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RESEARCH ARTICLE

MODIFICATION OF CARCINOGENESIS IN MICE WITH CHEMICALLY-INDUCED TUMORS

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ABSTRACT

Environmental pollution is an important problem of our time. Human activity leads to a significant accumulation of toxic and carcinogenic substances. Their combined effects can increase the risk for cancer. In an experiment on mice the effect application on skin benzo(a)pyrene (BP) at doses of 6 and 9 μg/mouse and sodium nitrite (SN) received by animals with water was studied at a concentration of 50 mg/l and 500 mg/l. The modifying effect of SN on carcinogenesis induced by BP was revealed, which was manifested by an increase of 2.4 ($p < 0.01$) times in the incidence of skin tumors under the action of maximum doses of BP and SN (9 μ g/mouse and 500 mg/l). SN stimulated increase of 2.8-3.5 times ($p < 0.01$) in hormone-dependent tumors (mammary gland + ovary + uterus). NS had a reinforcing effect on the increase in the multiplicity of tumors 1.4-1.6 times ($p < 0.05$). The stimulating effect of NN on carcinogenesis is possibly caused by the increased formation of reactive oxygen and nitrogen forms, as well as inhibition of the phagocytic function of blood neutrophils.

INTRODUCTION

The combinedeffect of several/numerous chemical compounds (including carcinogenic ones) on the human organism is considered to be typical of modern life. In this respect studies on application of carcinogens and environmental factors modulating the effect of the latter have received much attention, especially in cases of benzo((a)pyrene (BP) and nitrates/nitrites interaction.

BP is classified by experts of the International Agency for Research on Cancer (IARC) as belonging to the first group of carcinogenic factors (that is carcinogenic for humans) (IARC, Lyon, 2012), and in our country it is included in San Pin 1.2.2353-08[1]. BP is known to possess significant stability in the environment, and it enters human organism with air contaminated by polycyclic aromatic hydrocarbons, food products, tobacco smoke, etc. Cutaneous transport (especially in industrial conditions) may also be significant.

Nitrates and nitrites are ubiquitously found in the environment, being involved in nitrogen cycling in the biosphere and metabolic processes of living organisms. Excess of nitrates and nitrites may cause toxic and immunosuppressive effect in the organism (IARC, Lyon, 2010; Lundberg *et al*., 2008). Nitrites may react with secondary amines producing endogenously carcinogenic nitroso compounds. They also may be reduced to NO with subsequent formation of highly reactive nitrogen oxides (NO_2, N_2O_3, N_2O_4, $ONOO^-$) (IARC, Lyon, 2010; Thomas *et al*., 2008).

The efficiency of antitumor protection of the organism depends largely on the recent functional condition of the cells in the system of natural antitumor resistance of the organism: macrophages, neutrophils, NK cells, etc. Their phagocytic and cytotoxic activity is associated in particular with the formation of reactive oxygen species (ROS): superoxide anion radical, hydrogen peroxide, hydroxyl radical, singlet oxygen and nitrogen oxide (Berezhnaya and Chekhun, 2005; Potselueva *et al*., 2005). At the same time excessive production of ROS and active nitrogen forms (ANF) may cause DNA damage, modification the DNA-repairing enzymes, cause activation of

[1]Carcinogenic factors and the main requirements for anti cancer prevention. Sanitary-epidemiological rules and regulations. San Pin 1.2.2353-08M.: FederalCenter of hygiene and epidemiology Rospotrebnadzor. 2008. 31 p.

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oncogenic signal, pathways that may induce tumor initiation and progression (Thomas *et al*.,2008; Beda and Nedospasov, 2007; Muntane, 2010). Tumor cells under the effect of interleukin 1β interferon-γ, tumor necrose factor-α, bacterial lipopolysaccharide, heat shock proteins and other factors may also produce NO. The role of NO in tumor pathogenesis is more complex than previously thought. NO and its metabolites affect cell cycles regulation, modulate different events of carcinogenesis, including apoptosis, angiogenesis, invasion and metastasis (Muntane, 2010; Ilnitsky *et al*., 2007).

