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The effect of selenium, zinc and copper on the excretion of urinary modified nucleobases in rats treated with prostate cancer cells

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Abstract: Given the strong associations between diet and cancer risk, there is considerable scientific interest in determining whether dietary factors associated with prostate cancer cell implantation may influence epigenetic alternations. The aim of the research was to assess impact of selected trace elements (selenium, zinc and copper) on the kinetics of changes (10-13-14-21 week of life cycle of rats) in the level of 7-methylguanine, 3-methyladenine, 1-methylguanine and 8-oxo-guanine in the urine of rats with implanted prostate cancer cells (LNCaP). Modified nucleobases were determined by validated high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) method using multiple reaction monitoring (MRM) mode. In the presented model the implantation of rats with cancer cells did not affect the level of the examined biomarkers in the rats' urine. The level of methyl derivatives was statistically significantly reduced with the age of the examined rats. The implantation of rats with cancer cells results in the appearance of tumors in 71% of the rats obtaining the standard diet and respectively in 25% of those supplemented with selenium. Supplementation with selenium affects both the effectiveness of tumor induction and the concentration of 7-MeG, 3-MeA, 1-MeG and 8-oxoG in urine of the examined rats. These findings show that modified nucleosides can play an important role in cancer prevention.

Keywords: cancer, biomarkers, selenium

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1 Introduction

Epigenetic alternations have recently been recognized to play an important role in human pathologies, including cancer. It was established that they occur along with genetic mutations in cancer development and progression. Elevated levels of the methylated nucleosides and nucleobases were found in urine from patients suffering from breast [1,2], lung [3,4], ovarian [5], bladder [6], colon [7], and leukemia [8] cancer. Alternations in association/dissociation of specific proteins with methylated bases can disturb normal biological functions. It was found that the increased N-7 guanine methylation (7-MeG) in methyl-CpG pairs can alter the protein dissociation and the chromatin remodeling leading to increased gene expression [9]. Moreover, the majority of lesions created by alkylating agents, such as 3-methyladenine (3-MeA) strongly block (replication) synthesis [10-12]. Lesions such as 8-oxoG are established biomarkers of oxidative stress [13]. Both methylation and oxidation of guanine result in decreased interactions with methylated DNA binding protein and associated proteins [14]. The GC-TA transversions, potentially derived from 8-oxoG were observed *in vivo*, in the ras oncogene and the p53 tumor suppressor gene in lung and liver cancer [13]. Epigenetic alternations are reversible, which makes them a promising target for anticancer drugs and dietary components. In this article we wanted to find out whether or not the implantation of cancer cells exerts any effect on epigenetic and oxidative changes in the organism and how markers of these changes are affected by selected mineral supplements.

The aim of the present research was to assess the impact of selected trace elements (selenium, zinc and copper) on the kinetics of changes (10-13-14-21 week of life cycle of rats) in the level of 7-methylguanine, 3-methyladenine, 1-methylguanine and 8-oxo-guanine in the urine of rats with implanted prostate cancer cells. The studies of the impact of dietary components on the

growth and development of the neoplastic process and on selected biomarkers can be very important both in cancer prevention and in pharmacological treatment of prostate cancer and other prostate diseases. Prevention is a promising option for the control of cancer.

2 Materials and methods

2.1 Laboratory animals

Male Sprague-Dawley rats were obtained from the Animal Laboratory, Department of General and Experimental Pathology from the Medical University of Warsaw. The animals were kept under the standard conditions of the animal house with 12-h light-dark cycle at a temperature of 22°C. They had free access to food (standard diet: Labofeed H, Zurawia 19, 89–240 Kcynia, Poland) and water. Their diet was composed of the following compounds (per 1 kg): protein (210 g), fat (39.2 g), fibre (43.2 g), ash (55 g), carbohydrates (300 g), vitamin A (15,000 IU), vitamin D3 (1000 IU), vitamin E (90 mg), vitamin K3 (3 mg), vitamin B1 (21 mg), vitamin B2 (16 mg), vitamin B6 (17 mg), vitamin B12 (80 µg), pantothenic acid (30 mg), folic acid (5 mg), nicotinic acid (133 mg), Ca (10 g), P (8.17 g), Mg (3 g), K (9.4 g), Na (2.2 g), Cl (2.5 g), S (1.9 g), Fe (250 mg), Mn (100 mg), Zn (76.9 mg), Cu (21.3 mg), Co (2.0 mg), I (1.0 mg), and Se (0.5 mg).

