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Influence of occupational exposure to low-dose ionizing radiation on the plasma activity of superoxide dismutase and glutathione level

Uticaj profesionalne ekspozicije malim dozama jonizujućeg zračenja na aktivnost superoksid dismutaze i nivo glutationa u plazmi

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Abstract

Background/Aim. During exposure to low-level doses (LLD) of ionizing radiation (IR), the most of harmful effects are produced indirectly, through radiolysis of water and formation of reactive oxygen species (ROS). The antioxidant enzymes – superoxide dismutase (SOD): manganese SOD (MnSOD) and copper-zinc SOD (CuZnSOD), as well as glutathione (GSH), are the most important intracellular antioxidants in the metabolism of ROS. Overproduction of ROS challenges antioxidant enzymes. The aim of this study was to examine if previous exposure to low doses of IR induces adaptive response by means of stimulation of intracellular antioxidant defense system. **Methods.** We investigated a group of medical workers occupationally exposed to IR (n = 44), 29 male and 15 female. The controls (n = 33) consisted of medical workers not exposed to IR, 23 male and 10 female. The examinees from both groups worked in the same environment and matched in crucial characteristics. All measurements were performed by a calibrated thermoluminiscent dosimeter type CaF2:Mn. SOD activity and GSH content were measured spectrophotometrically in the plasma of both groups of medical workers. Half of each blood sample was irradiated by 2Gy of γ radiation, dose-rate 0.45 Gy/min, and the distance from the source of 74 cm. **Results.** The dosimetry results indicate that occupational doses were very low. Our results confirmed significantly higher SOD activity in the exposed vs. unexposed workers ($p < 0.00006$). SOD activity after irradiation of blood samples failed to show a significant difference between the exposed workers and the controls ($p = 0.905$), even the difference in each group before and after the irradiation was significant. In blood samples of the exposed workers expression of enzymes after the irradiation, was not as high as in the controls, or even in the case of the exposed in nuclear medicine personnel, SOD activity was decreased. There were no significant differences in the content of GSH between the groups. **Conclusion.** Our results pointed out that occupational exposure to low doses of IR compromised mitochondrial function. During occupational exposure, the activity of antioxidant enzymes was increased as a protection against the increased production of ROS. After high-dose irradiation dysfunction of mitochondrial system was noticed, suggesting the break-down of antioxidant defense and failure of an adaptive response. Therefore, the "chronic oxidative stress" might reduce antioxidant defense in the case of accidental exposure to high doses of IR. It could indirectly increase the incidence of some other "free radicals' diseases" in occupationally exposed personnel.

Key words:
occupational exposure; radiation, ionizing; antioxidants; superoxide dismutase; glutathione; oxidative stress.

Apstrakt

Uvod/Cilj. Najveći deo štetnih efekata jonizujućeg zračenja (JZ) nastalih tokom profesionalnog izlaganja malim dozama (MDJZ) nastaje indirekno, radiolizom vode i produkcijom kiseoničnih slobodnih radikala (ROS). Antioksidativni enzimi – superoksid dismutaza (SOD): mangan SOD (Mn-SOD), bakar-cink SOD (CuZn-SOD), kao i glutation najznačajniji su u metabolizmu i neutralizaciji ROS. Povećana produkcija ROS uzrokuje povećanje aktivnosti antioksidativnih enzima. Cilj rada bio je da se utvrdi da li dugotrajna ekspozicija MDJZ indukuje povećanje aktivnosti enzima, odnosno adaptivni odgovor. **Metode.** Ispitali smo grupu medicinskih radnika profesionalno izloženih JZ (n = 44), 29 muškaraca i 15 žena i grupu neeksponiranih radnika – kontrolna grupa (n = 33), 23 muškarca i 10 žena. Ispitanici obe grupe radili su u istim uslovima veštačke klimatizacije. Grupe su bile odgovarajuće po svim relevantnim karakteristikama. Lična dozimetrija urađena je kalibrisanim termoluminiscentnim dozimetrima. Aktivnost enzima i sadržaj glutationa određivani su spektrofotometrijski u plazmi obe grupe ispitanika. Pre određivanja, polovina svakog uzorka krvi ozračena je dozom od 2 Gy γ zračenja, brzinom doze od 0,45 Gy/min, sa udaljenosti 74 cm od izvora Co-60. **Rezultati.** Rezultati dozimetrije pokazuju da su profesionalne doze jonizujućeg zračenja bile veoma niske. Utvrđena je značajno veća aktivnost SOD kod eksponiranih u odnosu na neeksponirane osobe ($p < 0,00006$). Posle ozračivanja uzoraka krvi, nije bilo razlike u aktivnosti enzima između grupa ($p = 0,905$), iako je došlo do značajne promene aktivnosti

