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REDUCTION OF RADIATION-INDUCED NITRATIVE STRESS IN LEUCOCYTES AND KIDNEY CELLS OF RATS UPON ADMINISTRATION OF POLYPHENOLIC COMPLEX CONCENTRATES FROM RED WINE

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The research has shown that exposure to ionizing radiation at the dose of 30 cGy leads to the activation of NO-synthase way of nitrogen oxide synthesis, as well as to the accumulation of its stable metabolites and 3'-nitrotyrosine modified proteins in rat peripheral blood leucocytes and the renal cortical layer. NO-synthase activity was preserved at the control value through the consumption of red wine natural polyphenolic complex concentrates by the irradiated animals. The content of proteins modified by tyrosine nitration decreased in the early period of post-radiation exposure due to the influence of the investigated concentrate. Thus the ability of red wine natural polyphenolic complex concentrates to prevent adverse changes in L-arginine/NO system and, therefore, inhibit the development of nitrative stress induced by low doses of ionizing radiation has been proved experimentally.

Key words: *3'-nitrotyrosine modified proteins, NO stable metabolites, NO-synthase, polyphenols, X-rays.*

Introduction. Due to the growth of radioactive pollution of the biosphere caused by accidents at nuclear power plants, an increased number of air flights and intensive usage of ionizing radiation in medicine, the study of effects of low doses of ionizing radiation on living organisms, including humans and animals, is becoming more and more pressing [1, 2].

A considerable increase in free radical processes, the disorder of the redox state of cells and the development of oxidative stress, the main marker of which is an increase in superoxide anion ($O_2^{\cdot -}$), are the key events in biological systems under X-ray exposure conditions [3]. Under pathological conditions, including the action of ionizing radiation, diabetes and cardiovascular diseases, NO, which normally acts as a second messenger, is produced in excessive amounts and reacts with $O_2^{\cdot -}$, resulting in the formation of peroxynitrite ($ONOO^-$). $ONOO^-$, in turn, can significantly enhance the degradation of cellular structures by the modification of proteins (on tyrosine residue in particular), cause DNA damage, the induction of lipid peroxidation and disorder in cellular signaling, resulting in the development of nitrative stress [3–7]. A compensatory increase in NO-synthase activity and, consequently, the increased production of NO is also the result of $ONOO^-$ formation, adding to nitrative stress. For example, the increased formation of NO following irradiation is characteristic of such organs as the liver, lung, kidney, intestine, heart, brain and bone marrow and vascular endothelium [5–10]. In addition, it is well-known that within several hours of exposure to low doses of ionizing radiation inflammatory response is developed, activating leucocytes in vessels. The development of inflammatory processes can greatly complicate the handling of nitrative-oxidative stress, thus inflicting damage on the body. Accordingly, the search for new radioprotective compounds and new diagnostic approaches to detecting radiation damage is extremely important today.

The ability of phenolic groups in polyphenols to neutralize electrons of free radicals and form relatively stable phenoxyl radicals suggests they can be strong radioprotectors, since in such a way polyphenolic compounds stop radiation-induced oxidation chain reactions in cells [11]. It was shown that polyphenols can act as scavengers of reactive oxygen species (ROS), reactive nitrogen species (RNS) and lipoperoxyde radicals [12–16]. They are also capable of chelating transition of metal ions such as iron and copper, which play an important role in the initiation of free radical reactions [17]. In this way, polyphenols act in redox sensitive signaling cascades when preventing radiation-induced single-stranded DNA breaks [18] and inhibit the cytotoxic effects of $ONOO^-$ [19]. A lot of flavonoids such as quercetin, luteolin and catechins are considered to be even better antioxidants than vitamin C, vitamin E and β-carotene [17].

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As red wine polyphenols have anti-inflammatory, immunomodulating, antioxidant and detoxification properties both *in vitro* and *in vivo* [20–22], they can potentially be used as effective radioprotectors. However, the role of red wine polyphenols, and in particular their concentrated preparations, in the development of nitrative stress caused by low-dose radiation has not been sufficiently studied. Therefore, the main aim of this study was to identify the impact of natural polyphenolic complexes of wine (hereinafter called polyphenol complexes, PC) on indicators of radiation-induced nitrative stress under low-dose radiation in cells of the cortical layer of kidneys and lymphocytes of rats.

Materials and Methods. The experiments were conducted on Wistar outbred white rats 180–200 g in weight. All the procedures with the animals were conducted in accordance with General Principles of Animal Treatment, approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986). The animals were kept in the vivarium and had free access to food and water.

The experimental rats were divided into four groups: 1 – normal untreated control animals (hereinafter called C); 2 – animals that consumed PC concentrate with drinking water (hereinafter called C+PC); 3 – rats that were irradiated (hereinafter called R), 4 – animals that consumed PC concentrate with drinking water 10 days before and throughout the experiment after irradiation (hereinafter called R+PC). Indices were measured in 24, 48, 72 and 168 h after the irradiation.

