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PRODUCTION OF NITROGEN OXIDE DERIVATIVES UNDER THE INFLUENCE OF NO-SYNTHASE INHIBITORS AND NATURAL COMPOUNDS IN MICE WITH TRANSPLANTED TUMORS

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***Aim*: The aim of the present study was to investigate the dynamics of nitric oxide derivative (NOD) formation in mice with transplanted tumors and to analize whether synthetic NO-synthase inhibitors, NO-donors and natural compounds could modulate NOD synthesis. *Materials and Methods*: In the study $F_1(C_{57}BlxCBA)$, CBA/Lac, BDF and Balb/c mice were used. Endogenous NOD synthesis in mice with transplanted Ehrlich carcinoma (EC) and Lewis lung carcinoma (LLC) was estimated by measuring urine nitrates (NA) and nitrites (NI) excretion and their concentration in tumor tissue determined by cadmium-reduction method. *Results*: It is shown that EC development is accompanied by increased endogenous NOD formation whereas LLC growth — by its decrease. Total NOD excretion with urine in EC-bearing mice during tumor development was in the range of $1.1x10^{-7}$-$7.0x10^{-6}$ mol/kg body weight that was 1.7–6.8 times higher than that in LLC-bearing mice. Treatment of EC-bearing animals with N^{ω}-nitro-L-arginine and aminoguanidine resulted in decreased NOD formation causing moderate tumor growth retardation. Effect of treatment with nitroprusside was shown to be dependent on the rout of its administration and dosage. Treatment of EC-bearing mice with picnogenol, tannic acid, spirulina and paprika enriched with selenium resulted in tumor growth inhibition at the early stage of EC growth accompanied by stimulation of endogenous NOD formation. *Conclusion*: Regulation of endogenous NOD formation towards normal physiological levels or hyperproduction of these compounds may result in tumor growth suppression.**
***Key Words*: nitric oxide derivatives, NO-synthase inhibitors, natural antioxidants, tumors.**

The role of nitric oxide in tumor biology is ambiguous and is studied insufficiently. NO-production in human and animal organisms is based on enzymatic NO-synthase transformation of L-Arg-guanidine fragment in the reaction with oxygen [1, 2]. Long duration of NO-biosynthesis (10–100 fold longer than basic level) results in genotoxicity effect, induces dose-dependant DNA-destruction etc, leading to tumor development. Multifold increase in NO-production is considered to be a consequence of inducible NO-synthase activation (iNOS), being expressed in different cell types in normal state and pathology, including macrophages, microglyal cells, keratinocytes, hepatocytes, astrocytes, endothelial cells of blood vessels, epithelial cells and a variety of human tumor cells affected by cytokines: interleukin-1 and 2, interferon-γ or their combination with tumor necrosis factor-α or -β, etc [3–7].

Direction of NO effect is defined by many factors: concentration, exposition, reaction products with key reagents (oxygen and its active forms, carbon dioxide, etc) and targets (metals, thiol containing aminoacids, proteins, etc). Clinical investigations show that NOS expression in many tumor tissues is often disturbed. That is true for tumors of central nervous system, stomach, colon and mammary gland, and melanoma, where NOS activity is found to be elevated. A direct correlation is shown between iNOS expression in tissues of these tumors and the following parameters: stage of a disease, vessel development in tumor, high frequency of metastases, what result in poor prognosis [8–11]. Animal experiments revealed that during iNOS activation, utilization of enzyme selective inhibitors resulted in tumor growth suppression [12, 13]. Exogenous NO is shown to inhibit endogenous NO synthesis. Mechanisms of feedback include direct NOS inactivation via NO binding by hem-containing enzyme group and inhibition of m-RNA iNOS expression [14].

