MICE LUNG ADENOMA BIOASSAY FOR STUDIES OF POSSIBLE MODIFYING AGENTS IN CARCINOGENESIS

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Abstract. Pulmonary tumours of mice are often been used as a tool in cancer research. The aim of this paper is to investigate and consider the utility of mouse pulmonary adenomas induced by urethane model in cancer chemoprevention studies.

Three sets of experiments have been conducted on inbred mice (both sexes), total – 372 animals. Potential anticarcinogenic compounds – vitamin E, novel organoselenium compounds – D-glucosamine hydroselenate and original anticarcinogenic mixture which consisted of these ingredients: retinol acetate, α-tocopherol, riboflavin, sodium selenite and glucuronic acid were given chronically *per os*, carcinogen – urethane was given by intraperitoneal injections. The experiments lasted for 4 months.

The results of our studies showed that all tested compounds exert inhibitory effect on lung carcinogenesis induced by urethane. Lung adenoma can serve as a model for analysis of complementary agents – cofactors whether intensifying or inhibiting pulmonary carcinogenesis.

Keywords: urethane, D-glucosamine hydroselenate, vitamin E, original anticarcinogenic mixture, lung carcinogenesis, mice.

PELIŲ PLAUČIŲ ADENOMŲ MODELIS KANCEROGENEZĖS MODULIATORIŲ TYRIMAMS

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Santrauka. Plaučių navikai yra dažnas vėžio eksperimentinių tyrinėjimų objektas. Šio straipsnio tikslas – ištirti ir apžvelgti pelių plaučių adenomų, indukuotų uretanu, modelio panaudojimo galimybes vėžio chemoprofilaktiniams tyrimams.

Trys eksperimentų serijos atliktos naudojant linijines peles (abiejų lyčių), iš viso 372 gyvūnai. Potencialūs antikancerogeniniai junginiai – vitaminas E, naujas organinis seleno junginys D-gliukozamino hidroselenatas ir originalus mišinys, susidedantis iš retinolio acetato, α-tokoferolio, riboflavino, natrio selenito ir gliukurono rūgšties, buvo duodami pelėms *per os*, o kancerogenas uretanas švirkščiamas į pilvo ertmę. Bandymų trukmė – 4 mėnesiai.

Tyrimų rezultatai parodė, kad tirti junginiai slopino plaučių kancerogenezę, indukuotą uretanu. Pelių plaučių adenomų modelis yra tinkamas tyrinėjant potencialius kancerogenezę modifikuojančius (skatinančius ir slopinančius) veiksnius.

Raktažodžiai: uretanas, D-gliukozamino hidroselenatas, vitaminas E, originalus antikancerogeniniu veikimu pasižyminčių medžiagų kompleksas, plaučių kancerogenezė, pelės.

Introduction. The new direction of cancer prevention – chemoprevention – is based on the knowledge of multistep pathway of carcinogenesis. This process may be interrupted by some chemical factors: vitamins, antioxidants etc. Cancer chemoprevention is an still experimental method, though it is gradually implemented in the clinical practice. Especially murine models are important in cancer research as they help to evaluate novel preventive and therapeutic strategies (Céspedes et al., 2006; Workman et al., 2010). Mouse models are useful of their relatively low maintenance cost, short gestation period, and ease of genetic manipulation and of the extensive information available on their genetic background (Nandan and Yang, 2007; De Jong and Maina, 2010).

Pulmonary tumours of mice have often been used as a tool in cancer research (Shimkin et al., 1966; Shimkin and Stoner, 1975; Roomi et al., 2009). L.L.Griciūtė was the

first who began these studies in Lithuania (Griciūtė, 1955).

It is known that the lungs of mice are especially responsive to urethane (ethyl carbamate). Pulmonary adenomas induced by this carcinogen often are multiple and their number depends on the dose of urethane used (Fig. 1, 2).