Red wine was kindly donated for the research by «Magarach», National Institute for Vine and Wine. Concentrates were obtained by evaporating red wine on rotary evaporators, Laborota 4001 (Germany). The mass concentration of phenolic compounds in the tested concentrate was 59 g/l, of which polymeric compounds constituted 40 g/l and monomers – 19 g/l. The main components were caftaric, coutaric and gallic acids, catechins and quercetin.

The concentrates were consumed with drinking water, a daily dose being 12.5 mg of polyphenolic compounds per 1 kg of body weight, which corresponds to the theoretical average concentration of polyphenols in 300 ml of red wine (a daily recommended dose for a person weighing 70 kg).

The total polyphenolic content was standardized in wine and the concentrate to the gallic acid equivalent using Folin-Chokalteu reagent [23].

The rats were exposed to the single total radiation in a dose of 30 cGy by RUM-17 installation with the following parameters: a skin-focus distance of 95 cm, voltage of 130 kV, current of 10 mA, Cu 0.5 mm and Al 1.0 mm filters, the power of a dose – 8.3 $mGy \cdot s^{-1}$. The dose was controlled by a clinical dosimeter of 27012 type («Otto Shön», Germany).

The rats entered the surgical stage by ether anesthesia. Samples collection was carried out after the decapitation of the animals. The derived renal cortical layer was snap-frozen with liquid nitrogen and stored at –70 °C.

Blood was collected into porcelain cups. Heparin was used as an anticoagulant. Lymphocytes were separated on gradient of Histopaque-1083 (density of 1.083 g/ml) (10831, «Sigma», USA).

Determination of nitrites and nitrates. The samples were deproteinized by centrifugation at 14,000 rpm for 1 h at 4 °C with the addition of 96 % ethanol. 100 µl of VCl_3 was added to 100 µl of supernatant for the measurement of the total content of NO stable metabolites (NO_x), shortly followed by the addition of Griess` reagents (sulfanilamide (50 µl) and N-(1-naphthyl)ethylenediamine dihydrochloride (50 µl)). The Griess solution was premixed immediately prior to the application to the plate. Nitrites were measured in a similar manner. However, the samples were exposed exclusively to Griess reagents. In either case, the absorbance at 540 nm was measured using a plate reader (Epoch, «Bio-Tek», USA) following incubation (30 min) [24].

Determination of the total NO-synthase activity. NO-synthase activity was determined after the lysis of samples in the buffer containing 0.05 M Tris-HCl (pH 7.4), 0.25 M saccharose, 0.001 M EDTA, in which protease inhibitors (0.5 mM aprotinin (A1153, «Sigma», USA), 0.5 mM pepstatyn (P5318, «Sigma», USA) and 10 mM phenylmethanesulfonyl fluoride (P7626, «Sigma», USA)) were added directly before the process. After a 30-minute incubation at 4 °C the lysate was centrifuged at 14,000 rpm for 30 min. 10 mM HEPES buffer containing 1 M $MgCl_2$, 1 M $CaCl_2$, 3 mM L-arginine and 250 mM $NADPH+H^+$ was added to the supernatant. The samples were incubated at 37 °C for 30 min and the reaction was stopped by adding

96 % ethanol in 1:2 wt/vol ratio. Proteins were precipitated by centrifugation (at 20 °C, for 20 min at 2500 rpm). 100 μl of supernatant and 100 μl of Griess reagent were added to an eppendorf tube and incubated for 30 min at 37 °C. The samples were then transferred to microplates. Absorbance was measu-red at λ = 540 nm by a plate reader. Total enzyme activity was determined by the difference in nitrite formation [25].

Determination of protein concentration was carried out by the conventional Lowry method [26].

Western blot analysis of nitrated proteins. For the Western blot analysis, it was necessary to obtain tissue homogenates in RIPA buffer (1:10 wt/vol) (containing 50 mM Tris-HCl, pH 7.2; 150 mM NaCl; 1 % sodium deoxycholate; 0.1 % sodium dodecyl sulfate (SDS); 158 mM NaCl; 1 mM EGTA) containing protease/peptidase inhibitors (leupeptin (10 μg/ml), aprotinin (20 μg/ml), pepstatine (20 μg/ml), and phenylmethylsulfonyl fluoride (1 mM)) with aligned protein concentration. All the steps were performed at 4 °C. Proteins were separated on 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in Lemmli buffer system [21, 27]. The separated proteins were transferred onto nitrocellulose membrane by electroblotting, followed by processing of the blots with antibodies [28].

