

EKSPERIMENTINIAI TYRIMAI

Treatment of Lewis lung carcinoma by photodynamic therapy and glucan from barley

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Summary. *Objective.* During the photodynamic treatment, complement system is activated and tumor cells are opsonized with iC3b fragment. β -glucans can enhance cytotoxicity of iC3b-opsonized cells due to their specific interaction with complement receptor 3 (CR3; CD11b/CD18) on the surface of the effector cells. In contrast to microorganisms, tumor cells lack β -glucan as a surface component and cannot trigger complement receptor 3-dependent cellular cytotoxicity and initiate tumor-killing activity. This mechanism could be induced in the presence of β -glucans. This study aimed at determining the influence of coadministration of β -glucan from barley on the efficacy of photodynamic tumor therapy (PDT).

Material and methods. C57 Bl/6 female mice bearing Lewis lung carcinoma were used throughout the study. Mice were randomized into groups (15 in each group) and exposed to the treatment with intravenous Photofrin injection (dose, 10 mg/kg) and after 24 h following laser illumination, or with oral administration of β -glucan from barley at a dose of 400 μ g/mouse per day up to 5 days, or with their combination. Tumor growth dynamics and survival of the treated and untreated mice were monitored.

Results. Tumor volume in all treated groups was significantly lower ($P < 0.001$) than that in the control group. The most effective tumor growth suppression ($P = 0.033$) was achieved in mice treated with combination of PDT and β -glucan from barley as compared with PDT alone. The best survival was achieved in the same group, but difference was not significant as compared to the control group ($P = 0.143$) and to PDT alone group ($P = 0.319$).

Conclusions. The present study demonstrates that coadministration of β -glucan from barley can enhance efficacy of photodynamic therapy.

Introduction

Photodynamic therapy (PDT) is a treatment method that combines the administration of a light-sensitive drug and lesion-directed activation of the photosensitizer with visible light. The agent is absorbed by cells all over the body, but it stays in cancer cells longer than it does in normal cells. Approximately 24 to 72 hours after injection (1), when most of the agent leaves normal cells but remains in cancer cells, the tumor is exposed to light. The photosensitizer in tumor absorbs the light and produces reactive oxygen species (singlet oxygen and free radicals such as OH⁻, HO²⁻, and O²⁻) that destroys nearby cancer cells (2, 3). In addition to killing cancer cells directly, PDT appears to shrink or

destroy tumors in two other ways (1–4). The photosensitizer can damage blood vessels in tumor, thereby preventing the cancer from receiving necessary nutrients. In addition, photo-oxidative lesions produced by PDT are recognized as self-alteration by the host. It activates the immune system to attack the tumor cells, and major effectors, inflammation and acute-phase response, are mobilized. The complement system has an important role in this host response (5). Complement system is activated via alternative pathway during the post-PDT treatment (6). After several cascade reactions, it results in the covalent attachment of C3b to the cell surface, where then it is rapidly degraded into the fragments iC3b and C3dg. Then

these fragments bind to complement receptor 3 (CR3; CD11b/CD18) on leukocytes. The CR3 on human leukocytes does not trigger the killing of tumor cells coated with their ligand iC3b. But CR3 priming for cytotoxic function requires ligation of both, the I-domain and lectin-like domain of CR3 (7). CR3-DCC is normally reserved for yeast and fungi that have β -glucan as an exposed component of their cell wall (8). Yeast cell wall β -glucan binds to a C-terminal lectin domain of CD11b, and iCR3b binds to N-terminal I-domain binding site of CD11b. After this dual ligation, efficient cytotoxic degranulation and phagocytosis are primed. In contrast to microorganisms, tumor cells lack β -glucan as a surface component and cannot trigger CR3-dependent cellular cytotoxicity and initiate tumor-killing activity.

β -Glucans are naturally occurring polysaccharides. These glucose polymers are produced by a variety of plants such as oat, barley, and seaweed. β -Glucans are the constituents of the cell wall of certain pathogenic bacteria (*Pneumocystis carinii*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Histoplasma capsulatum*, *Candida albicans*) and fungi (*Saccharomyces cerevisiae*). It has been common knowledge in the scientific community that β -glucan is the most powerful stimulator of the immune system and a very powerful antagonist to both benign and malignant tumors; it lowers cholesterol and triglyceride level, normalizes blood sugar level, heals and rejuvenates the skin, and has various other benefits (9).

