studies, the results are most probably a consequence of the combination of NK cell activation by glucan and stimulation of some cytokine [such as IL-12 and interferon gamma ($\text{IFN}\gamma$)] release by Se.^[30]

Conclusion

Our data confirmed that Se supplementation used concomitantly with a high quality glucan can significantly improve its biological effects. Studies evaluating the exact biological mechanisms of action are currently in progress.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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