At the same time NO plays a positive role in defense reactions of an organism. Thus, NO secreted by nonspecific immunity cells, macrophages and neutrophils, shows oxidizing and antimicrobial properties and is responsible for phagocytes cytostatic and cytotoxic potential with respect to tumor cells [15]. There is another significant property of NO: apoptosis initiation, including that in transformed cells, due to violation of mitochondrial oxidative phosphorylation, ribonucleotide reductase metabolism, etc [16]. In some instances, when NO cell production is low (what promotes survival of transformed cells), it is proposed that the activity of iNOS should be restored with the use of medicinal agents or with gene therapy [7]. NO concentration in biological fluids of organism, tumor tissues, tumor microenvironment is shown to predict the activity of key proteins for carcinogenesis — such as p53, extracellular signal-regulated kinase (ERK), hypoxic inducible factor (HIF), Akt etc. [17]. Taking into account the above information a special scientific and practical interest will include investigations of NO biosynthesis

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Abbreviations used: AG – aminoguanidine; ASA – acetylsalicylic acid; CZ – L-carnozine; Dq – Diquertin; EC – Ehrlich carcinoma; GA – gallic acid; I-3-C – indol-3-carbinol; iNOS – inducible NO-synthase; LLC – Lewis lung carcinoma; L-NMMA – N^g-monomethyl-L-arginine; L-NNA – N^{ω}-nitro-L-arginine; NA – nitrates; NI – nitrites; NOD – nitric oxide derivatives; NP – nitroprusside; Pg – pycnogenol; Se-P – sweet pepper; Se-Sp – spirulina;TA – tannic acid; VS – vine stone.

levels upon the influence of NOS modulators and natural antioxidants in different models of tumor growth.

The aim of the present study was to investigate the dynamics of nitric oxide derivatives (NOD) formation in mice with transplanted tumors and to analize whether synthetic NO-synthase inhibitors, NO-donors and natural compounds could modulate NOD synthesis.

MATERIALS AND METHODS

322 male $F_1(C_{57}BIxCBA)$, CBA/Lac, BDF and Balb/c mice weighting 22–31 g were used in the work. Animals were fed with briquetted feed with constant access to water. All experimental procedures were conducted following the normative rules of bioethics.Ehrlich carcinoma (EC) and Lewis lung carcinoma (LLC) tumor strains were received from Russian Oncologic Scientific Centre of RAMS (Moscow, Russia). LLC and EC cells ($5x10^6$ or 10^6 cells per mice) were inoculated subcutaneously, in right armpit and right inguinal region.

The following reagents were used: N^g-monomethyl-L-arginine (L-NMMA), N^{ω}-nitro-L-arginine (L-NNA), N-[[3-(aminomethyl)phenyl]methyl]-ethanimidamide dihydrochloride (1400W), aminoguanidine (AG) (Sigma-RBI); L-carnozine (CZ) (BioChemika). Other reagents: nitroprusside (NP), acetylsalicylic acid (ASA), etc. were of Russian production. While choosing NOS modulators, chemical properties of compounds were taken into consideration [1].

Endogenous NOD formation in the organism was evaluated on two tumor growth models — LLC (60 mice) and EC (40 mice) by analysis of nitrate (NA) and nitrite (NI) excretion with urine per day and their concentration in tumor tissue.

Gathering of diurnal urine for NA and NI analysis of mice F_1(C57BIxCBA) with LLC was achieved before tumor transplantation and after it on the 2d, 9th, 16th, 21st and 30th day. NA and NI concentrations in tumor tissue were determined on the 14th, 21st, 28th and 32nd day of tumor growth.

Gathering of diurnal urine of Balb/c-mice with EC was performed before EC transplantation and after it on the 4th, 10th, 17th, 24th and 32nd day of tumor growth. NA and NI concentrations in tumor tissue of BDF mice with EC transplanted tumor were determined on the 32nd day of tumor growth.

