MODIFYING EFFECT OF VITAMIN E ON ADRIAMYCIN AND CYCLOPHOSPHAMIDE INDUCED GENOTOXICITY AND ANTIOXIDANT STATUS IN RATS

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Abstract. In the present study, we have evaluated the protective effect of vitamin E (VE) against adriamycin (AD) and cyclophosphamide (CP) induced genotoxicity using chromosome aberration and micronucleus assays in Wistar rat bone marrow cells. The level of lipid peroxidation product malondialdehyde, the activity of antioxidant enzymes catalase and superoxide dismutase were measured in blood serum and erythrocyte hemolysate to evaluate the antioxidant status in rats. VE (250 mg/kg body weight, b.w.) was administered *via gavage* once a day for 3 consecutive days. Single dose of AD (5 mg/kg b.w.) or CP (30 mg/kg b.w.) was delivered by the intraperitoneal route. Pre-treatment of rats with VE was conducted for two days before AD or CP injection and concomitantly with AD or CP on the 3rd day. VE was administered concomitantly with AD or CP injection on the 1st day and for two consecutive days in the post-treated groups. No beneficial effect of VE on the antioxidant status of rats was determined. VE decreased drug induced bone marrow toxicity in all experimental groups. Protective effect of VE against AD- and CP-induced genotoxicity was dependent on the treatment schedule (i.e. sequencing of VE treatment). VE was protective against AD-induced chromosome damage in animals pre-treated with VE (both chromosome aberration and micronucleus assays) and against CP-induced damage in animals post-treated with VE (micronucleus assay only).

Keywords: vitamin E, adriamycin, cyclophosphamide, chromosome aberration, micronucleus, rats.

Introduction. It is well documented that cancer chemotherapy is often associated with oxidative stress and fall in plasma concentrations of various antioxidants in treated patients. This may trigger various physiological side-effects and certain toxicities including DNA damage in normal tissues and development of secondary primary cancer (Elsendoorn et al., 2001). One of the most promising strategies to reduce oxidative stress and thus prevent or attenuate the subsequent side-effects is the combination of the drug delivery together with an antioxidant (Singal et al., 2000; Quiles et al., 2002). Many antioxidants have been assayed for their protective properties and ability to modulate the side effects of cytostatic drugs in different experimental models. However, the results are often equivocal and inconclusive reporting beneficial as well as adverse effects. For our study we selected vitamin E, which is considered to be one of the most important antioxidants to prevent oxidative injury of DNA and other cell structures (Brigelius-Flohe and Traber, 1999), and two cytostatic agents – adriamycin and cyclophosphamide, that represent different modes of genotoxic and antitumour activities.

Vitamin E is a collective name that covers eight different tocopherols and tocotrienols, with α-tocopherol being the most biologically active form (commonly terms "α-tocopherol" and "vitamin E" are used interchangeably). Vitamin E is known to protect DNA from free radicals attack by scavenging reactive oxygen species (ROS: peroxyl radicals, singlet oxygen and superoxide radicals) and preventing the propagation of free-radical reactions, e.g. lipid peroxidation, thus reducing the formation of reactive DNA-damaging products (Bisby et al., 1996; Brigelius-Flohé, 2009). Besides, vitamin E acts as a scavenger of nitric oxide, nitrogen dioxide and other reactive nitrogen species (RNS), which are also known to react with DNA and other biological molecules (Sandhu et al., 2000). Vitamin E has been found to inhibit mutagenesis *in vivo* and *in vitro.* It has been shown to be effective in reducing genotoxic effects of numerous compounds in various assays: cisplatin in Swiss mice bone marrow cells and spermatogonia *in vivo* (Choudhury and Jagdale, 2002), carbon tetrachloride in ovine peripheral blood lymphocytes *in vitro* (Šiviková et al., 2001), malondialdehyde, β-propiolactone, sodium azide, N-methyl-N'-nitro-N-nitrosoguanidine in some Ames test studies (Ajith et al., 2008), benzo[a]pyrene and N-nitrosomorpholine in rat hepatocytes and mice bone marrow cells (Slamenova et al., 2002; Slapšytė et al., 2011), zearalenone in cultured DOK, Vero and Caco-2 cells (Abid-Essefi et al., 2003). However, vitamin E has been reported to have pro-oxidant properties, depending on the cellular environment (Rietjens et al., 2002). The beneficial and/or adverse effects of vitamin E are, in fact, not yet completely elucidated. It also concerns the human epidemiological studies. There is epidemiological evidence of an association between increased intake of vitamin E and reduced morbidity and mortality from coronary artery disease, vitamin E supplementation has been reported to enhance immune response in healthy geriatric humans (Meydani et al., 1997; Virtamo et al., 1998; Singh et al., 2005). However, the results obtained from numerous placebo-controlled clinical and prospective cohort studies evaluating the protective effect and utility of vitamin E intake in reducing cancer risk are

contradictory and inconclusive (Patterson et al., 1997; Young and Lee, 1999). Nevertheless, vitamin E is considered to be a promising chemopreventive agent against prostate cancer (Thompson et al., 2009).