2.2 Experimental procedure

The experiment was conducted over 90 days. After the adaptation period (10 days - from 60 to 70 days of rats' age), the animals (n=63) were randomly divided into two experimental groups. In group 1 (study group) the rats were implanted prostate cancer cells, and in group 2 (control group) they were accommodated under the same conditions as those in Group 1, fed with the same diet, but with no implanted cancer cells. The cancer cells (LNCaP) were implanted intraperitoneally in the amount $1x10^6$ (in PBS 0.4 mL) to the rats at day 90 of their lifetime. The certified line of androgen-dependent human prostate cancer cells was obtained from ATTC bank (American Type Culture Collection, Menassas, VA).

The animals from both groups (study and control) were provided with the minerals by oral gavage in a solution:

- zinc 4.6 mg/mL (1.85 mg Zn (II)/day/rat) (as $ZnSO_4x7H_2O$ in aqueous suspension) (n=16),
- copper 0.639 mg/mL (0.256 mg Cu (II)/day/rat) (as $CuSO_4x5H_2O$ in aqueous suspension) (n=16),
- selenium 0.018 mg/mL (0.0072 mg Se/day/rat) (as Na_2SeO_4 in aqueous suspension) (n=16),

The rats were fed extra supplements suspended in water, 0.4 mL daily, from 70 days until 150 days of age when they were decapitated. The animals were fed only the standard diet (without supplementation) (n=15) received 0.4 mL of water. The doses of trace elements were selected based on the values used in the Labofeed H diet (extrapolated on the rats' body weight). According to the level of trace elements in the Labofeed diet, the rats were fed, via gavage, extra supplements of double dose of Zn, Se and Cu. The doses of selected minerals were chosen based on their levels in dietary supplements that are commonly used by people and are available at any pharmacy.

2.3 Urine samples

In order to obtain rat urine samples the animals were placed in individual metabolic cages for 24 h. For determination of the level of selected biomarkers, the urine from week 10, 13, 14 and week 21 of the rats' lifetime was used (that is, at the beginning of the experiment just before and after implantation of prostate cancer cells, and at the end of the experiment when the tumors appeared) (Figure 1). The obtained material was stored at the temperature of -70°C until the analysis was performed.

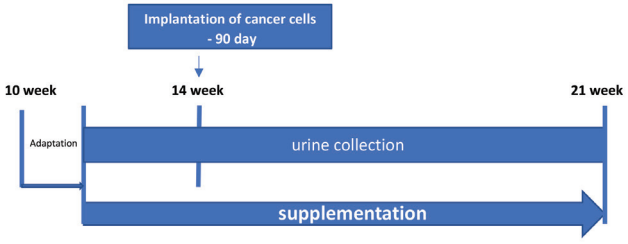

Figure 1: Scheme of the experimental procedure.

2.4 Chemicals

Reference standards i.e. 7-methylguanine, 3-methyladenine and 1-methylguanine, 8-oxoguanine as well as internal standard (tubercidin) were purchased from Sigma Aldrich (St Louis, Mo, USA).

2.5 Chromatographic determination

Modified nucleobases were determined by validated high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) method using multiple reaction

monitoring (MRM) mode on Agilent 1260 Infinity (Agilent Technologies, Santa Clara, CA, US) coupled to QTRAP 4000 (AB Sciex, Framingham, MA, US). Briefly, MRM transitions, declustering potential (DP), and collision energy (CE) for 7-methylguanine, 3-methyladenine, 1-methylguanine, 8-oxoguanine were (*m/z*) 166>79 (DP=96 V, CE=43 V), 150>123 (DP=86 V, CE=31 V) and 166>135 (DP=81 V, CE=31 V), 284>168 (DP=46 V, CE=19 V), respectively. Chromatographic separation was achieved using SeQuant® ZIC®-HILIC (50x2.1 mm, 5 µM, Merck) column. The column was maintained at 25°C at the flow rate of 0.5 mL min^{-1}. The mobile phases consisted of 20 mM ammonium acetate as the eluent A and acetonitrile with 0.2% formic acid as the eluent B. The gradient (%B) was as follows: 0 min 95%; 1 min 95%; 7 min 50%, 8 min 50%. The total run time was 15 min, and the injection volume was 5 µL. Urine samples (0.1 mL) prior to injection to LC were mixed with tubercidin (0.1 mL, 1 µg/mL) and acetonitrile (0.6 mL), vortexed in high speed (3 min) and centrifuged (5 min at 10,000 g).