Investigation of synthetic NOS inhibitors and NO donors effect on EC growth was carried out on 102 $F_1(C_{57}BIxCBA)$ and BDF mice. 7 groups of hybrid mice (9–10 animals per group) were formed. The control group was composed from 16 mice. BDF mice were divided into 2 groups, 10 mice per group. EC was transplanted to all mice subcutaneously. The intraperitoneal introduction was used for administration of an active compound solution, 0.1 ml, 5 days per week (except special cases discussed later) to animals from the 2–8th groups beginning from the 8th day after EC transplantation. In total, each animal received 14 doses. There were formed the following groups: 1) control, 2) treatment with 50 mg/kg L-NMMA, 3) 100 mg/kg L-NNA, 4) 140 mg/kg AG, 5) combined treatment with 140 mg/kg AG and 10 mg/kg ASA. The 6 group of animals received 4 mg/kg of NP on the 2nd and 3d day after EC transplantation and later subcutaneously 40 µg/kg of NP. The 7th group received subcutaneously 80 µg/kg of NP on the 8th day after EC transplantation. The 8th-group of mice received 1000 mg/kg of CZ with water since the second day after transplantation and during all the experiment.

The effect of selective inhibitor iNOS-1400W on EC growth was studied on 20 BDF mice with transplanted EC (group 9, control, n=10, and group 10, n=10, treated subcutaneously with 13 mg/kg 1400W inhibitor solution from day 4 after tumor transplantation. In total, 14 1400W administrations per animal were performed.

Investigation of effect of natural biologically active compounds was analyzed in CBA/Lac (n=50), $F_1(C_{57}BIxCBA)$ (n=50) and Balb/c (n=20) male mice using the following preparations: pycnogenol (Pg), containing >=60% of procyanidins (Biolandes, France), diquertin (Dq) with>90% of dihydroquercetin (Flavir, Russia), indol-3-carbinol (I-3-C) (Sigma), phenolic acids: gallic (GA), tannic (TA) and also Se-enriched paprika (Se-P, 1 mg Se/kg Mayak cultivar grown on sodium selenate containing 0.03% NPK-fertilizers) and algae spirulina-*Arthospira platensis* (Se-Sp, 1 g Se/kg; production of “Agro-Victoria” corporation, Russia). The preparations were given *per os* as water suspensions 2–3 weeks before EC transplantation and after it up to the end of experiment 5 times per week. The euthanasia of animals was performed under the light ester anesthetic.

Tumor growth inhibition (TGI) under the effect of tested compounds was estimated in tumor growth dynamics using mass and volume parameters in comparison with the control group according to [18].

For diural urine sampling animals were placed in exchange cages (5 per cage) for 24 h without feeding and with free water access. To exclude NI oxidation 0.3 ml of 30% sodium hydroxide solution was used. Removal of protein-carbohydrate component in urine water extract was achieved using $ZnS0_4$ and potassium ferricyanide.

On the 14th, 21st, 28th days after LLC transplantation (or 32nd day for EC) the euthanasia of animals under the light ester anesthetic was performed. Tumors were removed, homogenized and the samples of tissue extracts were obtained as follows: 1–4 g of homogenized tumor tissue were placed in a calibrated vessel, bidistilled water was added (tissue/water ratio= 1:10) and the resulting mixture was heated at 60° C during 15 min. To prevent NI destruction, pH of water suspension was adjusted to 7.2–7.4 using buffer solution. Then the mixure was cooled to room temperature, protein-carbohydrate component was removed by centrifugation for 15 min at 500 g. NI and NA content in all reagents, water and materials was controlled.

NI and NA concentration in urine and tumors was determined by spectrophotometric method using Griss reagent with prereduction of NA to NI by porous cadmium [19]. Optical density of colored solutions was determined on spectrophotometer SF-46.

Statistical analysis of the data (mean value±SD) was performed by standard methods using Student's *t*-test.

RESULTS AND DISCUSSION

EC growth in Balb/c mice was accompanied by increase of NA and NI excretion with urine (Table 1). By the end of experiment (day 32) urine NI and NA excretion increased by 3.6 ($p<0.01$) and 67.1 ($p<0.01$) times respectively compared to these parameters of animals with the initial tumor nods (day 4). NI excretion in EC-bearing mice was in the range $3.6x10^{-7}$—$1.3x10^{-6}$ mol/kg bw, and NA excretion — $8.5x10^{-8}$—$5.7x10^{-6}$ mol/kg bw. Total nitro compound excretion during EC growth increased by 15.5 fold (in NO_3^- equivalents). During this period mean tumor volume increased by 67.3 times. A positive correlation between EC volume and diurnal total NI and NA excretion with urine was revealed (r=0.99).

