EXPERIMENTAL STUDIES OF POSSIBLE MODULATIVE EFFECT OF β-GLUCAN ON MICE LUNG CARCINOGENESIS

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Abstract. β-glucans are naturally occurring polysaccharides that are found in baker`s yeast, oats, barley fibre as well as medicinal mushrooms. Literary data show that they produce various biological effects (immune modulating, anticarcinogenic, lipid and body weight lowering). In the present study, the possible modulative effect of β-glucan (from yeast) on lung carcinogenesis was evaluated. We used 224 BALB/c mice both sexes divided into 6 groups. During the experimental period, the animals were treated with aqueous solutions of β-glucan, with a dry weight of 100, or 500 μg/ml, respectively (solutions were offered to mice *ad libitum*). Lung tumours were induced by organotropically acting urethane (given by intraperitoneal injections 10 mg/mouse, twice a week, total dose 50 mg/mouse). After 4 months, all mice were killed by cervical dislocation. Lungs were examined macroscopically and microscopically. The results of our study showed that β-glucan from yeast did not significantly inhibit lung adenamogenesis induced by urethane. Considering the known beneficial effects of β-glucans in other assay systems, future efforts should direct at performing experiments to verify the actual efficacy of β-glucans or β-glucans containing compounds on chemically induced carcinogenesis.

Keywords: β-glucan, urethane, lung carcinogenesis, mice.

EKSPERIMENTINIAI BETA GLIUKANO GALIMO MODULIACINIO POVEIKIO PELIŲ PLAUČIŲ KANCEROGENEZEI TYRIMAI

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Santrauka. Beta gliukanai yra natūralūs polisacharidai, randami kepimo mielėse, avižose, miežiuose ir grybuose. Tyrimų duomenimis, jiems būdingas įvairus biologinis poveikis (imunomoduliacinis, antikancerogeninis, mažinantis lipidų koncentraciją ir kūno svorį). Šiuo darbu norėta nustatyti galimą moduliacinį beta gliukano (išskirto iš mielių) poveikį chemiškai indukuotai laboratorinių gyvūnų plaučių kancerogenezei. Naudotos 224 BALB/c linijos pelės (abiejų lyčių), suskirstytos į 6 grupes. Bandymo metu jos girdytos vandeniniu beta gliukano tirpalu (100 arba 500 μg/ml) *ad libitum*. Plaučių navikams indukuoti naudotas kancerogenas uretanas (švirkštas intraperitonealiai po 10 mg pelei du kartus per savaitę; suminė dozė – 50 mg pelei). Po 4 mėnesių gyvūnai dekapituoti. Jų plaučiai ištirti makroskopiškai ir mikroskopiškai. Bandymo rezultatai parodė, kad beta gliukanas statistiškai reikšmingai plaučių adenomogenezės, sukeltos uretanu *in vivo,* neslopino. Atsižvelgiant į tai, kad įvairūs beta gliukanai ar jų turintys junginiai yra naudingi kitose tyrimų (testų) sistemose, ateityje reikėtų atlikti daugiau bandymų ir išsiaiškinti jų veiksmingumą kancerogenezės metu.

Raktažodžiai: beta gliukanas, uretanas, plaučių kancerogenezė, pelės.

Introduction. Natural products brought to attention by complementary and alternative medicine, are undergoing scientific analysis and development to prevent cancer. A major problem in characterizing many natural products is that they represent a complex mixture of ingredients, each one of which may contribute to bioactivity (Hong et al., 2004; Vetvicka et al., 2007).

β-glucans are naturally occurring polysaccharides and are the constituents of the cell wall of certain pathogenic bacteria, yeast and fungi. They have been purified from baker‘s yeast, oats, barley fibre as well as mushrooms. β-glucans are present in natural yeast and mushrooms mainly as β-1.3,1.6-glucan, and as β-1.3, 1.4-glucan in oats and barley (Chang et al., 2006). Literary data show that β-glucans produce important biological effects, including its immune modulating, anticarcinogenic, lipid lowering effects, as well as its ability to reduce the blood sugar levels, bodyweight, and have other benefits (Ooi and Liu, 2000; Cheung et al., 2002; Kim et al., 2006; Akramienė et al., 2007; Mantovani et al., 2008; Chan et al.,

2009; Lin et al., 2009).