The aim of the present study was investigation of nitrite modifying effect on chemical carcinogenesis induced by benzo(a)pyrene and also several mechanisms of its realization in experiments on mice.

MATERIALS AND METHODS

Mice

The investigation was performed on 320 female mice F_1 ($CBAxC_{57}BL/6$), then F_1.Two-months-old mice from the "Stolbovaya" farm were used.

The design of the experiment

Six groups of 50 mice in each group were formed. Twenty animals were used as a control group to determine immunological parameters. The duration of the experiment was 144 weeks before the natural death of the animals.Daily interscapular region of the back of mice (previously wool from the back was cut) was treated with a solution of BP in acetone in doses of 6 or 9 μg/mouse for two courses of 12 weeks each. Nitrites animals received with drinking water throughout the experiment in concentrations of 50 and 500 mg / L. Solutions in vials were changed every 3-4 days.

Below is a detailed scheme of administration of the studied substances for each of the six groups.

The first groupreceived daily acetone application in the interscapular region of the back of miceduring two periods of 12 weeks andduring the whole experiment sodium nitrite water solution (SN) of 500 mg/l.

The second groupreceived daily skin application of BP 6 μg/mouse for two courses with 12 weeks duration. Total BP dose reached 144μg/mouse.

The third group received BP skin application similar to that of the second group andwater solution of nitrites (SN) of 50 mg/lduring the whole experiment.

The fourth groupreceived daily skin application of BP 9 μg/mouse for two courses with 12 weeks duration. Total BP dose reached 216μg/mouse.

The fifth group received the same amount of BPsimilar to that of thefourth groupand else during the whole experiment water solution of nitrites (SN) of 50 mg/l.

The sixth groupreceived daily skin application of BP 9 μg/mouse for twocourses with 12 weeks duration (total BP dose reached 216μg/mouse) andwater nitrite solution (SN) 500 mg/lduring the whole experiment.

Macroscopic and microscopic examinations

All animals that died during the experiment were subjected to macroscopic analysis for presence of tumor. After that, macroscopically changed organs and tissues dead mice were fixed in 10% buffered formaldehyde, embedded in paraffin and stained with haematoxylin and eosin (Merkulov, 1969).).

Quantitative and qualitative analysis of tumors

The IARC classification has been used to diagnose tumors in mice (Turusov,1979). The results were evaluated using the following parameters: number of animals at the time of identification of the first tumor (effective number), the number of animals with tumors (incidence), the number of all tumors taking into account their morphological characteristic and localization, coefficient of multiplicity (number of tumors per number of animals with tumors in each group).

Substances

Benzo(a)pyrene (Fluka, AG), Hb (Sigma, USA), luminol (Merck, Germany), zimozan (Sigma, USA) and reagents of domestic production with classification of "chemically pure" and "pure for analysis", were used in the investigation.

Luminol-dependent chemiluminescence

The functional activity of blood neurophylls (by registering the formation of ROS) was determined, using luminoldependantchemiluminescense(CL), registered at Biolumat device, model 9500 («Berthoold», Germany). It is known that blood chemiluminescence is generated predominantly by neitrophyls and to a lesser extent by monocytes (Haitov,2018; Deryagina *et al*., 2003). Zymozan was used as an activator of phagocytosis and it was opsonized by serum of 10-20 healthy donors in concentration of 15 mg/ml in analyzing sample. The number of leucocytes was estimated in Goryaev's camera, count leukocyte was determined in stained blood smears.

The Griss Method

Exogenic and endogenic nitrates and nitrites are shown to be excreted from the organism with urine (Cortas and Wakid, 1991). For urine collection, 5 mice from each group were placed in exchange cells for 24 hours, depriving the animal of food, with free access to bidistillated water. Determination of nitrites in urine was achieved, using Griss method; nitrates were pre-reduced to nitrites, using freshly prepared cadmium (Tsikas,2007).