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unutar svake grupe. Porast aktivnosti bio je manji kod prethodno eksponiranih osoba, a kod zaposlenih na nuklearnoj medicini došlo je do pada enzimske aktivnosti. Nije bilo razlike u sadržaju redukovanog glutationa među ispitivanim grupama. **Zaključak.** Rezultati našeg istraživanja ukazuju da profesionalna ekspozicija MDJZ kompromituje funkciju mitohondrija. Profesionalno izlaganje MDJZ dovodi do porasta aktivnosti antioksidativnih enzima kao odgovor na povišenu produkciju ROS. Posle izlaganja visokim dozama JZ dolazi do disfunkcije mitohondrijalnog sistema, kada je insuficijencija antioksidativne odbrane moguća, ali ne i dalja adaptacija. Stoga, "hronični oksidativni stres" izgleda da slabi antioksidativnu odbranu u mogućem akcidentalnom izlaganju visokim dozama JZ.

Ključne reči:
profesionalna izloženost; zračenje, jonizujuće; antioksidansi; dismutaza, superoksidna; glutation; stres, oksidativni.

Introduction

Biological effects of ionizing radiation (IR) are induced by two processes: direct - damaging of deoxyribonucleic acid (DNA) or indirect - generating free-radicals. During the exposure to low-level doses (LLD) of IR, the dominant way is the indirect one, which is based on kinetic energy transfer from the particles or photons to the existing molecules. This transfer induces radiolysis of water and formation of reactive oxygen species (ROS). The main product is a superoxide, which is transformed to other ROS products. This process is considered a cause of 70% of biological effects [1–6].

Parallel to ROS overproduction, cells are stimulated to increase their expression of antioxidants. The antioxidant enzymes – superoxide dismutase (SOD): manganese SOD (MnSOD), and copper-zinc SOD (CuZnSOD), as well as glutathione (GSH), are the most important intracellular antioxidants in the metabolism of ROS. In the cells exposed to IR a higher activity of the enzymes is noticed [7–10].

It should be noted that many conflicting findings of ROS overproduction due to irradiation, as well as activity/higher content of antioxidants are published, especially for chronic LLD exposure. It is also still unclear, if a previous exposure to LLD of IR induces an adaptive response, expressed as a lower rate of harmful effects during a later exposure to high doses [11–22].

These unsolved problems in radiobiology could affect medical practice in radiation protection of the occupationally exposed or protection of the people exposed in a contaminated environment. Considering the important role of ROS in certain diseases is not directly connected to radiation, as well as the role of antioxidants in cell-protective processes, the aim of this study was to examine if chronic/occupational exposure to LLD of IR induce higher activity/content of antioxidants and if they are really protective against ROS generated by chronic low or accidental high doses of IR.

Methods

We compared the activity of total SOD (tSOD), MnSOD, CuZnSOD, and GSH in the blood of people exposed to known low dose of low linear energy transfer (LET) IR and people not exposed to IR. Therefore, we investigated the group of medical workers occupationally exposed to IR (n = 44), 29 male and 15 female. The exposed medical workers were divided in two subgroups: Ex-exposed to x-rays in radiology, and En-exposed to γ-rays in nuclear medicine. The controls (n = 33) consisted of medical workers occupationally not exposed to IR, 23 male and 10 female. The examinees from both groups worked in the same environment and matched in crucial characteristics.

