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Original research paper

Role of germanium L-cysteine α-tocopherol complex as stimulator of some antioxidant defense systems in gamma-irradiated rats

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This study was conducted to evaluate the potency of the newly prepared germanium L-cysteine α-tocopherol complex [germanium dichloro tetrakis (L-cysteinyl-α-tocopherol amide) dichloride] as a protective agent against γ-irradiation-induced free radicals production and liver toxicity. Male Swiss albino rats were injected intraperitoneally with the germanium complex in a concentration of 75 mg kg^{-1} body mass per dose, for 6 successive doses, last dose administered twenty minutes pre-exposure to a single dose of whole body γ-irradiation of 6.5 Gy. Lipid peroxidation (LPx), nitric oxide (NO), glutathione (GSH) levels, and activity of the antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) were estimated in blood and liver. Blood total protein, cholesterol, triglyceride and α-tocopherol content were estimated as well. The results revealed that administration of germanium complex pre-irradiation resulted in significant ($p < 0.001$) improvement compared to the irradiated group in the level of hepatic and blood LPx. Hepatic GSH revealed a significant increase ($p < 0.001$), while its level showed no significant variation in blood. Also, the level of NO in blood and liver increased significantly ($p < 0.001$). On the other hand, pretreatment with the germanium complex normalized the activities of SOD, GPx and CAT in blood and liver when compared to the irradiated group. The study also documents a marked decrease in a blood triglyceride and cholesterol ($p < 0.001$) and a significant increase ($p < 0.001$) of α-tocopherol and total protein contents in blood. These biochemical changes were associated with marked improvement of histological status. Therefore, the germanium L-cysteine α-tocopherol complex may be a good candidate for ameliorating the changes induced by irradiation, which indicates the beneficial radio-protective role of this antioxidant agent.

Keywords: germanium L-cysteine α-tocopherol, γ-irradiation, liver, antioxidant enzymes

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There is growing evidence that radiation injury to living cells is, to a large extent, due to oxidative stress (1). The interaction of ionizing radiation with living cells induces a variety of reaction products and a complex chain reaction in which many macromolecules and their degradation products participate. Major biomarkers of oxidative damage to living cells are lipid peroxidation (LPx) products, DNA-hydroxylation and protein hydroxylation products (2). Efficient defense and repair mechanisms exist in living cells to protect against oxidant species (3). The antioxidative defense system is composed of methods to: transfer sensitive material to compartments better protected from the action of reactive species, complex transition metals, a potential source of electrons, thereby rendering them unreactive, inhibit vulnerable processes such as DNA replication, repair damaged molecules, initiate apoptosis, and, possibly most important considering the liability to internal and external modifying factors, activate antioxidant enzymes, and finally use a variety of direct free radical scavengers (4). Enzymes involved in antioxidative defense particularly well documented are superoxide dismutase, glutathione peroxidases and catalase (2). There is need for non-toxic and inexpensive drugs for clinical radiation protection. Antioxidant agents may help protect against chemically induced or radiation induced toxicity in rats (5). Previous studies have indicated that some commonly used antioxidants of plant origin include vitamin E, vitamin C, selenium, phenolic compounds, carotenoids and flavonoids (6).

Numerous biochemical compounds such as cysteine (7), germanium (8) and α-tocopherol (9) have been used individually to target oxygen and oxygen free-radicals in attempts to reduce radiation-induced damage. Various studies have suggested that germanium compounds may have a protective effect against liver injury and similar oxygen-enriching properties and rigorously documented antioxidant effects (8, 10). Ge-132 administered to patients undergoing radiotherapy for cancer offers protection against radiation-induced killing of white and red blood cells (10). Precise mechanisms describing how organic germanium can protect cells from radiation damage have not been elucidated. However, according to Asai (11), atoms of Ge-132 securely fasten to red blood cells and shelter these cells from oncoming electrons by diverting them around the atom. It is postulated that organic germanium could have affinity for blood cells; possibly owing to electronic attraction due to germanium's extended network shape and its ability to conduct electrons efficiently (10). α-Tocopherol is the most important lipid-soluble chain-breaking antioxidant in tissues and blood. This vitamin might protect cellular components against peroxidation damage *via* the free radical scavenging mechanism (9). In addition, cysteine is a potent antioxidant that has been shown to protect from oxidative stress. In particular, cysteine is known to increase the intracellular stores of glutathione, thereby enhancing endogenous antioxidant levels (12).

Few studies have observed the important role of some metals and/or their organocomplexes against detrimental effects of ionizing radiation. The present study was undertaken to investigate the possible radioprotective role of the prepared germanium L-cysteine α-tocopherol complex of the formula $Ge(C_{32}H_{50}NO_3S)_4Cl_4$ against whole body γ-irradiation induced alteration in the antioxidant system *in vivo*.

EXPERIMENTAL

Chemicals

All chemicals and reagents used were of the highest purity available and purchased from either Merck (Germany) or Sigma-Aldrich Chemie (Germany). Kits for total protein, cholesterol and triglyceride were supplied by Biocon Company (Germany).

