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Effect of irradiation on enzymes of antioxidative defense system in L929 cell culture in the presence of α-tocopherol-acetate

ABSTRACT

Background: *Cell damage induced by ionising radiation occurs through direct and indirect effects. Vitamin E as a natural antioxidant protects against radiation-induced cell damage. Enzymes of antioxidative defense system - superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), play the main role in endogenous cell protection from oxidative damage. The aim of this study was to examine whether a presence of α-tocopherol-acetate has the influence on activity of SOD, GSH-Px and CAT in L929 cell culture after the irradiation.*

Materials and methods: *Cell culture L929 samples were treated with α-tocopherol acetate (10^{-4}M) during one hour and thereafter cells were irradiated with X-rays (25 Gy). Activity of SOD, GSH-Px and CAT was determined at 1, 24, 48, 72 and 96 hours after irradiation.*

Results: *Irradiation in applied dose induces decrease in endogenous SOD activity but no changes in CAT and GSH-Px activity of L929 cells were observed, at 96 h after irradiation. Activity of SOD was significantly increased (p1'<0.05), CAT activity was significantly decreased (p1'<0.05), while no significant difference was found in activity of GSH-Px in irradiated and α-tocopherol acetate pre-treated L929 cells, in comaprison to irradiated but unprotected cells.*

Conclusion: *A treatment of L929 cells with α-tocopherol acetate before irradiation protects SOD, has no effect on GSH-Px and induces decrease of CAT activity after 96 hours of irradiation. As radiation exposure itself results in changes of antioxidant enzyme activities, observed changes in the presence of α-tocopherol acetate were discussed with respect to proper balance of antioxidative defense required for radioprotection.*

Key words: *Cell culture; Radiation; Alpha tocopherol-acetate; Antioxidative enzymes*

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INTRODUCTION

Potentially toxic reactive oxygen species are produced in normal cellular metabolism and in abundance of prooxidant states.

Oxidation, induced by free radicals, has long been known to play an important role in radiation effects and carcinogenesis as well (1,2).

Abbrevations used in text: p1= statistical significance between irradiated and control; p1'= statistical significance between irradiated and irradiated in the presence of α-tocopherol-acetate; p2= statistical significance between irradiated and control in single time point of experiment; p2'= statistical significance between irradiated and control in the presence of α-tocopherol-acetate in single time point of experiment

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The role of endogenous enzymes of antioxidative defense system in these pathologic oxidations was examined as well as the effect of exogenous antioxidants *in vivo* and *in vitro* (3-5). Mechanisms of oxidation include one - electron pathways involving radicals such as superoxide and hidroxyl radical, or by two electron pathways that include nonradical oxidants such as hydrogen peroxide (6,7).

Reactive oxygen species are degraded by the organized antioxidant enzyme system and by other antioxidants as well. Vitamin E is the main natural antioxidant in biological membranes. Its role in radioprotection and in carcinogenesis has been already proved (2, 8-10). Radioprotective effect of vitamin E is lower at a higher dose rate (e.g. 1Gy/min) than at dose rate of 0.2 Gy/min (8). There is evidence that radiation-induced lipid peroxidation is greater after lower dose rates compared to higher dose rates (6) and it is possible that comparatively greater protection would be observed at lower dose rates with natural antioxidants, such as vitamin E. It is evident that radioprotective effect of vitamin E includes complex mechanisms, particularly in cell culture (9,11) so we decided to examine activity of antioxidative enzymes in L929 cell culture after the irradiation in the presence of vitamin E.

MATERIALS AND METHODS

The L929 cell line (mouse fibroblasts) was routinely grown in 6-well tissue culture plates (Costar, USA) in DMEM (Sigma) supplemented with 10% heat- inactivated foetal calf serum (NIVNS) and antibiotics. Cells were cultured at 37°C in a humified 5% carbon dioxide atmosphere. Exponentially growing L929 cells were used throughout the assays (12).

Cells were seeded in 6-well culture plates at a concentration of 100 000/per well. All experiments were performed 72h after the seeding.

The samples were divided into three groups: 1. Control group (untreated samples) 2. Experimental group of irradiated samples 3. Experimental group of irradiated and α-tocopherol acetate pretreated samples.

For antioxidant treatment, the medium was

replaced with a fresh one, supplemented with alpha tocopherol-acetate at a concentration of 10^{-4} M, and incubation continued for one h at 37°C.

Cells were irradiated with a target dose of 25 Gy (dose specified in previous experiments, using a LINAC, Mevatron Siemens MD 7475 (10 MV X- rays), with a dose rate of 3 Gy/min and cultured up to 96h after the irradiation (13).