All the measurements were performed by a calibrated thermoluminiscence dosimeter (TLD) type CaF2:Mn. The values of TLD were read after 30 days, worn on the upper pocket, under the lead protective apron. The TLD had known radiological and thermal history, density of 3.18 g/cm^3, and were highly sensitive to low energy with a wide range of measurement (μGy–2 kGy). The accumulated radiation dose was calculated on the basis of individual (TL) dose records multiplied by the exposure time [23–25].

For measuring SOD expression a sample of 5 ml blood was taken in a sterile plastic test-tube prepared with 0.05 ml heparin and 0.1 ml ethylenediamine tetraacetic acid (EDTA). The samples were centrifuged at 3 000 g for 15 minutes to separate the plasma. The standard procedure was performed to separate hemolysate [26]. Half of each sample was poured in a sterile plastic test-tube placed in a Plexiglas container 15 × 15 cm, and irradiated by 60-Co source of γ-ray at room temperature. The employed radiation dose (challenge dose) was 2 Gy, dose-rate 0.45 Gy/min, and distance from the source 74 cm. All blood samples were kept frozen at -70 °C until the analyses performed simultaneously.

Activity of SOD was measured spectrophotometrically, as an inhibition of epinephrine autooxidation at 480 nm [27]. The analysis was performed in sodium carbonate buffer (50 mmol, ph 10.2; Serva, Feinbiochemica, Heilderberg, New York) containing 0.1 mmol EDTA [Sigma, St. Louis, United States of America (USA)], after additing 10 mmol epinephrine [Sigma, St. Louis, (USA)]. The activity of mitochondrial SOD (MnSOD) was measured in the same way after the addition of potassium cyanide (KCN), and the activity of cytosolic SOD (CuZnSOD) was calculated as a difference in tSOD and MnSOD. The activity of SOD was expressed as a number of international units per mg Hb (U/mg Hb). The international unit was defined as an activity which induces 50% inhibition of epinephrine autooxidation.

The reduced GSH was determined using 5.5-dithiobis-2-nitrobenzoic acid (DTNB), 36.9 mg in 10 ml of methanol, which reacted with aliphatic thiol compounds in Tris-HCl buffer (0.4 mol, pH 8.9), thus producing yellow-colored p-nitrophenol anion. Color intensity was used for spectrophotometric measurement of GSH concentration at 412 nm [28].

All the values were presented as the mean value ± standard deviation. Mann-Whitney U test, and Kruskal Wallis

test were used as nonparametric tests, with $p < 0.05$ considered statistically significant, and $p < 0.01$ highly significant. The correlation between dependent values (before and after the irradiation) was evaluated by Wilcoxon test. A correlation between SOD, GSH values and other possibly influencing parameters were evaluated by regression analysis.

Results

The tested groups were matched in significant parameters. The characteristics of the examinees are presented in table 1.

The results of personal dosimetry of the three subsequent years were analyzed and used to estimate mean doses. Mean annual radiation doses are presented in table 2.

The obtained results confirmed significantly higher tSOD activity in the workers occupationally exposed to LD of IR ($p < 0.00006$). The difference between the controls and the subgroups was significant ($p = 0.0006$, and $p = 0.0008$), while it was not significant between the subgroups ($p = 0.5981$).

The values of tSODo activity after the irradiation showed the lack of significant difference between the occupationally exposed workers and the controls ($p = 0.905$), as well as between the controls and the subgroups of the exposed workers ($p = 0.905$, $p = 0.897$, $p = 0.751$).

The difference between dependent values (tSOD and tSODo) was highly significant in the controls ($p = 0.00017$), but not in the exposed group, as well as in the subgroups ($p = 0.2829$, $p = 0.2171$, $p = 1.000$).