The level of the modified nucleosides and bases in urine was standardized by conversion to the creatinine level. The latter was determined in urine samples with a creatinine test (Hydrex, Warsaw, Poland) based on Jaffe's reaction.

2.6 Statistics

The Statistica 13.0 software (StatSoft, USA) was used for statistical analysis. The normal distribution of the data was tested using the Shapiro-Wilk method. For the normal data, the Student's test and ANOVA, followed by Tukey's test with unequal sample size were used for analysis. The non-normal data was analyzed with Mann-Whitney U nonparametric test. In order to verify the results of tumors incidence calculation of the relative risk (RR) was used. The results were considered statistically significant when $p<0.05$.

3 Results

As a result of the performed studies it was found out that the concentration of 7-methylguanine, 3-methyldenine and 1-methylguanine in urine of rats implanted and non-implanted with prostate cancer cells is statistically significantly reduced with the age of animals (week 10 → 21) concerning the rats obtaining the standard diet only (Figures 2-5). No such dependence was found in the case of determining the concentration of 8-oxoG. In the case of 8-oxoG, there were no statistically significant differences in the level of biomarkers of oxidative stress between week 10 and 21 of the lifetime of the examined rats. There were no statistically significant differences in the level of selected biomarkers in the urine of the rats implanted with cancer cells in comparison with the non-implanted ones, both in week 14, that is a week after implantation of cancer cells, and at the end of the experiment (week 21), that is 8 weeks after implantation of cancer cells. This allowed us to suppose that the implantation of cancer cells does not affect the concentration of the examined biomarkers in the urine of the rats (Figures 2-5).

It was found out that dietary supplementation of selenium affects both the effectiveness of tumor induction and the level of 7-MeG, 3-MeA, 1-MeG and 8-oxoG. The implantation of rats with cancer cells results in the appearance of tumors in 71% (5/7) of the rats obtaining the standard diet and respectively in 25% (2/8) of those supplemented with selenium. In the initial period after implantation with cancer cells (week 14) the supplementation of rats with Se resulted in the inhibition of oxidative stress, reduction of the concentration of 8-oxoG and modification of the level of the examined biomarkers of the methylation process (Table 2). No such dependence was found in the case of rats supplemented with Zn and Cu. Although the supplementation with both the above compounds also inhibited the induction and growth of cancer tumors, yet it did not significantly affect the examined biomarkers in urine collected both from week 14 and week 21 of the rats' lifetime (Tables 1 and 2). However, both zinc and copper were found to stimulate the methylation process just before the rats were implanted with cancer cells (week 13).

In the case of rats supplemented with Zn and Se, no decrease in the level of methyl derivatives in urine in week 21 was found as compared with week 10 (Figures 2-5) and in the case of supplementation with Zn for 4 weeks, a statistically significant increase was found in the level of 7-MeG, 3-MeA and 1-MeG in the group of rats that were not implanted with cancer cells (week 10 → 14). This mechanism may be important for delaying the ageing process of the organism.

4 Discussion

For many years modified nucleosides have been suggested as tumor markers. It was found that their levels are frequently elevated in patients with oncogenic disease. However, so far, few studies have been published concerning the kinetics of changes in the profile of the