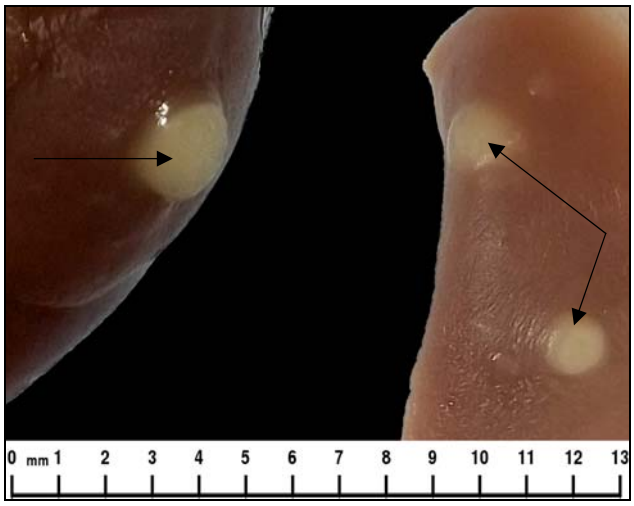

Fig. 1. **Gross lung specimens of urethane-induced mice tumours**

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 more pink colour of the normal lung parenchyma

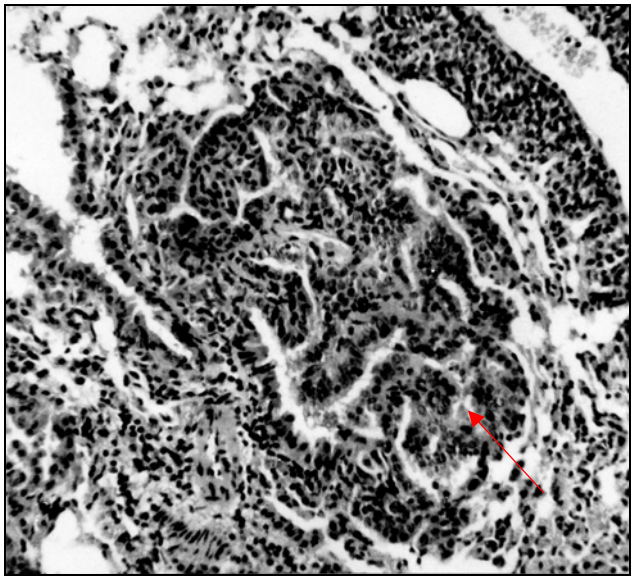

Fig. 2. **Urethane-induced mouse tumours, H&E**

Adenomas are characterized by well-differentiated cuboidal cells with irregular round nuclei and clear to lightly eosinophilic cytoplasm forming glandular or papillary structures

During the recent years, we carried out experiments using mice lung adenoma assay in studies of cancer modifying agents. The aim of this paper is to investigate and consider the utility of mouse pulmonary adenomas induced by urethane model in cancer chemoprevention studies.

Materials and methods. Animals: mice 8–10 weeks old, both sexes, and having a body weight of 20–24 g; mainly 20–30 (♂♀) animals in each group were used in the studies (total of 372 mice). The animals were obtained from the Animal Facility of State Research Institute Centre for Innovative Medicine (previous Institute of Immunology, Vilnius University). Experiment conditions were complying 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 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).

Exp. 1. Investigation of chemopreventive properties of vitamin E. The experiments have been carried out on BALB/C mice (142). The mice of this strain are of intermediate susceptibility for induction of lung tumours (Shimkin and Stoner, 1975). They were given urethane (commercial urethane, purum, Fluka, Buchs, Schweiz) in saline (100 µl) by intraperitoneal injections twice a week, single dose – 10 mg/mouse, total – 100 mg/mouse. Vitamin E (DL-α-Tocopherol, Merck, NJ, USA) 50 or 250 mg/kg b.w. was given by gavage (starting 1 week period before carcinogen injection) 3 times per week and thereafter during all the experiment (4 months).

Exp. 2. Investigation of chemopreventive properties of original novel selenium compound. D-glucosamine hydroselenate was synthesized in Institute of Oncology, Vilnius University. The experiments have been carried out on mice CBAxC57Bl (110). The mice of this strain are relatively resistant to induction of lung tumours (Shimkin and Stoner, 1975). They were given urethane by intraperitoneal injections twice a week, single dose – 10 mg/mouse, total – 50 mg/mouse. Selenium compound (1 or 10 mg/kg b.w.) was given *per os* chronically (dissolved in water, in aluminium foil-wrapped bottles to avoid light decomposition) *ad libitum* for 4 months.

