JBUON 2014; 19(2): 554-561
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Dose-dependent survival of K562 cells subjected to irradiation, time course of endogenous enzyme activity and protective effect of applied superoxide dismutase

Visnja Bogdanovic[1], Snezana Spasic[2], Lazar Rutonjski[3], Mihajla Djan[4], Jasminka Mrdjanovic[1]

[1]*University of Novi Sad, Medical Faculty, Oncology Institute of Vojvodina, Department for Experimental Oncology, Sremska Kamenica;* [2]*University of Belgrade, Centre for Chemistry ICHTM, Belgrade;* [3]*University of Novi Sad, Medical Faculty, Oncology Institute of Vojvodina, Department for Radiology, Sremska Kamenica;* [4]*University of Novi Sad, Faculty of Natural Sciences, Department for Genetics, Novi Sad, Serbia*

Summary

Purpose: *Irradiation-generated reactive species are proven to affect the cell survival and antioxidant enzyme levels. Radioresistance is a phenomenon which includes many cell mechanisms and signaling pathways. Superoxide dismutase (SOD) acts in and outside of cells after irradiation. The aim of this study was to determine LD_{50} (lethal dose for 50% of K562 cells), to monitor the effect of a chosen dose and exogenously applied superoxide dismutase (ExSOD) on the cell number and the activity of SOD, glutathione peroxidase (GSH-Px) and catalase (CAT).*

Methods: *The survival of irradiated (20-32.5 Gy) K562 cells was determined using the trypan-blue exclusion. Besides irradiated and non-irradiated cells (controls), another two groups of cells were treated with SOD (10^{-6} M) which then served as SOD-treated controls or were irradiated (30 Gy) one hour later. The number of cells and the activity of SOD, GSH-Px and CAT (using kinetic methods) were monitored after 1, 24, 48, 72 and 96 hrs in unirradiated, irradiated, SOD-treated and SOD-treated/irradiated experimental groups.*

Results: *K562 cells showed dose-dependent survival in the chosen range of doses. A dose of 30 Gy induced 50% cell mortality and increased the activity of all three investigated enzymes after 24 hrs. Pretreatment with SOD preserved the survival of irradiated cells and increased SOD, GSH-Px and CAT activity. ExSOD induced an increase of the activity of all examined enzymes.*

Conclusion: *A balanced enhancement in endogenous antioxidative activity may be the cause of the increased radioresistance of K562 SOD-pretreated cells.*

Key words: *antioxidative enzymes, irradiation, K562 cells, radioprotection*

Introduction

Oxidation, induced by reactive oxygen species (ROS), has long been known to play an important role in radiation effects and carcinogenesis as well [1-8].

Radioresistance of cells depends on its species, type of irradiation, delivered dose, different irradiation intervals and the kind of delivery (single or fractionated) [9,10]. Processes involved in radioresistance include various signal transduction pathways at nuclear and extranuclear levels [10-12]. Cell cycle arrest, induction of apoptosis and promotion of DNA-repair increase the radioresistance of cells subjected to irradiation *in vitro* and *in vivo* [13,14].

The role of endogenous antioxidative enzymes in defense from irradiation has been examined both *in vivo* and *in vitro* [6,7,15,16]. One important family of enzymes is the SOD [17,18]. They act inside and outside the cell, immediately after irradiation, and also in the post-irradiation

Correspondence to: Visnja Bogdanovic, PhD. University of Novi Sad, Medical Faculty, Oncology Institute of Vojvodina, Department for Experimental Oncology, Put doktora Goldmana 4, 21204 Sremska Kamenica, Serbia. Tel: +381 21 480 55 88,
E-mail: visnja.bogdanovic@gmail.com
Received: 26/11/2013; Accepted: 08/12/2013

Table 1. Experimental grouping schedule

Experimental group	*SOD pretreatment*	*Irradiation*	*Cell number*	*Protein concentration and SOD, GSH-Px, CAT activity*
U	-	-	+	+
U+SOD	-	+	+	+
I	+	-	+	+
I+SOD	+	+	+	+

U: unirradiated cells, U+SOD: unirradiated cells pretreated with SOD, I: irradiated cells, I+SOD: irradiated cells pretreated with SOD

period [19-21].

Beside their physiological generation inside the cell, superoxide radicals ($O2^{\cdot -}$) are ensued at irradiation through water radiolysis. Superoxide is short-lived and does not readily cross bio-membranes, but it is converted through SOD to longer-lasting and membrane-diffusible hydrogen peroxide (H_2O_2) [22]. Hydrogen peroxide has broad regulatory and oxidative roles and can be further degraded to water by other antioxidant enzymes, such as GSH-Px and CAT [23]. It is supposed that H_2O_2 has a precise turnout in the cell differentiation through its activity in signal transduction [9,24].

Some investigators reported about overexpression of copper (Cu), and manganese (Mn) SOD after irradiation, but most consistently known consequence of x-ray application is elevation in MnSOD [22,25]. The removal of superoxide radicals by MnSOD in the mitochondria is a critical step in preventing radiation-induced cell death [26]. Overexpression of MnSOD blocks mitochondrial membrane permeability and the release of cytochrome C, probably by decreasing the superoxide radical level [27]. Also, IL-1 and TNFα have been noticed to activate MnSOD expression on RNA and protein level [28,29].