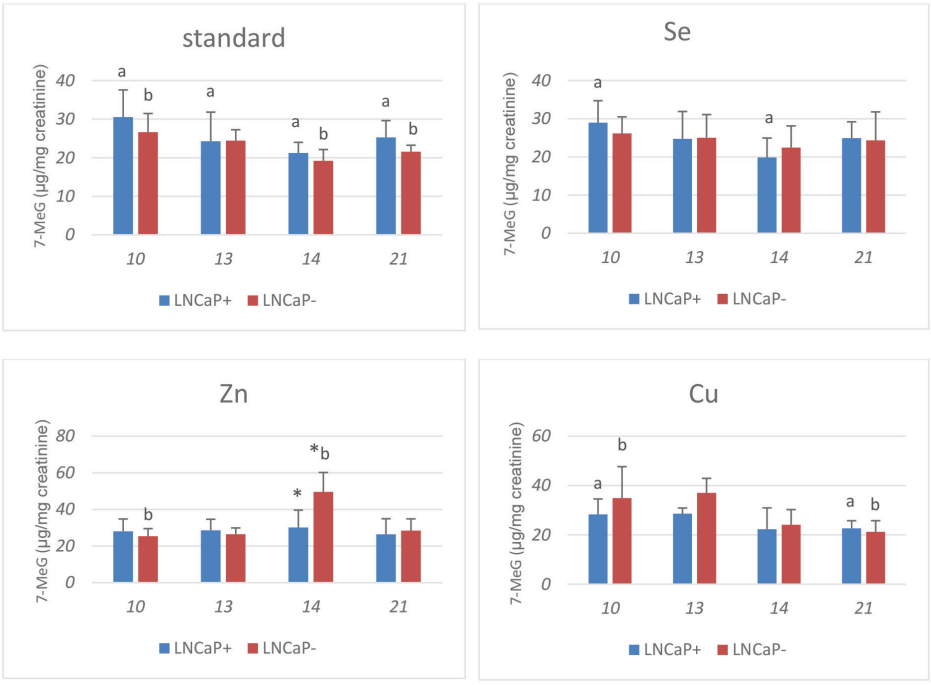

Figure 2: The effect of Se, Zn and Cu supplementation on the excretion of urinary 7- methylguanine (µg/mg creatinine) (10-13-14-21 week) in rats treated and non-treated with prostate cancer cells.

a – groups implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
b – groups non-implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
* - implanted versus non-implanted cancer cells rats

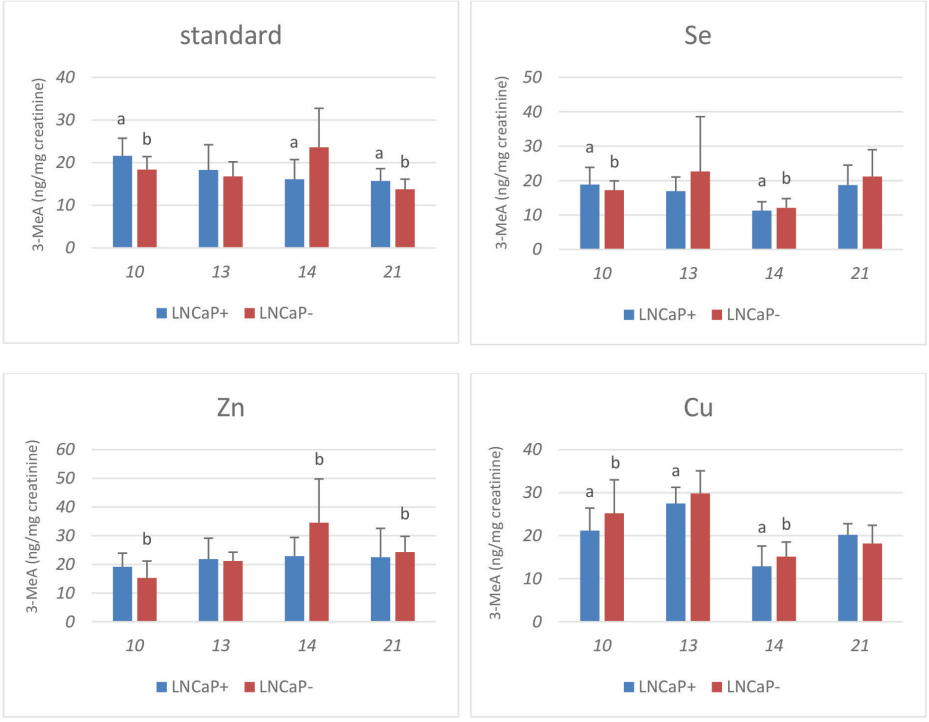

Figure 3: The effect of Se, Zn and Cu supplementation on the excretion of urinary 3-methyladenine (ng/mg creatinine) (10-13-14-21 week) in rats treated and non-treated with prostate cancer cells.

a – groups implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
b – groups non-implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
* - implanted versus non-implanted cancer cells rats