Exp. 3. Investigation of chemopreventive properties of original anticarcinogenic mixture. The mixture was prepared in Department of Drug Technology of previous Kaunas Medical Institute (now – Lithuanian University of Health Science). The mixture consisted of the following ingredients in the relative amounts indicated: retinol acetate, 24 µg; tocopherol, 3.2 mg; riboflavin, 8.1 mg; sodium selenite, 3 µg and glucuronic acid, 9.8 mg.

The experiments have been carried out on BALB/c mice (120). These animals were purchased from Rapolov Animal Facility (St. Petersburg, Russia). Mice were given urethane as described in exp. 2. The duration of experiments was 4 months.

In all experiments body weights, food and fluid consumption and general health status were monitored weekly. After 4 months all surviving mice were killed by cervical dislocation. Mice were autopsied and examined. Excised lungs were fixed in neutral buffered 10% formalin. After fixation, all only macroscopically noted lung tumours were counted by two independent experts in order to ensure the objectivity. Randomly selected tumours (3 samples from each group) were dissected for histological verification of their adenomatous nature. Lungs were 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 their histological type were considered.

Results and discussion. As the aim of our studies was to adopt pulmonary adenomas bioassay for cancer chemoprevention studies we examined several potential chemical compounds.

Exp. 1. In the first set of experiments urethane induced lung adenomas in all mice, therefore we didn't get the effect reflecting the influence of vitamin E on the incidence of adenomas (Table 1). However, it was found that vitamin E inhibited lung carcinogenesis induced by urethane: the average number of tumours per mouse was lower in comparison with a group given only carcinogen. We observed also dose-dependent effect: higher dose of vitamin E was more effective in comparison with lower dose ($P \leq 0.05$).

Table. 1. **Incidence and multiplicity of lung tumours in mice treated with urethane and vitamin E**

Groups	Number of animals	Number of animals with tumours (%)	Lung tumours/ mouse M±SD
Urethane ♂	15	15 (100)	8.3±2.0
♀	14	14 (100)	11.7±3.9
Total	29	29 (100)	10.0±3.0
Vit. E_1 + Urethane ♂	16	16 (100)	7.6±3.1
♀	14	14 (100)	10.1±2.8
Total	30	30 (100)	8.8±2.9
Vit. E_2 + Urethane ♂	15	15 (100)	6.8±2.8
♀	13	13 (100)	7.2±2.5
Total	28	28 (100)	7.0±2.7 *
Vit. E_1 ♂	10	0	–
♀	10	0	–
Total	20	0	–
Vit. E_2 ♂	10	0	–
♀	10	0	–
Total	20	0	–
Intact control ♂	7	0	–
♀	8	0	–
Total	15	0	–

Vit. E_1 – 50 mg/kg b.w.; Vit. E_2 – 250 mg/kg b.w; * $P \leq 0,05$

During the last decades, scientists have been focused in the role of vitamin E in cancer (reviewed in Mamede et al., 2012). Though many of these studies are contradictory, several studies show that vitamin E could be an important agent against lung cancer (Quin et al., 2005; Yang et al., 2010; Li et al., 2011). The anticarcinogenic activity of vitamin E may be exerted by several mechanisms. It is known that vitamin E has an antioxidant effect, is involved in the biosynthesis of heme and proteins, cell proliferation, tissue respiration, and other critical processes of tissue metabolism; vitamin E inhibits free radical reactions, prevents the formation of peroxides that damage cellular and subcellular membranes (Shibata et al., 2008; Baumeister et al., 2009; Comitato et al., 2009). Our previous studies showed the protective effect of vitamin E on benzo(a)pyrene induced chromosome damage in mice bone marrow cells (Slapsyte et al., 2011).