Preparation of the germanium L-cysteine α-tocopherol complex

L-cysteine (0.1 mol) and 0.1 mol of α-tocopherol in 25 mL of toluene were reacted in the presence of 1 mL *p*-toluene sulfonic acid as catalyst and refluxed until a stoichiometric amount of water was recovered in a Dean-stark trap. The product was then concentrated by rotator evaporation and three times recrystallized from acetone. Germanium tetrachloride (0.1 mol) was added slowly to L-cysteinyl-α-tocopherol (0.4 mol) dissolved in 200 mL acetone. The reaction mixture was heated to about 90 °C and poured into an evaporating dish to let the acetone evaporate until white crystals of the quaternary ammonium compound were formed. As shown in Table I and Fig. 1, the structure of the obtained compound germanium dichloro tetrakis (L-cysteinyl-α-tocopherol amide) dichloride was confirmed by elemental analysis on a Vario EL Elementar (Elementar Americas, USA), Fourier transform infrared FT-IR using KBr disks (ATI Mattson Genesis, USA), and ^{1}H NMR spectra Varian Gemini-spectrophotometer (Camberage Isotope Laboratory, UK) in DMSO. The melting point of the synthesized compound was determined in open capillary tubes (Toshniwal Electric, Japan).

Table I. Physicochemical and spectral analysis of the germanium L-cysteine α-tocopherol complex

Elemental analysis							
	C	H	N	S	Ge		
Calculated (%)	67.09	3.39	2.79	6.37	3.64		
Found (%)	67.64	3.97	3.05	6.64	3.31		
IR							
Function group	NH	SH	CH_2	CH_3	C=O	CH	Ge-N
ν (cm^{-1})	3328	2358	1489	1413	1724	1332	635
^{1}H NMR							
Function group	CH_3	CH_3-Ph	CH_2	CH	SH	NH	
δ (ppm)	2.35	1.53	1.30	3.77	1.50	2.00	
Molecular formula (M_r)	$Ge(C_{32}H_{50}NO_3S)_4Cl_4$ (2329.69)						
Melting point (°C)	> 300						

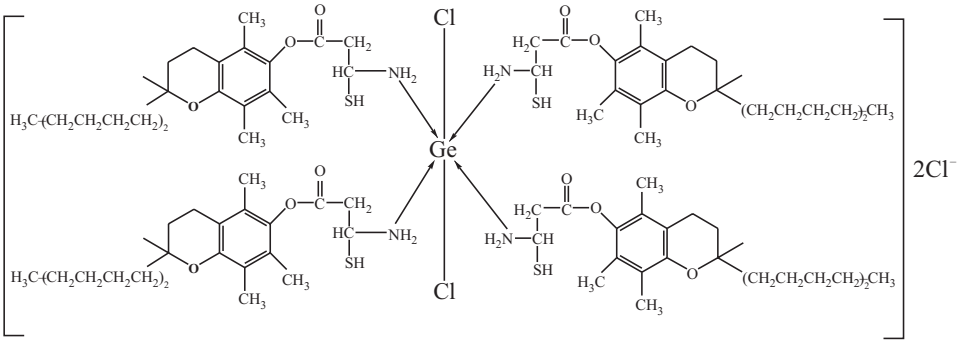

Fig. 1. Chemical structure of germanium, dichloro tetrakis (L-cysteinyl-α-tocopherol amide) dichloride.

Animals and in vivo *experiments*

Animal care and handling complied with the guidelines set by the World Health Organization, Geneva, Switzerland. Male Swiss albino rats weighing 100–120 g (6–8 weeks) obtained from the Egyptian Organization for Biological Products and Vaccines, Giza (Egypt), were used in this study. Rats were housed at a constant temperature (24 ± 2 °C) with alternating 12 hours light and dark cycles and fed standard laboratory food and water *ad libitum*.

Doses determination

In order to determine a non toxic dose of germanium L-cysteine α-tocopherol, the doses that caused acute toxicity were identified. Hence, adult male Swiss albino rats were subjected to a series of different concentrations ranging from 15 to 150 mg kg^{-1} body mass dissolved in sterile saline. These concentrations were then injected *i.p.* into 10 rats; each concentration and each dose were repeated six times. Acute toxicity doses were calculated using probability analysis according to Ghosh (13). Thus a non toxic dose of 75 mg kg^{-1} body mass was chosen for the subsequent study in an *in vivo* radioprotection experiment. Germanium L-cysteine α-tocopherol was dissolved in 0.9% saline and administered to rats *i.p.* in a concentration of 75 mg kg^{-1} body mass per dose for 6 successive doses based on the preliminary study. Last dose was administered twenty minutes before radiation exposure.