Cyclophosphamide (nitrogen mustard derivative) is a bifunctional alkylating agent, which requires metabolic activation to exhibit its mutagenic and cytostatic activities. It can induce monoadduct formation, depurination and depyrimidation as well as DNA-DNA and DNA-protein cross-links. Cyclophosphamide induces oxidative stress and is cytotoxic to normal cells. Generation of ROS by cyclophosphamide results in an enhanced lipid peroxidation and decrease in the activity of antioxidant defence system, and is one of the mechanisms by which cyclophosphamide and its metabolites exert their toxic effects in different tissues (Sulkowska et al., 1998). Adriamycin (also named doxorubicin) is a cytotoxic benzanthroquinone drug. It does not require enzymatic activation, and the principal mechanisms contributing to its genotoxic and cytostatic activity are considered to result from intercalation into the DNA molecule, inhibition of topoisomerase II activity, DNA alkylation and cross-linking (Gewirtz, 1999, and references therein). Besides, the quinone structure of adriamycin permits it to act as electron acceptor with subsequent formation of semiquinone free radicals, which may induce free-radical injury to DNA or interact with molecular oxygen to form hydroxyl radicals, superoxides, and peroxides. Adriamycin is well-known for its oxidative stress-mediated side effects in heart, lung, and kidney.

The present study was undertaken to investigate the possible beneficial effect of vitamin E against adriamycin- and cyclophosphamide-induced genotoxicity using chromosome aberration and micronucleus assays in Wistar rat bone marrow cells. Besides, the level of lipid peroxidation product malondialdehyde, the activity of antioxidant enzymes catalase and superoxide dismutase were measured in blood serum and erythrocyte hemolysate to evaluate the oxidant/antioxidant status of rats.

Materials and methods. *Chemicals.* Vitamin E (d-α-tocopherol acetate), foetal calf serum, thiobarbituric acid (TBA), nitro blue tetrazolium, May-Grünwald and Giemsa stains were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Adriamycin and cyclophosphamide were used in commercial forms 'Adriblastina' (Pharmacia&Upjohn S.p.A., Italy) and 'Endoxan' (ASTA Medica AG, Gemany), respectively.

Animals and treatment schedule. Male albino Wistar rats aged 9–10 weeks and having body weight of 240–280 g, were used in the present study. The animals were supplied from the Animal Facility of the Department of Immunology, State Research Institute Centre for Innovative Medicine. Experiment conditions were in compliance with good laboratory practices and with the Law of the Republic of Lithuania on the Care, Keeping and Use of Animals as well as secondary legislation – Order of the State Food and Veterinary Service of the Republic of Lithuania "On Veterinary Regulations on Breeding, Handling and Transportation of Laboratory Animals" and "On the Use of Laboratory Animals in Scientific Experiments" (Law of the Care, Welfare and Use of Animals, 2002). The animals were acclimatized for one week before the study; they were housed under stable conditions of temperature, humidity and a light/dark cycle (12 h/12 h) with free access to standard commercial pellet diet and drinking water. They were housed 6 animals per cage and randomly divided into 10 groups.

The animals were treated with a single dose of adriamycin (AD, 5 mg/kg body weight, b.w.) or cyclophosphamide (CP, 30 mg/kg b.w.) by the intraperitoneal route. The appropriate amount of AD or CP was adjusted in 0.5 ml/200 g b.w. in sterile physiological saline just before injection. AD or CP was administered 24 h before the sacrifice of rats in the AD_{24} and CP_{24} groups, and 72 h before sacrifice in the AD_{72} and CP_{72} groups. Vitamin E (VE, 250 mg/kg b.w.) was administered *via gavage* once a day for 3 consecutive days. VE dose was adjusted in 1 ml/200 g b.w. in sunflower oil. Pre-treatment of rats with VE was conducted for two days before AD or CP injection and concomitantly with AD or CP on the 3rd day in the VE+AD_{24} and VE+CP_{24} groups. Groups AD_{72}+VE and CP_{72}+VE comprised VE post-treated animals: VE was administered concomitantly with AD or CP injection on the 1st day and for two consecutive days. Animals in the VE group received vitamin E once a day for 3 consecutive days. Controls comprised vehicle treated animals.