The immunomodulating effect of β-glucan is probably associated with the activation of cytotoxic macrophages and T-helper as well as natural killer cells and with the activation of cytotoxic macrophages and the promotion of T lymphocyte differentiation and activation, for the alternative complement pathway (Bohn et al., 1995). β-Glucans are modulators of both humoral and cellular immunity (Vetvicka et al., 2007). It has been shown that β-glucans from yeast activate macrophages and induce interleukin (IL-6) and tumour necrosis factor *in vitro*, promoting vascular permeability and stimulating the classic complement pathway (Tokunaka et al., 2000).

Recently, several works *in vitro* and *in vivo* have demonstrated that β-glucans have protective activity against various mutagenic agents (Mantovani et al., 2008). For instance, β-glucans have protective effect against genotoxicity and cytotoxicity when administered along with such drugs as cyclophosphamide, adriamycin and cisplatin. Besides showing a protective effect against several chemical agents, β-glucans can act as antioxidants and prevent damage by H_2O_2 and other reactive species (Krizkova et al., 2006).

So the results of many studies indicate significant protective antioxidant, antimutagenic and antigenotoxic activities of the polysaccharides and imply their potential application in cancer prevention.

Results of the most clinical research done in Japan showed that yeast β-glucan can also enhance the effect of anticancer chemotherapy or radiation therapy and have positive effect on the survival and quality of life of cancer patients (Torisu at al., 1990; Nakazato et al., 1994).

The aim of the present study was to investigate modifying potential of β-glucan extracted from yeast on lung carcinogenesis induced by urethane in mice model.

Materials and methods. Chemicals: β(1.3/1.6) glucans 85% extract from yeast (pulsis, CHEMEX Hamburg GmbH, Germany); commercial urethane (purum, Fluka, Buchs, Schweiz).

Animals and treatment: 224 BALB/c mice (8 weeks old, both sexes, and having a body weight of 20–24 g) were used in the study. The animals were obtained from Animal Facility of State Research Institute Centre for Innovative Medicine. Experiment conditions were in compliance with Good laboratory practices and with the Law of the Republic of Lithuania on the Care, Keeping and Use of Animals as well as secondary legislation – Order of the State Food and Veterinary Service of the Republic of Lithuania "On Veterinary Regulations on Breeding, Handling and Transportation of Laboratory Animals" and "On the Use of Laboratory Animals in Scientific Experiments" (Law of the Care, Welfare and Use of Animals, 2002). The protocol was approved by State Food and Veterinary Service/Ethical Committee for Animal Research (protocol No. 0202, 2009).

The animals were acclimatized for one week before the study; they were housed under constant conditions of temperature, humidity and light/dark cycle (12 h/12 h). Commercial pellet diet and fresh drinking water were provided *ad libitum*.

The mice were randomly allocated into 6 groups (Fig. 1).