At the beginning of the experiment, rats were divided into four groups of 8 rats each. The first was the control group treated *i.p.* with 0.9% saline, the second group was exposed to γ-irradiation, the third group was treated with germanium L-cysteine α-tocopherol compound alone, the fourth group was treated with germanium L-cysteine α-tocopherol pre-exposed to γ-irradiation.

Irradiation

Whole body γ-irradiation was performed at the National Centre for Radiation Research and Technology, Atomic Energy Authority (NCRRT), Cairo, Egypt, using caesium-

-137 in a Gamma cell-40 Irradiator (Atomic Energy of Canada Limited, Canada). Animals were irradiated at an acute single dose level of 6.5 Gy delivered at a dose rate of 0.65 Gy min^{-1}.

Samples collection

Samples were collected after 7 days post-irradiation from 16 hours fasting animals. Whole blood was collected by heart puncture after light anesthesia using heparinized syringes. Part of whole blood was used for determination of lipid peroxidation (LPx), nitric oxide (NO), glutathione (GSH) levels, and activity of the antioxidant scavenger enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT); blood total protein, cholesterol, triglyceride and α-tocopherol contents were also estimated. Liver was dissected out and divided into two parts. One part was used for assays of GSH, CAT, GPx, SOD, LPx, and NO. Liver homogenates were prepared in ice-cold phosphate buffer (0.1 mol L^{-1}, pH 7.4) using a Potter-Elvehjem homogenizer (Labimex, Poland) to give a 10% homogenate. The other part of the liver was used for a histopathological study by fixation in 10% formalin in saline, then embedded in paraffin wax, serially sectioned and stained according to Conn *et al.* (14) using a standard hematoxylin and eosin method.

Analytical procedures

LPx in blood and tissues was ascertained by the formation of thiobarbituric acid reactive substances (TBARS) and measured as described by Conrad *et al.* (15). GSH content was determined according to Beutler *et al.* (16). SOD, CAT, and GPx activities were quantitated according to Minami and Yoshikawa (17), Johansson and Borg (18), Paglia and Valentine (19), respectively. NO was determined according to the methods of Moshage *et al.* (20). Total protein, cholesterol and triglycerides contents were assessed spectrophotometrically using reagent kits obtained from Biocon Company (Germany). Determination of α-tocopherol was carried out according to the method of Baker and Frank (21).

Statistical analysis

Student's *t*-test was used for data evaluation. The data were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

In the present study, whole body γ-irradiation strongly initiates the process of LPx as indicated by the formation of TBARS in both blood and liver, reduction in the level of GSH and increased formation of NO, as shown in Table II. The results for LPx show that, γ-irradiated rats exhibited a significant ($p < 0.001$) increase in TBARS level in liver (from 129.65 ± 9.62 to 176.63 ± 7.08 μg g^{-1}) and in blood (from 32.25 ± 1.86 to 49.81 ± 4.04 μg mL^{-1}) as compared to the control. Administration of germanium L-cysteine α-tocopherol pre-irradiation significantly decreased ($p < 0.001$) the level of TBARS in liver to 140.62 ± 9.56 μg g^{-1}

Table II. The effect of germanium L-cysteine α-tocopherol on blood reduced glutathione (GSH), nitric oxide (NO), and lipid peroxidation (TBARS) levels, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity[a]

Group	GSH		NO		TBARS		CAT		SOD		GPx	
	Liver (mg g^{-1})	Blood (mg mL^{-1})	Liver (μmol g^{-1})	Blood (μmol mL^{-1})	Liver (μmol g^{-1})	Blood (μmol mL^{-1})	Liver (μmol min^{-1} g^{-1})	Blood (μmol min^{-1} mL^{-1})	Liver (nmol min^{-1} g^{-1})	Blood (nmol min^{-1} mL^{-1})	Liver (μmol min^{-1} g^{-1})	Blood (μmol min^{-1} mL^{-1})
Control	1.09 ± 0.12	51.38 ± 3.38	1.32 ± 0.10	9.67 ± 0.67	129.65 ± 9.62	32.25 ± 1.86	16.27 ± 1.42	7.45 ± 0.82	43.30 ± 0.57	1.07 ± 0.07	25.21 ± 3.10	11.02± 1.47
Radiation	0.78 ± 0.09[b]	41.7 ± 4.56[b]	1.88 ± 0.06[b]	24.25 ± 1.89[b]	176.63 ± 7.08[a]	49.81 ± 4.04[b]	8.76 ± 1.22[b]	6.40 ± 0.83	39.03 ± 0.28[b]	0.38 ± 0.01[b]	7.75 ± 1.32[b]	5.61± 0.45[b]
Protector	1.20 ± 0.16[c]	47.00 ± 3.39	1.41 ± 0.12[c]	11.00 ± 1.38[c]	131.28 ± 12.97[b]	29.16 ± 1.84[c]	18.86 ± 1.17[c]	8.49 ± 1.62	42.23 ± 0.20[c]	0.98 ± 0.03[c]	30.52 ± 1.77 [b,c]	9.41± 0.95[c]
Protector+ radiation	1.37 ± 0.13[c]	44.29 ± 5.02	1.48 ± 0.06[c]	13.50 ± 1.75[b,c]	140.62 ± 9.56[c]	39.92 ± 4.16[c]	19.76 ± 1.21[b,c]	11.23 ± 1.19[b,c]	48.64 ± 0.08[b,c]	0.66 ± 0.03[b,c]	24.01 ± 2.76 [b,c]	7.72± 0.87 [b,c]