Colchicine (2 mg/kg b.w.) was injected i.p. 90 min. before sacrifice, which occurred 24 h after the last dose administration. Both femurs were dissected from each animal: one femur was used for the chromosome aberration and the other for micronucleus analysis.

Chromosome aberration assay. Bone marrow cells were flushed from the femur with 0.55% potassium chloride hypotonic solution, incubated for 25 min. at 37 °C and then centrifuged at 150 x *g* for 8 min. Cells were fixed in methanol-glacial acetic acid (3:1). Slides were prepared by a flame-drying procedure and then stained with 5% Giemsa stain. Slides were coded and scored blind by the single scorer at a magnification of 1000x (Nikon ECLIPSE E200, Japan). Only well-spread metaphases with 42±1 chromosomes were used for the analysis. The frequencies of chromosome aberrations were estimated in 100 metaphases per animal. Aberrations were recorded as individual types according to Savage's (1976) classification but for convenience were grouped as chromatid breaks (ctb), chromatid exchanges (cte), chromosome breaks (csb) and chromosome exchanges (cse). Gaps were counted but not included into statistical analysis.

Micronucleus assay. The bone marrow micronucleus assay was conducted following the standard procedure (Schmid, 1975). Bone marrow cells were flushed from the femur with 2 ml of heat-inactivated foetal calf serum, mixed thoroughly to obtain a fine suspension and centrifuged for 5 min. at 150 x *g*. The supernatant was discarded and the cell pellet was carefully re-suspended. At least two smears per animal were prepared and

allowed to air dry prior to fixation with methanol. The slides were stained in May-Grünwald solution followed by Giemsa, coded and scored blind by the same scorer under magnification of 1000x (Jenaval, Zeiss, Germany). The 2000 polychromatic erythrocytes (PCEs, immature erythrocytes) were scored per animal for the frequency of micronucleated cells (MNPCEs) in each of the 6 animals per dosage group. Bone marrow toxicity was monitored by a decrease of PCE to total erythrocytes (referred as PCE/(PCE+NCE) ratio, where NCE - normochromatic erythrocytes). To assess bone marrow toxicity, 1000 erythrocytes per animal were analysed.

Biochemical analysis. The basic procedures for biochemical analyses were described in our previous works (Surinenaite et al., 2006). Briefly, the level of malondialdehyde (MDA) was measured in blood serum by the thiobarbituric acid (TBA) assay based on TBA reaction with MDA resulting in the release of spectrophotometrically detectable (at 532 nm) colour complexes. Superoxide dismutase (SOD) catalytic activity was evaluated spectrophotometrically (at 540 nm) measuring the rate of inhibition of the reduction of nitro blue tetrazolium in erythrocyte hemolysate. Catalase (CAT) activity evaluation was based on the ability of hydrogen peroxide to form coloured complexes with ammonium molybdate.

Statistical analysis. All data were processed with SPSS 12.0 statistical software (SPSS Inc., Chicago, IL, USA). All values were expressed as mean ±S.E.M. of six animals per group. The data were analyzed using one-way analysis of variance (ANOVA) followed by Post Hoc LSD test for comparison between the various groups. Bivariate Pearson's correlation coefficients were used to investigate the relationships between the parameters indicating genotoxicity and those concerning the antioxidant status. Differences were considered significant if $P< 0.05$.

Results. The results of the chromosome aberration assay are presented in Table 1. A slight though insignificant increase of chromosome aberrations was determined in animals treated with VE when compared with the controls (2.17 *vs.* 1.57, P>0.05). As expected, animals treated with AD and CP showed a high frequency of chromosome aberrations as well as abnormal metaphases. However, the level of chromosome aberrations in animals from the CP_{72} group (i.e. sacrificed 72 h after CP injection) was markedly lower when compared with the CP_{24} group (i.e. sacrificed 24 h after CP injection), and did not significantly differ from the level determined in the controls (P>0.05). In all treatments, the most frequent chromosome aberrations observed were chromatid breaks. Besides, CP was a potent inducer of chromatid exchanges also. These results agree with those reported in other investigations, which showed that chromatid breaks are the changes most frequently induced by adriamycin and cyclophosphamide *in vivo* (Antunes and Takahashi, 1998). VE had no protective effect against CP-induced chromosome damage. However, chromosome aberration analysis revealed protective effect of VE against AD-induced clastogenicity. Significant decrease of chromosome aberration frequency was determined in animals pre-treated with VE before AD injection (9.57 *vs.* 5.5, P<0.02).