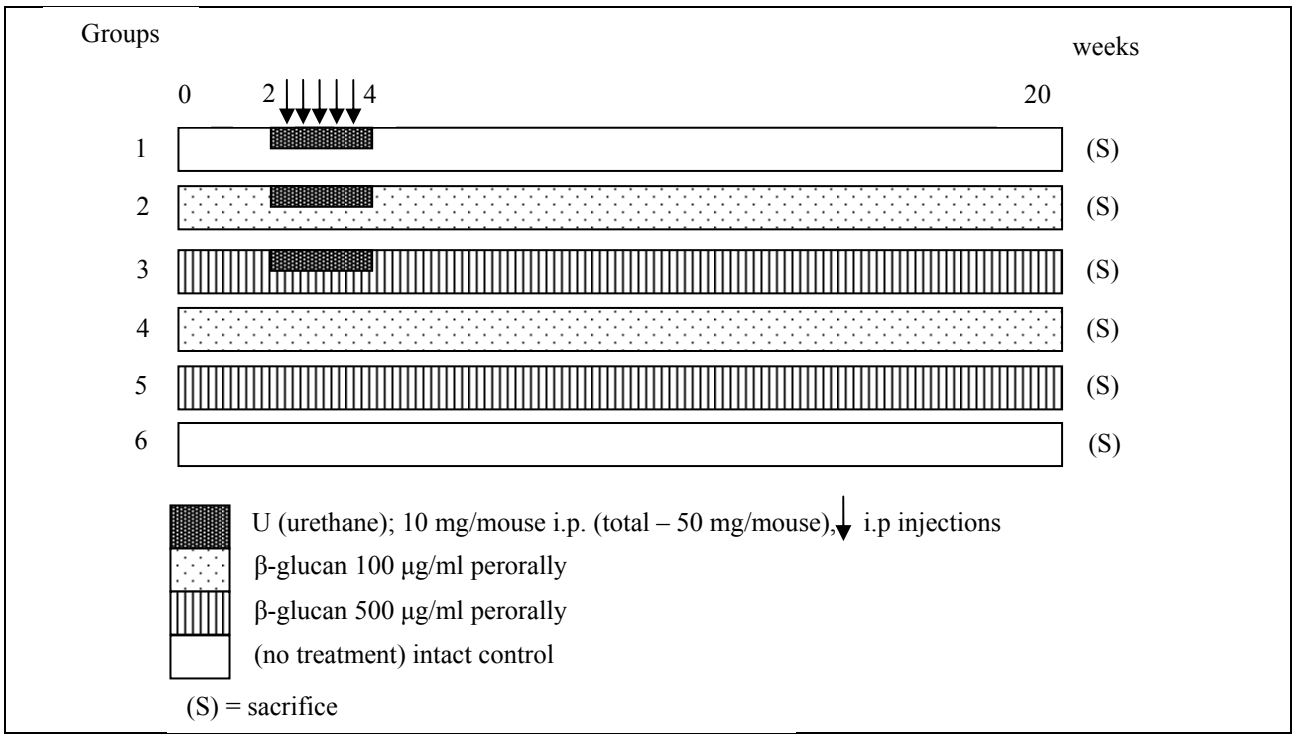

Fig. 1. **Experimental design**

The doses of tested compounds were chosen according to our previous experience and literary data (Barauskaite and Griciute, 1986; Uleckiene and Domkiene, 2003; Didziapetriene and Uleckiene, 2008; Akramiene, 2011).

Urethane was given 10 mg/mouse twice a week by intraperitoneal injections (total dose of 50 mg/mouse). During the experimental period animals were treated with aqueous solution of β-glucan (starting 2 weeks prior carcinogen administration), with a dry weight of 100, and 500 μg/ml respectively. The solutions were offered to animals *ad libitum*, in aluminium foil-wrapped bottles to avoid light decomposition. They were the sole source of drinking fluid. The body weight was recorded weekly. After 4 months, all surviving mice were killed by cervical dislocation. Mice were autopsied and examined. Lungs were fixed in 10% formalin. After fixation, all only macroscopically noted lung tumours were counted by two independent experts in order to ensure the objectivity. The part of lung material with visible tumours was dehydrated in alcohols, embedded in paraffin, and 5-μ sections were stained with hematoxylin and eosin (H&E).

The results were statistically processed using Student`s *t*-test. Mean number of tumours per tumour-bearing mouse, percentage of animals with tumours, time to develop tumours, and histological type of them were considered.

Results and discussion. In this study, we used mouse lung adenoma assay which is one of assays used for the investigation of possible cancer chemopreventive agents (Mikchailova et al., 2002; Uleckiene and Domkiene, 2003; Vesnushkin et al., 2006; Didziapetriene and Uleckiene, 2008). We examined BALB/c mice characterized by high sensitivity to pulmonotropic carcinogen urethane. It is known that the carcinogenic effect of urethane directly depends on its dose and can be modified by influence of potential anticarcinogenic agents. Adenomas may be found in either lung, in any lobe, are often situated just beneath the pleura and are recognized by their nodular, pearly, grey-white appearance contrasting with the pinker colour of the normal lung parenchyma (Fig. 2).

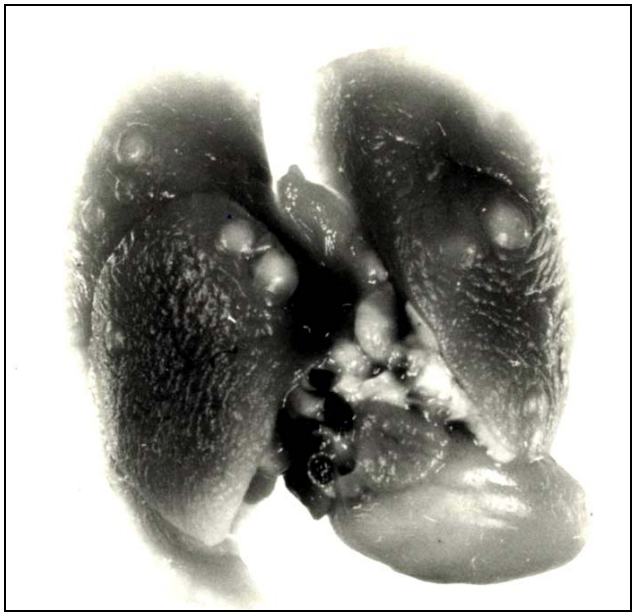

Fig. 2. **Mouse lungs with adenomas** (macroscopic view)

The results on compounds currently being tested in the experiments on mice are shown in Table 1.