[a] Each value represents mean ± SEM (n = 8).
[b] Significantly different from control group ($p < 0.001$), [c] Significantly different from irradiated group ($p < 0.001$).

and in blood to 39.92 ± 4.16 μg mL^{-1} when compared to the irradiated group. In addition, γ-irradiation caused a significant decrease in the GSH level ($p < 0.001$) compared to the normal group; the level of GSH in both blood and hepatic tissue decreased from 1.09 ± 0.12 to 0.78 ± 0.09 μg g^{-1} in liver and from 51.38 ± 3.38 to 41.70 ± 4.56 μg mL^{-1} in blood. Treatment with germanium L-cysteine α-tocopherol before radiation significantly improve ($p < 0.001$) the hepatic GSH level compared to the irradiated group to 1.37 ± 0.13 μg g^{-1}, while blood GSH level showed no significant variation. Bump *et al.* (22) reported that GSH is a versatile protector and executes its radioprotective function through free-radical scavenging, restoration of the damaged molecules by hydrogen donation, reduction of peroxides, and maintenance of protein thiols in the reduced state. Further, lower depletion of blood and liver GSH levels in irradiated animals might have been due to higher GSH availability, which enhanced the capability of cells to cope with the free radicals generated by radiation. The significant reduction in liver GSH due to radiation could be due to an enhanced utilization of the antioxidant system during detoxification of the free radicals generated by radiation accompanied, with depletion of glutathione with enhanced lipid peroxidation (6).

The results of NO in blood and hepatic tissue for different studied groups show that in γ-irradiated group the NO level exhibited a significant ($p < 0.001$) increase as compared with control, the level of NO in liver increased from 1.32 ± 0.10 to 1.88 ± 0.06 μg g^{-1} and in blood from 9.67 ± 0.67 to 24.25 ± 1.89 μg mL^{-1}. When the animals were protected before radiation the level of NO was restored to the control level; normaly it was significantly ($p < 0.001$) reduced as compared to the irradiated group (liver NO level decreased to 1.48 ± 0.06 μg g^{-1} and blood level dropped to 13.50 ± 1.75 μg mL^{-1}). The increased production of NO in the irradiated group is due to induction of NO synthase enzyme, which is considered to be absent under physiological conditions and induced by

radiation (23). Superoxide anion ($O^{2\bullet-}$) can attack NO to form peroxynitrite ($ONOO^-$) anion, which may oxidize many biological molecules including sulfhydryls (such as glutathione), iron sulfur centers and lipids (24).

The effect of germanium L-cysteine α-tocopherol on the blood and hepatic antioxidant enzymes CAT, SOD and GPx activity of rats exposed to γ-irradiation is shown in Table II. As compared to the control group, the irradiated group revealed significant decline ($p < 0.001$) in activities of antioxidant enzymes SOD and GPx in blood and liver. Hepatic SOD decreased from 43.30 ± 0.57 to 39.03 ± 0.28 nmol min^{-1} g^{-1} and in blood from 1.07 ± 0.07 to 0.38 ± 0.01 nmol min^{-1} mL^{-1}; hepatic GPx decreased from 25.21 ± 2.48 to 7.75 ± 1.32 μmol min^{-1} g^{-1} and in blood from 11.02 ± 1.47 to 5.61 ± 0.45 μmol min^{-1} mL^{-1}. Hepatic CAT activity showed a significant decrease ($p < 0.001$) from 16.27 ± 1.42 to 8.76 ± 1.22 μmol min^{-1} g^{-1} while its activity was not significantly changed in blood. On the other hand, the pretreatment with germanium L-cysteine α-tocopherol normalized the activities of CAT, SOD, and GPx in blood and liver compared to the irradiated group: the activity of SOD in liver increased back to 48.64 ± 0.08 nmol min^{-1} g^{-1} and to 0.66 ± 0.03 nmol min^{-1} mL^{-1} in blood, hepatic GPx increased to 24.01 ± 2.76 μmol min^{-1} g^{-1} and to 7.72 ± 0.87 μmol min^{-1} mL^{-1} in blood, hepatic CAT increased to 19.76 ± 1.21 μmol min^{-1} g^{-1} and to 11.23 ± 1.19 μmol min^{-1} mL^{-1} in blood. Oliinyk *et al.* (25) mentioned that the inhibition of antioxidant systems in blood and tissues of animals is accompanied by an increase in lipid peroxide products after irradiation exposure. Also, Ueda *et al*. (26) mentioned that when the oxidative damage is extreme as a result of irradiation, ROS scavenging enzymes are degraded. In support of these results, we found a decline in GPx in liver of rats exposed to irradiation. It is hypothesized that the enzyme inactivating action of ROS or lipid peroxides induced by irradiation can overcome enzyme synthesis capacity. Also, the decline in SOD activity recorded in mice exposed to radiation may be due to the loss of mitochondria, which leads to a decrease in total MnSOD activity in different tissues of rats (26). Pretreatment with the germanium complex revealed improvement in the activities of antioxidant enzymes, which can be explained by the fact that germanium can enhance the activities of catalase and SOD and inhibit lipid peroxidation in the liver of γ-irradiated mice (27).