Table 1. **Frequencies of chromosome aberrations in Wistar rat bone marrow cells after treatment with adriamycin** (AD), **cyclophosphamide** (CP) **and vitamin E** (VE)

Treatment group	Aberrant metaphases, % ± S.E.M.	Chromosome aberrations[a] /100 cells, mean ± S.E.M.				
		ctb	cte	csb	cse	Total
Control	1.57±0.29	1.14±0.34	0	0.43±0.20	0	1.57±0.29
VE	2.17±0.40	1.50±0.59	0	0.66±0.33	0	2.17±0.40
CP_{24}	28.14±1.87[b]	36.00±5.16	7.29±1.21	3.86±1.44	0	47.14±6.54[b]
VE+CP_{24}	29.4±1.47[b]	24.20±4.24	17.80±2.06	4.00±0.82	0	46.00±5.46[b]
CP_{72}	2.43±0.29	1.57±0.20	0	0.86±0.26	0	2.43±0.29
CP_{72}+VE	3.00±0.36	2.17±0.31	0	0.83±0.31	0	3.00±0.36
AD_{24}	9.29±0.97[b]	8.00±0.95	0.57±0.29	0.86±0.34	0.14±0.14	9.57±1.07[b]
VE+AD_{24}	5.17±0.87[b,c]	3.50±0.56	0.67±0.33	1.33±0.33	0	5.50±0.93[b,c]
AD_{72}	12.00±1.19[b]	9.57±1.17	1.00±0.44	2.00±0.34	0	12.57±1.09[b]
AD_{72}+VE	10.5±0.67[b]	7.17±1.66	0.50±0.22	3.33±0.80	0.5±0.49	11.5±1.06[b]

Notes: [a]ctb, chromatid breaks; cte, chromatid exchanges; csb, chromosome breaks; cse, chromosome exchanges. [b]P<0.0001 when compared with the controls. [c]P<0.02 when compared with the AD_{24} group.

The results obtained in the micronucleus test are summarized in Table 2. A slight though insignificant decrease of MNPCEs was determined in animals treated with VE when compared with the controls (0.18 *vs.* 0.22, P>0.05). In addition, there were no signs of VE cytotoxicity (i.e. no significant decline of the proportion of PCEs to total erythrocytes). Treatment of animals with the tested drugs resulted in significant increase of MNPCEs in the CP_{24} (P<0.0001), CP_{72} (P<0.002) and AD_{24} (P<0.0001) groups. Both CP and AD have caused

toxicity to the bone marrow as indicated by significant decrease of PCE frequency in the above mentioned groups (P<0.0001). No PCEs were observed in the AD_{72} group indicating inhibition of erythropoiesis. Micronucleus assay revealed protective effect of VE against both CP and AD induced chromosome damage. This protective effect was significant against CP-induced damage in animals post-treated with VE (0.66 *vs* 0.31, P<0.01) and against AD-induced damage in animals pre-treated with VE (1.51 *vs* 0.72, P<0.01). No PCEs were observed in the AD_{72} group and thus it is impossible to make any definite conclusion about the effect of VE post-treatment on the changes of MNPCEs. Besides, VE revealed protective effect against drug induced bone marrow toxicity. The proportion of PCEs to total erythrocytes increased in all VE-treated groups and this increase was significant when compared to the adequate drug-treated animals (P<0.0001).

Table 2. **Bone marrow toxicity and frequencies of micronucleated polychromatic erythrocytes** (MNPCEs) **in Wistar rats after treatment with adriamycin** (AD), **cyclophosphamide** (CP) **and vitamin E** (VE)

Treatment group	MNPCE, % ± S.E.M.	PCE / (PCE+NCE), ± S.E.M.
Control	0.22±0.03	0.82±0.03
VE	0.18±0.02	0.79±0.06
CP_{24}	2.66±0.28[a]	0.32±0.03[c]
VE+CP_{24}	2.03±0.33	0.66±0.03[d]
CP_{72}	0.66±0.08[b]	0.37±0.04[c]
CP_{72}+VE	0.31±0.03[e]	0.81±0.04[d]
AD_{24}	1.51±0.16[a]	0.35±0.03[c]
VE+AD_{24}	0.72±0.22[e]	0.69±0.04[d]
AD_{72}	n.d.	n.d.
AD_{72}+VE	0.38±0.08	0.77±0.05

Notes: [a]P<0.0001, [b]P<0.002, [c]P<0.03 when compared with the controls; [d]P<0.0001, [e]P<0.01 when compared with the adequate drug-treated group; n.d., not determined (cytotoxic effect).

