Journal of
APPLIED
BIOMEDICINE

J. Appl. Biomed.
6: 195–201, 2008
ISSN 1214-0287
www.zsf.jcu.cz/jab

ORIGINAL ARTICLE

Changes in selected parameters of the antioxidant system in radiation damage to the organism

Leoš Navrátil[1, 4], **Jaroslav Racek**[2], **Renata Havránková**[1], **Ladislav Beránek**[3], **Zuzana Freitinger Skalická**[1], **Hana Siffnerová**[1], **Eva Kantorová**[1]

[1]University of South Bohemia, Faculty of Health and Social Studies, Department of Radiology and Toxicology, České Budějovice, Czech Republic
[2]Charles University, Medical Faculty, Institute of Clinical Biochemistry and Hematology, Plzeň, Czech Republic
[3]University of South Bohemia, Faculty of Education, Department of Informatics, České Budějovice, Czech Republic
[4]Charles University, 1. Medical Faculty, Institute of Biophysics and Informatics, Praha, Czech Republic

Received 1st October 2008.
Revised 8th December 2008.
Published online 12th December 2008.

Summary
The target of the present work was a study of the oxidation-reduction system of the organism damaged with ionizing radiation. The parameters chosen for examination were: the superoxide dismutase activity in erythrocytes, the concentration of reduced glutathione in erythrocytes, the activity of glutathione peroxidase in the total blood, the concentration of malondialdehyde in the blood plasma, and the antioxidant capacity of blood plasma. These parameters were monitored in four groups (patients after radiotherapy, volunteers whose blood was submitted to *in vitro* irradiation, employees of a nuclear power plant, and a group of healthy persons). A statistically significant decrease in the GSHPx activity was measured in patients after radiotherapy and in employees of the nuclear power plant. Results of processing the data obtained by the method of multidimensional analysis of variance suggest that the dynamics of the changes is comparable in the group of patients after radiotherapy and employees of the nuclear power plant on the one hand, and in the control group and group of healthy volunteers whose blood was subjected to *in vitro* irradiation, on the other. The study demonstrated changes concerning certain parameters of the oxidation-reduction system after the action of ionizing radiation.

Key words: antioxidant system – blood plasma – ionizing radiation

✉ Leoš Navrátil, University of South Bohemia, Faculty of Health and Social Studies, Department of Radiology and Toxicology, Matice školské 17, 370 01 České Budějovice, Czech Republic
leos.navratil@volny.cz
☎ +420 387 730 342

INTRODUCTION

Considerable attention is currently being paid to research into the organism oxidation-reduction system for diagnostic and therapeutic purposes. This also applies to studying interactions between this system and damage to the organism with ionizing radiation. Protection of the organism

against oxidative damage is based on a system in which antioxidants and whole groups of these substances interact with each other. Functions of one antioxidant frequently present conditions for the effects of some other link in the system. The antioxidant system as a whole is adapted to changes of reduction-oxidation conditions of the organism and environment.

Superoxide dismutase (SOD) is an enzyme providing removal of the most common free radical, superoxide (O_2^-). The danger of the superoxide inheres in the production of considerably harmful reactive forms of oxygen – *inter alia* of hydrogen peroxide, the hydroxylic radical, and peroxynitrite or hypochlorous acid. The most dangerous product – the hydroxylic radical – has a short biological half-life (of order of magnitude 10^{-9} s). No effective mechanism for its removal is available and living organisms aim at "prevention" with the aim of removing excess superoxide and thus preventing the production of these hydroxylic radicals. The oxidative stress accompanied by superoxide production induces SOD synthesis; in this process, the SOD type, reacting by enhanced synthesis, depends on the subcellular location of free radical production (Yanagisawa et al. 1997, Burlakova and Naidikh 2004).

Glutathione peroxidase (GSHPx) is an enzyme catalyzing glutathione oxidation, in the course of which reduced glutathione is converted into oxidized glutathione. This enzyme can, however, be active only in the presence of sufficient amounts of reduced glutathione (GSH), and this case can occur only if the pentose cycle delivers sufficient amounts of reductive equivalents in the form of NADPH (Kumar et al. 1986). The reduced glutathione (GSH) is known to exert at least partial protective effects on erythrocytes, provided that it decomposes hydrogen peroxide and fatty acid hydroperoxides in reactions catalyzed with GSH peroxidase (Steinerová et al. 1995, Racek et al. 1999).