Exp. 2. In the second set of experiments we used lower dose of carcinogen (in comparison with exp. 1) and strain of mice which is less responsible to induction of lung tumours. Chronic oral consumption of novel organoselenium compound was effective against urethane induced lung carcinogenesis: D-glucosamine hydroselenate reduced multiplicity of tumours per mouse by 44–50% ($p < 0.05$), as well as inhibited incidence of tumours as shown in Fig. 3.

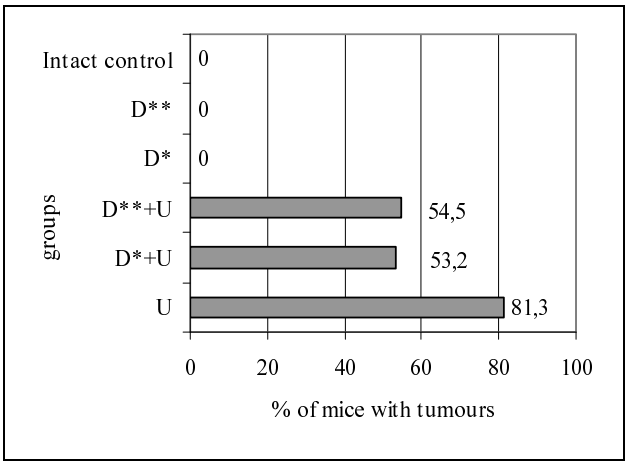

Fig. 3. **Incidence of lung adenomas in mice given urethane** (U) **and D-glucosamine hydroselenate** (D)
D*–- 1 mg/kg b.w., D** – 10 mg/kg b.w. (*per os*)

The majority of animal and clinical studies as well as our studies show selenium compounds (both organic or inorganic) as cancer chemoprevention agents, the efficacy of which depends on the stage of carcinogenesis, chemical form of selenium, formulation and dosages (Schrauzer, 2000; Uleckienė et al., 2005; Jackson and Combs, 2008; Yan and DeMars, 2011). Selenium exerts its anticarcinogenic effect by multiple mechanisms. It is known that some of the glutathione peroxidases, which are antioxidant enzymes, require the presence of selenium as an essential cofactor, thus suggesting that low selenium intake impairs antioxidant defences. Other mechanisms for selenium and its compounds anticarcinogenicity

include: alteration of carcinogen metabolism, cell cycle regulation, immune surveillance, cancer cell migration and angiogenesis, cell death programming (Jackson and Combs, 2008).

Exp. 3. The results of studies showed that original mixture (retinol acetate, 24 µg; α-tocopherol, 3.2 mg; riboflavin, 8.1 mg; sodium selenite, 3 µg and glucuronic acid, 9.8 mg) statistically significant reduced multiplicity of lung adenomas induced by urethane. The inhibitory effect was 20 % ($p<0.05$). Our previous studies have demonstrated that this mixture inhibits renal tumours induced by N-nitrosodimethylamine in rats. The similar anticarcinogenic effect was observed in animal models using other carcinogens (N-nitrososarcosine, N-metil-N-benzilnitrosamine, 7,12-dimethylbenzantracene) (personal communication).

The effect of dietary intake of various mixtures nutrients is widely studied in clinical trials as well as in experiments *in vivo* (Zhang et al., 2008; Olsen et al., 2009; Park et al., 2010; Black, 2010; Molnar et al., 2012). Recently new data on the effect of nutrient mixtures on lung tumorigenesis have been published. For instance, it was shown that nutrient mixture consisted of several ingredients – vitamin C (as ascorbic acid and as Mg, Ca, and palmitate ascorbate), L-lysine, L-proline, L-arginine, N-acetylcysteine, standardized green tea extract, selenium, cooper, manganese has inhibitory potential on the development of mouse lung tumours induced by urethane (Roomi et al., 2009). Inhibition of lung cancer growth was observed in mice given dietary mixed tocopherols (Lambert et al., 2009). In the other studies using various models the inhibitory effect of γ-tocopherol-rich mixture of tocopherols against lung tumorigenesis and the growth of xenograft tumours of human lung cancer cells were demonstrated (Lu et al., 2010). On the other hand, combined treatment with quercetin and vitamin E did not demonstrate any effect greater than that due to vitamin alone in smoking-induced lung tumour in mice (Yang et al., 2008). Further studies are necessary to determine the underlying mechanisms for the anticancer activity if such activity occurs in other models of cancer.

