

Antioxidant Dose Response in Human Blood Cells Exposed to Different Types of Irradiation

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The research work was conducted to investigate the changes of superoxide dismutases (SODs) activity in human blood cells irradiated with 22.6 MeV protons, and to compare the results with examinations performed with x- and γ- rays. The activity of SOD was significantly lowered after 1 Gy of proton beam and increase of dose further decreases total SOD activity. The effect of proton beam is same in the cases of cytosol SOD (CuZnSOD) and mitochondrial SOD (MnSOD), which means that both defense lines are disturbed already after 1Gy proton beam irradiation, which is very important in the radio therapy of tumor cells. This dose response is compared to dose of x- or γ - irradiation, the difference is obvious. The increased MnSOD activity is induced by *in vitro* irradiation with 2 Gy of x - rays and after 4 Gy of MnSOD activity is on the control level again. The measured activity of mitochondrial SOD shows that the most striking fall after irradiation with 3 Gy or 4 Gy of γ-rays.

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Introduction

As it is known, exposure of cells to ionizing radiation produces reactive oxygen species (ROS) that are associated with radiation-induced cytotoxicity (Yamaguchi *et al.*, 1994). The antioxidant (AO) enzymes, MnSOD and CuZnSOD are of major importance in scavenging the reactive oxygen species. The radiation induced changes in SOD activity might provide the cells a way of defending itself against the oxygen radicals (St Clair *et al.*, 1992). Olinski *et al.* (1998) reported that enzymes represent the key component of the defense mechanism of cells and tissue protection from harmful effects of free reactive radicals, which influence the genome and other bio-molecules of cells. The exposure of cancer patients to therapeutic doses of ionizing radiation causes base modifications in genomic DNA of lymphocytes. Sun *et al.* (1998) investigated the role of antioxidant enzymes on ionizing radiation resistance by analyzing cell morphology, cell viability, and cellular radio-resistance in stably transfected CHO cell lines over-expressing MnSOD, CuZnSOD and glutathione peroxidase (GSH-Px). He found that over-expression of human MnSOD substantially protected cells from ionizing radiation injury. The results suggested that MnSOD may play a central role in protecting cells against reactive oxygen species injury during ionizing radiation exposure among MnSOD, CuZnSOD and GSH-Px.

Hardmeier *et al.* (1997) irradiated radiation-resistant (RR) and radiationsensitive (RS) mice and studied SOD, catalase (CAT) and GSH-Px at the transcriptional and activity level. The findings of higher SOD and CAT activities in RR than in RS animals could point to a role for these AO enzymes for the process of radiation sensitivity. Singh *et al.* (1996) studied levels of antioxidants in haemolysates from breast cancer patients after chemo- and radiotherapy. They showed that levels of antioxidant glutathione and antioxidant enzymes such as SOD, CAT and glutathione-S-transferase were decreased. Punnonen *et al.* (1995) examined the effects of chronic UVB irradiation on the human epidermis. Chronic UVB irradiation was shown to be accompanied by induction of epidermal SOD activity *in vivo*, while the activities of the other antioxidant enzymes were not significantly changed.

Joksić *et al.* (2000) suggest that oxidative stress conditions related to irradiation exposure trigger an antioxidant response that includes change in the activity of SOD. The main aim of this investigation is to compare antioxidant dose response in human blood cells, particularly in harvested lymphocytes, after irradiation with proton beam in relation to x and γ -irradiation.

Materials and Methods

Irradiation: The proton irradiation was performed with the 15 MV Tandem Accelerator in INFN - Laboratori Nazionali del Sud, Catania. The experiment was performed with a 3.5 cm diameter round beam. The dose homogeneity was obtained by scattering the protons through a 250 μm -thick lead foil and 150- μm thick brass foil located 155 and 210 cm from the distal part of the collimator, respectively. For dose response heparinized whole blood samples obtained from healthy donors were irradiated in plastic chambers (outer dimensions 28 x 28 x 5 mm³) in a central portion of a un-modulated beam at the beginning of the peak. Dose response was evaluated using quantification of activity of SOD employing doses from 1 - 4 Gy at dose rate 1 Gy min⁻¹. Within the whole experiment the dose rate varied about 4%.

For purposes of comparison aliquot of 1.5 ml of whole blood obtained from donors in a sterile plastic test-tubes placed in a plexiglas container 15 x 15 cm² and irradiated using 300 kVp x-rays, 10 mA, 2.7 mm CuHVT and ⁶⁰Co as a source of γ -rays. The radiation doses employed were 1 - 4 Gy, the

dose-rate 1 Gy min⁻¹, the dimensions of the radiation field were 20 x 20 cm², and the distance from the source 74 cm. All samples were irradiated at room temperature (IAEA, 1986).