NOD excretion by $F_1(C_{57}BlxCBA)$ mice with transplanted LLC demonstrated certain peculiarities (Table 2). One day after LLC inoculation maximal excretion values of NI and NA were 1.9 times higher than the respective parameters of healthy animals, and were constantly higher by 22.2–70.5% versus control at all time points during LLC growth.

NI concentration in LLC tumor tissue (Table 3) was in the range of $2.8–4.6x10^{-6}$ mol/kg, increasing monotonously during tumor development whereas NA concentration in the majority of samples was negligible. Total NA+NI content in tumor tissue did not exceed $5.3x10^{-6}$ mol/kg and did not depend on tumor development.

Total amount of NI and NA in EC tumor tissue isolated from BDF mice on day 32 was 5.5 times higher ($p<0.01$) than their maximal total content in LLC tumor tissue (Table 3).

Investigation of the effect of synthetic compounds capable to modulate NOS activity has shown that intraperitoneal administration of L-NNA (100 mg/kg), AG (140 mg/kg), AG (140 mg/kg)+ASA (10 mg/kg), to hybride mice resulted in statistically significant EC growth inhibition (Table 4). The earliest and stable TGI was demonstrated for NNA (39%, $p<0.01$). AG caused low TGI (14–22%, $p<0.05$) but in combination with ASA it inhibited EC growth more efficiently (20–42%, $p<0.05$). NP effect depended on the dose and rout of administration: subcutaneous administration

Table 1. Urine NI and NA excretion in dynamics of Ehrlich carcinoma growth in Balb/c mice[a)]

Parameter	Day of tumor growth					
	0	4	10	17	24	32
Tumor volume, mm^3	No tumor	49±21	720±117	1480±197	2189±809	3300±462
NO_2^- excretion	0	$(3.6 \pm 2.6)\times10^{-7}$	$(4.5 \pm 1.09)\times10^{-7}$	$(5.8 \pm 4.64)\times10^{-7}$	$(1.0 \pm 0.53)\times10^{-6}$	$(1.3 \pm 0.55)\times10^{-6}$
NO_3^- excretion	$1.1 \pm 0.44\times10^{-7}$	$(8.5 \pm 9.9)\times10^{-8}$	$(8.9 \pm 3.0)\times10^{-7}$	$(3.3 \pm 1.1)\times10^{-6}$	$(3.1 \pm 0.3)\times10^{-6}$	$(5.7 \pm 2.97)\times10^{-6}$
Total NI and NA excretion (mol/kg bw)	$(1.1 \pm 0.44)\times10^{-7}$	$(4.45 \pm 3.29)\times 10^{-7}$	$(1.34 \pm 0.38)\times10^{-6}$	$(3.88 \pm 1.41)\times10^{-6}$	$(4.1 \pm 0.73)\times10^{-6}$	$(7.0 \pm 3.63)\times10^{-6}$

Note. [a)] mean values ± SD (n=10).

Table 2. Urine NI and NA excretion of $F_1(C_{57}BlxCBA)$ mice bearing Lewis lung carcinoma

Days of LLC growth / Group of animals	NI and NA excretion[a)], mol/kg bw			Comparison with control[b)]
	NO_2^-	NO_3^-	Total NI and NA	
2	$(2.94 \pm 1.12)\times10^{-7}$	$(3.42 \pm 0.15)\times10^{-6}$, $p<0.01$	$(3.71 \pm 0.20)\times10^{-6}$, $p<0.01$	+91.2
9	0.0	$(7.67 \pm 2.31)\times10^{-7}$, $p<0.01$	$(7.67 \pm 2.31)\times10^{-7}$, $p<0.01$	-60.5
6	$(5.93 \pm 7.9)\times10^{-8}$	$(5.11 \pm 4.0)\times10^{-7}$, $p<0.01$	$(5.7 \pm 4.14)\times10^{-7}$, $p<0.01$	-70.5
21	0.0	$(1.18 \pm 0.36)\times10^{-6}$	$(1.18 \pm 0.36)\times10^{-6}$	-39.2
30	0.0	$(1.51 \pm 0.43)\times10^{-6}$	$(1.51 \pm 0.43)\times10^{-6}$	-22.2
Control (healthy animals)	$(2.6 \pm 1.04)\times10^{-7}$	$(1.68 \pm 0.85)\times10^{-6}$	$(1.94 \pm 1.12)\times10^{-6}$	0.0