Table 3 presents the activities of superoxide dismutase (SOD) and catalase (CAT) and the levels of malondialdehyde (MD) in rat blood serum. Gavage of animals with VE resulted in significant decrease of SOD activity when compared with the controls (1.4 *vs.* 1.96, P<0.02). Treatment with CP or AD significantly decreased SOD activity in animals from the CP_{72} and AD_{72} (P<0.02) groups. Post-treatment of CP- or AD-injected animals with VE revealed a slight though insignificant increase of SOD activity in CP_{72}+VE and AD_{72}+VE groups when compared with the adequate drug-treated groups. However, our results showed that pre-treatment of animals with VE (groups VE+CP_{24} and VE+AD_{24}) significantly decreased SOD activity when compared with the adequate drug-treated groups.

Gavage with VE alone did not cause any obvious changes of CAT activity. Treatment with the tested drugs significantly decreased CAT activity in the CP_{24} and AD_{72} groups (P<0.02). Co-treatment with VE did not reveal any significant changes of CAT activity in VE co-treated animals when compared with the adequate drug-treated groups.

Table 3. **Superoxide dismutase** (SOD) **and catalase** (CAT) **activity and malondialdehyde** (MDA) **level in Wistar rat erythrocyte hemolysate and blood serum after treatment with adriamycin** (AD), **cyclophosphamide** (CP) **and vitamin E** (VE)

Treatment Group	SOD, $Ux10^4$/ml	CAT, nmol/l/min	MDA, nmol/l
Control	1.96±0.12	63.26±2.02	21.50±1.99
VE	1.40±0.16[a]	62.37±2.31	28.46±1.72[a]
CP_{24}	1.83±0.23	52.11±1.47[a]	21.35±4.12
VE + CP_{24}	0.50±0.08[b]	54.00±1.82	36.70±0.45[c]
CP_{72}	1.19±0.13[a]	64.87±2.35	13.96±1.54[a]
CP_{72} + VE	1.43±0.1	63.78±1.69	29.13±2.14[c]
AD_{24}	2.13±0,24	56.03±2.83	18.35±3.33
VE+ AD_{24}	0.60±0.08[b]	47.8±5.39[a]	35.04±2.67[c]
AD_{72}	1.03±0.17[a]	51.43±2.62[a]	24.08±3.38
AD_{72} + VE	1.44±0.16	59.4±2.75	22.05±3.17

Notes: [a]P<0.02 when compared with the controls; [b]P<0.0003, [c]P<0.02 when compared with the adequate drug-treated group.

MDA level in the drug-treated groups fluctuated in the range between 13.96 nmol/l (CP_{72} group) and 24.08 nmol/l (AD_{72}) and did not exceed or was even lower when compared with the control level. However, gavage with VE resulted in significant increase of MDA level in all VE treated groups when compared with the controls or the adequate drug-treated groups (with the only exception of AD_{72}+VE group).

Discussion. In the present study, we evaluated two cytogenetic endpoints, such as chromosome aberrations and micronuclei, in the same animal. Rats are generally used for chromosome aberration assessment and rarely in the micronucleus assay, mainly because granules from ruptured leukocytes are thought to resemble micronuclei and contaminate bone marrow smears. There are only few reports on the concurrent analysis of these two endpoints in the same animal (Krishna and Theiss, 1995; Slapsyte et al., 2007). This approach has several advantages, first of all such as reducing overall animal usage and correlating genotoxicity results from different endpoints. In the present study, we established a significant correlation between the frequency of aberrant metaphases and MNPCEs on an individual animal basis (taking all rats used in the study together, independent of the treatment group). Animals that had higher aberrant cells also had increased MNPCE frequencies (r=0.756, P<0.0001). Our data are in good agreement with results of Krishna and Theiss (1995), who also indicated a clear relationship between these two endpoints in the same animal (correlation coefficient of 0.9). In general, these findings

confirm the utility of simultaneous assessment of *in vivo* chromosome aberration and micronucleus endpoints.