Statistics

Data obtained were treated statistically as based on the Student criteria, Mann-Whitney U test and χ^2 (Gubler, 1978).

RESULTS

The results of the study of the combined action of BP and nitrites (SN) on females of F_1 hybrids are presented in Tables 1 and 2. The morphological characteristics of tumors are presented in Table 3. The number of animals with tumors in all groups reached 69-88%. Although number of animals with tumors were lower in the groups with joint supplementation of BP and nitrites than those receiving only BP but the total amount of tumors in the first case was higher on 13.8-32. 0% than in the second. The coefficient of multiplicity increased only when both reagents were applied and it was not changed for the

animals with sole BP treatment, regardless of the dose (Table 1). Under the action of BP (6 µg /mouse) and SN (50 mg/l), the multiplicity index increased by 43.2 % compared to the action of one BP. In mice receiving maximum doses of BP (9 µg/mouse) and SN (500 mg/l), the increase in the multiplicity index was even more significant and amounted to 61.4%($p<0.05$).

Table 1 The effect of sodium nitrite (SN) on incidence and multiplicity of tumors induced by benzo(a)pyrene (BP) in $F_1(CBAxC_{57}BL/6)$ mice

Group no. Treatment	Numbers of mice	Effective number	Mice with tumors number/%[a]	Number of tumors (malignant)	Coefficient of multiplicity
I SN (500 mg/l + acetone	50	43	30/70.0	38(30)	1.27
II BP (6 µg/mouse)	50	50	44/88.0	58(51)	1.32
III BP (6 µg/mouse)+ SN (50 mg/l)	50	46	35/76.0	66(54)	1.89*
IV BP (9 µg/mouse)	50	45	38/84.0	50(44)	1.32
V BP (9 µg/mouse +SN (50 mg/l)	50	45	31/69.0	61(50)	1.97**
VI BP(9 µg/mouse)+ SN (500 mg/l)	50	40	31/ 78.0	66(52)	2.13**

[a] – %, calculated to the effective number
* – difference between III and II groups is statistically significant ($p<0.05$);
** – difference of V and VI groups compared to the IV group is statistically significant ($p<0.05$).

As can be seen from the Table 2, the highest concentration of nitrites (500 mg/L) increased the carcinogenic effect of BP (9 µg/mouse) on the skin by 57.9%.

Table 2 Effect of combined administration of benzo(a)pyrene (BP) and sodium nitrite (SN) on number and localization of tumors in $F_1(CBAxC_{57}BL/6)$ mice

Localization of tumors	Groups					
	I	II	III	IV	V	VI
	Tumors (number/%)[a]					
Skin	1/2.3	5/10.0	5/10.9	9/20.0	10/22.2*	19/47.5*
Liver	6/13.9	9/18.0	12/26.0	8/17.7	8/17.7	11/27.5
Hormone dependant organs (ovary+ uterus+mammary gland)	4/9.2	5/10.0	13/28.1**	4/8.8	14/31.1**	10/25.0***
Lung	1/2.3	3/6.0	4/8.7	3/6.6	2/4.4	4/10.0
Kidney	0/0	2/4.0	2/4.3	1/2.3	1/2.2	1/2.5
Haematopoietic system	26/60.4	34/68.0	30/65.2	25/55.5	26/57.7	21/52.5
Total number of mice with tumors (numder/%)	30/70.0	44/88.0	35/76.0	38/84.0	31/69.0	31/78.0

Note:
[a] % - calculated to the effective number;
Group 1– SN(500 mg/l) + acetone;
Group 2– BP (6 µg/mouse);
Group 3– BP (6 µg/mouse) + SN(50 mg/l);
Group 4– BP (9 µg/mouse);
Group 5 group – BP (9 µg/mouse) + SN(50 mg/l);
Group 6– BP (9 µg/mouse) + SN(500 mg /l).