Glucan from barley is a low-molecular-weight β -glucan with mixed (1 \rightarrow 3)- and (1 \rightarrow 4)- β -linkage in the backbone. It has been shown in vitro that barley β -glucan bind to CR3 (10). Although it is reported that oral administration of β -glucan derived from barley can greatly enhance the activity of antitumor monoclonal antibodies in xenograft models (11).

This study aimed at determining the influence of coadministration of β -glucan from barley on the PDT outcome due to enhancing the cytotoxicity of effector cells on iC3b-opsonized tumor cells.

Material and methods

Animals and tumor model

C57 Bl/6 female mice (age, 8–10 weeks; body weight, 19–22 g) obtained from the Immunology Institute, Lithuania, were used throughout the study. Mice were subcutaneously injected with 0.2 mL of five-fold diluted Lewis lung carcinoma (LLC) tumor mass suspension in a right groin. Ten days after implantation, tumors reached the volume of 400–600 mm³ and were exposed to treatment. Tumor volume (TV) was determined by measuring the tumor diameter

with vernier calipers, and it was calculated according to the formula:

$$TV=L \times W \times H \times \pi / 6,$$

where L is length, W is width, and H is height of the tumor.

All animal procedures were performed in accordance with the guidelines established by the Lithuanian Animal Care Committee, which approved the study.

Photosensitizer

Photofrin (porfimer sodium, a kind gift from Axan Pharma Inc., Mont-Saint-Hilaire, Quebec, Canada) was dissolved in 0.9% sodium chloride solution and used at a concentration of 10 mg/kg. It was injected intravenously in tumor-bearing mice 24 h before the tumors were exposed to light treatment.

Laser illumination

The tumors (400–600 mm³ in diameter) were illuminated with light from diode laser (Institute of Oncology, Vilnius University, Lithuania) at 630 \pm 2 nm and at a fluence rate of 160 mW/cm² for 15 min, reaching a dose of 200 J/cm². During light treatment, individual animals were anaesthetized.

Glucan

β -Glucan from barley (powder, >95%, Sigma-Aldrich, Germany) was dissolved in phosphate-buffered saline (PBS) and administered orally at a dose of 400 μ g/mouse (volume, 0.2 mL) every day up to 5 days, starting on the day of intravenous injection of Photofrin.

Experimental design

Mice were coded and randomized into four groups (15 in each group): control group, mice did not receive any treatment; PDT group, Photofrin was injected intravenously 24 h prior to laser illumination, which was performed as described above; GL-Barley group, β -glucan (GL) from barley was administered orally at a dose of 400 μ g/mouse per day up to 5 days; PDT+GL-Barley group, Photofrin was injected intravenously 24 h before laser illumination, which was performed as described above, and β -glucan from barley was administered orally at a dose of 400 μ g/mouse per day up to 5 days starting on the day of Photofrin injection.

Data analysis

GraphPad Prism 3.0 software was used for the statistical analysis. The results of tumor response were

statistically analyzed using two-way ANOVA. Data are given as mean \pm standard deviation. Mean tumor size over time between two groups was tested for significant difference using the Fisher's F test. The Kaplan-Meier method was used for survival analysis. The level of significance of the differences between the survival curves was assessed by Gehan's Wilcoxon test. Differences were considered significant when $P < 0.05$.

Results

To evaluate the treatment effect in LLC tumor-bearing mice, we measured tumor volume every second day starting on the day of exposure to treatment until the end of the experiment. The pilot experiments revealed no antitumor activity when light without Photofrin or Photofrin without light was applied (data not shown). Mean tumor volume in all treated groups was significantly lower ($P < 0.001$) than that in the control group, as it is shown in Fig. 1. Both treatment regimens, administration of PDT alone and β -glucan from barley alone, have shown a significant efficacy ($P < 0.001$) in tumor growth suppression in LLC tumor-

bearing mice as compared with untreated mice. However, treatment with β -glucan from barley alone was more effective than treatment with PDT alone, but the difference was not significant ($P = 0.145$). The most effective tumor growth suppression was achieved in mice treated with combination of PDT and β -glucan from barley, and the difference was significant ($P = 0.033$) as compared with PDT alone. The difference in tumor volumes between these treatment groups appears on the day 2 and lasts until the day 14 after the exposure to the treatment (Table).