Fig. 2 shows that the levels of serum α-tocopherol and total protein in rats exposed to whole body γ-irradiation declined markedly ($p < 0.001$) compared to the control group; the level of blood α-tocopherol decreased from 4.34 ± 0.24 to 3.48 ± 0.23 μg per 100 mL and the level of blood total protein decreased from 5.57 ± 0.19 to 4.12 ± 0.28 mg per 100 mL. On the other hand, pretreatment with the germanium L-cysteine α-tocopherol complex prior to irradiation caused significant elevation ($p < 0.001$) in the level of α-tocopherol and total protein compared to the irradiated group up to 4.63 ± 0.20 μg per 100 mL and to 5.25 ± 0.18 mg per 100 mL, respectively. γ-Irradiation can damage or inactivate proteins by two different mechanisms. First, it can rapture the covalent bonds in target protein molecules as a direct result of a photon depositing energy into the molecule. Second, it can act indirectly, like with a water molecule, producing free radicals and other non-radical reactive oxygen species that are in turn responsible for most (99.9%) of the protein damage (28). In addition, the depletion in the vitamin E level can be explained by the fact that vitamin E is a natural component of cell membranes; it reacts quickly with peroxyl radicals and forms a tocopheroxyl radical, *i.e.*, a sacrificial molecule with which the peroxyl radicals preferentially react, rather than with biological molecules, thus preventing damage to cell structures (29).

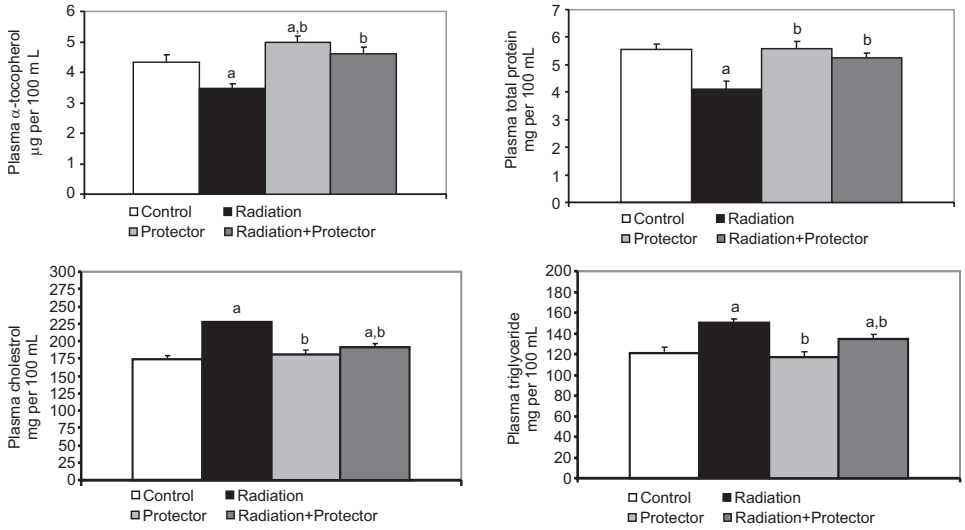

Fig. 2. The effect of germanium L-cysteine α-tocopherol complex on the level of blood α-tocopherol, total protein, cholesterol and triglycerides of rats exposed to γ-irradiation (mean ± SEM, $n = 8$). [a] Significantly different from control group; [b] from irradiated group ($p < 0.001$).

Blood cholesterol and triglyceride levels in rats exposed to γ-irradiation were significantly increased ($p < 0.001$) compared to the control group; the level increased from 174.25 ± 4.61 to 227.50 ± 4.65 mg per 100 mL and from 120.50 ± 5.78 to 150.00 ± 6.44 mg per 100 mL, respectively. However in the group treated with germanium L-cysteine α--tocopherol prior to irradiation, both cholesterol and triglyceride values recorded a significant decrease ($p < 0.001$) compared to the irradiated group, normally to 190.75 ± 6.41 mg per 100 mL and to 135.00 ± 4.94 mg per 100 mL, respectively. Significant elevation of total cholesterol in plasma of irradiated animals was explained by Bok *et al.* (30), who mentioned that irradiation increases activation of HMG-CoA reductase enzyme, the key regulatory enzyme in the reaction of the cholesterol synthesis process. The increase in plasma triglycerides in rats exposed to γ-irradiation may be attributed to inhibition of the activity of lipoprotein lipase. This result was consistent with the observation of Sedlakova *et al.* (31), who mentioned that lipoprotein lipase activity decreases post irradiation exposure in adipose tissues giving rise to hypertriglyceridemia.