Table 1

Characteristics of the examinees

Characteristics	Exposed	Controls	*p*
Mean age (years)	45.00±6.81	44.18±6.31	0.77
Gender [n (%)]			
female	15 (34.10)	10 (30.30)	0.73
male	29 (65.90)	23 (69.70)	
Alcohol consumption [n (%)]	27 (61.36)	15 (45.45)	0.1653
Smoking habit [n (%)]	19 (43.18)	13 (39.39)	0.738
Smoking duration (years)	9.068±11.979	8.938±12.732	0.988
Cigarettes per day	8.29±10.83	6.06±8.72	0.527
Dietary style	"National cousin"	"National cousin"	
Supplements	no	no	

Table 2

Mean annual radiation doses

Dose* (mS/v)	Ex (x̄±SD.)	Range	En (x̄±SD.)	Range	*p*
D_{y1}	3,315±3,2731	0,100–11,200	0,752±0,6558	0,100–2,360	$p = 0,0013$
Dy_2	3,145±3,2503	0,060–10,700	0,716±0,5856	0,200–2,230	$p = 0,0296$
Dy_3	2,151±2,1705	0,020–7,560	0,651±0,4839	0,140–1,910	$p = 0,0250$
Dy_{mean}	2,870±2,7842	0,150–9,203	0,706±0,5578	0,147–2,167	$p = 0,0289$

* radiation dose of the three subsequent years (Dy); Ex-workers exposed to x-rays in radiology; En-workers exposed to γ-rays in nuclear medicine

The mean values of tSOD activity before and after (tSODo) irradiation are presented in figure 1.

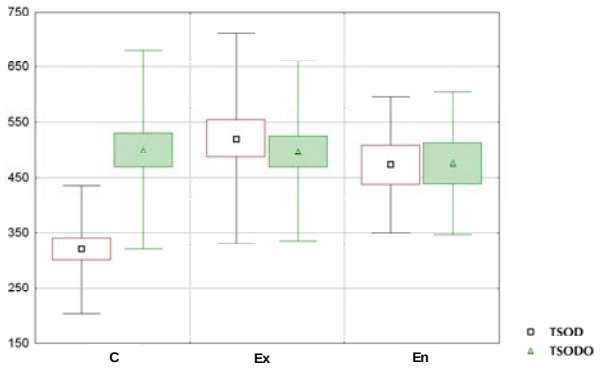

Fig. 1 – Mean values of total superoxide dismutase before (tSOD) and after (tSODo) irradiation

C – the controls; Ex – workers exposed to x-rays in radiology; En – workers exposed to γ-rays in nuclear medicine

The mean values of MnSOD activity before and after (MnSODo) irradiation are presented in figure 2.

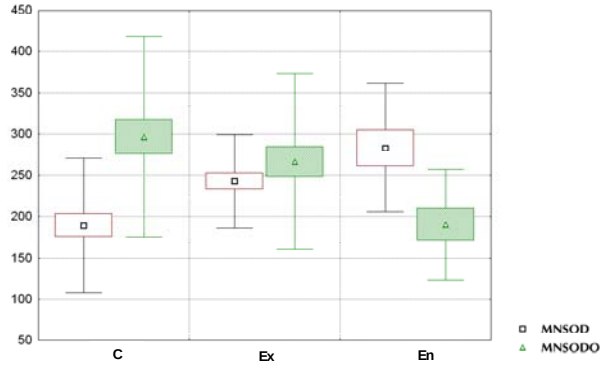

Fig. 2 – Mean values of mitochondrial superoxide dismutase before (MnSOD) and after (MnSODo) irradiation

C – the controls; Ex – workers exposed to x-rays in radiology; En – workers exposed to γ-rays in nuclear medicine

The obtained results confirmed significantly higher MnSOD activity in the workers occupationally exposed to LD of IR vs. the controls ($p = 0.0105$). The difference between the controls and the subgroups was significant ($p = 0.0105$, $p = 0.024$), while it was not significant between the subgroups, but was very close to it ($p = 0.0613$).

The values of MnSODo activity after irradiation showed the lack of significant difference between occupationally exposed workers and the controls ($p = 0.358$), but it was significant between the controls and the subgroups En, as well as between the subgroups ($p = 0.005$, $p = 0.030$).

The difference between dependent values (MnSOD and MnSODo) was highly significant for the controls and the subgroup En ($p = 0.00158$ and $p = 0.0076$, respectively), but not in the whole exposed group and the subgroup Ex ($p = 0.4488$, and $p = 0.3126$).

The mean values of CuZnSOD before, and after (CuZnSODo), irradiation are presented in figure 3.