Cell suspension was obtained using 0.25 % trypsin in EDTA. Cells were peletted (10 minutes /1200 rpm), resuspended in saline and samples were frozen (-20°C) and thawed (37°C) for three times. The cytosol fraction was obtained by sample centrifugation (10 minutes /10 000 rpm).

The protein concentration (14), and the activity of antioxidant enzymes SOD (15), GSH-Px (16), and CAT (17) were determined at 1, 24, 48, 72 and 96h of the experiment.

Kolmogorov-Smirnov test was used for the statistical analysis of the obtained results. A p1 value represents the statistical significance between the total experimental groups, and p2 value represents the statistical significance between groups in every single point of the experiment. A p value less than 0.05 ($p<0.05$) was taken as the highest level of statistical significance.

RESULTS

The SOD activity in the control group was the lowest in the first hour of the experiment, but later on growing trend was observed. The maximal activity was reached at 72 h (Figure 1). In the group of irradiated samples steadily decrease of SOD activity was noticed, with highest activity in the first hour after the irradiation.

The SOD activity in irradiated and vitamin E pretreated group was significantly lower in the first hour, in comparison with other two groups. The SOD activity however, increased and was kept at a level around 35 U/mg up to the end of the experiment.

In the control, unirradiated samples the changes in the GSH-Px activity, were similar to those previously observed (3). GSH-Px activity in the irradiated group increased in the first 24h and thereafter sharply decreased (Figure 2).

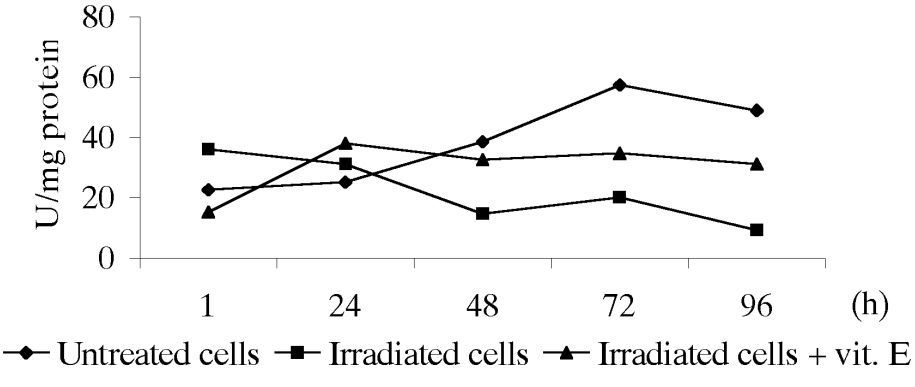

Figure 1. The influence of α-tocopherol-acetate pretreatment in irradiated cells on the SOD activity

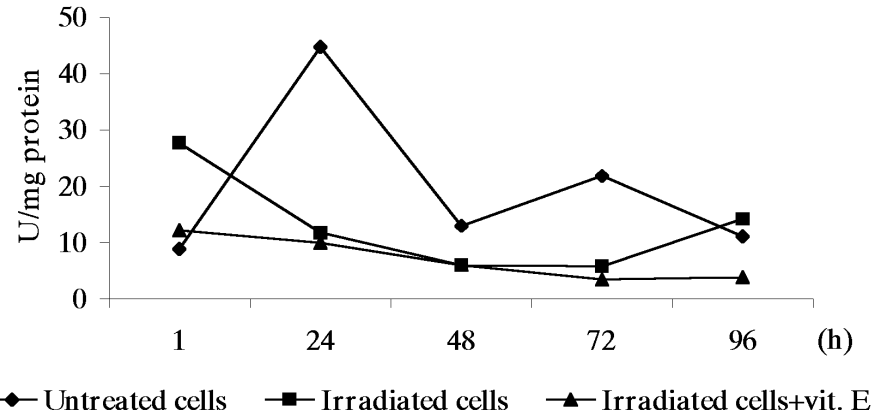

Figure 2. The influence of α-tocopherol-acetate pretreatment in irradiated cells on the GSH-Px activity