In general, in three sets of our experiments urethane-challenged mice developed only benign lung tumours. Histological examination revealed adenomas which were characterized by well-differentiated cuboidal cells with irregular round nuclei and clear to slightly eosinophilic cytoplasm, forming glandular or papillary structures. No tumours were evident in the livers, kidney, and spleen in any of the mice.

Thus, what could be learned from these experiments? Firstly, we can mention that the induction of primary pulmonary tumours is relatively rapid; with highly active carcinogen, such as urethane, grossly visible nodules can be identified on the surface of the lungs within 3 months, although longer periods are preferred in order that the tumours may attain larger size. The tumours are easily recognized and have a typical appearance grossly and under the microscope. The multiplicity of tumours, directly related to the dose of the carcinogen, is an important quantitative dividend.

There are, of course, certain real or assumed drawbacks to the pulmonary tumour bioassay technique. Perhaps the most important ones are the belief that these are not truly malignant tumours and those they have no close counterpart in human pathology. Since with the more susceptible strains, all mice eventually develop such tumours (Shimkin et al., 1966).

According to the literary data, in recent years the mouse lung adenomas model has been mainly used for investigation of cancer chemopreventive properties of various agents, for instance: N-acetylcysteine, 2-amino-2-thiazoline salt (Simkeviciene et al., 2002), myelopeptide-2 (Mikhailova et al., 2002), β-carotene (Uleckiene and Domkiene, 2003), selenium compounds (Uleckiene et al., 2005), melatonin (Vesnushkin et al., 2006), original nutrients mixtures (Roomi et al., 2009), β-glucan (Zabulyte et al., 2012).

All studies have been conducted on mice, and although the animal lung cancer mirrors human lung cancer, there is usual limitation regarding translation of animal model to practical use in humans. During last decade, a rapid progress in developing new animal models for lung cancer chemoprevention studies was observed (Zheng and Takano, 2011). However, lung adenoma assay is a classical example and could depend on selection of the question we are planning to answer. Due to evaluation of our previous investigations, we are continuing our studies using urethane-induced lung tumour model for assessment of possible cancer modifying agent such as sodium valproate.

Conclusions.

1. All tested compounds showed cancer chemopreventive activity: vitamin E, novel organoselenium compound-D-glucosamine hydroselenate and original anticarcinogenic mixture (retinol acetate, α-tocopherol, riboflavin, sodium selenite, and glucuronic acid) were found to inhibit lung adenomogenesis in mice.
2. Mice lung adenoma model is useful to assess the efficacy of potential chemopreventive agents.

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Literature

1. Baumeister P., Korn G., Berghaus A., Matthias C., Harréus U. Chemopreventive action of dexamethasone and alpha-tocopherol in oxidative stressed cells. Cancer Detect Prev. 2009. 32(5–6). P. 452–457.

2. Black H.S. Interaction of ascorbic acid and tocopherol on beta-carotene modulated carcinogenesis. Hemoglobin. 2010. 34(3). P. 284–290.

3. Céspedes M.V., Casanova I., Parreño M., Mangues R. Mouse models in oncogenesis and cancer therapy. Clin Transl Oncol. 2006. 8(5). P. 318–329.

4. Comitato R., Nesaretnam K., Leoni G., Ambra R., Canali R., Bolli A., Marino M., Virgili F. A novel mechanism of natural vitamin E tocotrienol activity: involvement of ERbeta signal transduction. Am J

Physiol Endocrinol Metab. 2009. 297(2). P. E427–437.

5. De Jong M., Maina T. Of mice and humans: are they the same? - Implications in cancer translational research. J Nucl Med. 2010. 51(4). P. 501–504.