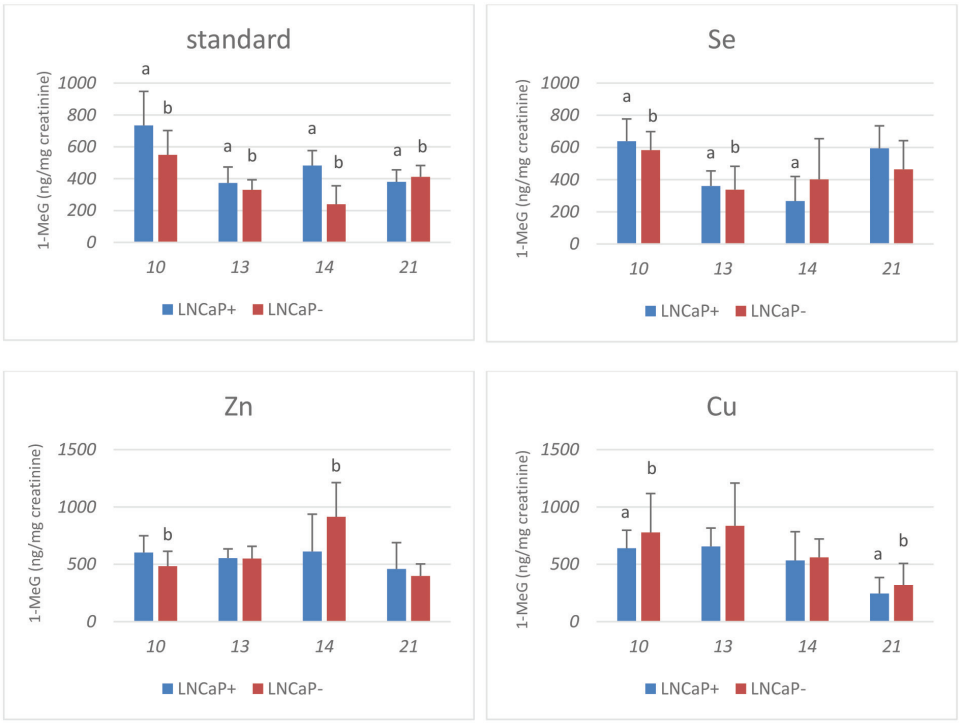

Figure 4: The effect of Se, Zn and Cu supplementation on the excretion of urinary 1-methylguanine (ng/mg creatinine) (10-13-14-21 week) in rats treated and non-treated with prostate cancer cells.

a – groups implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
b – groups non-implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
* - implanted versus non-implanted cancer cells rats