Membranes were incubated with the primary antibody (monoclonal antibody to 3-nitrotyrosine, N5538, «Sigma», USA) in the blocking buffer for 2 h followed by extensive washing. Anti-mouse IgG conjugated with horseradish peroxidase was used as the secondary antibody (AP308P, «Millipore», USA). The incubation with the secondary antibody was performed for 1 h, after which the membrane was washed with PBS/0.1 % Tween-20 (5 times for 3 min).

Immunoreactive bands on the membranes were detected using ECL Detection Reagents («Millipore», USA). The time of exposure of the membranes on X-ray film depended on the intensity of chemiluminescence and lasted on average 5–15 min. The X-ray film was developed in a standard 1-phenyl-3-pyrazolidinone-hydroquinone developer and fixed with acid fixer.

The membranes were stripped in 25 mM glycine-HCl, pH 2.5 buffer containing 1 % SDS and reprobed with β-actin antibody to confirm equal protein loading [21].

Statistical analysis of the research results was carried out using Origin Pro. The calculation of basic statistical parameters was performed by direct quantitative data obtained from the study (arithmetic mean – M, the standard deviation of the arithmetic mean – m).

To assess the reliability of the difference between statistical characteristics of the two alternative data sets, we performed Student's t-test. The difference was considered significant under $p \geq 0.95$ (the level of significance $P < 0.05$).

Results. NOS activity decreased 1.7 times in 48 h ($P < 0.05$), 1.5 times in 72 h ($P < 0.01$) and 1.3 times in 168 h ($P < 0.05$) in rat peripheral blood lymphocytes under PC consumption in the control animals. The following changes were noted while studying the state of L-arginine/NO system in the lymphocytes under irradiation. NOS activity decreased 1.1 times compared with the control in 24 h ($P < 0.05$). Later the index increased 1.4 times in 48 h, 1.6 times in 72 h ($P < 0.01$) and 1.1 times in 168 h ($P < 0.05$). NOS activity in rat lymphocytes against the background of exposure to ionizing radiation and PC consumption increased 1.3 times in 24 h ($P < 0.01$), decreased 1.2 times on the second day ($P < 0.05$), twice on the third day and 1.3 times in 168 h ($P < 0.01$) compared to the irradiated animals (Table 1).

After irradiation, NO_2^- content decreased 1.8 times ($P < 0.05$) in 24 h in lymphocytes, whereas it increased 1.5 times in 48 h and 1.6 times in 72 h ($P < 0.05$) compared to the control. After irradiation, NO_x^- content increased 1.3 times in 24 h ($P < 0.05$), 1.6 times in 48 and 72 h ($P < 0.01$), 1.4 times in 168 h ($P < 0.05$) compared to the control. Under PC treatment, in 24 h after irradiation, NO_x content increased 1.2 times ($P < 0.05$), whereas the content of NO_2^- increased 1.6 times ($P < 0.05$). An increase in NO_3^- content 1.2 times ($P < 0.01$) was found only in 48 h compared to the irradiated animals (Table 1).

The content of 3′-nitrotyrosine modified proteins in lysates of rat lymphocytes after exposure to ionizing radiation increased by 37 % in 48 h ($P < 0.05$), by 111 % in 72 h and by 74 % in 168 h ($P < 0.01$) (Fig. 1, *c–h*). Under irradiation and PC consumption, there was a slight decrease in the investigated parameter on the second day of the experiment, and a 1.3-fold decrease on the third and seventh days ($P<0.01$) compared with the parameter under irradiation (Fig. 1, *e*, *f*).

In peripheral blood leucocytes of the four experimental groups of animals, we observed the presence of a dominant protein with a molecular mass of approximately 35 kDa. It is interesting that after irradiation there were two distinct bands corresponding to proteins with molecular mass of 40–32 kDa. It provides evidence that under irradiation different proteins are modified than in the control. Moreover, PC concentrate affecting the total content of nitrated proteins is not able to cause changes in the targets of those post-translational modifications.

However, low doses of ionizing radiation do not always lead to death of immunocompetent cells. They often only change their population structure and activate these cells [29, 30]. Irradiated leucocytes synthesize large amounts of ROS, RNS and intercellular signaling molecules (cytokines) [31]. Consequently, this causes irreversible disturbance at the organism level. We studied the state of L-arginine/NO system in kidney tissue, since kidneys provide homeostasis preservation in blood through the production and release of cytokines, chemokines and hematopoietic factors [32]. Although kidneys are considered to be sufficiently resistant to irradiation, the imbalance of biochemical reactions in renal cells leads to the impairment of the whole body, particularly in the early post-radiation period [5].