Blood culture and enzyme assays: Three hours after irradiation lymphocyte cultures were established for each blood sample irradiated with protons and lymphocyte cultures from each dose of x- and γ -irradiated samples. Cultures containing 0.5ml whole blood, 8ml of RPMI-1640 medium supplemented with 20% of fetal calf serum, 2mM L-glutamine and 2.4 μg ml⁻¹ phytohemagglutinin were kept for 48 hours at 37°C (Moorhead *et al.*, 1960). After 48 hours cells were spun down, the medium and serum removed. Cells were washed two times in PBS, centrifuged 300 x g for 10 min. The pellet was resuspended in approx. 500 μl of the remaining solution and kept frozen at -70°C before it was used for the subcellular fractionation. The procedure of McCord and Fridovich (1969) was used with minor modifications. Hemoglobin was removed by adding chloroform and ethanol (De La Torre *et al.*, 1996) and after centrifugation during 10 min. on 1200 x g, the activity of SOD was measured by the method of Misra and Fridovich (1972). The reaction of auto-oxidation of epinephrine to adrenochrome was performed in 3 ml of 0.05 M Na₂CO₃ at pH 10.2 and inhibition was monitored at 480nm. After assaying the total SOD activity, the samples were treated with 4mM KCN in order to inhibit cytosolic SOD (Geller and Winge, 1983). The values thus obtained and the differences between the two measurements were considered as MnSOD and CuZnSOD, respectively. Protein concentration was determined by the method of Lowry *et al.* (1951). The results were analyzed by Student's t-test. Differences between means were considered significant at p < 0.05.

Results

The specific activity of MnSOD and CuZnSOD in lymphocyte cultures following irradiation with different doses (1-4 Gy) of x-rays, γ -rays or protons have been shown in Table 1. The activity of MnSOD in lymphocytes culture, found to be 20.9 \pm 1.9 units mg⁻¹ protein, was markedly increased (p < 0.05) following irradiation with 2 Gy of x-rays (Fig. 1). The activity of CuZnSOD was slightly decreased only after doses of 3Gy and 4 Gy of x-irradiation (Fig. 2).

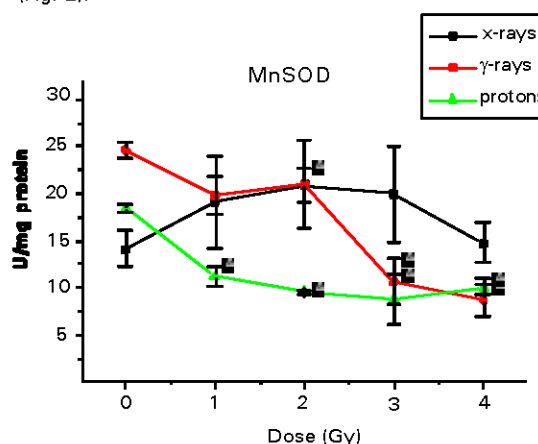


Fig. 1: MnSOD activity in lymphocyte cultures; unirradiated controls vs. irradiated samples with dose of 1, 2, 3 or 4 Gy respectively. The number per each group is four (n=4). The lines represent mean values \pm SEM. *p < 0.05

Table 1: Specific activity of MnSOD and CuZnSOD in lymphocyte cultures

Activity of enzyme (U mg ⁻¹ protein)	Dose (Gy)	x-rays	γ-rays	Protons
MnSOD	0	14.1 ± 1.9	24.5 ± 0.9	18.4 ± 0.5
	1	19.0 ± 4.9	19.7 ± 2.1	11.2 ± 1.2 *
	2	20.9 ± 1.9 *	20.9 ± 4.6	9.6 ± 0.2 *
	3	19.9 ± 5.1	10.6 ± 2.5 *	8.7 ± 2.7 *
	4	14.8 ± 2.2	8.7 ± 1.7 *	10.1 ± 0.8 *
CuZnSOD	0	75.4 ± 11.0	60.3 ± 18.0	138.1 ± 9.1
	1	81.1 ± 13.5	46.6 ± 9.3	83.9 ± 11.0 *
	2	76.8 ± 13.4	43.5 ± 5.3	69.5 ± 4.2 *
	3	58.3 ± 8.8	47.2 ± 10.2	44.2 ± 6.3 *
	4	53.4 ± 10.5	30.4 ± 8.5	34.1 ± 11.8 *

* p < 0.05

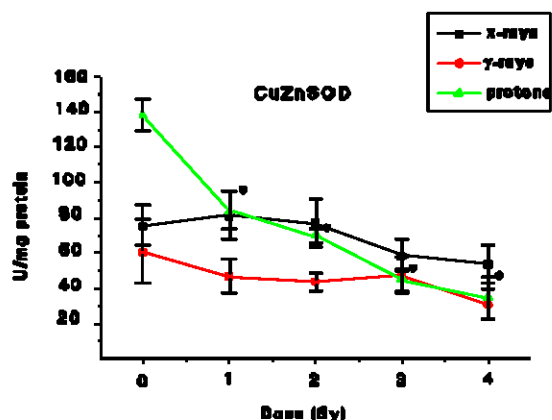


Fig. 2: CuZnSOD activity in lymphocyte cultures; unirradiated controls vs irradiated samples with dose of 1, 2, 3 or 4 Gy respectively. The number per each group is four (n = 4). The lines represent mean values ± SEM. *p < 0.05