* the differences between groups V, VI and V are statistically significant ($p<0.01$);
** the differences between groups III, and II, V and IV are statistically significant ($p<0.01$);
*** the difference between groups VI and IV is statistically significant ($p<0.01$).

The incidence ofskin tumors (Squamous cell carcinoma + papiloma) was 47.5% in the mice of group VI exceeded 2.1 times ($p<0.01$) similar parameter for the mice of the Vgroup, treated with the same BP dose, but 10 times lower concentration of nitrites (50 mg/l) (Table 2).The number of malignant skin tumors in the mice treated with BP alone or in combination with nitrites consisted 60.0% - 77.8% of the total tumors amount (Table 3).

Table 3 Histological types of tumors in $F_1(CBAxC_{57}BL/6)$ mice treated with benzo (a) pyrene (BP) and sodium nitrite (SN)

Tumor type	Experimental groups						
	I SN (500 mg/l + acetone	II BP (6 µg/mouse)	III BP (6 µg/mouse) +SN (50 mg/l)	IV BP (9 µg/mouse)	V BP (9 µg/mouse) + SN (50 mg/l)	VI BP (9 µg/mouse) + SN (500 mg/l)	Total
Benign tumors (number)							
Hepatoma	5	3	5	2	3	5	23
Pulmonary adenoma	1	2	2	1	1	2	9
Papiloma of the skin	1	2	2	2	4	7	18
Uterus(leiomyoma)	1	–	3	1	3	–	8
Total (Benign)	8	7	12	6	11	14	58
Malignant tumors (number)							
Hepatocellular carcinoma	1	4	6	5	5	6	27
Hemangioendo-telioma of the liver	–	2	1	1	–	–	4
Haemoblastoses	26	34	30	25	26	21	162
Squamous cell carcinoma	0	3	3	7	6	12	31
Adenocarcinoma mammary gland	1	2	5	2	5	4	19
Ovariantumors (cystadenoma)	2	2	3	1	3	3	14
Uterinesarcoma (Other tumors)Pulmonary and kidney adenocarcinomas	–	1	2	–	3	3	9
	–	3	4	3	2	3	15
Total (Malignant)	30	51	54	44	50	52	281
Total (Benign+ Malignant)	38	58	66	50	61	66	339

Note. (−) - tumors are nor registered.

The number of the animals with hormone-dependant tumors (mammary gland, uterus and ovary - total) among mice treated with BP and nitrites was 2.5-3.5 times higher ($p<0.01$), than in the animals which were treated with BP alone (Tables 2, 4). Comparison of the latent period of the development of skin and mammary gland tumors failed to reveal any statistically significant difference between the groups with BP or its combination with nitrites. Comparison of life expectancy in different groups revealed clear dose-dependent tendency of its reduction for mice, receiving nitrites, that indicates typical high toxicity of nitrites (especially at high doses). Thus, the life expectancy of mice treated with the highest doses of BP + nitrites was reduced by 13% ($p<0.05$) compared to mice treated only with BP (9 µg/mouse). Data by other authors indicate a high incidence of spontaneous tumors including those hormone dependent (mammary gland, uterus, ovaries) in females of F_1 hybrids $(CBAxC_{57}BL/6)$ mice. Investigation of oncological characteristics of F_1 hybrids mice, demonstrates the tumor rate equal to 86.5 %, multiplicity indexof 1.34, hemaolastosis rate of 70.0 %, that of the mammary gland tumors – 22.0 %, liver tumors – 19.0 %, lung tumors − 7.0 %, uterine tumors – 2% (Krasnova *et al*., 2005). In this study the life expectancy of mice varied from 9.5 (41 weeks) to 36 months (157 weeks). Unfortunately, the results of other few studies differ significantly, which makes it difficult to correctly compare our results with the data of "historical control" (the frequency of spontaneous tumors in F_1 mice in previous years).