Studies on biological protection afforded by ExSOD included cell lines and experimental animals, as well as humans in clinical investigations [21,30-33]. Various strategies such as elevating the level of SOD inside and/or outside the cells have been employed [32,34]. ExSOD has beneficial effects in cases of postirradiation-induced dermatitis, fibrosis and diseases such as rheumatoid arthritis (RA), Crohn's disease and progressive systemic sclerosis (PSS) [20,35].

In this study, we determined LD_{50} of K562 cell line and examined the 96-hrs cell survival and the activity of three major antioxidative enzymes (SOD, GSH-Px and CAT) after irradiation with 30 Gy, as well as their activity when the cells were pretreated with SOD.

Methods

The K562 cell line (human erythroleukemia) was routinely grown in 6-well tissue culture plates (Costar, USA) in RPMI 1640 medium (Sigma, New York, USA) supplemented with 2mM glutamine, 10% heat-inactivated foetal calf serum (NIVNS, Veterinary Institute, Novi Sad, Serbia), 100 IU/ml penicillin (ICN, USA) and 100 µg/ml streptomycin (ICN, USA). Cells were cultured at 37 °C in a humidified 5% carbon dioxide atmosphere. Exponentially growing K562 cells were used throughout the assays [13].

The cells were seeded in culture flasks at a number of $0.85 x 10^6$/40 mL RPMI 1640 medium.

In the first part of the experiment, the cells were irradiated with 20-32.5 Gy, using a LINAC, Mevatron Siemens MD 7475, 10 MV X-rays, with a dose rate of 3 Gy/min and cultured up to 96 h after irradiation [15]. The trypan blue exclusion test (DET) was performed by mixing 50µL of cell suspension with 200 µL of 0.1% trypan blue solution in 0.9% NaCl. After 2 min of incubation at room temperature, the number of viable (unstained) cells was determined using a Burker–Turk hemocytometer [36].

The number of surviving cells was calculated by dividing the mean number of living cells at each experimental group of samples by the mean number of living cells of the unirradiated control group at each point of the experiment. Survival diagrams were plotted as the percent of the surviving fraction of cells. All counts were repeated four times.

After selection of the irradiation dose (30 Gy), the samples were divided into four groups: 1. Control group (unirradiated samples); 2. Experimental group of unirradiated SOD-treated samples; 3. Experimental group of irradiated samples; and 4. Experimental group of irradiated and SOD-pretreated samples (Table 1).

For the antioxidant treatment of cells, the RPMI 1640 cell culture medium was supplemented with SOD (Peroxinorm CN953745 lot 17, Pliva, Zagreb, Croatia) at 10^{-6} M concentration, and the incubation continued for one hour at 37 °C.

The chosen experimental groups were irradiated with 30 Gy, as previously stated.

The number of living cells was determined by DET according to Black, as previously described [36].