The antioxidative capacity of the blood plasma (AOC) is expressed as a sum of all the substances exerting antioxidative effects in the blood plasma and their concentration is given in mmol/l of water-soluble vitamin E (Trolox) exerting the same antioxidant activity (Lefevre et al. 1998).

The non-enzyme peroxidation of lipids is induced by a non-specific factor that is frequently pathological. It comprises a mixture of different products including hydrocarbons (ethane, pentane) and toxic aldehydes malondialdehyde (MDA) and 4-hydroxynonenal that are strongly bound to proteins, thus modifying their viability and function. Because of this, the degree of peroxidation of lipids can be assessed based on the MDA level (Štípek 2000).

The disturbance of the equilibrium between the reduction and removal of reactive forms of oxygen and nitrogen is referred to as the oxidative stress. It can be induced by an excess production of reactive forms of oxygen and nitrogen, insufficient functioning of the antioxidative protecting system or by a combination of these two failures. The excess production of reactive forms of oxygen is encountered in certain metabolic situations, after reoxygenation of tissues subsequent to ischemia, after intake of xenobiotics exerting oxidative-reductive action and also after further stimuli. It is easy to understand that these pathological conditions also include the damage to the organism with ionizing radiation. The few published studies, which include a work that appeared as early as 1980 (Czapski 1980), suggest that in this field, there is a lack of work considering the relationships between radiation dose and the mechanism of radiation action on the organism, and also later changes resulting from damage to the organism (Wang et al. 2003, Butomo et al. 2004).

Data in the literature are certainly not uniform in the investigation of the SOD activity after irradiation. Some works describe decreases in the activity e.g. in the kidneys after a whole-body exposure to a dose of 15 Gy in guinea pigs (Bukan et al. 2003) or in the rat blood plasma after doses of 2 x 360 cGy (Russanov et al. 1979, Kaya et al. 1999) and after lower doses of 20 cGy (Yamaoka et al. 1994). In contrast, other works describe increased activities in the rat myocardium (after a whole-body dose of 18 Gy (Jin et al. 2005) or in the rat hypothalamus (Kandasamy et al. 1993).

There are also controversial results of evaluating GSHPx activity. Bukan et al. (2003) describe a decrease in the activity in the kidneys of the guinea pig after exposure to a whole-body dose of 8 Gy and above, and similarly – in other authors – in the rabbit blood after a whole-body dose of 5.5 Gy (Deger et al. 2003), in the rabbit hypothalamus (Kandasamy et al. 1993) and in the rat blood plasma (Kaya et al. 1999). In contrast, after essentially lower radiation doses (20 cGy) the activity increases (Yamaoka et al. 1994).

The GSH concentration is closely correlated with the GSHPx activity. Absolute values in particular tissues and blood plasma are characterized by their considerable spread (Bhattathiri et al. 1994) and thus, mainly the dynamics of post-irradiation changes should be investigated. Deger et al. (2003) described a decrease in the concentration in the blood of

rabbit, Kaya et al. (1999) described a decrease in the rat plasma (Kojima et al. 1998), whereas Groen et al. (1996) observed no changes in the concentration in erythrocytes. A relationship between the radiation fractionation and GSH concentration was described by Jadhav et al. (1998). Elevations of concentrations in the brain tissues up to 4 hours after exposure to a dose of 50 cGy or in the RAW 264.7 cell tissue culture after doses of 25 and 100 cGy was described by Kojima et al. (1998, 2000). Relationships between GSH concentrations and radioresistance of N 10 cell tissue culture were considered by Yanagisawa et al. (1997).

Higher MDA concentrations in the blood plasma were observed after the total body exposure of rats to a dose of 5.5 Gy (Deger et al. 2003) and, in contrast, lower concentrations were observed in rats after a dose of 18 Gy (Jin et al. 2005). Concentrations of MDA were increased in the liver of rats after fractionated irradiation (Marchenko et al. 1998). The dependence of the MDA concentration in pig thrombocytes after their exposure to gamma-radiation doses of 10 to 100 Gy was described by Wachowicz et al. (1984).