Table. Significance of the difference in tumor volumes comparing mice receiving photodynamic therapy and photodynamic therapy + β -glucan from barley

| Days after exposure | F | P |
|---------------------|-------|--------|
| 2 | 8.72 | 0.007 |
| 4 | 12.73 | 0.0014 |
| 8 | 7.69 | 0.013 |
| 10 | 6.08 | 0.022 |
| 14 | 3.54 | 0.081 |
| 16 | 2.45 | 0.149 |

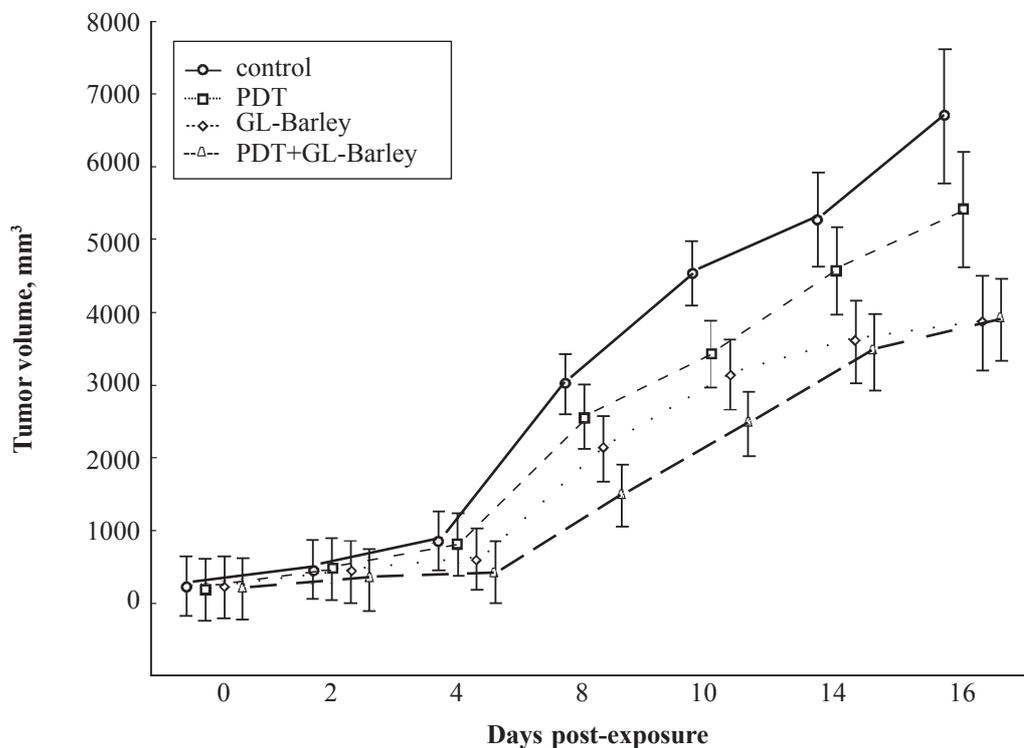


Fig. 1. Growth dynamics of Lewis lung carcinoma tumors after photodynamic therapy and/or treatment with β -glucan from barley

Control group – untreated mice; PDT group – intravenous Photofrin injection and after 24h following laser illumination; GL-Barley group – β -glucan from barley was administered orally at a dose of 400 $\mu\text{g}/\text{mouse}$ per day up to 5 days starting on the day of Photofrin injection; PDT+GL-Barley – intravenous Photofrin injection and after 24 h following laser illumination in combination with β -glucan from barley, which was administered orally at a dose of 400 $\mu\text{g}/\text{mouse}$ per day up to 5 days starting on the day of Photofrin injection.