Light microscopic examinations of liver sections of control animals exhibited normal constructions (Fig. 3a) while liver sections of rats exposed to γ-irradiation showed liver fibrosis and necrosis with mononuclear leucocytic inflammatory cells, infiltrating the dilated portal vein in the portal area associated with proliferation of diffuse Kupffer cells. Liver section exhibited tissue degeneration and lymphocyte infiltration, and vascular degeneration of the hepatocytes (Fig. 3b). In contrast, the irradiated animals pretreated with germanium L-cysteine α-tocopherol showed regeneration and amelioration of the hepatocytes to the normal structure (Fig. 3d). Furthermore, the microscopic structure of hepatic cells in the group of rats treated with germanium L-cysteine α-tocopherol

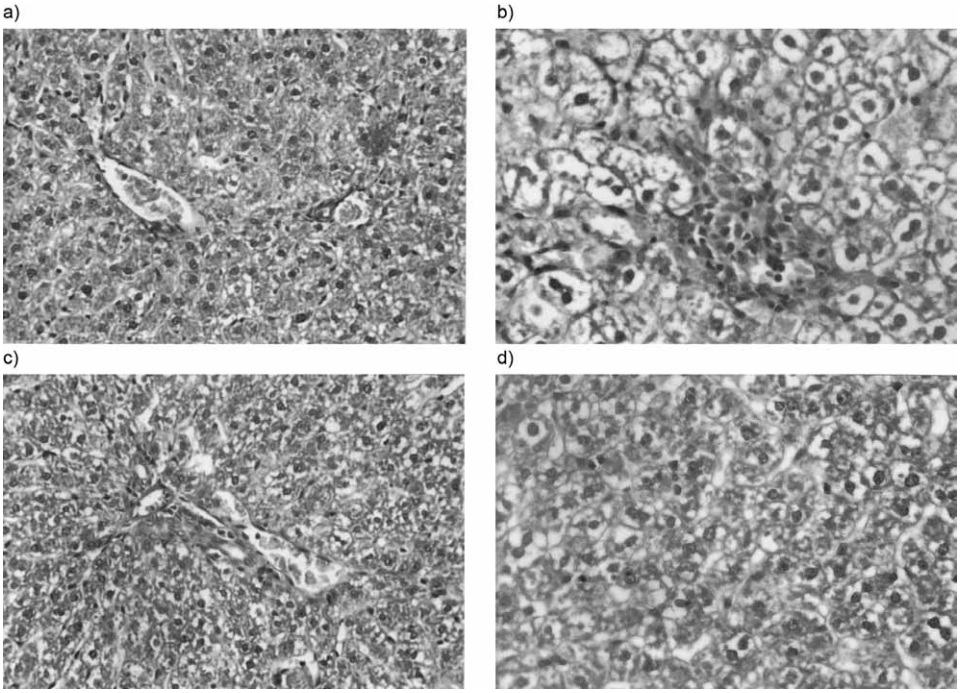

Fig. 3. The hepatoprotective effect of germanium L-cysteine α-tocopherol on the histopathological changes resulting from the whole body exposure of rats to γ-irradiation. Liver sections were stained with hematoxylin and eosin (original magnification 400x). a) control rat liver showing normal architecture; b) irradiated group showing liver fibrosis and necrosis with mononuclear leucocytic inflammatory cells, cellular infiltration in portal area associated with proliferation of diffuse Kupffer cells, tissue degeneration and lymphocyte infiltration; c) rats treated with germanium L-cysteine α-tocopherol showing normal shape like the control hepatic cells; d) irradiated group treated with germanium L-cysteine α-tocopherol pre-irradiation showing signs of improvement towards normal architecture.

alone showed the normal shape like the control hepatic cells (Fig. 3c). This finding is supported by several authors. Rodemann and Bamberg (32) reported that fibrosis is a common sequel of both cancer treatment by radiotherapy and accidental irradiation, which has been described for many tissues, including skin, lung, heart and liver. The underlying mechanisms of the radiation-induced fibrosis can be seen as a multicellular process involving various interacting cell systems in the target organ, resulting in the fibrotic phenotype of the fibroblast/fibrocyte cell system. Germanium L-cysteine α-tocopherol complex exerts a radical scavenging effect and can prevent radical generation immediately after irradiation, probably by inhibiting the chain reaction of membrane lipid peroxidation that follows, and thus protect membrane lipids from peroxidation damage. This effect is also confirmed by improvement in the histological changes in the group of rats treated with the germanium L-cysteine α-tocopherol complex pre-irradiation, as compared to the irradiated group.