Table 4 The incidence of hormone-dependent tumor in F_1(CBAxC_{57}BL/6) mice treated with benzo(a)pyrene (BP) and sodium nitrite (SN)

Groups (doses)	Effective number	Number of mice with tumors				
		Mammary gland		Ovary+ uterus		Total (ovary+ uterus + mammary gland) Number (%)
		Number	%	Number	%	
I 500 mg/l SN+acetone	43	1	2.3	3	6.9	4 (9.2)
II BP (6 µg/mouse)	50	2	4.0	3	6.0	5 (10)
III BP (6 µg/mouse+50mg/l SN	46	5	10.8	8	17.3	13 (28.1)*
IV BP (9µg/mouse)	45	2	4.4	2	4.4	4 (8.8)
V BP (9µg/mouse)+50 mg/l SN	45	5	11.1	9	20.0	14 (31.1)**
VI BP (9µg/mouse)+500 mg/l SN	40	4	10.0	6	15.0	10 (25)***

Note:
* the difference from Group II is statistically significant (p<0.01);
** the difference from Group III is statistically significant (p<0.01);
*** the difference from Group IV is statistically significant (p<0.01).

Simultaneously with the study of the modifying effect of nitrites on carcinogenesis, a study was conducted to study some mechanisms that could contribute to the realization of the potentiating action of sodium nitrite. In particular,changes in the functional activity of immune blood cells and endogenous formation of nitric oxide metabolites in mice treated with BP or its combination with SN were studied. The study of blood cell composition revealed an increase in the total number of leukocytes in mice exposed to BP or its combination with SN. When using the highest dose of BP in mice, leukocytosis was observed compared with intact animals. Chemiluminescent determination of spontaneous activity of blood neutrophils showed an increase in the formation of 1.5-5.5 times the active forms of oxygen (ROS) by cells as a result of effect BP or its combination with sodium nitrite (Table5).

Table 5 Chemiluminescence determination of the formation of the active forms of blood oxygen (of mouse crosses) in F_1(CBAxC_{57}BL/6) miceunder a combined effect of benzo(a)pyrene (BP) and sodium nitrite (SN)

Groups, doses	Number of mice	Total dose/ mouse		Chemiluminescence, pulse/min. per 10^3 neutrophils, M±SD		Coefficient of phagocytosis stimulation
		BP, µg	SN, g	spontaneous	Phagocytosis-dependent	
III BP (6 µg / mouse) + 50 mg/l SN	6	144	0.154	69.6±12.4**	374.4±51.5**	5.4
IV BP (9 µg /mouse)	6	216	0	18.4±3.5	85.8±11.8	4.7
V BP (9 µg /mouse) + 50 mg/l SN	6	216	0.154	30.9±11.6	146.4±41.1	4.7
VII Intact controls	6	0	0	12.6±2.5	128.2±10.1	10.2

Note: **- p<0,01 (compared with controls according to the Student test)

Nevertheless, statistically significant difference was revealed only in the mice of the III group (6 µg BP/mouse + 50 mg NS/l) compared to the control group of animals. The animals of this group showed an increase of 2.9 times (P<0.01) in phagocytosis-dependent chemiluminescence (PDC) of blood cells during their stimulation, while the mice of other experimental groups were characterized by values close to the control ones. Comparison of phagocytosis stimulation coefficients (ratio of PDC to the value of spontaneous chemiluminescence (SCL) revealed a decrease in the coefficient by 47.1-53.9% for the blood neutrophils of mice, exposed to BP or his combination with nitrites compared to control animals.