It has been observed that after irradiation the activity of NOS in the rat renal cortical layer increased

Table 1. **The content of NO stable metabolites and the activity of NO-synthase in rat leucocytes**

Rodent group	Total content of stable NO metabolites (nmol/mg protein)	Nitrite-anion	Nitrate-anion	NO-synthase activity (nmol NO_2^-/min · mg protein)
		nmol/mg of protein		
		24 h		
C	33.57 ± 1.07	10.38 ± 1.47	23.19 ± 1.84	0.72 ± 0.03
C + PC	26.98 ± 1.35[a]	12.26 ± 1.05	14.72 ± 1.82[b]	0.60 ± 0.06
R	35.75 ± 1.18	5.92 ± 1.08*	29.83 ± 1.29*	0.66 ± 0.004*
R + PC	41.51 ± 1.85[c]	9.76 ± 1.33[c]	31.75 ± 2.23	0.84 ± 0.02[d]
		48 h		
C	27.87 ± 1.49	6.04 ± 1.65	21.83 ± 1.37	0.70 ± 0.01
C + PC	34.52 ± 3.17	7.36 ± 0.78	27.16 ± 2.02	0.39 ± 0.06[a]
R	43.28 ± 2.07**	9.10 ± 0.96*	34.18 ± 1.82**	0.95 ± 0.01**
R + PC	51.19 ± 1.67	8.95 ± 1.86	42.24 ± 1.12[d]	0.83 ± 0.06[c]
		72 h		
C	32.24 ± 2.31	7.37 ± 1.92	24.87 ± 3.28	0.81 ± 0.01
C + PC	25.27 ± 2.94	9.36 ± 2.30	15.90 ± 1.73[a]	0.55 ± 0.14[b]
R	54.85 ± 2.22**	13.83 ± 1.13*	41.03 ± 2.82**	1.32 ± 0.02**
R + PC	53.42 ± 1.65	9.29 ± 2.03	44.12 ± 2.13	0.64 ± 0.01[d]
		168 h		
C	28.16 ± 4.56	11.32 ± 2.74	16.84 ± 1.61	0.79 ± 0.03
C + PC	37.72 ± 1.67[a]	9.12 ± 1.68	28.60 ± 2.64[a]	0.62 ± 0.09[a]
R	32.04 ± 0.99	9.17 ± 1.18	22.87 ± 1.73*	0.89 ± 0.01*
R + PC	34.16 ± 2.73	10.55 ± 1.83	23.61 ± 0.92	0.68 ± 0.004[d, e]

Note. Here and Table 2, Fig. 1, 2 data are mean ±S.E.M, $n = 6–11$. * Difference between control (C) and irradiation (R) ($P < 0.05$); ** Difference between control (C) and irradiation (R) ($P < 0.01$); [a] – difference between control (C) and control with PC consumption (C + PC) ($P < 0.05$); [b] – difference between control (C) and control with PC consumption (C + PC) ($P < 0.01$); [c] – difference between irradiation (R) and PC consumption against the background of irradiation (R + PC) ($P < 0.05$), [d] – difference between irradiation (R) and PC consumption against the background of irradiation (R + PC) ($P < 0.01$); [e] – difference between PC consumption against the background of irradiation (R + PC) and control with PC consumption (C + PC) ($P < 0.01$).