CONCLUSIONS

The presented data provide evidence that treatment of rats with germanium L-cysteine α-tocopherol prior to whole body γ-irradiation exerts a beneficial antioxidant protective effect through maintaining high blood levels of reduced glutathione and enhancing the activities of antioxidant enzymes (CAT, SOD and GPx). It also offers considerable protection against radiation induced liver injury by inhibiting the chain reaction of membrane lipid peroxidation. According to the foregoing results, we suggest that the radioresistant effects of germanium L-cysteine α-tocopherol could be possibly used as radioprotector against the treatment side effects induced by radiotherapy. The results of further experiments that can be extended to other organs could be used for planning a more effective therapy program combining radiation therapy and radioprotection.

REFERENCES

1. S. S. Wallace, Enzymatic processing of radiation-induced free radical damage in DNA, *Rad. Res.* **150** (Suppl. 5) (1998) S60-S79.
2. L. L. De Zwart, J. H. N. Meerman, J. N. M. Commandeur and N. P. E. Vermeulen, Biomarkers of free radical damage applications in experimental animals and in humans, *Free Rad. Biol. Med.* **26** (1999) 202–226.
3. Y. Deger, S. Dede, A. Belge, N. Mert, T. Kahraman and M. Alkan, Effects of X-ray radiation on lipid peroxidation and antioxidant systems in rabbits treated with antioxidant compounds, *Biol. Trace Elem. Res*. **94** (2003) 149–156.
4. P. A. Riley, Free radicals in biology: Oxidative stress and the effects of ionizing radiation, *Int. J. Radiat. Biol*. **65** (1994) 27–33.
5. M. L. Sagrista, A. E. Garcia, M. Africa De Madariaga and M. Mora, Antioxidant and pro-oxidant effect of the thiolic compounds *N*-acetyl-L-cysteine and glutathione against free radical-induced lipid peroxidation, *Free Radical Res.* **36** (2002) 329–340.
6. G. C. Jagetia and T. K. Reddy, Modulation of radiation-induced alteration in the antioxidant status of mice by naringin, *Life Sci.* **77** (2005) 780–794.
7. A. Badaloo, M. Reid, T. Forrester, W. C. Heird and F. Jahoor, Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition, *Am. J. Clin. Nutr.* **76** (2002) 646–652.
8. M. K. Yang and Y. G. Kim, Protective role of Ge-132 against paraquat-induced oxidative stress in the livers of senescence-accelerated mice, *J. Toxicol. Environ. Health A* **58** (1999) 289–297.
9. C. K. Chow, Vitamin E and oxidative stress, *Free Rad. Biol. Med*. **11** (1991) 215–232.
10. S. Goodman, Therapeutic effect of organic germanium, *Med. Hypotheses* **26** (1988) 207–215.
11. K. Asai, *Mechanism of Action, Miracle Cure Organic Germanium*, Japan Publications Inc. Tokyo 1980, pp. 350–375.
12. K. Jayalakshmi, M. Sairam, S. B. Singh, S. K. Sharma, G. Ilavazhagan and P. K. Banerjee, Neuroprotective effect of N-acetyl cysteine on hypoxia-induced oxidative stress in primary hippocampal culture, *Brain Res.* **1046** (2005) 97–104.
13. M. N. Ghosh, *Toxicity Studies, Fundamentals of Experimental Pharmacology*, Scientific Book Agency, Calcutta 1984, pp. 153-190.