In the literature, we found only one mention concerning an enhanced AOC activity at a statistically significant level in rabbits after their total body X-ray exposure to a dose of 5.5 Gy (Deger et al. 2003).

With respect to the oxidation-reduction system, there are interesting works by Kalinina et al. (1999) examining GSH/GSHPx levels in the fetal liver cells, myocardium and erythrocytes, studies investigating changes in SOD and GSH in persons irradiated in Chernobyl depending on their age (Vartanyan et al. 2004), or works demonstrating radioprotective effects of green tea based on oxidation-reduction system parameters (Wang et al. 2003).

MATERIAL AND METHODS

The blood was sampled from persons divided into four groups (A, B, C, D). In all the subjects, 9 ml of the blood was sampled from the cubital vein into a heparin solution.

For a one-month period, the participants took no antioxidants, they did not alter their regimen and none of them was administered with medicinal products.

Group A comprised of patients, who had experienced radiotherapeutic treatment with the use of radiation from a linear accelerator for malignant diseases of the lung. The blood of patients was sampled after the 8^{th}–9^{th} fraction of irradiation with single doses of 2 Gy.

The total radiation dose ranged between 6 and 46 Gy with a dose rate of 2.5 Gy/min. Patients on simultaneous chemotherapy or immunotherapy were eliminated from the group.

Group B included healthy volunteers, whose blood was exposed to a radiation dose of 2 Gy 60 min after sampling. ^{60}Co was employed as a gamma-ray source with a kerma value of 0.78 ± 0.04 Gy.

Group C consisted of employees of the Temelín Nuclear Power Plant (a nuclear power plant near the city of České Budějovice). At the time of sampling, the employees had been working at the power plant for different periods of time and there were also differences in the description of their jobs. The evaluated annual individual effective dose never exceeded 5 mSv in any employee, and the allowable annual limit accepted in the Czech Republic is of 50 mSv.

Group D was a control group. The samples were acquired from healthy persons.

The following parameters were evaluated in all the samples: the superoxide dismutase activity in erythrocytes (the result was related to 1 g of hemoglobin) (SOD); the reduced glutathione concentration in erythrocytes (GSH); the glutathione peroxidase activity in the total blood (the result was related to 1 g of hemoglobin) (GSHPx); the malondialdehyde concentration in the blood plasma (measured as thiobarbiturate acid reactive substances, TBARS) (MDA), and the plasma antioxidant capacity (AOC). This is a sum of all the substances exerting antioxidative effects present in the blood plasma; their concentration is expressed in mmol/l of water-soluble vitamin E (Trolox) with the same antioxidative activity.

For the determination of SOD, GSHPx and AOC, sets available from the company Randox Laboratories (Crumlin, Great Britain) were employed; a set from the company OXIS (Portland, USA) was used for GSH determination, and the MDA concentration was determined by photometry on a microtitration plate (Yarmonenko and Vaison 2004).

Data are expressed as means ± SD. We used the two-sided t-test at a significance level $2\alpha = 0.05$. The method of the multidimensional analysis of variance (MANOVA) was employed with a subsequent use of the cluster analysis.

Table 1. **Mean values of concentrations of substances evaluated or activities of enzymes in the samples tested**

Group	n	SOD (U/g Hb)	GSH (mmol/l)	GSHPx (U/g Hb)	MDA (μmol/l)	AOC (mmol/l)
A	18	1271.2 ± 107.12	1.88 ± 0.20	49.3 ± 12.83	1.74 ± 0.41	1.38 ± 0.21
B	10	1391.6 ± 113.30*	1.84 ± 0.35	64.3 ± 14.17*	1.93 ± 0.47	1.49 ± 0.07
C	32	1251.7 ± 114.99^{+}	1.99 ± 0.23	58.9 ± 13.74*	1.76 ± 0.34	1.57 ± 0.19*
D	21	1303.9 ± 187.22	1.87 ± 0.26	68.8 ± 9.89*§	1.89 ± 0.33	1.28 ± 0.19*$^{+\S}$

Values are given as means ± SD; n, number of patients
* statistically significant as compared with the group A
$^{+}$ statistically significant as compared with the group B
§ statistically significant as compared with the group C

RESULTS

The mean values measured and standard deviations of all the parameters of interest are summarized in Table 1; for statistical significance of the differences in results of the parameters studied based on the use of the t-test see Tables 2 to 6. Similar results concerning differences between particular groups studied were also obtained with the use of the method of the multidimensional analysis of variance (MANOVA) with a subsequent cluster analysis. Based on calculated p-values, it is possible to consider the clusters of corresponding groups as two-dimensional.