To obtain the cytosolic fraction, the cells were cen-

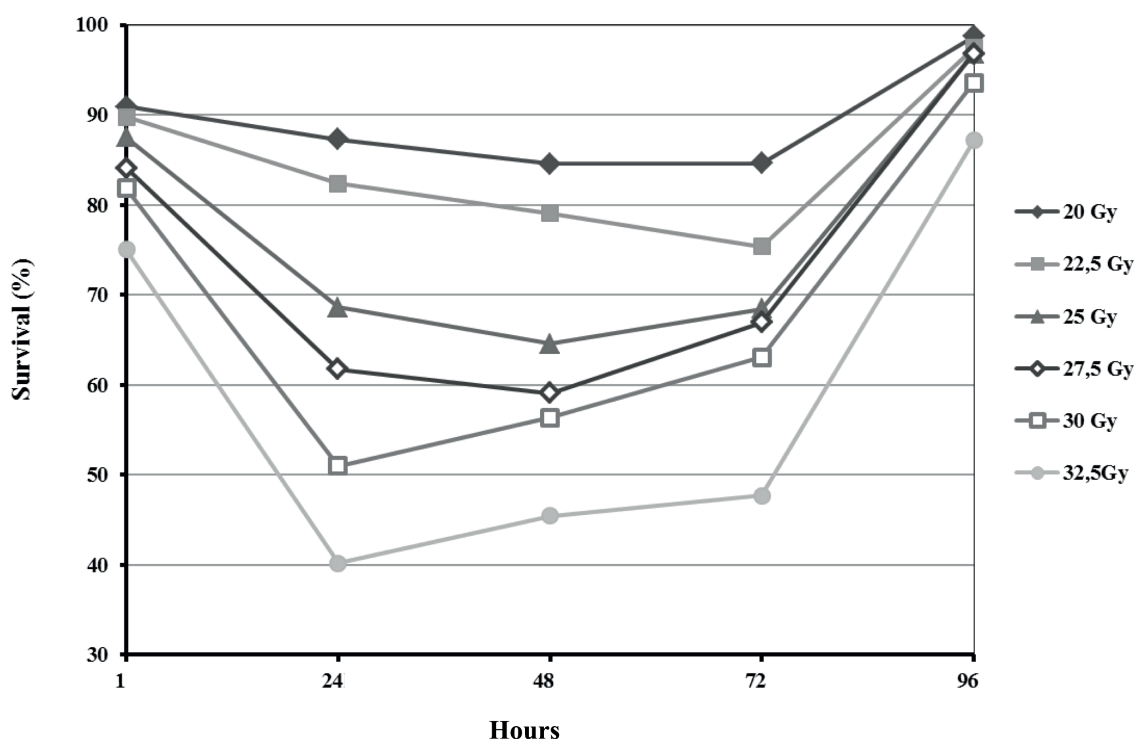

Figure 1. Dose and time dependent survival of K562 cells subjected to irradiation (20 and 32.5 Gy) and cultured up to 96 hrs. Results from a single experiment are shown and are representative of two further independent experiments.

trifuged (10 min/1200 rpm), resuspended in saline and then frozen (–20 °C) and thawed (37 °C) for three times and finally centrifuged (10 min/10,000 rpm).

The protein concentration [37] and the activity of antioxidant enzymes SOD [38], GSH-Px [39], and CAT [40] were monitored in all experimental samples 1,24,48,72 and 96 hrs post irradiation.

Statistics

All the experiments were performed in triplicate and enzyme analysis in quadruplicate. Results from a single experiment are shown and are representative of 2 further independent experiments. The results were expressed as mean±standard deviation of the mean (SDM).

The data were analyzed by multivariate analysis of variance (MANOVA) followed by Duncan's test at 0.05 significance level to compare the means using the SPSS 13.0 for Windows.

Results

The survival of K562 cells was strongly dependent on the experimental time and the applied irradiation doses (Figure 1). High survival at all applied doses was registered in the 1st and the 96th hrs after irradiation. In the 1st h of the experiment, irradiation had an insignificant influence on survival, and the same was true for the 96th h when the cells had fully recovered from irradiation effects. LD_{50} of K562 cells was achieved with 30 Gy 24 hrs after irradiation.

Cells incubated with ExSOD and irradiated with 30 Gy grew in a similar fashion with the control cells up to 72 hrs after initiation of the experiment (Figure 2). At 96 hrs, a greater number of ExSOD-treated cells was found to survive when compared with untreated cells (Figure 2). The cells, preincubated with ExSOD prior to irradiation, showed increased survival at 24 and 48 hrs postirradiation when compared to irradiated cells (Figure 2). However, this was not seen at 72 and 96 hrs (Figure 2).

The activity of total endogenous SOD was elevated at all time points when cells were treated with ExSOD (Figure 3). This observation was also true for cells that were preincubated with ExSOD prior to irradiation (Figure 3). In the absence of ExSOD treatment, total endogenous SOD activity was increased only at 24 hrs postirradiation, but its activity decreased at later time points (Figure 3).

In the control, non-irradiated samples, changes in the GSH-Px activity were similar to those previously observed. Only at 24 hrs after irradiation, an increase in the activity of GSH-Px was observed (Figure 4). In cells treated with ExSOD, a substantial increase in GSH-Px activity was shown at 96 hrs (Figure 4).