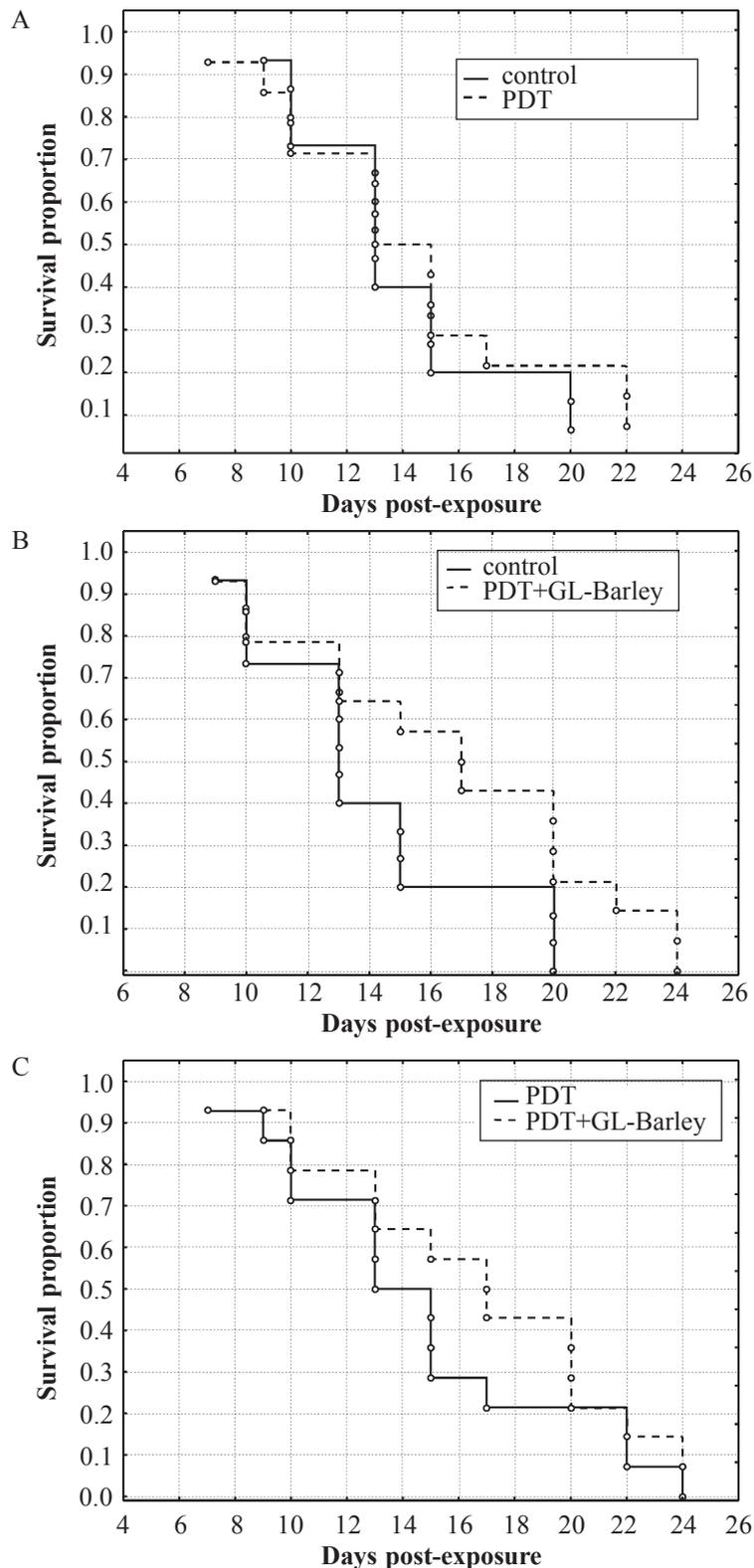


Fig. 2. Survival curves of Lewis lung carcinoma tumor-bearing mice after photodynamic therapy and/or treatment with β -glucan from barley

A – control group vs. PDT group; B – control group vs. PDT+GL-Barley group; C – PDT group vs. PDT+GL-Barley group. Control group – untreated mice; PDT – intravenous Photofrin injection and after 24 h following laser illumination; GL-Barley – β -glucan from barley was administered orally at a dose of 400 $\mu\text{g}/\text{mouse}$ per day up to 5 days starting on the day of Photofrin injection; PDT+GL-Barley – intravenous Photofrin injection and after 24 h following laser illumination in combination with β -glucan from barley, which was administered orally at a dose of 400 $\mu\text{g}/\text{mouse}$ per day up to 5 days starting on the day of Photofrin injection.

There was no difference in the survival of the LLC tumor-bearing mice treated with PDT alone as compared with untreated mice (Fig. 2A). The best survival was achieved in the group of mice treated with combination of PDT and glucan regimen, but difference was not significant as compared to the control group ($P=0.143$) and PDT alone group ($P=0.319$) (Figs. 2B and 2C).