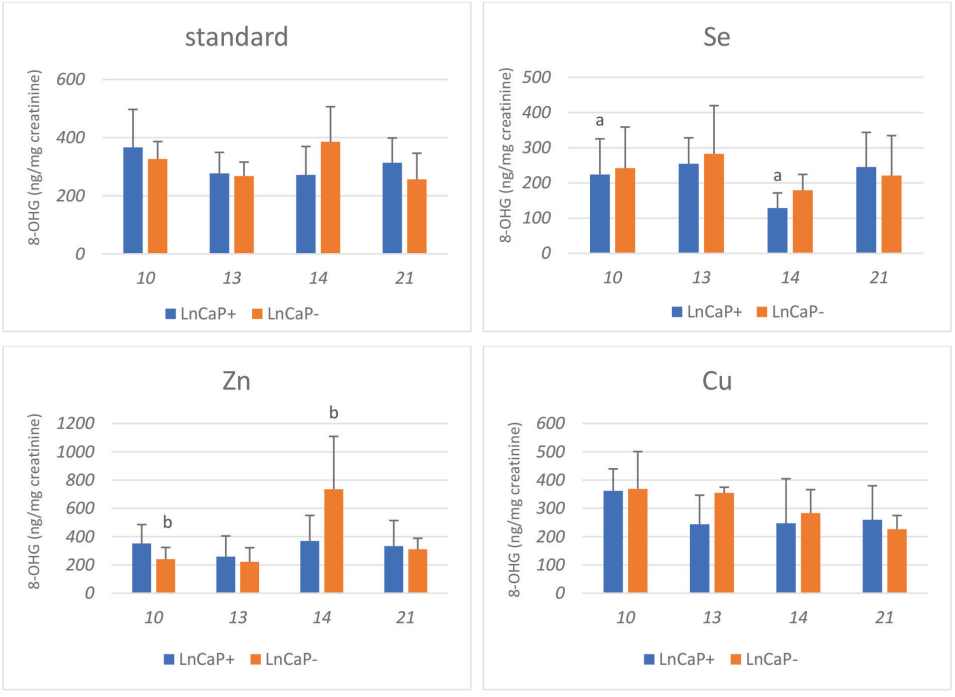

Figure 5: The effect of Se, Zn and Cu supplementation on the excretion of urinary 8-oxyguanine (ng/mg creatinine) (10-13-14-21 week) in rats treated and non-treated with prostate cancer cells.

a – groups implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
b – groups non-implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).
* - implanted versus non-implanted cancer cells rats

above-mentioned biomarkers at the early step of tumor growth as well as the assessment of the impact of selected dietary components on this process.

As a result of the performed studies it was shown that the concentration of 7-methylguanine, 3-methyldenine and 1-methylguanine in the urine of the rats with implanted and non-implanted prostate cancer cells is statistically significantly reduced with the age of rats (week 10 → 21) (the rats obtaining the standard diet only) (Figures 2-5). Similar results were obtained by other authors [15-18]. Those authors suggest that the mechanisms responsible for removing methylated bases and DNA repair become less efficient with age. They think that such differences can result among other things from the changes in chromatin structure. Washington et al. [15] showed that the activity of N-glycosylase of 3-methyladenine (MAG) and methyltransferase of O-6-methylguanine (MGMT) is much lower in elderly rats as compared with young individuals. That is why the exposure of older rats to mutagenic substances causing an increase of the DNA methylation level is an important factor leading to the development of the neoplastic process. Gaubatz and Tan [16] revealed

Table 1: Tumor induction in relation to supplementation.

kind of supplementation	Tumor incidence (%) (number animals that developed tumors)
standard diet (n=7)	5/7 (71%)
Zn (4.6 mg/mL) (n=8)	4/8 (50%)
Cu (0.639 mg/mL) (n=8)	3/8 (38%)
Se (0.018 mg/mL) (n=8)	2/8 (25%)[a] (p=0.001)

a – groups implanted cancer cells; group fed standard diet versus groups fed diet with addition of trace elements (p≤0.05).

Table 2: Summary of the changes in relative levels of metabolites in urine of rats supplemented with Se, Zn and Cu and statistical analysis A) 7-methylguanine; B) 1-methylguanine; C) 3-methyladenine; D) 8-oxo-guanine

A)

kind of supplementation	10 week		13 week		14 week		21 week	
	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-
standard	-	-	-	a	a	a	-	a
selenium	-	-	-	↓c	↓b	↓b	-	-
zinc	-	-	-	↓d	↑a↑b	↑a↑b↑d	-	↑a↑d
copper	-	-	-	↑a↑c↑d	-	↓d	-	↓d

B)

kind of supplementation	10 week		13 week		14 week		21 week	
	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-
standard	-	-	a	a	A	a	a	-
selenium	-	-	↓b↓c	↓b↓c	↓a↓b↓c	↓b	↑a↑c	-
zinc	-	-	↑a↑b↓d	↑a↑b↓d	↑b	↑a↑b↑d		-
copper	-	-	↑a↑c↑d	↑a↑c↑d	↑c	↑a↓d	↓c	-

C)

kind of supplementation	10 week		13 week		14 week		21 week	
	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-
standard	-	-	a	a	A	a	a	a
selenium	-	↓c	↓c	-	↓a↓b	↓a↓b	-	↑a
zinc	-	↓d	-	↑a↓d	↑b↑d	↑b↑d	-	↑a↑d
copper	-	↑c↑d	↑a↑c	↑a↑d	↓d	↓d	↑a	↑a↓d

D)

kind of supplementation	10 week		13 week		14 week		21 week	
	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-	LNCaP+	LNCaP-
standard	a	-	-	a	A	a	-	-
selenium	↓a↓b↓c	↓c	-	-	↓a↓b	↓a↓b↓c	-	-
zinc	↑b	↓d	-	↓d	↑b	↑a↑b↑d	-	-
copper	↑c	↑c↑d	-	↑a↑d	-	↑c↓d	-	-