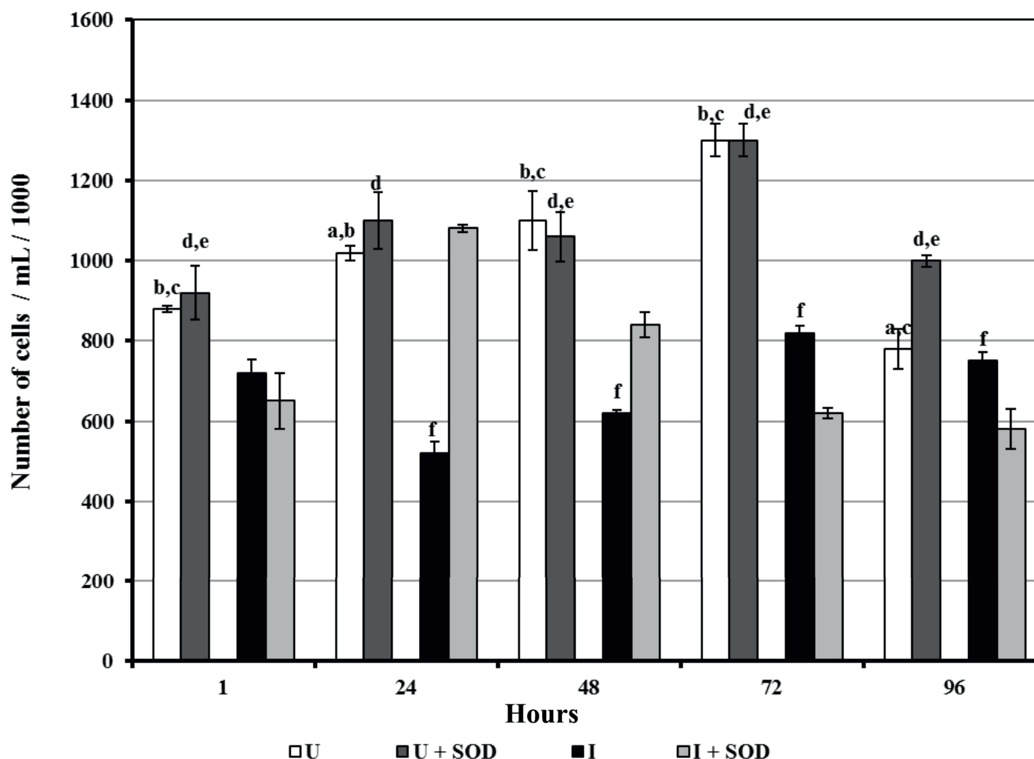

Figure 2. Long-term survival of K562 cells subjected to irradiation, incubated with SOD or a combination of both treatments. U: unirradiated cells; U+SOD: unirradiated cells pretreated with SOD; I: irradiated cells; I+SOD: irradiated cells pretreated with SOD. Results from a single experiment are shown and are representative of two further independent experiments. Means pairs followed by different letters are significantly different ($p<0.05$) by Duncan's test, n = 4. Legend: a) U↔U+SOD; b) U↔I; c) U↔I+SOD; d) U+SOD; e) U+SOD↔I+SOD; f) I↔I+SOD.

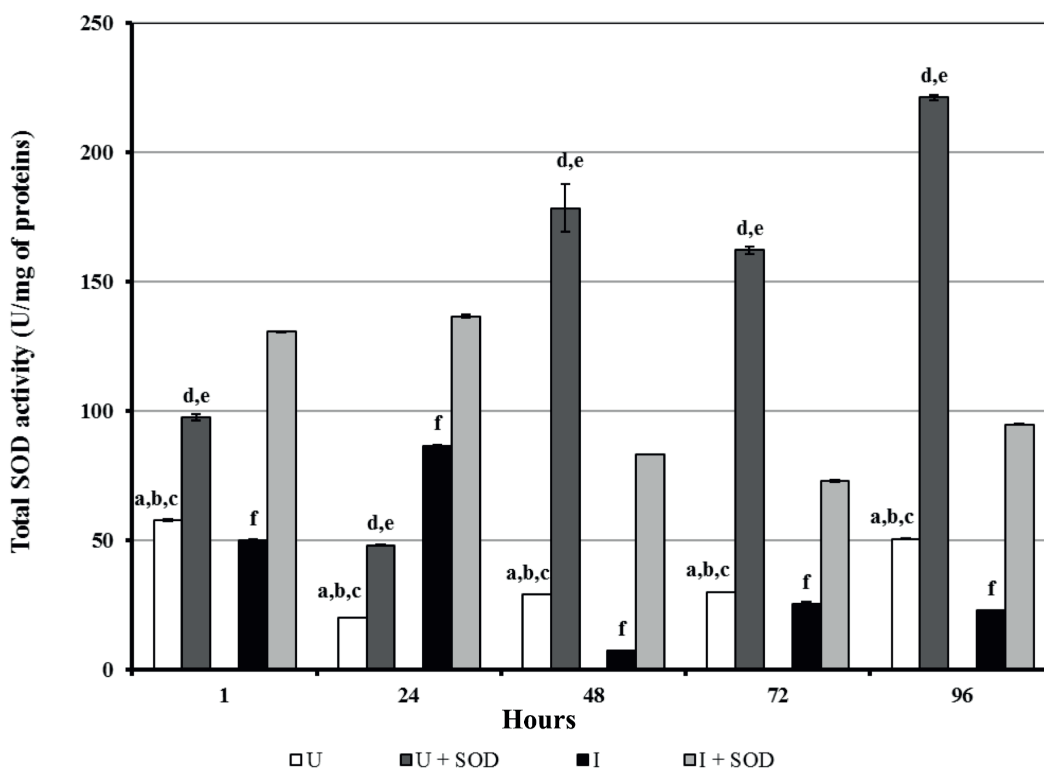

Figure 3. Time course of total endogenous SOD activity in K562 cells subjected to irradiation, incubated with SOD or a combination of both treatments. U: unirradiated cells; U+SOD: unirradiated cells pretreated with SOD; I: irradiated cells; I+SOD: irradiated cells pretreated with SOD. Results from a single experiment are shown and are representative of two further independent experiments. Means pairs followed by different letters are significantly different ($p<0.05$) by Duncan's test, n = 4. Legend: a) U↔U+SOD; b) U↔I; c) U↔I+SOD; d) U+SOD; e) U+SOD↔I+SOD; f) I↔I+SOD.