Note. [a)] mean values ± SD (n=10); compared to the control. [b)] (-) – % of NOD decrease; (+) – % of NOD increase.

Table 3. NI and NA concentration in tumor tissue of mice bearing LLC or EC

Mice (days of tumor growth)[a)]	NI and NA concentration, mol/kg tissue		
	NO_2^-	NO_3^-	Total NI and NA
F_1 mice with LLC (14)	$(2.8 \pm 1.1)\times10^{-6}$	0.0	$(2.8 \pm 1.1)\times10^{-6}$
F_1 mice with LLC (21)	$(3.70 \pm 1.5)\times10^{-6}$	$(1.6 \pm 0.6)\times10^{-6}$	$(5.3 \pm 1.98)\times10^{-6}$
F_1 mice with LLC (28)	$(4.6 \pm 1.5)\times10^{-6}$	0.0	$(4.6 \pm 1.5)\times10^{-6}$
BDF mice with EC (32)	$(1.19 \pm 0.37)\times10^{-6}$	$(1.72 \pm 0.38)\times10^{-5}$	$(2.91 \pm 0.42)\times10^{-5}$

Note. [a)] mean values ± SD (for 10 animals).

Table 4. Effect of NO-synthase inhibitors on Ehrlich carcinoma growth and endogenous production of NOD derivatives in $F_1(C_{57}BlxCBA)$ and BDF mice

Groups of EC-bearing mice, compounds (dose, mg/kg bw)	Tumor growth inhibition (TGI), %*	NO_2^- excretion, mol/kg bw*	NO_3^- excretion, mol/kg bw*	Total NI and NA, mol/kg bw (days of EC growth)	Effect on NOD excretion, %
1. Control	0	$(0.68 \pm 0.25)\times10^{-6}$	$(6.8 \pm 2.24)\times10^{-6}$	$(7.48 \pm 2.57)\times10^{-6}$ (22)	0
2. L-NMMA (50)	0	$(1.5 \pm 0.62)\times10^{-6}$ $p<0.01$	$(5.5 \pm 1.59)\times10^{-6}$	$(7.0 \pm 2.02)\times10^{-6}$ (22)	-6
3. L-NNA (100)	18–39 $p<0.05$; $p<0.01$	$(0.79 \pm 0.33)\times10^{-6}$	$(2.71 \pm 1.03)\times10^{-6}$ $p<0.01$	$(3.50 \pm 1.27)\times10^{-6}$ $p<0.01$ (22)	-53
4. AG (140)	18–22 $p<0.05$	$(1.02 \pm 0.36)\times10^{-6}$	$(4.53 \pm 1.81)\times10^{-6}$ $p<0.05$	$(5.55 \pm 2.19)\times10^{-6}$ (22)	-26
5. AG (140) + ASA (10)	20–42 $p<0.05$; $p<0.01$			**	**
6. EC + NP (4)	+(28–124) $p<0.05$; $p<0.01$	$(0.47 \pm 0.15)\times10^{-6}$	$(1.12 \pm 0.33)\times10^{-5}$ $p<0.01$	$(1.17 \pm 0.36)\times10^{-5}$ $p<0.01$ (22)	+56
7. NP (0.08)	21–26 $p<0.05$; $p<0.01$			**	**
8. CZ (130)	21–29 $p<0.05$; $p<0.01$	$(1.78 \pm 1.0)\times10^{-6}$	$(2.92 \pm 1.17)\times10^{-6}$ $p<0.01$	$(4.70 \pm 1.86)\times10^{-6}$ $p<0.05$ (22)	-37
9. Control	0	$(0.35 \pm 0.17)\times10^{-6}$	$(4.83 \pm 2.12)\times10^{-6}$	$(5.18 \pm 2.35)\times10^{-6}$ (22)	0
10. 1400W (13.0)	0	$(0.14 \pm 0.13)\times10^{-6}$ $p<0.01$	$(3.74 \pm 1.35)\times10^{-6}$	$(3.88 \pm 1.49)\times10^{-6}$ (22)	-25