The quantitative determination of NO oxidized derivatives (nitrates and nitrites) in daily urine is an integral indicator of endogenous biosynthesis of NO, necessary for maintenance of physiological and immune reactions, and also NO biosynthesis by tumor cells.)). In the urine can also be present residual non metabolized nitrites coming from drinking water. Daily nitrates excretion with urine by animals of experimental groups (at the 98th week of the experiment) exceeded 2.8-10.0 times (p<0.01) the similar value for intact mice (Table 6).). At the 110 weeks nitrates excretion reached the highest value – 564.8±107.3 µg/kg body mass in mice, treated with BP+ nitrites. Nitrites were detected in small or trace amounts only in urine of mice with joint application of BP and nitrites (III and V groups).

Table 6 Excretion of nitrates and nitrites with the daily urine in F_1 (CBAxC_{57}BL/6) mice treated with benzo(a)pyrene (BP) and sodium nitrate (SN)

Groups, doses	Number of mice	Total dose/mice		Content of NO_2^- and NO_3^- in the daily urine, µg /kg body weight	
		BP µg	NN g	NO_2^-	NO_3^-
I 500 mg/l SN+ acetone	5[a]	0	1.37	0	46.5±7.9**
	10[б]	0	1.54	0	110.3±17.6
II BP (6 µg /mouse)	5 [a]	144	0	0	168.8±28.7**
	10 [б]	144	0	0	176.9±26.5
III BP (6 µg/ mouse) + 50 mg/l SN	5 [a]	144	0.137	0	87.1±13.1**
	10 [б]	144	0.154	0.02±0.03	158.4±22.8
IV BP (9 µg /mouse)	5 [a]	216	0	0	60.0±12**
	10 [б]	216	0	0	75.6±16.7
V (9 µg /mouse) +50 mg/l SN	5 [a]	216	0.137	0	72.1±13.0**
	10 [б]	216	0.154	0.2±0.02	222.3±40.0
VI BP (9 µg /mouse)+500 mg/l SN	5 [a]	216	1.37	0	90.0±20.7**
	10 [б]	216	1.54	0	564.8±107.3
VII Intact, controls	5[a]	0	0	0	16.9±2.5
	10[б]	0	0	0	traces

Note: [a] the results were obtained on the 98th week of the experiment; [b] the results were obtained on the 110th week of the experiment
** - the difference from group VII[(a)] is statistically significant (p<0.01).

DISCUSSION

This study is the final in a series of research of the possible modifying effect of nitrites/nitrates on process blastomogenesis. Three experimental models were previously used: chemical carcinogenesis (induction of tumors by urethane and 1.2-dimethylhydrazine), viral blastomogenesis − (virus-induced leukemia) and transplantation blastomogenesis (transplantable tumors of different strains) (Ilnitsky *et al.*, 1993, 1997, 1997, 2004).

Potentiating effect of nitrites on carcinogenesis caused by urethane and 1.2-dimethylhydrazine was demonstrated (Ilnitsky *et al*., 1997, 2004). The effect was manifested in moderate increase of the incidence of tumors, including localizations specific to the action of these carcinogens.

Thus, we have sufficient evidence available of various tumor models and animal strains, which confirms the ability of nitrites to potentiate carcinogenesis. Dose-dependent effect was not always present (Ilnitsky *et al*., 1997). In some cases, the potentiating effect depended on the sex of animals and was more pronounced in female mice than in males (Yurchenko *et al*., 1986).

The data presented in this article confirm the previous conclusions about the moderate potentiating effect of nitrites on carcinogenesis induced by different blastomogenic factors (Ilnistky *et al*., 1993, 1997, 1997, 2004). If we consider that the potentiating effect of SNon skin carcinogenesis can be considered expected, the increase in the number of hormone-dependent tumors with the combined action of SN and BP may have its special explanation.In a previous study were shown nitrites to affect directly or indirectly the concentration and activity of certain hormones of the reproductive system in rats, in particular, of the follicle stimulating the hormone, chorionic gonadotropin, testosterone, etc. (Savitskiy, 2003). Nitrites may cause a genotoxic effect on mammal cells. Thus, incubation of the rat lung cells with nitrites in the culture causes disruption of the normal course of mitosis, chromosome damage in the cells, emergence of foci of multilayer growth with morphologically altered cells, on the basis of which a transplantable tumor strain was created(Osinkovskaya, 1987).