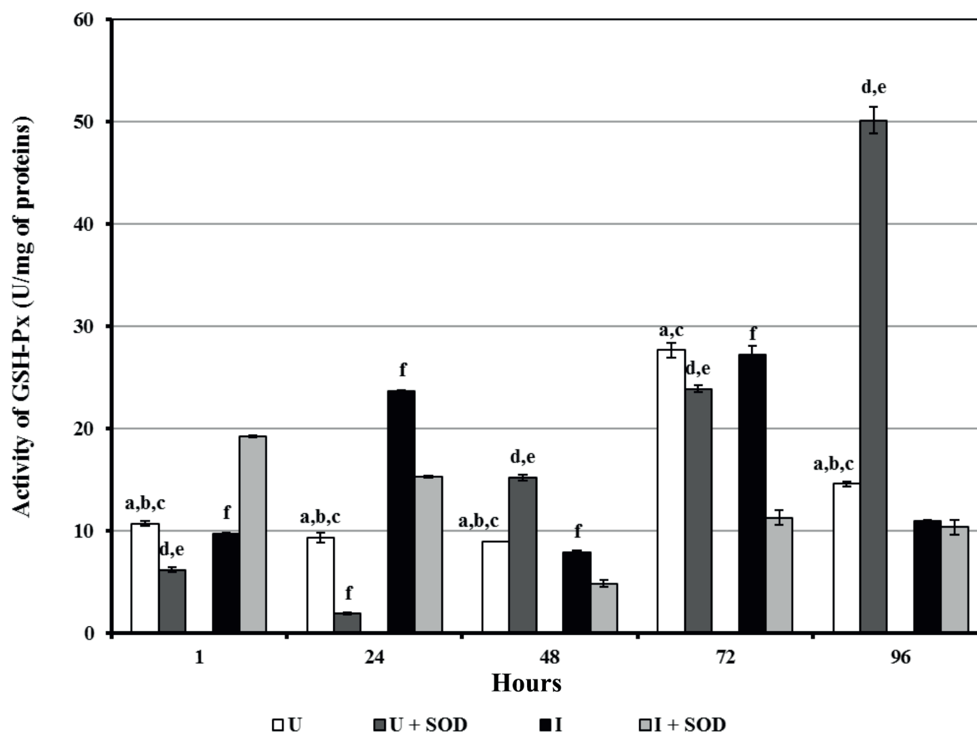

Figure 4. Time course of endogenous GSH-Px activity in K562 cells subjected to irradiation, incubated with SOD or a combination of both treatments. U: unirradiated cells; U+SOD: unirradiated cells pretreated with SOD; I: irradiated cells; I+SOD: irradiated cells pretreated with SOD. Results from a single experiment are shown and are representative of two further independent experiments. Means pairs followed by different letters are significantly different ($p<0.05$) by Duncan's test, n = 4. Legend: a) U↔U+SOD; b) U↔I; c) U↔I+SOD; d) U+SOD; e) U+SOD↔I+SOD; f) I↔I+SOD.

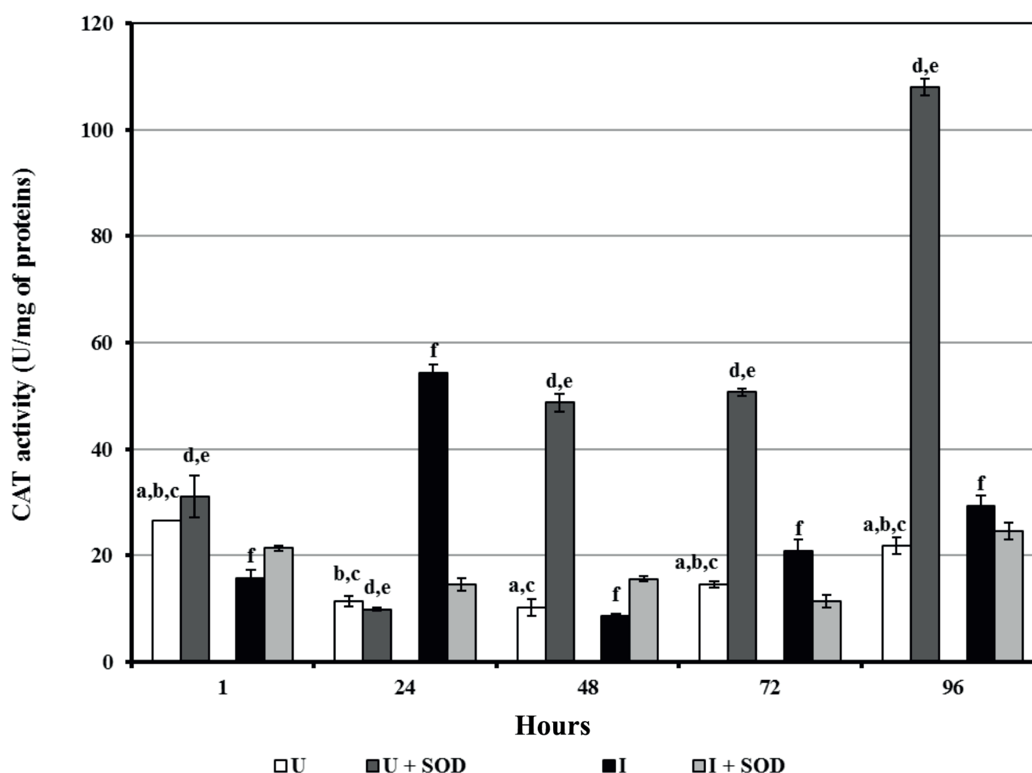

Figure 5. Time course of endogenous CAT activity in K562 cells subjected to irradiation, incubated with SOD or a combination of both treatments. U: unirradiated cells; U+SOD: unirradiated cells pretreated with SOD; I: irradiated cells; I+SOD: irradiated cells pretreated with SOD. Results from a single experiment are shown and are representative of two further independent experiments. Means pairs followed by different letters are significantly different ($p<0.05$) by Duncan's test, n=4. Legend: a) U↔U+SOD; b) U↔I; c) U↔I+SOD; d) U+SOD; e) U+SOD↔I+SOD; f) I↔I+SOD.