Note. *- compared with respective the control; +( )- increase in tumor growth; **-not determined; N^g-monomethyl-L-arginine (L-NMMA); N^{ω}-nitro-L-arginine (L-NNA); AG – aminoguanidine; ASA – acetylsalicylic acid; NP – nitroprusside; CZ – L-carnozine. 1–8 groups – mice $F_1(C_{57}BlxCBA)$. 9–10 groups – mice BDF.

of 80 µg/kg of NP resulted in 26% EC growth inhibition ($p<0.05$) but intraperitoneal administration of higher dose (4 mg/kg) on days 2 and 3 after EC transplantation stimulated tumor growth up to 124% ($p<0.01$). In mice treated with L-NNA and AG tumor growth inhibition by 53% ($p<0.01$) and a decrease of NOD formation by 26% were recorded.

It should be pointed out that L-NMMA often used in investigations as iNOS inhibitor (50 mg/kg) did not affect EC growth while N0D excretion with urine was close to that of control animals. Subcutaneous administration of 1400W, selective iNOS inhibitor (13 mg/kg), did not cause statistically significant EC tumor depression though NOD excretion was decreased by 25.0%.

Investigation of natural preparation effect on EC growth has shown that prolonged *per os* consumption of 150 mg PG/kg and 75 mg I-3-C/kg caused statistically significant TGI by 52% and 56% respectively ($p<0.01$). During the whole experiment Pg effect was stable and resulted in 31% decrease of tumor mass ($p<0.01$). GA (100 mg/kg), TA (100 mg/kg) and Se-Sp (1 g/kg) statistically significantly increased the latent period of tumor nod formation by 1.4–1.6 times and inhibited tumor growth during two weeks after EC transplantation. At the early stages of EC development TGI was equal to 91% (GA), 78% (TA), 75% (Sp), 89% (Se-Sp) and 65% (Se-P) ($p<0.05$).

Total NI and NA excretion detected on days 14, 21 or 23 of EC growth in control group of animals was 6.4×10^{-6} mol/kg bw (F_1 mice); 1.11×10^{-5} mol/kg bw (CBA/Lac mice) and 6.29×10^{-6} mol/kg bw (Balb/c mice) (Table 5). Pg, Dq, TA, Sp and Se-P administration resulted in increase of NOD excretion by 44.2% ($p<0.05$), 47.3% ($p<0.05$), 27.7%, 65.6% ($p<0.01$) and 114.6% ($p<0.01$) accordingly compared to the control group of animals. Only GA inhibited NO formation by 32% ($p<0.05$) on day 14 of EC growth whereas I-3-C and Se-Sp did not affect NO biosynthesis.

Thus, the results have shown that EC growth is accompanied by increased NOD formation whereas LLC tumor growth is characterized by depression of NOD biosynthesis. It should be noted that NO endogenous synthesis determination via NOD urine excretion is an integral parameter including also the amount of NOD, being formed from nitrogen oxide, necessary for normal function of cardiovascular, immune, endocrine and nervous systems. NO synthesis by constitutive NO-synthase isoforms is considered not to be accompanied by toxic effects of NO and its derivatives as concentration levels capable to cause necessary physiological reactions are in nano-to micromolar range [20]. There are few data on NO levels in animal tumor tissues. Study of human breast cancer MCF7 cells has shown that the activity of several key proteins (HIF-1α, ERK, and p53) is regulated by distinct threshold concentrations of nitric oxide. At low steady-state concentrations of NO (<50 nmol), ERK phosphorylation was induced via a guanylate cyclase-dependent mechanism. HIF-1α accumulation was associated with an intermediate amount of NO (>1.0×10^{-7} M), whereas p53 serine 15 phosphorylation occurred at considerably higher levels (>3.0×10^{-7} M) [17].