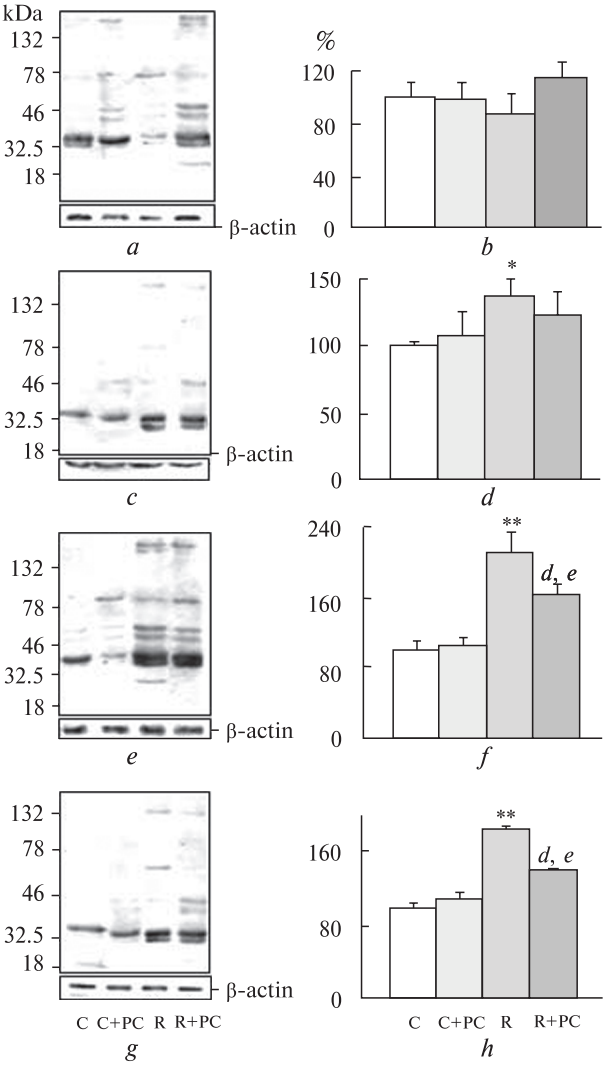

Fig. 1. Western blot analysis of 3′-nitrotyrosine modified proteins in leucocyte lysates under irradiation and PC consumption: 24 (*a*), 48 (*c*), 72 (*e*) and 168 (*g*) h after irradiation. The total nitrotyrosine content is shown in percent (control is taken as 100 %), (*b*), (*d*), (*f*), (*h*) respectively. *, **, *d*, *e* – see Note Table 1

1.7 times in 24 h, 1.8 times in 48 h, twice in 72 h and 1.5 times in 168 h compared to the control ($P < 0.01$). NOS activity was reduced throughout the experiment for the animals consuming PC concentrate after irradiation compared with the irradiated animals (1.5 times in 24 h ($P < 0.05$), 1.9 times in 48 h and 1.7 times in 72 h ($P < 0.01$)) (Table 2).

The content of NO metabolites may grow under the employed experimental conditions due to probable activation of NOS-independent ways of NO synthesis, among which the most important are the activation of nitrate- and nitritereductase, and cell xanthine oxidase.

Owing to the elevated NOS activity under irradiation, the content of NO_x increased 3.5 times in 24 h, 2.5 times in 48 h, 1.8 times in 72 h and 2.8 times in 168 h ($P < 0.01$). It should be noted that in 24 h the content of NO_3^- increased 3.7 times ($P < 0.05$), whereas the content of NO_2^- reduced 2.5 times compared to the control ($P < 0.01$) (Table 2). In the case of the combined action of PC and ionizing radiation, we observed a decrease in NO_x content 1.9 times in 24 h and 1.6 times in 168 h ($P < 0.01$), NO_3^- content 1.9 times in 24 h, 1.2 times in 48 h ($P < 0.05$) and 1.6 times in 168 h ($P < 0.01$) compared to the irradiated animals. Under PC consumption, NO_2^- increased in content twice ($P < 0.01$) on the first post-radiation day, whereas on the second and third days no changes in the content of the metabolite were noted (Table 2).

After irradiation, the level of proteins nitrated on tyrosine residues in the rat renal cortical layer increased by 17.5 % in 24 h ($P < 0.05$), by 47 % in 48 h, by 44 % in 72 h and by 50 % in 168 hrs ($P < 0.01$) compared to the control indexes. Under PC treatment against the background of irradiation, a downward trend in the indexes was shown in 24 and 48 h after the irradiation and a decrease by 44 % in 72 h and by 47 % in 168 h ($P < 0.01$) was reported compared with the indexes of the irradiated animals (Fig. 2, *a*–*f*).

The presence of dominant proteins (molecular mass of 35–40 kDa) was revealed in kidney lysates taken from all the four groups of animals. They also show an increase in nitrated proteins with molecular mass of approximately 80 kDa throughout all the four days of the experiment after irradiation and in those with molecular mass of 50 kDa on the second, third and seventh days after irradiation.

Discussion. There are a lot of studies aimed at determining the effects of oxidative-nitrative stress in tissues affected by high doses of ionizing radiation. Futhermore, it is often suggested that damage induced by low doses of irradiation cannot be detected and are likely not to exist [33, 34]. Such fundamentally different approaches to the interpretation of the effects of different doses of radiation are increasingly criticized. Therefore, our research was focused on studying the effects of ionizing radiation at the dose of 30 cGy on indices that

characterize oxidative-nitrative stress in the renal cortical layer and more radiosensitive peripheral blood leucocytes.

One of the key reasons of negative effects of radiation is NO hyperproduction, which *in vivo* is formed in the reaction catalyzed by NOS or some pathways independent of this enzyme. It is known that nitrates and nitrites are directly reduced to nitric oxide under appropriate reductases or xanthine oxidase. Nitrites may further be exposed to disproportionation to form NO.

The obtained results indicate that under irradiation the functioning of L-arginine/NO system is impaired in rodent tissues leading to emergence and further deepening of nitrative stress.