CAT activity showed a similar trend when compared to the activity of GSH-Px. A substantial increase in CAT activity was only found 24 h postirradiation (Figure 5). CAT activity was greater in SOD-treated cells than in untreated cells at 48, 72 and 96 hrs.

Discussion

It is well known that leukemic cells have a higher vulnerability to ionizing radiation than normal mononuclear cells [9]. On the contrary, some authors report very potent radioresistance capacity of leukemic/ K562 cells [41,42]. In the present investigation, K562 cells showed significant resistance to irradiation in a dose-dependent manner, with a LD_{50} of 30 Gy 24 h postirradiation.

The basic low SOD activity and overproduction of O_{2^-} in cancer cells may render them highly dependent on SOD for survival [43]. Exogenous sources of superoxide generation, such as irradiation, have additional deleterious effect. Irradiation-caused inhibition of SOD leads to free radical damage of membranes and apoptosis of cancer cells [43]. In our experiment, the number of K562 cells irradiated with 30 Gy was reduced to 50% after 24 hrs. Later on, recovery of the number of surviving cells was noticed. Twenty-four hrs postirradiation was the critical time point for finishing of the apoptotic processes, as well as for enhancement of the enzyme activity. After the initial decrease due to irradiation, all of three major enzymes were strongly activated in the survived cell population to defend cells from oxidative damage. We registered a significant increase in SOD, GSH-Px and CAT activity 24 hrs after irradiation. This is in correlation with investigations which confirmed that antioxidant mechanisms are activated around 24hrs after irradiation, thus eliminating the levels of ROS by metabolizing them to neutral products [21,44].

Exogenously added SOD does not penetrate readily into the cells, but it may scavenge superoxide radicals outside the cell and therefore serves as a first line of defense against irradiation [5]. In the experiment, the added Ex-SOD was supposed to serve as a scavenger of exogenous superoxide radicals and producer of H_2O_2. In SOD-pretreated and irradiated group, the results implied that the survival in this group was at the same level as in the control group during the whole experiment.

Also, it was noticed in our study that preincubation with SOD protects cells from injury immediately after irradiation. In the 1st h after the X-ray delivery, there was a significant increase in the activity of all monitored enzymes in the groups pretreated with SOD in comparison to the irradiated groups. These results support the hypothesis that SOD may act around and within the irradiated cells by scavenging the short-lived superoxide radicals produced at the time of irradiation, as well as those produced as a result of oxidative metabolism after the exposure.

Furthermore, accumulating evidence suggests that reactive oxygen species (ROS) are not only injurious byproducts of cellular aerobic metabolism but also essential participants in cell signaling and regulation. MnSOD, which may be induced by free radical challenge, is synthesized in the cytosol and post-translationally modified for transport into the mitochondria [45]. In the cells exposed to adverse conditions, increased activity of MnSOD is a survival factor that holds mitochondrial integrity [3,46]. Increased MnSOD increases the concentration of endogenously produced H_2O_2. At 24th h postirradiation, the intracellular production of H_2O_2 was a secondary event [21]. It was shown that intracellular H_2O_2 is involved in enhancing/modulating the activity of NF-kB in a cell type-specific manner [47]. The response of NF-kB to H_2O_2 depends on time, concentration, and the regime of exposure [48] . Thus, it seems that exogenous SOD produces H_2O_2, which, according to Adzic et al., induces MnSOD expression and increases the intracellular H_2O_2 production, triggers NF-kB thereby forming a positive feed-forward loop changing the redox potential towards oxidation [49].

It is well known that small changes in the redox potential towards oxidation may significantly elevate the proliferative capacity of malignant cells [50]. In response to that schedule, an increase in the antioxidative enzymes SOD, GSH-Px and CAT activities led to restore a reduced state of the cell. Together with SOD and GSH-Px, CAT constitutes the primary defense against ROS and may provide resistance to the effects of chemotherapy and ionizing radiation. The obtained results were in agreement with these findings.

In conclusion, it may be reported that K562 cells show significant resistance to irradiation in a dose-dependent manner. Irradiation at the applied dose of 30 Gy has a strong effect on survival with LD_{50} 24 hrs postirradiation. After the primary inhibition due to irradiation, all of the three major enzymes are activated together to repair cells from oxidative injury. The results indicate that pretreatment with SOD protects cells from irradiation-induced apoptosis.

However, because the balance of the enzymes is of utmost importance for oxidative protection, every impact on this equilibrium- in this case in excess of SOD in the absence of irradiation- may lead to misbalance, which should change the response in terms of sensitivity to oxidative stress. This condition might make cells vulnerable to further injury.

Acknowledgement

We are thankful to Prof.Gordana Grubor-Lajsic and to Dr. Gordana Bogdanovic for their kind support, and to Prof. Mihajlo Spasic for cooperation and editorial assistance with the manuscript.