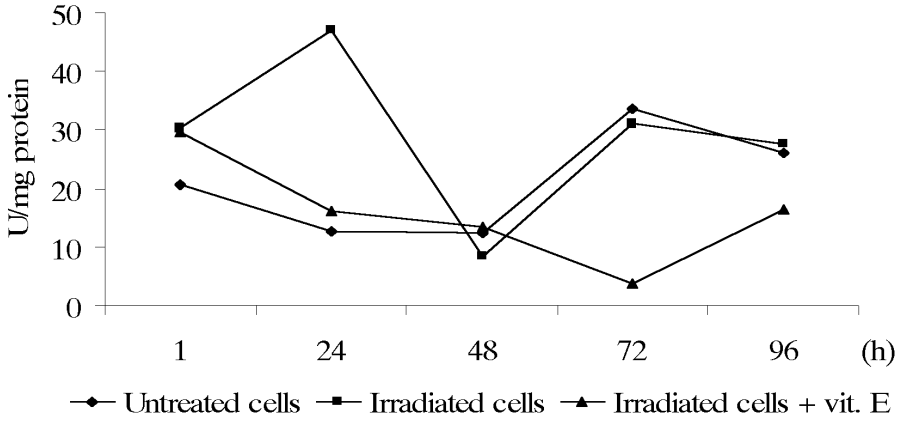

Figure 3. The influence of α-tocopherol-acetate pretreatment in irradiated cells on the CAT activity

A decrease of GSH-Px activity in irradiated and vitamin E pretreated group was observed at 24 and 48 hour, but it was not statistically significant in comparison with irradiated cells.

A significant difference in CAT activity was found only after 24 hours of the experiment between the control and the irradiated group ($p2<0.05$) (Figure 3). CAT activity was significantly different between irradiated and irradiated and α-tocopherol-acetat pretreated group ($p1'<0.05$), at the end of the experiment. Immediately after the irradiation the activity was almost identical, and after that, it showed completely different course.

DISCUSSION

Radiation exposure itself results in changes in antioxidant enzyme levels: most consistently reported is elevation in Mn SOD (18). In our experiment, SOD activity has increased one hour after the irradiation in comparison with control. During the experiment (24, 48, 72 and 96 hours after the irradiation) the activity was the same in both groups being even lower in irradiated than in controls at the end of the experiment. These changes in cell culture SOD activity after the irradiation suggest that events after the irradiation are dynamic, including interaction between primarily formed free radicals, secondary formed once and cell response at the level of enzyme synthesis. Our results correspond well with a fact that transformed, dedifferentiated cell lines have lower capability of SOD synthesis than normal ones (19-21). Remacle and coworkers (22) have found that enzyme system of fibroblasts in culture is remarkably sensitive to short oxidative stress such as x-irradiation. A protective role of vitamin E in our experiment was evident 24 hours after the irradiation. The SOD activity has not changed in that group during the experiment,

which is in consistence with the results of Lopez - Tores et al. (23). Prevention of SOD inactivation during the experiment, which is evident in irradiated group protected with tocopherol acetate, may be due to previously found a long window of protection provided by most natural antioxidants (18).

A decrease in GSH-Px activity in mouse tissues was observed in the postirradiation period (24). There was less depression when the diet was supplemented with either selenium or vitamin E. The application of α-tocopherol acetate induce increase in GSH-Px activity in transformed cell lines such as L929 (10). Contrary to that, in our experiments, GSH-Px activity was not changed with addition of α-tocopherol acetate in comparison with irradiated cells, the result which was consistent with Leist et al. (25) who had not found the influence of α-tocopherol acetate on GSH-Px activity.

Although it is probable that antioxidant enzymes, GSH-Px , SOD, and CAT, are important in providing protection from radiation exposure, the proper balance of the enzymes in specific cells and the whole organism required for maximum radioprotection is far from clear. For example, a large increase in MnSOD in some model systems may have radiosensitizing effects rather than a protective (26), probably related to the inability of cell to cope with overproduction of hydrogen peroxide (H_2O_2) or hydroxyl radical ($\dot{O}H$). In our experiment, CAT activity in irradiated group is significantly higher than in group with applied α-tocopherol acetate which may be correlated with former radiosensitive effect of SOD protection.

CONCLUSION

In our experiment application of α-tocopherol acetate protects SOD activity from inhibition due to irradiation and eliminate time related changes in GSH-Px and CAT activities after the irradiation point up that proper balance of enzymes in specific cells is of utmost importance for radiation protection.

REFERENCES

1. Yarmonenko SP. Radiobiology of Humans and Animals. Moscow: Mir Publishers, 1988.

2. Whitaker SJ. DNA Damage by Drugs and Radiation: What is Important and How is it Measured? Eur J Cancer 1992;28:273-6.

3. Bogdanović G, Pražić B, Kerenji A, Kuzmanović Z, Nikolić V. Aktivnost antioksidativnih enzima u ozračenoj kulturi ćelija K562: eksperimentalni *in vitro* model. Informacije o karcinogenima 1993:43-6.

4. Jovanović A. Uticaj selena na antioksidativni sistem eritrocita i jetre šarana. Magistarski rad. Novi Sad: Univerzitet u Novom Sadu, 1993.