Vitamin E is one of the most extensively studied antioxidants. The results obtained in previous studies demonstrate that vitamin E exerts protective effect against genotoxicity induced by various chemical agents in different test subjects. The present work was performed in order to evaluate the protective effect of VE against AD- and CP-induced genotoxicity. AD and CP are known to generate ROS which result in an enhanced lipid peroxidation and a decrease in the activity of antioxidant defence system, and this is considered to be one of the mechanisms by which both drugs and their metabolites exert their toxic effects in different tissues. In the present study, enzymatic activities of SOD and CAT and the level of MDA were determined in order to trace changes of antioxidant status in rats. Significant correlation was determined between the activities of CAT and SOD and the level of MDA on an individual animal basis: the Pearson correlation coefficient for MDA versus SOD was r=–0.328 (P=0.011), and MDA versus CAT was r=–0.444 (P=0.003). Our results show that gavage of animals with VE alone and VE pre-treatment of CP or AD injected animals significantly decreased SOD activity. Co-treatment with VE did not reveal any significant changes of CAT activity. However, significantly higher MDA levels were determined in all VE co-treated groups (except AD_{72}+VE) when compared with the controls and adequate drug-treated groups. This can indicate slight exacerbation of the antioxidant status of VE treated animals. Our results are somewhat different from those obtained by Thabrew et al. (1999) who found that oral VE supplementation (10, 45 or 200 mg/kg b.w.) proportionally decreased the production of MDA and increased the activity of SOD by red blood cells of adriamycin-treated mice. VE showed a positive effect on the induction of SOD and CAT in activity yeast depending on its concentration (Bronzetti et al., 2001). However, it is necessary to notice that vitamin E has been reported to have pro-oxidant properties, depending on the cellular environment. Under conditions of increasing oxidative stress, increased level of a-tocopherol may result in increased levels of a-tocopherol radicals which can initiate the processes of lipid peroxidation by themselves. This prooxidant action of VE is inhibited by co-antioxidants, which can reduce the radical back to VE when antioxidant networks are balanced (Rietjens et al., 2002).

Our results demonstrated that protective effect of VE against AD- and CP-induced genotoxicity was dependent on the treatment schedule (i.e. sequencing of VE treatment) and the drug used. We determined protective effect of VE against AD-induced chromosome damage in animals pre-treated with VE (both chromosome aberration and micronucleus assays) and against CP-induced damage in animals post-treated with VE (micronucleus assay only). VE revealed protective effect against drug induced bone marrow toxicity in all experimental groups. Our results are in agreement with those obtained by Antunes and Takakashi (1998), who demonstrated that VE reduced the total number of AD-induced chromosomal aberrations at the three-studied concentrations (100, 200 and 400 mg/kg b.w.), with the best result for the lowest one. However, some studies performed using alkaline single cell gel electrophoresis (comet assay) have not found beneficial effect after the use of VE. The results obtained by Blasiak et al. (2002) revealed that VE potentiate damage to DNA induced by idarubicin (analogue of adriamycin) in both normal human peripheral lymphocytes and leukemic cells. VE was shown to enhance the herbicide paraquat genotoxicity in cultured anuran leukocytes (Hanada, 2011). Similar results were obtained by Kontek et al. (2010) in their work aimed to study the modulatory effect of VE and other vitamins on the irinotecan-induced genotoxicity in human lymphocytes from healthy and cancer patients.

In our study, we have not revealed correlation between MDA level or SOD activity and chromosome damage on an individual animal basis. The Pearson correlation coefficient for aberrant metaphases versus MDA was r=0.1301 (P=0.303) and versus SOD activity 0.0594 (P=0.655). Correlation coefficient for MNPCEs versus MDA was r=0.003 (P=0.980) and versus SOD r=0.111 (P=0.432). However, significant correlation was determined between the CAT activity and the frequency of aberrant metaphases (r=–0.388, P=0.002) as well as MNPCEs (r=–0.438, P=0.001).

In literary sources it is emphasised that vitamins have different behaviour in relation to the concentration used and the genetic system employed, and this determines the fact that mechanisms of action of vitamins are not well known yet. Bearing in mind that in our study SOD and CAT activities in both cytostatic-treated groups remained unchanged or were even reduced and MDA level was increased when compared to the representative groups without VE treatment, it is difficult to explain protective (antigenotoxic) effect of VE on the ground of its antioxidative abilities only. The present data probably could support the idea that VE inhibition of the genotoxic response might involve diverse mechanisms, including inhibition of drug metabolism (Costa and Nepomuceno, 2006), mutagen detoxification by binding to active electrophilic metabolites, and activation of DNA repair mechanism (Konopacka et al., 1998).