The first lung tumours (three adenomas) were observed on the 11th week in mice (♂) which were found dead in a group given β-glucan (500) + urethane.

Table 1. **Incidence and multiplicity of lung tumours in BALB/c mice treated with urethane and β-glucan**

Groups No.	Treatment	Number of animals		Mice with tumours (%)	Lung tumours/ mouse ±SD
		initial	effective		
I.	Urethane	♂ 20	20	20 (100)	5.3±1.3
		♀ 20	20	20 (100)	4.7±1.4
		Total 40	40	40 (100)	5.0±1.4
II.	β-glucan (100) + Urethane	♂ 21	21	21 (100)	4.6±1.4
		♀ 20	20	20 (100)	3.7±0.9
		Total 41	41	41 (100)	4.2±1.2
III.	β-glucan (500) + Urethane	♂ 20	20	20 (100)	5.1±1.5
		♀ 20	20	20 (100)	3.9±1.1
		Total 40	40	40 (100)	4.5±1.3
IV.	β-glucan (100)	♂ 22	22	0	–
		♀ 20	20	0	
		Total 42	42	0	
V.	β-glucan (500)	♂ 21	21	0	–
		♀ 20	19	0	
		Total 41	40	0	
VI.	Intact control	♂ 10	10	0	–
		♀ 10	10	0	
		Total 20	20	0	

At the end of experiment, lung tumours were found in every mouse given carcinogen.

Fewer tumours were observed in the groups given lower dose of β-glucan, but the results were not statistically significant in comparison with group I (only urethane).

β-Glucan used in higher dose also did not show significant inhibitory effect on lung tumours development.

The most tumours in mice lungs (various groups) were 0.5 mm or less in diametre. Histological examination showed only benign adenomas in the lungs (Fig. 3).

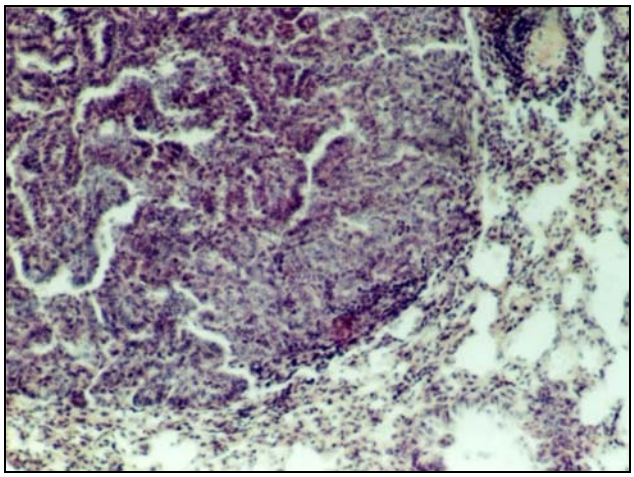

Fig. 3. **Mouse lung adenoma induced by urethane.** H&E; x140

No tumours were found in groups IV–VI.

No differences in the mean final body weight, body weight gain, and liquid and food consumption were observed among the experimental groups at the end of the 20th week, independent of β-glucan-treatment.

According to the literary data, β-glucans possess anticarcinogenic activity variable. Studies have been done using β-glucans extracted from various sources (yeast, mushrooms, barley etc.) *in vitro* and *in vivo* (Kogan et al., 2008; Mantovani et al., 2008; Chan et al., 2009).

Studies of two β-glucans extracted from *S.cerevisae* demonstrated that both inhibit the growth of mammary carcinoma and B16 melanoma cells, as well as increase the survival of mice with subcutaneous tumour implants (Luzio et al., 1979). A synthetic β-glucan was found to have cytostatic effects in mice against sarcoma 180 cells. Investigations *in vitro* and *in vivo* of β-glucan from *Letinula edodes* have demonstrated that it has a strong antitumor activity against sarcoma 180 (Zhang et al., 2005).

Suppressing effects of daily oral supplementation of β-glucan extracted from *Agaricus blazei* Murill have manifested on spontaneous and peritoneal disseminated lung metastasis in mouse model (Kobayashi et al., 2005). It was shown that β-glucan reduces pulmonary metastasis of murine Lewis lung carcinoma 3LL cells and peritoneal disseminated metastasis of human ovarian cancer HRA cells and inhibits the growth of these metastatic tumours in lung or peritoneal cavity; and in an *in vivo* experimental metastasis assay, however, the oral supplementation of β-glucan after intravenously tumour cell inoculation did not reduce the number of lung tumour colonies.