It is known that irradiation causes DNA damage, including strand breaks, destruction of nitrous bases and cross-linking. This leads to apoptosis induction in radiosensitive cells, including lymphocytes [30]. The regulation of cell survival or death is achieved through a cascade of signaling reactions, the key event of which is protein kinase activation. Owing to its redox chemistry, NO stimulates radiation-induced signaling through the activation of two superfamilies of protein kinases – mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3' kinase (PI3`K). The consequence of this stimulation is the activation of p53 protein by its phosphorylation. The activation of MAPK, PI3`K and protein kinase B (PKB) with further accumulation of activated p53 are factors causing inhibition of iNOS gene expression following irradiation [31, 35, 36]. They possibly lead to a decrease of the total NOS activity on the first day after irradiation in the dose of 30 cGy. The possible reason for the observed increase in NOS activity in peripheral blood lymphocytes on the second and third days following irradiation is perhaps the activation of gene transcription coding of the murine double minute 2 (Mdm2) factor by a low dose of radiation.

Table 2. **The content of NO stable metabolites and the activity of NO-synthase in the renal cortical layer of rats**

Rodent group	Total content of stable NO metabolites. (nmol/mg protein)	Nitrite-anion	Nitrate-anion	NO-synthase activity (nmol NO_2^-/min · mg protein)
		nmol/mg of protein		
		24 h		
C	12.37 ± 2.83	0.88 ± 0.04	11.49 ± 2.1	0.041 ± 0.014
C + PC	28.45 ± 2.86[a]	0.47 ± 0.02[a]	27.98 ± 3.05[b]	0.043 ± 0.012
R	43.09 ± 6.85**	0.35 ± 0.10**	42.74 ± 9.68**	0.070 ± 0.005*
R + PC	22.75 ± 2.49[d]	0.71 ± 0.04[d]	22.04 ± 4.90[c]	0.048 ± 0.008[c]
		48 h		
C	12.85 ± 1.30	0.56 ± 0.11	12.29 ± 2.90	0.039 ± 0.003
C + PC	28.42 ± 2.01[a]	0.92 ± 0.05[a]	27.50 ± 2.11[b]	0.038 ± 0.0004
R	31.50 ± 1.10**	0.76 ± 0.04*	30.74 ± 1.15**	0.071 ± 0.006**
R + PC	26.77 ± 2.09	0.84 ± 0.07	25.93 ± 2.02[c]	0.037 ± 0.005[d]
		72 h		
C	18.95 ± 3.85	0.87 ± 0.09	18.08 ± 1.84	0.046 ± 0.010
C + PC	29.22 ± 1.89[b]	0.88 ± 0.09	28.34 ± 1.83[a]	0.036 ± 0.002
R	34.40 ± 3.81**	1.56 ± 0.58	32.84 ± 4.83**	0.090 ± 0.009**
R + PC	36.08 ± 1.78	1.07 ± 0.03	35.01 ± 1.81	0.054 ± 0.004[d]
		168 h		
C	14,52 ± 2,15	0,72 ± 0,04	13,80 ± 1,32	0,041 ± 0,007
C + PC	15,24 ± 1,72	0,84 ± 0,06[a]	14,40 ± 1,05	0,039 ± 0,010
R	41,24 ± 2,51**	1,33 ± 0,09**	39,91 ± 1,37*	0,062 ± 0,014**
R + PC	26,15 ± 1,08[d, e]	0,96 ± 0,10[c]	25,19 ± 0,46[d, e]	0,046 ± 0,009