14. H. J. Conn, M. A. Darrow and V. M. Emmel, *Staining Procedure Used by Biological Stain Commission*, 2nd ed., Williams & Wilkins Co., Baltimore 1960, pp. 200–245.
15. C. C. Conrad, D. T. Grabowski, C. A. Walter, S. Sabla and A. Richardson, Using $MT^{-/-}$ mice to study metallothionein and oxidative stress, *Free Radical Biol. Med.* **28** (2000) 447–462.
16. E. Beutler, O. Duron and B. M. Kelly, Improved method of the determination of blood glutathione, *J. Lab. Clin. Med.* **61** (1963) 882–888.
17. M. Minami and H. Yoshikawa, A simplified assay method of superoxide dismutase, *Clin. Chim. Acta* **92** (1979) 337–342.
18. L. H. Johansson and L. A. H. Borg, A spectrophotometric method for determination of catalase activity in small tissue sample, *Anal. Biochem.* **174** (1988) 331–336.
19. D. E. Paglia and W. N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, *J. Lab. Clin. Med.* **70** (1967) 158–169.
20. H. Moshage, B. Kok, J. R. Huizenga and P. L. M. Jansen, Nitrite and nitrate determinations in plasma: A critical evaluation, *Clin. Chem.* **41** (1995) 892–896.
21. H. Baker and O. Frank, *Determination of α-Tocopherol, Clinical Vitaminology*, Willy Whitefeather, New York 1968, pp. 172–210.
22. E. A. Bump and J. M. Brown, Role of glutathione in the radiation response of mammalian cells *in vitro* and *in vivo*, *Pharm. Ther.* **47** (1990) 117–136.
23. P. Lestaevel, D. Clarençon, A. Gharib, A. Peinnequin, R. Cespuglio, P. Gourmelon, A. Alonso, J. D. Laval and E. Multon, Nitric oxide voltammetric measurements in the rat brain after gamma irradiation, *Rad. Res.* **160** (2003) 631–636.
24. R. Radi, J. S. Beckman, K. M. Bush and B. A. Freeman, Peroxynitrite oxidation of sulfhydryls. The cytosolic potential of superoxide and nitric oxide, *J. Biol. Chem.* **266** (1994) 4244–4250.
25. B. V. Oliinyk, V. A. Baraboi, S. A. Oliinyk and N. O. Horchakova, Effect of splenosid on lipid peroxidation process and glutathione antioxidant system in rats exposed to fractionated radiation, *Ukr. Biokhim. Zh.* **73** (2001) 73–77.
26. T. Ueda, Y. Toyoshima, T. Moritani, K. Ri, N. Otsuki, T. Kushihashi, H. Yasuhara and T. Hishida, Protective effect of dipyridamole against lethality and LPx in liver and spleen of the ddY mouse after whole-body irradiation, *Int. J. Rad. Biol.* **69** (1996) 199–204.
27. Y. S. Park, Y. G. Kim, J. C. Chang and D. Y. Kim, Radioprotective effects of red ginseng extracts on antioxidants and lipid peroxidation of the liver in γ-irradiated mice, *Korean Biochem. J.* **26** (1993) 184–191.
28. E. S. Kempner, Effects of high-energy electrons and gamma rays directly on protein molecules, *J. Pharm. Sci.* **90** (2001) 1637–1646.
29. R. Wolfa, D. Wolfb and V. Ruoccoc, Vitamin E: The radical protector, *J. Eur. Acad. Dermatol.* **10** (1998) 103–117.
30. S. Bok, S. Lee, Y. Park, K. Bae, K. Son, T. Jeong and M. Choi, Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and aryl CoA: Cholesterol transferase are lower in rats fed Citrus Peel extract or a mixture of Citrus bioflavonoids, *J. Nutr.* **129** (1999) 1182–1185.
31. A. Sedlakova, K. Borikova and T. Ahlers, Changes in lipoprotein lipase activity in the adipose tissue and heart on non-lethally X-irradiated rats, *Physiol. Bohemoslov.* **35** (1986) 400–405.
32. I. Rodemann and S. K. Bamberg, Cellular basis of radiation-induced fibrosis, *Radiother. Oncol.* **35** (1995) 83–90.

S A Ž E T A K

Uloga kompleksa germanija s L-cysteinom i α-tokoferolom kao stimulatora antioksidativnog obrambenog sustava štakora izloženih gama-zračenju

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U radu je procjenjivan kompleks germanija s L-cisteinom i α-tokoferolom [germanijev diklortetrakis (L-cisteinil-α-tokoferol amid) diklorid] kao zaštitno sredstvo protiv slobodnih radikala induciranih γ-zračenjem hepatotoksičnosti. Mužjacima švicarskih albino štakora davan je intraperitonealno kompleks germanija, u 6 sukcesivnih doza po 75 mg kg^{-1} tjelesne mase, posljednja doza dana je dvadeset minuta prije izlaganja cijelog organizma jednokratnoj dozi γ-ozračivanja od 6,5 Gy. U krvi i jetri praćena je razina lipidne peroksidacije (LPx), dušikovog(II) oksida (NO), glutationa (GSH), aktivnost antioksidativnih enzima glutation peroksidaze (GPx), superoksid dismutaze (SOD) i katalaze (CAT), te količina ukupnih proteina u krvi, kolesterola, triglicerida i α-tokoferola. Rezultati su pokazali da je primjena germanijevog kompleksa značajno ($p < 0,001$) poboljšala koncentraciju jetrenih i krvnih LPx. Koncentracija GSH u jetri je značajno porasla ($p < 0,001$), dok se njegova razina u krvi nije značajno promijenila. Koncentracija NO u krvi i jetri značajno se smanjila ($p < 0,001$). S druge strane, prethodna obrada s kompleksom germanija normalizirala je aktivnost SOD, GPx i CAT u krvi i jetri u odnosu na ozračenu skupinu. Istraživanja su također pokazala značajno smanjenje triglicerida i kolesterola ($p < 0,001$) i značajno povećanje ($p < 0,001$) α-tokoferola i ukupnih proteina u krvi. Te biokemijske promjene su povezane s izraženim poboljšanjem histoloških promjena u odnosu na ozračenu skupinu. Opisani kompleks germanija mogao bi se kao antioksidativno sredstvo potencijalno upotrijebiti za spriječavanje promjena uzrokovanih zračenjem.

Ključne riječi: germanijev L-cistein -tokoferol kompleks, γ-zračenje, jetra, antioksidativni enzimi

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