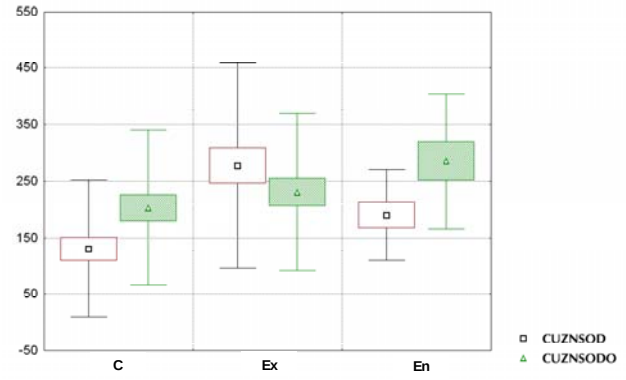

Fig. 3 – Mean values of copper – zink superoxide dismutase before (CuZnSOD) and after (CuZnSODo) irradiation
C – the controls; Ex – workers exposed to x-rays in radiology; En – workers exposed to γ-rays in nuclear medicine

The obtained results confirmed significantly higher CuZnSOD activity in the workers occupationally exposed to LD of IR vs. the controls ($p = 0.001$). The difference between the controls and subgroups Ex and En was significant, too ($p = 0.00001$, $p = 0.015$), while it was insignificant between the subgroups ($p = 0.205$).

The values of CuZnSODo activity after the irradiation showed the lack of significant difference between the occupationally exposed workers and the controls ($p = 0.386$). The difference was significant between the controls and the subgroup En ($p = 0.0040$), but it was not significant between the controls and the subgroup Ex, as well as between the subgroups ($p = 0.386$, and $p = 0.170$, respectively).

The difference between dependent values (CuZnSOD and CuZnSODo) was highly significant for the controls and the subgroup Ex ($p = 0.01614$ and $p = 0.0496$, respectively), but not in the whole exposed group and subgroup En ($p = 0.4984$ and, $p = 0.0843$, respectively).

The mean values of GSH before and after the irradiation are presented in figure 4.

The obtained result showed the lack of significant difference between the occupationally exposed workers and the controls ($p = 0.599$), as well as the controls and exposed subgroups ($p = 0.599$, and $p = 0.837$, respectively), and between the subgroups ($p = 0.705$).

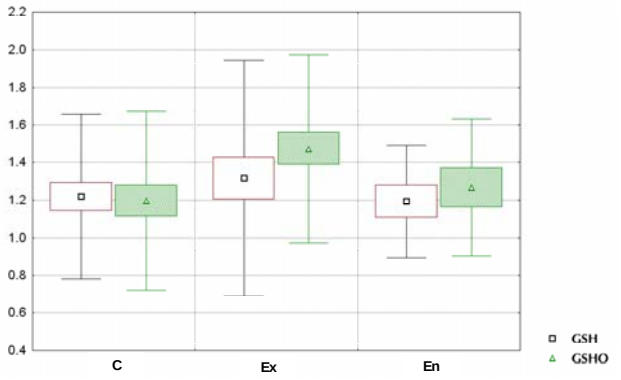

Fig. 4 - Mean values of reduced glutathione before (GSH) and after (GSHo) irradiation
C – the controls; Ex – workers exposed to x-rays in radiology; En – workers exposed to γ-rays in nuclear medicine

The values of GSH activity after the irradiation showed the lack of significant difference between the occupationally exposed workers and the controls ($p = 0.086$), as well as between the controls and the subgroups ($p = 0.08$ and ($p = 0.411$, respectively), as well as between the subgroups ($p = 0.170$).

There was no significant difference in glutatione content in any of the examined correlations ($p = 0.6358$, $p = 0.1047$, $p = 0.1206$, $p = 0.5829$), respectively.

Discussion

Exposure of the cells to many exogenous harmful factors can result in overproduction of ROS. One of them is IR. Radiation generates the same species of activated oxygen as they occur spontaneously (superoxide, hydrogen peroxide, and hydroxyl radical) [1, 29–31].