5. Simović M, Spasić BM, Michelson AM. Free radicals in human myocardial reperfusion injury. Life Chem Rep 1995;12:227-70.

6. Chow CK. Vitamin E and oxidative stress. Free Radical Biol Med 1991;11:215-32.

7. Nam SY, Cho CK, Kim SG. Correlation of increased mortality with the suppression of radiation - inducible microsomal epoxide hydrolase and glutathione S - transferase gene expression by dexamethasone: effects on vitamin C end E - induced radioprotection. Biochem Pharmacol 1998;56:1295-304.

8. Srinivasan V, Weiss JF. Radioprotection by vitamin E:Injectable vitamin E administred alone or with WR 3689 enhanced survival of irradiated mice. Int J Radiat Oncol Biol Phys 1992;23:841-6.

9. Halpner AD, Handelman GJ, Harris JM, Belmont CA, Blumberg JB. Protection by vitamin C of loss of vitamin E in cultured rat hepatocytes. Arch Biochem Biophys 1998;359:305-9.

10. Cerutti P, Ghosh R, Oya Y, Amstad P. The role of the cellular antioxidant defense in oxidant carcinogenesis. Environ Health Perspect 1994:102.

11. Hu WL, Goldring CE, Rao NR, Rice-Evans C, Burdon RH, Diplock AT. Variable alpha - tocopherol stimulation and protection of glutathione peroxidase activity in established and malignant fibroblasts. Biofactors 1992;4:47-9.

12. Kojić V, Lazić S, Kljajić R, Jovičin M, Krmpot Lj, Milanov D et al. The influence of new product FCS-Novi Sad on the growth of cultured cells. Sažetak. In: XXXIII Kancerološka nedelja. Beograd, 7-8 novembar, 1996.

13. Bogdanović V. Uticaj jonizujućeg zračenja na antioksidativni sistem maligne i nemaligne ćelijske linije. Magistarski rad. Novi Sad: Univerzitet u Novom Sadu, 2000.

14. Lowry OH, Rosenbrough AL, Farr AL, Randall RJ. Protein Measurement with the Folin phenol Reagent. J Biol Chem 1951;193:265-7.

15. McCord JM, Fridovich I. The Reduction of Cytochrome C by Milk Xantine Oxidase. J Biol Chem 1968;243:5753-60.

16. Paglia DE, Valentine NW. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidases. J Lab Clin Med 1967;70:74-7.

17. Clauborne A, Greenwald RA. Catalaze Activity. CRC Handbook of Methods for Oxygen Radical Research 1984;283-4.

18. WeissJF, Landauer MR. Radioprotection by antioxidants. Ann NY Acad Sci 2000;899:44-60.

19. Michelis C, Raes M, Houbion A, Remacle J. Association of antioxidant systems in the protection of human fibroblasts against oxygen derived free radicals. Free Rad Res Comms 1991;14:323-34.

20. Conti M, Couturier M, Lemonnier A, Lemonnier F. Effects of alpha-tocopherol on antioxidant enzyme activity in human fibroblast cultures. Int J Vitam Nutr Res 1993;63:71-6.

21. Sandstrom BE, Carlsson J, Marklund SL. Selenite - induced variation in glutathione peroxidase activity of three mammalian cell lines: no effect on radiation - induced cell killing or DNA strand breakage. Radiat Res Feb 1989;117:318-25.

22. Remacle J, Michiels C, Raes MF. The importance of antioxidant enzymes in cellular aging and degeneration. EXS 1992;62:99-108.

23. Lopez - Torres M, Thiele JJ, Shindo Y, Han D, Packer L. Topical application of alpha - tocopherol modulates the antioxidant network and diminishes ultraviolet - induced oxidative damage in murine skin. Br J Dermatol 1998;138:207-15.

24. Jakobs AJ Rankin WA , Srinivasan V and Weiss JF. Effects of vitamin E and selenium on glutatione peroxidase activity and survival of irradiated mice. Proceedings of the 7th International Congress of Radiation Research. Broerse JJ, Berendsen GW, Kal HB, van der Kogel AL, eds. Amsterdam: Martinus Nijhoff Publishers, 1983:D5-15.

25. Leist M, Raab B, Maurer S, Rosick U, Brigelius - Flohe R. Conventional cell culture media do not adequatly supply cells with antioxidants and thu facilitate peroxide- induced genotoxicity. Free Radical Biol Med 1996;21:297-306.

26. Scott MD, Meshnick SR, Eaton JW. Superoxide dismutase amplifies organismal sensitivity ionising radiarion. J Biol Chem 1998;264:2498-501.