In our experiment endogenous formation of NOD in EC mice beginning from the 9th to the 30th day of tumor growth was in the range 1.1×10^{-7}–7.0×10^{-6} mol/kg bw and for LLC-mice — 5.7×10^{-7}–3.71×10^{-6} mol/kg bw. At the same time the total NI and NA concentration in tumor tissue was 2.91×10^{-5} mol/kg, while NOD concentration in LLC tumor did not exceed 5.3×10^{-6} mol/kg tissue. It is obvious that mean NOD concentration in EC and LLC tumors is significantly higher than concentrations necessary for activation of proteins responsible for cells proliferation and apoptosis.

One can assume that the ability of NOS inhibitors (L-NNA and AG) to suppress tumor growth is related to their ability to depress iNOS activity [21]. Also it has been shown that NP could have oppositely directed effects and stimulate tumor growth at the relatively high doses (4 mg/kg) and suppress it at lower doses (80 µg/kg). There are the data demonstrating that in tumor microenvironment with low content of glucose and oxygen tumor cells may be more sensitive to high concentrations of NO and peroxinitrite that normal ones. That is why in several cases NO donors are considered to be effective for promotion of chemo- and radiotherapy efficiency [16]. However, we have shown that EC has been developing against the augmented NOD biosynthesis and double intra-peritoneal injection of a high NP dosage the next day after EC inoculation, which only stimulated tumor growth.

Table 5. Effect of natural antioxidants on Erlich carcinoma growth and endogenous production of NO derivatives (NOD) in CBA/Lac, $F_1(C_{57}Bl \times CBA)$ and Balb/c mice

Groups EC mice, compounds (dose, mg/kg bw)	Parameters				
	Tumor growth inhibition (TGI), or increase in tumor growth (+), %*	NO_2^- excretion, mol/kg bw*	NO_3^- excretion, mol/kg bw*	Total NI and NA mol/kg bw, (days of EC growth)	Effect on NOD excretion, %
CBA/Lac (control)	0	$(2.3 \pm 0.9) \times 10^{-6}$	$(0.88 \pm 0.24) \times 10^{-5}$	$(1.11 \pm 0.30) \times 10^{-5}$ (21)	0
CBA/Lac Pg (150)	31–52 $p<0.01$	$(3.2 \pm 1.1) \times 10^{-6}$	$(1.28 \pm 0.56) \times 10^{-5}$ $p<0.05$	$(1.6 \pm 0.67) \times 10^{-5}$ $p<0.05$ (21)	+44.2
CBA/Lac VS (300)	(+) 45 $p<0.05$	$(2.1 \pm 0.8) \times 10^{-6}$	$(1.06 \pm 0.32) \times 10^{-5}$	$(1.27 \pm 0.38) \times 10^{-5}$ (21)	+14.4
CBA/Lac Dq (150)	0	$(3.2 \pm 1.2) \times 10^{-6}$	$(1.32 \pm 0.51) \times 10^{-5}$ $p<0.05$	$(1.64 \pm 0.61) \times 10^{-5}$ $p<0.05$ (21)	+47.8
CBA/Lac I-C (75)	27–56 $p<0.01$	$(2.3 \pm 0.9) \times 10^{-6}$	$(0.92 \pm 0.33) \times 10^{-5}$	$(1.15 \pm 0.42) \times 10^{-5}$ (21)	+3.6
F_1 (control)	0	$(0.94 \pm 0.38) \times 10^{-6}$	$(5.46 \pm 1.69) \times 10^{-6}$	$(6.4 \pm 1,9) \times 10^{-6}$ (14)	0
F_1 GA (100)	27–91 $p<0.05$; $p<0.01$	$(1.02 \pm 0.44) \times 10^{-6}$	$(3.33 \pm 1.17) \times 10^{-6}$ $p<0.01$	$(4.35 \pm 1.48) \times 10^{-6}$ $p<0.05$ (14)	-32.0
F_1 TA (100)	24–78 $p<0.01$	$(1.34 \pm 0.50) \times 10^{-6}$	$(6.83 \pm 2.73) \times 10^{-6}$	$(8.17 \pm 3.04) \times 10^{-6}$ (14)	+27.7
F_1 Sp (1000)	41–75 $p<0.05$; $p<0.01$	$(1.5 \pm 0.5) \times 10^{-6}$ $p<0.05$	$(0.91 \pm 0.25) \times 10^{-5}$ $p<0.01$	$(1.06 \pm 0.28) \times 10^{-5}$ $p<0.01$ (14)	+65.6
F_1 Se-Sp (1000)	41–89 $p<0.05$; $p<0.01$	$(1.42 \pm 0.58) \times 10^{-6}$	$(4.43 \pm 1.9) \times 10^{-6}$	$(5.85 \pm 2.35) \times 10^{-6}$ (14)	-8.6
Balb/c (control)	0	$(0.87 \pm 0.30) \times 10^{-6}$	$(5.42 \pm 1.52) \times 10^{-6}$	$(6.29 \pm 1.73) \times 10^{-6}$ (23)	0
Balb/c Se-P (1000)	38–65 $p<0.05$; $p<0.01$	$(0.9 \pm 0.4) \times 10^{-6}$	$(1.26 \pm 0.54) \times 10^{-5}$ $p<0.01$	$(1.35 \pm 0.57) \times 10^{-5}$ $p<0.01$ (23)	+114.6