References

1. Whitaker SJ. DNA damage by drugs and radiation; What is important and how is it measured? Eur J Cancer 1992;28:273-276.
2. Schmidt-Ullrich RK, Dent P, Grant S, Mikkelsen RB, Valerie K. Signal transduction and cellular radiation responses. Radiat Res 2000;153:245-257.
3. Motoori S, Hideyuki JM, Masaaki E et al. Overexpression of mitochondrial manganese superoxide dismutase protects against radiation-induced cell death in the human hepatocellular carcinoma cell line HLE. Cancer Res 2001;61:5382-5388.
4. Kang MA, So YI, Simons AL, Spitz DR, Ouchi T. DNA damage induces reactive oxygen species generation through the H2AX-Nox1/Rac1 pathway. Cell Death Disease 2012;3:1-8.
5. Michelson AM, Puget K. Cell penetration by exogenous superoxide dismutase. Acta Physiol Scand 1980;492:67-80.
6. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-6055.
7. Simović M, Spasić BM, Michelson AM. Free radicals in human myocardial reperfusion injury. Life Chem Rep 1985;12:227-270.
8. Southgate TD, Sheardi V, Milsom MD et al. Radioprotective gene therapy through retroviral expression of manganese superoxide dismutase. J Gene Med 2006;8:557-565.
9. Nevoie A, Pascariu M, Jitaru D et al. Investigation of apoptosis in normal and leukemic cells induced by x-ray irradiation. Digest J Nanomaterials Biostructures 2011;6:259-264.
10. Haro H, Haro KJ, Scott AC et al. Mechanisms of resistance to high and low linear energy transfer radiation in myeloid leukemia cells. Blood 2012;120:2087-2097.
11. Bourguignon MH, Gisone PA, Perez MR et al. Genetic and epigenetic features in radiation sensitivity Part I: Cell signaling in radiation response. Eur J Nucl Med Mol Imaging 2005;32:229-246.
12. Yu H. Typical Cell Signaling Response to Ionizing Radiation: DNA Damage and Extranuclear Damage. Chin J Cancer Res 2012;2:83-89.
13. Zhao Y, Cui Y, Han J, Ren J, Wu G, Cheng J. Cell division cycle 25 homolog c effects on low-dose hyper-radiosensitivity and induced radioresistance at elevated dosage in A549 cells. J Radiat Res 2012;5:686-694.
14. Grace MB, Singh VK, Rhee JG, Jackson WE, Kao TC, Whitnaill MH. 5-AED enhances survival of irradiated mice in a G-CSF-dependent manner, stimulates innate immune cell function, reduces radiation-induced DNA damage and induces genes that modulate cell cycle progression and apoptosis. J Radiat Res 2012;88:296-310.
15. Halliwell B, Gutteridge JMC (Eds). Oxidative stress: adaptation, damage, repair and death. In: Free Radicals in Biology and Medicine (3rd Edn). Oxford University Press, Oxford, United Kingdom, 1999, pp 246-350.
16. Noaman E, Zahran AM, Kamal AM, Omran MF. Vitamin E and selenium administration as a modulator of antioxidant defense system: biochemical assessment and modification. Biol Trace Elem Res 2002;86:55-64.
17. Fridovich I. Superoxide radical and superoxide dismutase. Annu Rev Biochem 1985:64:87-112.
18. Azzam EI, Toledo SM, Spitz DR, Little JB. Oxidative Metabolism Modulates Signal Transduction and Micronucleus Formation in Bystander Cells from Particle-irradiated Normal Human Fibroblast Cultures. Cancer Res 2002;62:5436-5442.
19. Rabbani ZN, Anscher MS, Folz RJ et al. Overexpression of extracellular superoxide dismutase reduces acute radiation induced lung toxicity. BMC Cancer 2005; 59 doi:10.1186/1471-2407-5-59.
20. Manzanas GA, Carrizosa MC, Ocana VC et al. Superoxidase dismutase (SOD) topical use in oncologic patients: treatment of acute cutaneous toxicity secondary to radiotherapy. Clin Transl Oncol 2008;10.3:163-167.
21. Floratou K, Giannopoulou E, Antonacopoulou A et al. Oxidative stress due to radiation in CD34+ hematopoietic progenitor cells: protection by IGF-1. J Radiat Res 2012;5:672-685.
22. Brieger K, Schiavone S, Miler FJ, Krause KH. Reactive oxygen species: from health to disease. Swiss Med Wkly 2012;142:136-159.
23. Robbins D, Zhao Y. Oxidative Stress Induced byMn-SOD-p53 Interaction: Pro- or Anti-Tumorigenic? J Signal Transd 2012;1-13.