Measured activity of MnSOD showed that the most striking fall after *in vitro* irradiation with 3 Gy (10.6 ± 2.5) and 4 Gy (8.7 ± 1.7) of γ-rays in comparison to unirradiated control (24.5 ± 0.9) (Fig. 1). The doses of 1 - 4 Gy of γ-rays was not significantly changed the activity of cytosol SOD (Fig. 2). The activity of both SOD was markedly suppressed by the irradiation with dose of 1 Gy of proton beam unirradiated vs. irradiated (Fig. 1) and CuZnSOD: 138.1 ± 9.1 vs. 83.9 ± 11.0 units mg⁻¹ protein respectively (Fig. 2). The increase of the dose further decreases of CuZnSOD activity (Fig. 2).

Discussion

Antioxidants reduce the oxidative damage which is produced by the radiation stress. Vitamin E, uric acid, SOD and albumin protect the stress-induced oxidative damage in plasma and liver, however, few studies have been directed to stress-induced brain oxidative damage (Kiecolt-Glaser *et al.*, 1985). Some of the enzymes factors determine how normal blood cells respond to ionizing radiation. Special emphasis is given to recent insights into the antioxidant mechanisms underlying the intrinsic radiosensitivity or radioresistance of blood cells. The modulation of SOD activity in radiation dose response could point to a role for these AO enzymes for the process of radiation sensitivity (Rosen *et al.*, 1999). The dose response after the irradiation with protons is significantly different in relation to the dose response to x and γ irradiation. The dose of 1Gy proton beam already breaks down the AO defense system, which represents the convenience in radiotherapy of

different types of tumors. If the surplus of SO is not removed as a consequence hydroxyl radicals are produced which are very reactive and toxic. Free radicals attacks on biomolecules such as DNA, proteins and lipids and they initiate lipid peroxidation and the release of hydrolytic enzymes. After the destruction of cellular components, the tumor cells are bound to die. Thus, it was shown that the activities of SOD, CAT, GPx, glutathione reductase (GR) and glutathione transferase (GST) in red blood cells, in patients with oral cancer showed a significant decrease, representing the lack of AO defense. Radiotherapy induces lipid peroxidation by inactivating the AO enzymes, thereby rendering the system inefficient in management of the free radical attack (Sabitha and Shyamaladevi, 1999).

Following *in vitro* irradiation with 2 Gy of x-rays the activity of SOD is induced, which means that the AO defense system is preserved. In the case with γ-rays with dose of 3 Gy or 4 Gy significantly decreased only MnSOD activity, but the activity of CuZnSOD was not affected by these radiation doses. Bravard *et al.* (1999) also investigated the involvement of human lymphoblast AO enzymes in adaptive response to 3 Gy γ-radiation triggered by a low-dose (0.02 Gy) exposure 6 hours earlier. Soon after irradiation the activities of MnSOD, GST, Gpx, and CAT were slightly higher in adapted than in non-adapted cells. Increased activities of AOE observed soon after the dose of 3Gy γ-rays would result in a rapid scavenging of radicals and consequently less damage in adapted cells. The authors concluded that due to the moderate alterations of AOE, the activation of AO defenses would only partly contribute to the protective mechanism underlying the radioadaptation of lymphoblasts. The homeostasis of cells and adaptation to irradiation should be governed by oxidative balance. Therefore, 2 Gy doses of x-rays or γ-rays, which are used in radiotherapy enable the creation of the radio-resistance in healthy cells, especially in tumors cells, which have the powerful AO defense and repair system (Lindegard *et al.*, 1996; Thames *et al.*, 1989). On the other hand, the AO system is disturbed already after 1 Gy proton beam irradiation, which indicates on the advantage of proton beam in the radiotherapy of tumor cells in relation to x and γ irradiation.

Some of the clinical and genetic factors that may predispose patients to the development of complications of radiotherapy. Thus, the molecular and cellular basis of radiosensitivity is very important as a predictive assay for estimate the dose and quality of radiotherapy. Implications for understanding how normal tissues and tumors respond to therapeutic radiation introduce the concept of new quality in radiotherapy (Hellman and Weichselbaum 1994; Vijayakumar and Chen 1995). This study have led to the optimization of radiation treatment schedules.

The results suggested that SODs, particularly MnSOD, to have a role in clinical radiotherapy, appropriate scheduling of new type of therapies must take into account of their dose-dependent effects in order to achieve clinical radiosensitization of tumor cells (Suit and Urie 1992; Jones *et al.*, 1995; Pajović *et al.*, 2000).

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