In our previous experiments peformed on mice β-glucans extracted from various sources show benefit during photodynamic treatment (PDT) (Akramienė et al., 2009, 2010, 2011). Other researchers also reported that branched high molecular weight β-glucan potentiates PDT (Krosl and Korbelic, 1994). It was shown in our studies that growth of Lewis lung carcinoma tumour is suppressed more when treated by PDT in combination with β-glucan than in tumours treated by photodynamic therapy alone. β-Glucans enhance the tumour response to PDT resulting in pronounced necrosis of PDT-treated tumours and suppression of the DNA damage repair system. Co-administration of β-glucans does not prolong survival of Lewis lung carcinoma tumour bearing mice, treated by PDT. The expression level of proliferating cell nuclear antigen (PCNA) in Lewis lung carcinoma tumour, treated by PDT is decreased with co-administration of β-glucan and PCNA expression in Lewis lung carcinoma tumour, treated by β-glucans alone is lower than in Lewis lung carcinoma tumours treated by PDT alone. β-glucan from yeast in comparison with β-glucan from barley or Laminarin (a storage polysaccharide of the marine brown algae *L.digitata*) was most effective to modulate Lewis lung carcinoma tumour response to PDT. These studies agree with recent observation that apart from the presence of the (1→3), (1→6)-β linkage, the size and complexity of β-glucan are important for the interaction with human cells (Lin et al., 2009).

In other study on xenografts in SCID mice it was shown that Rituximab therapy of lymphoma is enhanced by orally administered β-glucans from barley (Modak et al., 2005). Survival of mice with disseminated lymphoma was significantly increased in the combination group as compared to other treatment group.

In contrast to well-established antitumour activity of *Agaricus blazei* or other β-glucans observed in tumour-transplantable models less data exist on the modifying potential of these substances on chemical carcinogenesis models and the data are controversial (Barbisan et al., 2002).

In experiments on rats it was demonstrated that *Agaricus blazei* (10 %) has chemopreventive influence on the promoting phase of chemical hepatocarcinogenesis process (Pinheiro et al., 2003).

In other studies, it was shown that previous treatment with aqueous extract of *Agaricus blazei* exerts a hepatoprotectivive effect on both liver toxicity and on the initiation stage of hepatocarcinogenesis induced by moderately but not severe toxic dose of diethylnitrosamine (DENA) (Barbisan et al., 2002).

The hepatoprotective effect of *Agaricus blazei* aqueous extracts could be related to a modifying influence on DENA metabolism, thus, reducing its liver toxicity, mutagenicity, and carcinogenicity. Being potent antioxidants they are capable of inhibiting isoenzymes of cytochrome P450 family, enzymes which are involved during carcinogenesis.

However in other study treatment with *Agaricus blazei* during the post-initiation stage of carcinogenesis did not alter the development of rat DENA-initiated hepatic preneoplastic foci (Barbisan et al., 2003). The absence of a beneficial effect of this mushroom extract on altered foci of hepatocytes could be due to the carcinogenesis step evaluated in that study. The possibility exists that the extracts could be effective in later stages of rat liver carcinogenesis.

Our present results indicate that β-glucan does not have a modifying influence on mice chemical lung carcinogenesis.

Summarising literary data and our findings it is important to consider that tested extracts may be not a multipotential and broad-spectrum edible item for cancer prevention. However the possibility exists that the potential of these substances to inhibit carcinogenesis may be manifested in some target organs other than lungs. The effect may depend also on both the dose of the chemopreventive agent and on carcinogen used.

Furthermore, depending on the source the β-glucans are structurally different, therefore it is suggested that they may influence tumour growth differently (Wang et al., 2004). For instance, correlation of structure to antitumour activities of five derivatives of β-glucans from *Poria cocos sclerotium* was observed. This showed that good water solubility, relatively high chain stiffness, and moderate molecular mass of the derivatives in aqueous solution contribute beneficial to enhancement of antitumour activity (Wang et al., 2004).

So, the data show that due to various chemical composition and physical properties β-glucans exert different anticarcinogenic activity. The mechanism of its action remains still unclear. Further studies should be focused on the investigation of β-glucans structure and anticarcinogenic activity, elucidation of their antitumour mechanism at the molecular level, and improvement of their various biological activities by chemical modifications.

Conclusions. The present results indicate that β-glucan from yeast does not have a modifying influence on mice lung adenomagenesis induced by urethane under the conditions of the current study. Considering the known beneficial effects of β-glucans in other assay systems, future efforts should direct at performing experiments to verify the actual efficacy of β-glucans or β-glucans containing compounds on chemically induced carcinogenesis.

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