24. Chenais B, Andriolo M, Guiraud P, Belhoussine R, Jeannesson P. Oxidative stress involvement in chemically induced differentiation of K562 cells. Free Rad Biol Med 2000;1:18-27.
25. Veldwijk MR, Herskind C, Sellner L et al. Normal-tissue radioprotection by overexpression of the copper-zinc and manganese superoxide dismutase genes. Strahlenther Onkol 2009;185:517-523.
26. Epperly MW, Gretton JE, Sikora CA et al. Mitochondrial Localization of Superoxide Dismutase Is Required for Decreasing Radiation-Induced Cellular Damage. Radiat Res 2003;160:568-578.
27. Epperly MW, Sikora CA, De Filippi SJ et al. Manganese Superoxide Dismutase (SOD2) Inhibits Radiation-Induced Apoptosis by Stabilization of the Mitochondrial Membrane. Radiat Res 2002;157:568-577.
28. Eastgate J, Moreb J, Nick HS, Suzuki K, Taniguchi N, Zucali JR. A role for manganese superoxide dismutase in radioprotection of hematopoietic stem cells by interleukin-1. Blood 1993;81:639-646.
29. Brown CO, Salem K, Wagner B et al. Interleukin-6 counteracts therapy-induced cellular oxidative stress in multiple myeloma by up-regulating manganese superoxide dismutase. Biochem J 2012;444:515-527.
30. Edeas MA, Peltier E, Khalfoun Y, Lindenbaum A. Immunocytochemical study of uptake of exogenous carrier-free copper-zinc superoxide dismutase by peripheral blood lymphocytes. Cell Mol Biol 1996;42:1137-1143.
31. Lefaix JL, Delanian S, Leplat JJ et al. Successful treatment of radiation-induced fibrosis using and Mn-SOD: An experimental study. Int J Radiat Oncol Biol Phys 1996;35.2:305-312.
32. Secilmis M, Kiroglu O, Ogulener N. Role of superoxide dismutase enzymes and ascorbate in protection of nitrergic relaxation against superoxide anions in mouse duodenum. Acta Pharmacol Sin 2008;29:687-697.
33. Trott DW, Gunduz F, Laughlin MH, Woodman CR. Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. J Appl Phys 2009;106:1925-1934.
34. Veldwijk MR, Trah J, Wang M et al. Overexpression of Manganese Superoxide Dismutase Does Not Increase Clonogenic Cell Survival Despite Effect on Apoptosis in Irradiated Lymphoblastoid Cells. Radiat Res 2011;176:725-731.
35. Niwa Y, Somiya K, Michelson AM, Puget K. Effect of liposomal-encapsulated superoxide dismutase on active oxygen-related human disorders. A preliminary study. Free Rad Commun 1985;2:137-153.
36. Black MC, Berenbaum B. Factors affecting the dye exclusion test for cell viability. Exp Cell Res 1985;3:9-13.
37. Lowry OH, Rosenbrough AL, Farr AL, Randall RJ. Protein Measurement with the Folin phenol Reagent. Bio Chem 1951;193:265-267.
38. Mc Cord JM, Fridovich I. The Reduction of Cytochrome C by Milk Xanthine Oxidase. J Biol Chem 1968;243:5753-5760.
39. Paglia DE, Valentine NW. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidases. J Lab Clin Med 1967;7:74-77.
40. Clauborne A, Greenwald RA (Eds). Catalase Activity. CRC Handbook of Methods For Oxygen Radical Research. Boca Raton, Fl, 1984, pp 283-284.
41. Bogdanović G, Pražić B, Kerenji A, Kuzmanović Z, Nikolić V. The activity of antioxidative enzymes in irradiated K562 cell culture-Experimental in vitro model. Inform Kancer 1993;10:43-46 (in Serbian).
42. Bogdanović V, Stankov K, Icevic et al. Fullerenol $C_{60}(OH)_{24}$ effects on antioxidative enzymes activity in irradiated human erythroleukemia cell line. J Radiat Res 2008;49:321-327.
43. Huang P, Feng L, Oldham E, Keating MJ, Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. Nature 2000;407:390-395.
44. Spitz DR, Azzam EI, Li JJ et al. Metabolic oxidation/reduction reactions and cellular responses to ionizing radiation: a unifying concept in stress response biology. Cancer Metastasis Res 2004;23:311-322.
45. Holley AK, Bakthavatchalu V, Velez-Roman JM, St Clair DK. Manganese Superoxide Dismutase: Guardian of the Powerhouse. Int J Mol Sci 2011;12:7114-7162.
46. Veldwijk MR, Herskind C, Laufs S, Zeller WJ, Fruehauf S, Wenz F. Recombinant adeno-associated virus 2-mediated transfer of the human superoxide dismutase gene does not confer radioresistance on HeLa cervical carcinoma cells. Radiother Oncol 2004;72:341-350.
47. Tolando R, Jovanović A, Brigelius–Flohe R, Ursini F, Maiorino M. Reactive oxygen species and proinflammatory cytokine signaling in endothelial cells: effect of selenium supplementation. Free Radic Biol Med 2000;28:979-986.
48. Bajić A, Spasić M, Andrjus PR et al. Fluctuating vs. Continuous Exposure to H2O2: The Effects on Mitochondrial Membrane Potential, Intracellular Calcium, and NF-kB in Astroglia. PLoS One 2013; 8:e76383. doi:10.1371/journal.pone.0076383
49. Adzić M, Nićiforović A, Vucić V et al. Systemic NF-kappa B activation in blood cells of breast cancer patients. Redox Rep 2011;11:39-44.
50. Dayem AA, Choi HY, Kim JH, Cho SG. Role of Oxidative Stress in Stem, Cancer, and Cancer Stem Cells. Cancers 2010;2:859-884.