Besides the quantity, some experiments indicate that radiation-induced ROS are larger, distributed by the particle track, and produced in a shorter time, which makes them more harmful to cells. Low-doses of IR challenge the antioxidant enzymes and induce the increase of their activity. In the case of subsequent exposure to high doses, it could be considered as stress response, and the whole process as adaptation. If it is so, it should result in a lower rate of harmful effects through mobilization of protective mechanisms (antioxidant defense, DNA repair, proliferation of immune-competent cells) and intensifying mechanism against already produced damage (apoptosis, removal of damaged cells by immune system) [15, 32–34].

The results of cellular research projects could not be clinically applied because multicellular organisms have evolved additional supracellular responses to radiation damage in order to limit the damage and keep homeostasis. Supracellular-tissue response is not a simple sum of cellular responses and is often significantly different from a single cell response. It could be increased by the bystander effects and genomic instability or decreased by the adaptation. Addition-

ally, the effects of chronic exposure to low doses vs. acute exposure are different, too. Therefore, the effects of chronic exposure to low doses, as occupational exposure, should be studied for the improvement of radiation protection [1, 35, 36].

Data from the literature on oxidative stress and antioxidants in occupationally exposed personnel are limited and describe elevated production of ROS. Investigation of occupational exposure to IR in flying crews, as from KLM company, confirmed oxidative stress induced by radiation [37]. We have previously published results of a study revealing that occupational exposure of medical personnel to very low doses of IR induces oxidative stress, measured through the overproduction of superoxide and malondialdehyde [38]. This overproduction is involved in various pathological processes, ranging from aging to malignancy [34, 39–42].

Our results confirm significantly higher activity of tested antioxidant enzymes (tSOD, MnSOD, CuZnSOD) in the examinees exposed to IR. These results are in accordance with the results of other authors [37]. In the exposed group the activity of enzymes decreased with cumulative doses and with age [43]. The activity of MnSOD was highly correlated with the activity of CuZnSOD and GSH ($p = 0.05$).

Even SOD activity was significantly different, there were no significant differences in the content of GSH in our groups. Basic level of GSH was influenced by many factors. In both groups, it was depleted in smokers and it was in accordance with other investigations [7, 37, 43]. In the exposed persons, GSH level was inversely correlated with doses and exposure time, which could be correlated with long-lasting oxidative stress. GSH, as a main source for regeneration of other antioxidants, especially for the regeneration and increase of SOD, was significantly correlated with mitochondrial function presented through MnSOD activity ($p = 0.05$). This correlation indicated that sensitivity of the cell to ROS induced by IR is limited by the GSH level, as it was the case in some other form of oxidative stress [17, 44, 45].

High-dose irradiation induced overexpression of SOD enzymes in both groups, more in the controls than in the exposed workers and after that, there were no significant differences between them. The only difference was a decrease in MnSODo activity in the En subgroup. Our results turned out to show that MnSODo activity depended on age and smoking which correlates with literature date [43]. The activity of MnSODo was in correlation with that of other enzymes after the irradiation, as well as activity of enzymes before a high-dose irradiation. Most off all, the activity of MnSODo highly significantly inversely correlated with cumulative dose of IR ($p = 0.00$). It could be considered as confirmation of the previous conclusion that a long-lasting exposure to LLD of IR is a factor of reducing the protective capacity for subsequent low or high doses.

Although the level of GSHo increased after a high-dose irradiation, it was only slight and insignificant. GSH level significantly correlated with age, smoking, previous occupational exposure, production of ROS, GSH and MnSOD before irradiation.

Enzymes investigated in this study are very important for many physiological and pathological processes, especially aging and cancerogenesis. Therefore, the "chronic oxidative stress" which reduces antioxidant defense, as nonspecific, could indirectly increase the incidence of some other "free radicals' diseases" in occupationally exposed personnel [46–55].

Conclusion

Our results pointed out that occupational exposure to low doses of IR compromised mitochondrial function. During occupational exposure, the activity of antioxidant enzymes was increased as a protection against the increased production of ROS. After high-dose irradiation dysfunction of mitochondrial system was noticed, suggesting the breakdown of antioxidant defense and failure of an adaptive response. Therefore, the "chronic oxidative stress" might reduce antioxidant defense in the case of accidental exposure to high doses of IR. It could indirectly increase incidence of some other "free radicals' diseases" in occupationally exposed personnel.

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