In combination ionizing radiation with nitrites the level of radiation-induced chromosomal rearrangements in thespermatocytes of mice increased significantly (Suchko and Malenchenko, 1992). It is possible to assume the existence of several other mechanisms determining the potentiating effect of nitrites on carcinogenesis, since nitrites and nitrates have an adverse effect on many systems of the living organism (Landberg *et al*., 2008). The cytotoxic effect of nitrites is manifested in the frequency of hemic hypoxia, with violations of redox reactions affecting nicotinamide nucleotides - coenzymes of a large number of dehydrogenases (Ridnour *et al*, 2004). Our earlier results proved the ability of nitrites to induce formation of compounds with radical properties (Deryagina, 2003). Study of mechanism of nitrites interaction with hemoglobin revealed the formation of intermediate radical compounds: superoxide anion radical, nitrosyl radical, ferric radical. There is also a possibility of NO formation as result of NO reduction with hem-containing proteins (deoxyhemoglobin − HHb, deoxy form of myoglobin, cytochrome oxidase, cytochrome P-450) (Stepuro *et al*, 1997). Radicals and highly reactive nitrogen oxides may damage DNA and demonstrate pronounced nitrosation properties, that may increase number DNA mutations and/or cause biochemical modification of numerous proteins, inducing tumor progression (Muntane and De la Mata, 2010; Choudhari *et al*., 2013). Moreover, nitrites may cause suppression effect on phagocytic activity of immune cells: neutrophils and macrophages (Deryagina *et al*., 2003).

In the present study, animals that were treated with BP or its combination with nitrites showed an increase in spontaneous formation of ROS by blood neutrophils, which could also initiate free radical processes. However, the phagocytic function of the blood neutrophils in these mice may be impaired, as evidenced by the decrease in the absolute value of the calculated phagocytosis stimulation coefficient. Along with this, stimulation of the formation of oxidized NO (derivatives) products − nitrates and nitrites in mice with tumors was recorded. Increased formation of NO and its metabolites in mice with tumors is usually explained by activation of the enzyme – inducible NO-synthase (iNOS) in immune and tumor cells in response to proinflammatory cytokines and other factors. (Ilnitsky *et al*., 1993; Muntane and De la Mata, 2010; Choudhary *et al*., 2013). Depending on the concentration, duration of exposure and nature cells secreting radicals, nitric oxide exhibits pro or antitumor effect(Thomas *et al*., 2008; Muntane and De la Mata, 2010). Data on the participation of nitric oxide in the formation of new vessels in the tumor and surrounding tissues, as well as the disaggregating effect on tumor cells were obtained(Cheng *et al*., 2014). Along with this in several studies a therapeutic potential of NO donors is revealed, capable to increase the efficiency of radio and chemotherapy (Bonavida, 2015).

CONCLUSION

Sodium nitrite (SN) administered to mice with water in concentrations corresponding 50 mg/l and 500 mg/l in water causes a moderate potentiating effect on carcinogenesis induced by skin application of BP. In all groups of animals treated with a combination of BP (6 and 9 μg) with nitrites (50 mg/l and 500 mg/l) thefrequency and coefficient of multiplicity of hormone dependant tumors (mammary gland, uterus and ovary in total) were statistically significantly higher than in groups treated with BP alone. The number of skin tumors (with a predominance of malignant) was significantly higher in the group of mice that received the highest dose of BP (9 μg), as well as the highest concentration of nitrites (500 mg/l), corresponding to 100 maximum permissible levels. Increased formation of ROS, metabolites of nitric oxide and inhibition of phagocytic function of blood neutrophils may contribute to the potentiation of carcinogenesis under the action of nitrites.

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