Note: Each group contained 10 mice. Natural antioxidants: pycnogenol (Pg), diquertin (Dq), indol-carbinol (I-C), phenolic acids: gallic acid (GA) and tannic acid (TA), vine stone (VS) and Se-enriches plants: sweet pepper (Se-P), spirulina (Se-Sp); * — compared with respective control.

Lack of L-NMMA-dependent inhibition of NOD endogenous formation can be explained by peculiarities of its metabolism. It is known that NOS converts L-NMMA to N-hydroxy-N-methyl-L-agrinine, which inhibits the enzyme irreversibly. But in certain tissues and cells especially in hypoxic conditions, L-NMMA metabolism may be accompanied by the formation of L-arginine or may possess other peculiarities [1].

Taking into account the NOD endogenous formation in a model of EC tumor growth, its 25–53% reduction upoin the use of L-NNA, AG and 400W appears to be insufficient to attain its normal physiological level. In conditions of developing tumor acceptable iNOS blocking will not be able to produce stable defense response of an organism, but may retard tumor growth. At the same time significant depression of iNOS activity may have also negative consequences. It is known that N0 is formed in the oxidation reaction of L-arginine, which is also a precursor of endogenous synthesis of polyamines. This supposes the existence of an integrated mechanism of nitric oxide and polyamine synthesis regulation. Polyamines are known to stimulate mammalian cells proliferation and potentiate carcinogenesis. The results show that blocking of NO-synthase may cause pretumor changes in intestine due to the decreased NO release and induction of ornitine-decarboxylase — an enzyme of polyamine synthesis [22].

A study of the effect of natural compounds with pronounced antioxidant properties on tumor growth and endogenous NOD formation has revealed that the use of 4 from 9 tested compounds resulted in tumor suppression on the background of stimulation of NOD formation. One can suppose that such effect could be related not only to direct reactions with free radicals so typical for antioxidants but also to other mechanisms involved in stimulation of detoxification defense systems through antioxidant responsive elements present in the promoter region of genes inducible upon oxidative and chemical stresses [23].

In conclusion, our study has demonstrated that EC development is accompanied by increased endogenous NOD formation while LLC growth — by depression of NOD synthesis. Modulators of NO-synthase activity (L-NNA and AG) decreased NOD formation, inducing simultaneously moderate, but stable decrease of tumor development in EC-bearing mice. The use of NP had oppositely directed effects on EC growth dependent on the dose and the rout of administration. Most of natural antioxidants tested demonstrated antitumor activity at the early stage of EC growth stimulating endogenous NOD formation.

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