6. Griciūtė L.L. K voprosu o blastomogennom deistvii uretana: Eksperimentalnije opucholi liogkich. Avtoreferat Dis. Kand. Med. Nauk. 1955. 14 P. (in Russian).

7. Jackson M.I., Combs G.F.Jr. Selenium and anticarcinogenesis: underlying mechanisms. Curr Opin Clin Nutr Metab Care. 2008. 11(6). P. 718–726.

8. Lambert J.D., Lu G., Lee M.J., Hu J., Ju J., Yang C.S. Inhibition of lung cancer growth in mice by dietary mixed tocopherols. Mol Nutr Food Res. 2009. 53(8). P. 1030–1035.

9. Li G.X., Lee M.J., Liu A.B., Yang Z., Lin Y., Shih W.J., Yang C.S. δ-tocopherol is more active than α - or γ -tocopherol in inhibiting lung tumorigenesis *in vivo*. Cancer Prev Res (Phila). 2011. 4(3). P. 404–413.

10. Lu G., Xiao H., Li G.X., Picinich S.C., Chen Y.K., Liu A., Lee M.J., Loy S., Yang C.S. A gamma-tocopherol-rich mixture of tocopherols inhibits chemically induced lung tumorigenesis in A/J mice and xenograft tumour growth. Carcinogenesis. 2010. 31(4). P. 687–694.

11. Mamede A.C., Tavares S.D., Abrantes A.M., Trindade J., Maia J.M., Botelho M.F. The role of vitamins in cancer: a review. Nutr Cancer. 2011. 63(4). P. 479–494.

12. Mikhailova A.A., Kirilina E.A., Morozova O.V., Turusov V.S. Effect of myelopeptide-2 on the development of spontaneous and urethane-induced tumors in mice. Bull. Exp. Biol. Med. 2002. 133 (1). P. 68–70.

13. Molnár J., Serly J., Pusztai R., Vincze I., Molnár P., Horváth G., Deli J., Maoka T., Zalatnai A., Tokuda H., Nishino H. Putative supramolecular complexes formed by carotenoids and xanthophylls with ascorbic acid to reverse multidrug resistance in cancer cells. Anticancer Res. 2012. 32(2). P. 507–517.

14. Nandan M.O., Yang V.W. Genetic and chemical models of colorectal cancer in mice. Curr Colorectal Cancer Rep. 2010. 6(2). P. 51–59.

15. Olsen A., Halkjaer J., van Gils C.H., Buijsse B., Verhagen H., Jenab M., Boutron-Ruault M.C., Ericson U., Ocké M.C., Peeters P.H., Touvier M., Niravong M., Waaseth M., Skeie G., Khaw K.T., Travis R., Ferrari P., Sanchez M.J., Agudo A., Overvad K., Linseisen J., Weikert C., Sacerdote C., Evangelista A., Zylis D., Tsiotas K., Manjer J., van Guelpen B., Riboli E., Slimani N., Bingham S. Dietary intake of the water-soluble vitamins B1, B2, B6, B12 and C in 10 countries in the European Prospective Investigation into Cancer and Nutrition. Eur J Clin Nutr. 2009. 63. Suppl 4. P. S122–149.

16. Park Y., Spiegelman D., Hunter D.J., Albanes D., Bergkvist L., Buring J.E., Freudenheim J.L., Giovannucci E., Goldbohm R.A., Harnack L., Kato I., Krogh V., Leitzmann M.F., Limburg P.J., Marshall J.R., McCullough M.L., Miller A.B., Rohan T.E., Schatzkin A., Shore R., Sieri S., Stampfer M.J., Virtamo J., Weijenberg M., Willett W.C., Wolk A., Zhang S.M., Smith-Warner S.A. Intakes of vitamins A, C, and E and use of multiple vitamin supplements and risk of colon cancer: a pooled analysis of prospective cohort studies. Cancer Causes Control. 2010. 21(11). 1745–1757.

17. Quin J., Engle D., Litwiller A., Peralta E., Grasch A., Boley T., Hazelrigg S. Vitamin E succinate decreases lung cancer tumor growth in mice. J Surg Res. 2005. Vol. 127(2). P. 139–143.