Conclusion. Our results demonstrated that protective effect of vitamin E against adriamycin- and cyclophosphamide-induced genotoxicity was dependent on the treatment schedule (i.e. sequencing of VE treatment) thus indicating the importance to tailor antigenotoxicity treatment schedule to the individual class of drugs. Vitamin E was protective against adriamycin-induced chromosome damage in animals pre-treated with vitamin E (both chromosome aberration and micronucleus assays) and against cyclophosphamide-induced damage in animals post-treated with vitamin E (micronucleus assay only). Vitamin E decreased drug induced bone marrow toxicity in all experimental groups. No beneficial effect of vitamin E on the antioxidant status of rats was determined.

References

1. Abid-Essefi S., Baudrimont I., Ouanes Z., Mobio T.A., Anane R., Creppy E.E., Bacha H. DNA fragmentation, apoptosis and cell cycle arrest induced by zearalenone in cultured DOK, Vero and Caco-2 cells: prevention by vitamin E. Toxicology. 2003. 192(2–3). P. 237–248.

2. Ajith T.A., Ann M., Thomas J. Evaluation of comparative and combined antimutagenic potential of vitamin C and vitamin E using histidine mutant Salmonella typhimurium strains. Indian J. Clinical Biochemistry. 2008. 23(1). P. 24–28.

3. Antunes L.M.G., Takahashi C.S. Effects of high doses of vitamins C and E against doxorubicin-induced chromosomal damage in Wistar rat bone marrow cells. Mutat. Res. 1998. 419(1–3). P. 137–143.

4. Bisby R.H., Johnson S.A., Parker A.W. Quenching of reactive oxidative species by probucol and comparison with other antioxidants. Free Radic. Biol. Med. 1996. 20. P. 411–420.

5. Blasiak J., Gloc E., Wozniak K., Mlynarski W., Stolarska M., Skorski T., Majsterek I. Genotoxicity of idarubicin and its modulation by vitamins C and E and amifostine. Chem. Biol. Interact. 2002. 149(1). P. 1–18.

6. Brigelius-Flohé R. Vitamin E: the shrew waiting to be tamed. Free Radic. Biol. Med. 2009. 46(5). P. 543–554.

7. Brigelius-Flohe R., Traber M.G. Vitamin E: function and metabolism. FASEB J. 1999. 13(10). P. 1145–1155.

8. Bronzetti G., Cini M., Andreoli E., Caltavuturo L., Panunzio M., Croce C.D. Protective effects of vitamins and selenium compounds in yeast. Mutat. Res. 2001. 496(1–2). 105–115.

9. Choudhury R.C., Jagdale M.B. Vitamin E protection from/potentiation of the cytogenetic toxicity of cisplatin in Swiss mice. J. Chemother. 2002. 14(4). P. 397–405.

10. Costa W.F., Nepomuceno J.C. Protective effects of a mixture of antioxidant vitamins and minerals on the genotoxicity of doxorubicin in somatic cells of *Drosophila melanogaster*. Environm. Mol. Mutagen. 2006. 47(1). P. 18–24.

11. Elsendoorn T.J., Weijl N.I., Mithoe S., Zwinderman A.H., Van Dam F., De Zwart F.A., Tates A.D., Osanto S. Chemotherapy-induced chromosomal damage in peripheral blood lymphocytes of cancer patients supplemented with antioxidants or placebo. Mutat. Res. 2001. 498. P. 148–158.

12. Gewirtz D.A. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochem. Pharmacol. 1999. 57(7). P. 727–741.

13. Hanada H. D1-α-tocopherol enhances the herbicide 1,1'-dimethyl-4,4'-bipyridium dichloride (paraquat, PQ) genotoxicity in cultured anuran leukocytes. Hereditas. 2011. 148(4–5). P. 118–124.

14. Konopacka M., Widel M., Rzeszowska-Wolny J. Modifying effect of vitamins C, E and β-carotene against γ-ray-induced DNA damage in mouse cells. Mutat. Res. 1998. 417(2–3). P. 85–94.

15. Kontek R., Drozda R., Sliwinski M., Grzegorczyk K. Genotoxicity of irinotecan and its modulation by vitamins A, C and E in human lymphocytes from healthy individuals and cancer patients. Toxicol. Vitro. 2010. 24(2). P. 417–424.

16. Krishna G., Theiss J.C. Concurrent analysis of cytogenetic damage in vivo: a multiple endpoin-multiple tissue approach. Environ. Mol. Mutagen. 1995. 25(4). P. 314–320.