Discussion

PDT induces the activation of complement system in host defense via alternative pathway mainly. C3 is the key component in the cascade of complement system activation. The cleavage of C3 results in generation of C3b, a part of which becomes attached to the cell surface – tumor cells become opsonized with iC3b fragment. The effector cells, such as leukocytes, NK cells, and macrophages, then can be attracted to attach to these cells. Analysis of C3 content in PDT-treated tumor revealed a marked increase in the levels of this protein peaking at 3 h after therapy and remaining highly elevated until 24 h post-PDT, and the potential for complement activation via alternative pathway remained unchanged until a significant increase at 24 h post-PDT, which persisted up to 72 h post-PDT (6). Therefore, this period is essential for incorporation of β -glucan into the binding of iC3b-opsonized tumor cell to CR3 on the effector cell to have a dual ligation of this receptor and to cause effective cytotoxicity.

The results of our study support our suggestion

that coadministration of (1 \rightarrow 3),(1 \rightarrow 4)- β -glucan from barley can significantly enhance a suppressive effect of PDT on tumor growth in tumor-bearing mice. It was observed that treatment of LLC tumor-bearing mice with β -glucan from barley alone significantly suppressed the tumor growth as compared with untreated mice as well. It can be explained that due to subcutaneous inoculation of the tumor cells to mice, antibodies start to be produced. The binding of these antibodies to the surface of tumor cells activates classical complement pathway, which can also be enhanced by the presence of β -glucan (11, 12). Further studies are needed in order to understand the mechanism of β -glucan action on tumor cells following PDT treatment. In addition, other types of β -glucans, such as (1 \rightarrow 3),(1 \rightarrow 6)- β -glucans, should be tested, because antitumor and antimetastatic effect has been shown in several studies with these β -glucans from different sources (9, 13).

Conclusion

The present study demonstrates that coadministration of β -glucan from barley enhances efficacy of photodynamic therapy by suppressing the tumor growth but not prolonging survival of Lewis lung carcinoma-bearing mice.

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Luiso (Lewis) plaučių karcinomos gydymas fotodinaminio metodu ir β -gliukanu, išskirtu iš miežių

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Raktažodžiai: β -gliukanas, komplemento receptorius 3, fotodinaminis navikų gydymas, Luiso plaučių karcinoma.

Santrauka. *Tyrimo tikslas.* Po fotodinaminio poveikio aktyvuojama komplemento sistema ir naviko ląstelės opsonizuojamos iC3b fragmentu. β -gliukanas sustiprina ciotoksinį poveikį iC3b fragmentu opsonizuotoms ląstelėms dėl jo sąveikos su komplemento receptoriumi 3 (CD11b/CD18), esančiu efektorinių ląstelių paviršiuje. Skirtingai nei mikroorganizmai naviko ląstelės savo membranos sudėtyje neturi β -gliukano, todėl negali aktyvinti nuo minėto receptoriaus priklausomo citotoksiškumo ir sukelti navikinės ląstelės žūtį. Šis mechanizmas gali būti indukuotas esant β -gliukanui. Šio tyrimo tikslas – nustatyti β -gliukano, išskirtu iš miežių, įtaką fotodinaminio navikų gydymo veiksmingumui.

Tyrimo medžiaga ir metodai. Šio tyrimo metu naudotos C57Bl/6 pelės. Jos buvo skirstytos į grupes (n=15) ir taikyta fotodinaminė terapija (FDT) (fotofrinas injekuotas į veną (dozė – 10 mg/kg), o po 24 val. navikai švitinti lazerio šviesa) arba β -gliukanu, išskirtu iš miežių, po oda – 400 μ g/d. pelei, iš viso penkias dienas, arba jų darinys. Stebėta navikų augimo dinamika ir pelių išgyvenamumas.

Rezultatai. Navikų tūriai, palyginus su kontroline grupe, buvo mažesni visose gydomosiose grupėse ($p < 0,001$). Veiksmingiausiai navikų augimas buvo slopinamas pelėms, kurios buvo gydytos FDT kartu su β -gliukanu. Rezultatas statistiškai reikšmingai ($p = 0,033$) skyrėsi palyginti su grupe, kur pelės buvo gydytos tik FDT. Nepaisant to, kad, taikant kombinuotą gydymą gyvūnai išgyveno ilgiausiai, tačiau statistiškai reikšmingo skirtumo nenustatyta palyginti su kontrolinės grupės gyvūno gyvenimo trukme ($p = 0,143$) bei FDT ($p = 0,319$) grupėse.

Išvados. Tyrimo duomenys rodo, kad β -gliukanas, išskirtas iš miežių, gali didinti fotodinaminio navikų gydymo veiksmingumą.

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