18. Roomi M.W., Roomi N.W., Kalinovsky T., Rath M., Niedzwiecki A. Chemopreventive effect of a novel nutrient mixture on lung tumorigenesis induced by urethane in male A/J mice. Tumori. 2009. 95(4). P. 508–513.

19. Schrauzer G.N. Anticarcinogenic effects of selenium. Cell Mol Life Sci. 2000. 57(13–14). P. 1864–1873.

20. Shibata A., Nakagawa K., Sookwong P., Tsuzuki T., Oikawa S., Miyazawa T. Tumor anti-angiogenic effect and mechanism of action of delta-tocotrienol. Biochem Pharmacol. 2008. 76(3). P. 330–339.

21. Shimkin M.B., Stoner G.D. Lung tumors in mice: application to carcinogenesis bioassay. Adv Cancer Res. 1975. 21. P. 1–58.

22. Shimkin M.B., Weisburger J.H., Weisburger E.K., Gubareff N., Suntzeff V. Bioassay of 29 alkylating chemicals by the pulmonary-tumor response in strain A mice. J Natl Cancer Inst. 1966. 36. P. 915–935.

23. Simkeviciene V., Straukas J., Uleckiene S. N-acetil-l-cysteine and 2-amino-2-thiazoline N-acetyl-l-cysteinate as a possible cancer chemopreventive agent in murine models. Acta Biol Hung. 2002. 53(3). P. 293–298.

24. Slapšytė G., Mierauskienė J., Uleckienė S., Didžiapetrienė J. Modifying effect of vitamin E and ethanol on benzo[a]pyrene induced chromosome damage in mice bone marrow cells. Veterinarija ir zootechnika. 2011. 53(75). P. 56–60.

25. Uleckiene S., Didziapetriene J., Griciute L., Sukeliene D. Evaluation of original selenium-containing compounds for potentially chemopreventive properties in experimental lung carcinogenesis. Trace Elem & Electrol. 2005. 22(1). P. 33–36.

26. Uleckiene S., Domkiene V. Investigation of ethyl alcohol and beta-carotene effect on two models of carcinogenesis. Acta Biol Hung. 2003. 54(1). P. 89–

93.

27. Vesnushkin G.M., Plotnikova N.A., Semenchenko A.V., Anisimov V.N. Melatonin inhibits urethane-induced carcinogenesis tumors in murine lung. Vopr. Onkol. 2006. 52 (2). P. 164–168.

28. Workman P., Aboagye E.O., Balkwill F., Balmain A., Bruder G., Chaplin D.J., Double J.A., Everitt J., Farningham D.A., Glennie M.J., Kelland L.R., Robinson V., Stratford I.J., Tozer G.M., Watson S., Wedge S.R., Eccles S.A. Guidelines for the welfare and use of animals in cancer research. Br J Cancer. 2010. 102(11). P. 1555–1577.

29. Yan L., Demars L.C. Dietary supplementation with methylseleninic acid, but not selenomethionine, reduces spontaneous metastasis of Lewis lung carcinoma in mice. Int J Cancer. 2011. doi: 10.1002/ijc.27355.

30. Yang C.S., Lu G., Ju J., Li G.X. Inhibition of inflammation and carcinogenesis in the lung and colon by tocopherols. Ann N Y Acad Sci. 2010. Vol. 1203. P. 29–34.

31. Zabulytė D., Jonauskienė I., Uleckienė S., Akramienė D., Matusevičius P., Didžiapetrienė J. Experimental studies of possible modulative effect of β-glucan on mice lung carcinogenesis. Veterinarija ir zootechnika. 2012. 59(81). P. 86–91.

32. Zhang S.M., Cook N.R., Albert C.M., Gaziano J.M., Buring J.E., Manson J.E. Effect of combined folic acid, vitamin B6, and vitamin B12 on cancer risk in women: a randomized trial. JAMA. 2008. 300(17). P. 2012–2021.

33. Zheng H.C., Takano Y. NNK-Induced lung tumors: a review of animal model. J Oncol. 2011. 2011:635379. P. 1–8.

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