Marks indicate the direction of the changes, i.e, ↑ for increase; ↓ for decrease, as indicated by the statistical analysis (p<0.05):
a – standard vs Se, Zn, Cu; **b** – Se versus Zn; **c** – Se versus Cu; **d** – Zn versus Cu

that the activity of the N-methylpurine-DNA glycosylase, responsible for eliminating 7-methylguanine from the mammalian genome, is not deficient in senescent liver tissue. Moreover, the expression and activity of DNMT1 decrease with age, which inhibits the non-invasive (post-replicative) DNA methylation and is thus responsible for global DNA hypomethylation. On the other hand, the expression of DNMT3a and DNMT3b increases, which means that local DNA hypermethylation is increased de novo [19,20]. As a result of the above processes the control of transcription of genes encoding DNA methyltransferases can lead to global hypomethylation and local hypermethylation connected with age. Age-depending hypermethylation was found in numerous genes, including APC, AXIN2, DKK1,ESR1, HPP1, IGF2, N33, p16, SFRP1, SFRP2 and SFRP4 in healthy cells of the epithelium of the large intestine [21,22]. Local DNA hypermethylation increases with age, whereas global level of DNA hypermethylation decreases with age, which was also confirmed by our investigations [18].

The problem discussed in the present study is the effect of the changes of the global model of DNA methylation on the course of cancer transformation. Elevated levels of modified nucleosides were found in urine from patients suffering from various types of cancer [1-8]. Concentrations of cytidine, 1-methyladenosine, 1-methylguanosine, N2N2-dimethylguanine, 2-methylguanosine, N6-methyladenosine, 5-methyluridine in urine of patients with cancer were elevated significantly [2,7,23]. Seidel et al. [23] revealed that the levels of cytidine, 1-methyladenosine, N2,N2-dimethylguanine and 2-pyridone-5-carboxamide-N1-ribofuranoside excreted by patients with more advanced cancer (stage 2) was higher than that of patients with primary cancer (stage 1). The obtained results showed that there were no statistically significant differences in the level of the examined biomarkers in urine of patients with primary cancer as compared with the control group (healthy ones). Zheng et al. [2] showed that the mean nucleoside concentrations from patients with benign breast tumors were significantly lower than those of breast cancer patients. By using 13 nucleoside concentrations as data vectors for principal component analysis, 73% of breast cancer patients were correctly identified from healthy controls, while only 20% of patients with benign breast tumors were indistinguishable from breast cancer patients [2]. It results from our investigations that the implantation of rats with cancer cells does not affect the level of the examined biomarkers in urine. No statistically significant differences were found in the level of selected biomarkers in the urine of the rats implanted with cancer cells as compared with non-implanted ones, both in week 14 (i.e. a week after implanting cancer cells) and at the end of the experiment (week 21, i.e. 8 weeks after implanting cancer cells). On the basis of the above-presented results a question arises if the disturbances in epigenomic functioning is the cause or the effect of the development of cancer. The level of modified nucleosides depends on the stage of cancer advancement in the organism.

In the last few years considerable progress has been observed in investigations into better understanding of the dependence of the diet and expression of certain genes and their influence on the initiation and progression of multiple diseases. The reversibility of epigenetic transformations raises hope that some dietary components can be used both in prevention and treatment of certain diseases, especially cancer. The use of Se is particularly promising for prostate cancer, based on the observation of inverse association of prostate cancer risk and Se status. However, some epidemiologic and other data suggest differential effects in men and women and there are hints that selenium supplements might even have harmful effects, this especially being the case in certain populations [24-33]. Additional research is needed to assess whether selenium may affect the risk of cancer in individuals with specific genetic backgrounds or nutritional status, and to determine how the various chemical forms of selenium compounds, various doses may have different effects on cancer risk. A number of studies showed the effect of Se status or dietary supplementation on global and specific DNA methylation as well as the expression of activity of DNA methyltransferases (DNMT). The DNA-methyltransferases (DNMT) are essential enzymes enabling the methylation process. The studies employing rodents and cell lines showed that Se decreased the expression of DNMT1 and inhibited its activity in colon, liver and breast cancer [34]. This topic was also elucidated in a human study that found a significant inverse association of plasma Se and global DNA methylation in leukocytes. Xiang et al. [35] found that the tumor suppressor genes APC (adenomatous polyposis coli), which are silenced in prostate tumors, due to hypermethylation of their promoters, were demethylated and re-expressed in LNCaP cells after selenite-treatment. The use of SelMet caused the promoter demethylation and re-expression of GSTP1. The other mechanisms of Se influence include reduction of HDAC activity, an increase of histone acetylation and of the activity of p21 promoter, as well as the inhibition of the cell cycle and stimulation of apoptosis in the cells of colon cancer [36,37]. Our studies also showed that there is a relationship between the activity of Se via the DNA hypomethylation process and a decrease of