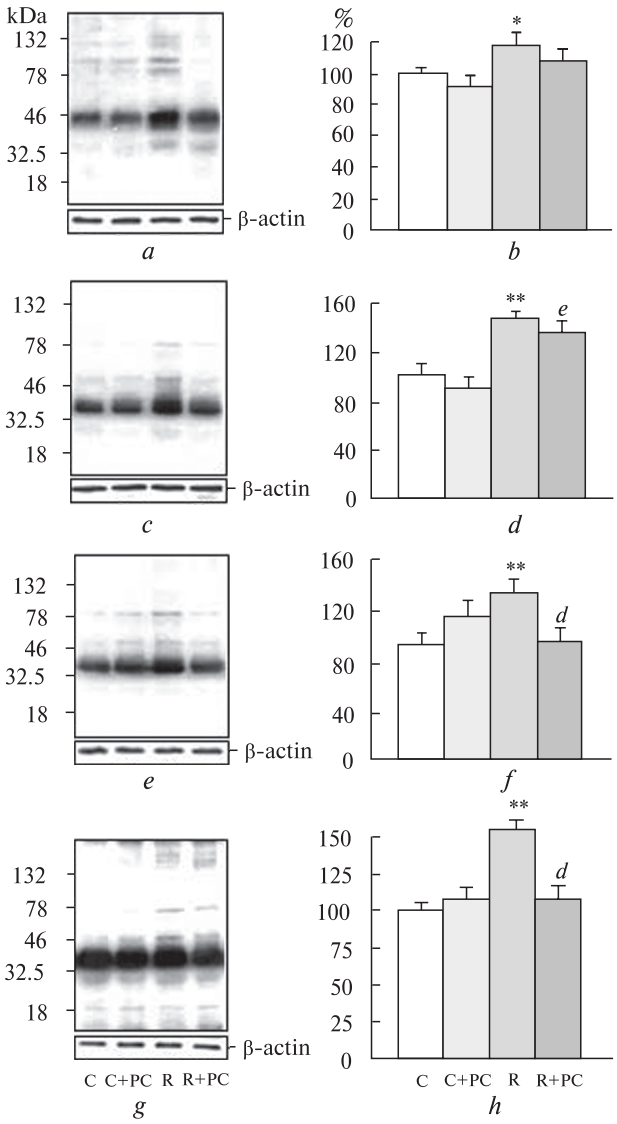

Fig. 2. Western blot analysis of 3′-nitrotyrosine modified proteins in the kidney cortical layer under irradiation and PC consumption: 24 (*a*), 48 (*c*), 72 (*e*) and 168 h (*g*) after irradiation. The total nitrotyrosine content (control is taken as 100 %) is shown, (*b*), (*d*), (*f*), (*h*) respectively

This protein is a E3 ubiquitin-protein ligase, which induces p53 proteasome-mediated degradation and increases iNOS activity [31, 35, 36]. Increased total NOS activity, which is above the control level, may be caused by the activation of signaling pathways that control the activity of iNOS at transcriptional and posttranscriptional levels [37].

Oxidation of L-arginine was activated in the rat renal cortical layer, which was testified by increased NOS activity throughout the experiment and, consequently, by an increase in NO_x content.

NO_x content also increased in all the stages of the experiment in immunocompetent cells. Interestingly, in 24 h after irradiation, NO_2^- content decreased against the background of a significant increase in both NO_3^- levels in lymphocytes and renal cells. It is known that nitrite-anion is a product of NO spontaneous oxidation in physiological conditions under normal oxygenation. It should be taken into consideration that nitrate-anion is produced in two ways – through the oxidation of nitrite-anion or simultaneously from ROS and RNS through $ONOO^-$ degradation:

$$ONOO^- + H^+ \rightarrow HNO_3^- \rightarrow H^+ + NO_3^-$$
$$ONOO^- + H^+ \rightarrow ONOOH \rightarrow {}^{\cdot}NO_2^- + {}^{\cdot}OH.$$

Therefore, an increase in NO_3^- content under irradiation shows not only the activation of NO synthesis by iNOS but also an increase of ROS generation, and thus the development of oxidative stress [38, 39].

PC consumption caused the reduction of total NOS activity throughout the three days of the experiment following irradiation in renal cell lysates. It is known that polyphenolic compounds are able to inhibit the iNOS mRNA translation, the synthesis of which was induced by lipopolysaccharide, interleukin-1 and TNF-α (tumor necrosis factor α) [40, 41]. This mechanism may occur also under inhibition of iNOS activity by polyphenolic compounds, which was increased after the exposure to low doses of radiation.

A decrease in NOS activity under consumption of PC concentrate caused the reduction of the total content of NO stable metabolites in 24 h in lysates of renal cortical layer cells. This may be considered evidence of the fact that red wine polyphenols affect NO enzymatic synthesis, inhibiting radiation-induced NOS activity growth. It should be noted that the content of NO_3^- decreased and the content of NO_2^- increased compared to the irradiated animals. It is quite probable that the reason for this is the activation of nitrate reductase, which catalyzes the recovery of NO_3^- to NO_2^- and simultaneously slows down reverse reactions – the oxidation of NO_2^- to NO_3^-. In 48 and 72 h after irradiation, these indices were higher than control values. Judging from our results, other pathways of NO and NO_x production are activated in excretory

system tissues under irradiation. The activation of nonenzymatic NO formation in the reaction of NO_2^- and NO_3^- under relevant reductase maybe the first way of NO_2^- content growth, whereas NO release from its depot (nitrosylated proteins) is the second way [42]. The process of NO release from nitrosothiols is catalyzed by two main denitrosylases related to redox pathways [43–47]. The first enzyme catalyzes transnitrosylation to reduced glutathione (GSH) forming S-nitrosoglutathione (GSNO). Accumulated GSNO undergoes enzymatic denitrosylation involving GSNO reductase (GSNOR) [43, 45]. The second denitrosylase regulates posttranslational modification of proteins through thioredoxin (Trx) involvement. Consecutive reactions are coupled to Trx reductase and NADPH [44].

Similarly, in peripheral blood lymphocytes, NO_2^- and NO_3^- content increased only on the first day after irradiation under PC consumption. In later periods of the experiment, these indices remained at the level of irradiated animals.