17. Meydani S.N., Meydani M., Blumberg J.B., Leka L.S., Siber G., Loszewski R., Pedros M.C., Diamond R.D., Stollar B.D. Vitamin E supplemetation and in vivo immune response in healthy elderly subjects. JAMA. 1997. 277. P. 1380–1386.

18. Patterson R.E., White E., Kristal A.R., Neuhouser M., Potter J.D. Vitamin supplements and cancer risk: The epidemiologic evidence. Cancer Cause Control. 1997. Vol. 8(5). P. 786–802.

19. Quiles J.L., Huertas J.R., Battino M., Mataix J., Ramirez-Tortosa M.C. Antioxidant nutrients and adriamycin toxicity. Toxicology. 2002. 180(1). P. 79–95.

20. Rietjens I.M.C.M., Boersma M.G., de Haan L., Spenkelink B., Awad H.M., Cnubben N.H.P., van Zanden J.J., van der Woude H., Alink G.M., Koeman J.H. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ.Toxicol. Pharmacol. 2002. 11(3–4). P. 321–333.

21. Sandhu J.K., Haqqani A.S., Birnboim H.Ch. Effect of dietary vitamin E on spontaneous or nitric oxide donor-induced mutations in a mouse tumor model. J. Nat. Cancer Inst. 2000. 92. P. 1429–1433.

22. Savage J.R. Classification and relationships of induced chromosomal structural changes. J. Med. Genet. 1976. 13. P. 103–122.

23. Schmid W. The micronucleus test. Mutat. Res. 1975. 31. P. 9–15.

24. Singal P.K., Li T., Kumar D., Danelisen I., Iliskovic N. Adriamycin-induced heart failure: mechanism and modulation. Mol. Cell Biochem. 2000. 207(1–2). P. 77–85.

25. Singh U., Devaraj S., Jiala I. Vitamin E, oxidative stress, and inflammation. Annu. Rev. Nutr. 2005. 25.

P. 151–174.

26. Šiviková K., Piešova E., Dianovsky J. The protection of Vitamin E and selenium against carbon tetrachloride-induced genotoxicity in ovine peripheral blood lymphocytes. Mutat. Res. 2001. 494(1–2). P. 135–142.

27. Slamenova D., Chalupa I., Robichova S., Gabelova A., Farkasova T., Hrusovska L., Bacova G. Effect of dietary intake of vitamin A or E on the level of DNA damage, chromosomal aberrations, and micronuclei induced in freshly isolated rat hepatocytes by different carcinogens. Nutr. Cancer. 2002. 42(1). P. 117–124.

28. Slapsyte G., J. Mierauskiene, V. Morkunas, G. Prasmickiene, J. Didziapetriene. Modifying effects of sodium selenite on adriamycin and cyclophosphamide induced chromosome damage and changes of antioxidant status in rats. Trace Elem. Electroly. 2007. 24. P. 235–243.

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

30. Sulkowska M., Sulkowski S., Skrzydlewska E., Farbiszewski R. Cyclophosphamide-induced generation of reactive oxygen species. Comparison with morphological changes in type II alveolar epithelial cells and lung capillaries. Exp. Toxicol. Pathol. 1998. 50(3). P. 209–220.

31. Surinenaite B., Kazbariene B., Prasmickiene G., Krikstaponiene A., Didziapetriene J., Jankevicius F., Characiejus D. Surgical stress induced alterations of antioxidative and immune system parameters. Biologija. 2006. 2. P. 76–79.

32. Thabrew M.I., Samarawickrema N., Chandrasena L.G., Jayasekera S. Effect of oral supplementation with vitamin E on the oxido-reductive status of red blood cells in normal mice and mice subject to oxidative stress bychronic administration of adriamycin. Ann. Clin. Biochem. 1999. 36. P. 216–220.

33. Thompson I.M., Tangen C.M., Goodman P.J., Lucia M.S., Klein E.A. Chemoprevention of prostate cancer. J. Urol. 2009. 182(2). P. 499–507.

34. Virtamo J., Rapola J.M., Ripatti S., Heinonen O.P., Taylor P.R., Albanes D., Huttunen J.K. et al. Effect of vitamin E and beta carotene on the incidence of primary nonfatal myocardial infarction and fatal coronary heart disease. Arch. Intern. Med. 1998. 158(6). P. 668–675.

35. Young K.J., Lee P.N. Intervention studies on cancer. Eur. J. Cancer Prev. 1999. 8(2). P. 91–103.

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