the risk of tumor growth. The supplementation of rats with selenium affects both the effectiveness of tumor induction and the levels of 7-MeG, 3-MeA and 1-MeG in urine. In the initial period after the rats were implanted with cancer cells (week 14) the supplementation with Se inhibited oxidative stress, reduced the concentration of 8-oxoG in urine and also decreased the levels of the examined biomarkers of the methylation process. It is interesting to note that this relationship was not found in the case of zinc and copper. Wycherly et al. [38] showed that high levels of N-nitrosodiethylamine and high levels of selenite intake each increased concentration of 8-hydroxy-2'-deoxyguanosine in rat liver DNA.

Zinc plays the role of a cofactor for several enzymes of methionine cycle transsulfuration pahway, which is a key pathway creating the donors of methyl groups such as S-adenosyl-L-methionine (SAM) or betaine. Betaine-homocystein methyltransferase and methionine synthase are the other enzymes whose activity depends on the presence of zinc [39]. Serine hydroxymethyltransferase which is the main enzyme metabolizing folic acid and transferring methyl groups from serine to the methionine cycle is regulated by zinc-dependent transcription factors, including transcription factor 1. The above data provide strong foundations to state that zinc is an important dietary component which participates in maintaining the correct state of methylation in the cells. Zinc deficiency can result in the deficiency of methyl groups, similar to the case of a low supply of other donors of methyl groups such as folic acid. In the performed studies the supplementation of rats with zinc resulted in inhibition of the initiation and growth of tumors but it did not significantly affect the examined biomarkers of the DNA methylation process. The anti-cancer effect of zinc is associated with its antioxidant properties, the influence of zinc on the immune system, transcription factors, cell differentiation and proliferation, and many other factors [40]. Due to the multitude of functions performed by this element, it can be assumed to play a leading role in defending against the initiation and promotion of tumors. Many patients with cancer, especially of the lungs, breast, head and neck, have a decreased level of zinc in the blood [41,42]. It is interesting to note that in this research the supplementation of rats with Zn for a period of 4 weeks resulted in a statistically significant increase of the concentration of 7-MeG, 3-MeA and 1-MeG in the group of rats that were not implanted with cancer cells (week 10 → 14). This can be an important mechanism for prolongation of the process of ageing of the organism. It can also have protective activity on the stage of initiation of the cancer process.

Similarly as it was in the case of zinc, the supplementation of rats with copper did not significantly affect the examined biomarkers of the DNA methylation process. However, few reports are available as concerns the effect of copper on the processes occurring on the epigenetic level in neoplastic diseases. Further studies concerning copper exposure on epigenetic factors are needed. There is no consensus about the cell death pathway induced by Cu. The results suggested that Cu induces the activation of apoptosis through caspase dependent and independent pathways [43,44]. Copper is generally considered as a prooxidant metal which participates in Fenton-like reaction. One of the mechanisms of the pro- and anti-cancer activity of copper involves its ability to produce a strongly reactive hydroxyl radical responsible for oxidative damage to DNA strands and also peroxidation of cell membrane lipids [45,46]. However, in the performed studies the supplementation of rats with copper did not affect the level of 8-oxoG.

Summing up the results presented in this work the following conclusions can be drawn. The level of methyl derivatives was statistically significantly reduced with the age of the examined rats. In the presented model the implantation of rats with cancer cells did not affect the level of the examined biomarkers in the rats' urine. Supplementation with selenium reduced both the effectiveness of tumor induction and the concentration of 7-MeG, 3-MeA, 1-MeG and 8-oxoG in the urine of the examined rats.

Conflict of interest: Authors state no conflict of interest

Ethical approval: The research has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

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