It is known that the content of nitrated proteins increases in cells injured by ionizing radiation due to excessive production of NO and $ONOO^-$ through growing iNOS activity. Current strategies aimed at limiting the cytotoxin formation are based on using a variety of herbal compounds that have the ability to scavenge RNS *in vitro* such as vine and wine phenolic compounds [5].

Our findings suggest that exposure to low doses of ionizing radiation leads to an increase in nitrotyrosine-modified proteins – key markers of nitrative stress in leucocytes and the rat renal cortical layer. We showed a reduction of this index in 72 and 168 h under combined effects of PC and X-rays. An increase in nitrotyrosine-modified protein content was also observed in lymphocytes under irradiation. PC introduction against the background of irradiation caused a decrease of this index on the third day compared to irradiation. Under the action of radiation, we detected an increase in nitrotyrosine-modified protein content due to an intensified processes of $ONOO^-$ formation. PC treatment has an inhibitory effect on protein nitration through NOS inhibition and $ONOO^-$ neutralization [48]. The most significant decrease of 3`-nitrotyrosine modified proteins both in leucocytes and rat renal cortical layers on the third day after irradiation under PC consumption may be an indication of the activation of cellular signaling pathways by these compounds, the denitration process being one of their links. It is widely accepted that protein nitration is a reversible process involved in intracellular signaling, along with phosphorylation. However, the mechanism of denitration is not clearly understood [49–51].

The ability of polyphenols to act as antioxidants and activate the endogenous antioxidant defense system in various organs such as the liver, kidneys, heart and brain is biologically important [19, 16, 18, 52–54]. All these data and our results confirm the ability of wine polyphenolic compounds to prevent the development of oxidative-nitrative stress caused by exposure to low doses of ionizing radiation.

Conclusion. Our results provide molecular mechanisms of nitrative stress development based on disorders in L-arginine/NO system and justify the use of wine polyphenolic complexes to prevent the development of radiation-induced changes and restore the physiological status of the organism after exposure to low doses of radiation.

ОСЛАБЛЕНИЕ ПРОЦЕССОВ РАДИОИНДУЦИРОВАННОГО НИТРАТИВНОГО СТРЕССА В ЛЕЙКОЦИТАХ И КЛЕТКАХ ПОЧКИ КРЫС ПРИ ВВЕДЕНИИ КОНЦЕНТРАТА ПОЛИФЕНОЛЬНОГО КОМПЛЕКСА ИЗ КРАСНОГО ВИНОГРАДНОГО ВИНА

М. Сабадашка, Н. Сибирная

Установлено, что действие ионизирующего излучения в дозе 30 сГр приводит к активации NO-синтазного пути синтеза оксида азота, накоплению его стабильных метаболитов и 3′-нитротирозин-модифицированных протеинов в лейкоцитах периферической крови и корковом слое почки крыс. Введение облученным животным концентрата природного полифенольного комплекса из красного виноградного вина способствовало сохранению активности NO-синтазы на уровне контрольных значений. При введении исследуемого концентрата содержание протеинов, модифицированных нитрованием по остаткам тирозина, снижалось в ранний пострадиационный период как в лейкоцитах, так и клетках коркового слоя почки. Нами экспериментально подтверждена способность концентрата полифенольного комплекса из виноградного вина корректировать негативные изменения системы L-аргинин/NO и подавлять развитие нитративного стресса, индуцированного малыми дозами ионизирующего излучения.

ПРИГНІЧЕННЯ ПРОЦЕСІВ РАДІОІНДУКОВАНОГО НІТРАТИВНОГО СТРЕСУ У ЛЕЙКОЦИТАХ ТА КЛІТИНАХ НИРКИ ЩУРІВ ПРИ ВВЕДЕННІ КОНЦЕНТРАТУ ПОЛІФЕНОЛЬНОГО КОМПЛЕКСУ З ЧЕРВОНОГО ВИНОГРАДНОГО ВИНА

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Встановлено, що дія іонізуючого випромінювання у дозі 30 сГр призводить до активації NO-синтазного шляху синтезу оксиду нітрогена та накопичення його стабільних метаболітів і 3′-нітротирозин-модифікованих протеїнів у лейкоцитах периферичної крові та корковому шарі нирки щурів. Введення опроміненим тваринам концентрату природного поліфенольного комплексу з червоного виноградного вина сприяло збереженню активності NO-синтази на рівні контрольних значень. Виявлено, що вміст протеїнів, модифікованих нітруванням за залишками тирозину, знижувався у ранній пострадіаційний період як у лейкоцитах, так і у клітинах коркового шару нирки за впливу досліджуваного концентрату. Отже, експериментально підтверджено здатність концентрату поліфенольного комплексу з виноградного вина коригувати негативні зміни системи L-аргінін/NO і, таким чином, пригнічувати розвиток нітративного стресу, індукованого малими дозами іонізуючого випромінювання.

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