Mahalanobis distances of pairs of particular groups were calculated. We use the Mahalanobis distance to classify to which clusters (or classes of groups with similar properties) groups belong. A group belongs to a respective cluster when its Mahalanobis distance from that cluster is minimal.

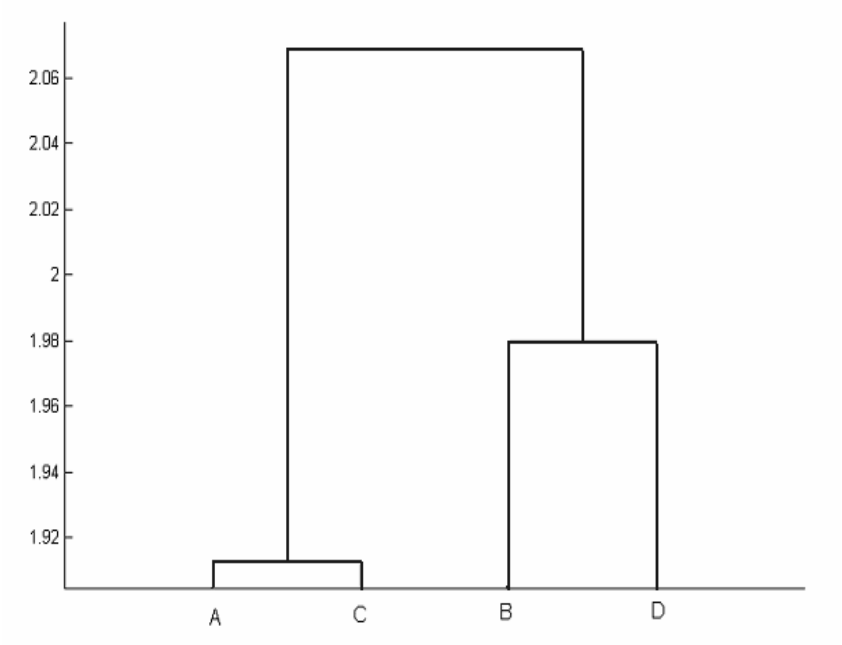

Fig. 1: **Dendrogram of groups studied with the Mahalanobis distance of pairs of particular groups.**

A dendrogram based on calculated distances is presented in Fig. 1. The groups form two clusters of groups of similar properties – the first cluster includes groups A and C, the second cluster includes groups B and D.

DISCUSSION

Relevant relationships can be observed when comparing the results presented here with data from the literature.

In the evaluation of the SOD activity in erythrocytes, we observed no statistically significant differences in the groups studied in comparison with controls. The highest mean value was measured in a group in which the blood was exposed to *in vitro* irradiation after taking the blood samples (group B). However, this result can be hardly considered as very important, since the activity was measured in erythrocytes and these elements themselves are not capable of proteosynthesis.

No statistically significant changes were observed in the evaluation of the GSH concentration in the blood plasma. Groen et al. (1996) also observed no changes in erythrocytes after radiotherapy in patients with lung carcinoma.

The highest GSHPx activity values were measured in controls and in the *in vitro* irradiated blood samples. A statistically significant decrease in activity was found both in patients after radiotherapy and employees of the Temelín Nuclear Power Plant. These changes can be explained by a pentose cycle inhibition and they are in agreement with results obtained by Bukan et al. (2003). In patients after radiotherapy, it is possible to expect these changes; the cause of the changes in employees of the Nuclear Power Plant Temelín is unclear, since even in their case histories, there were no important data that could explain these results.

Also no statistically significant changes were observed when evaluating the MDA concentration in the blood plasma. In all the groups studied, there is a minimum variance of the values in agreement with relevant assumptions, since ionizing radiation does not affect the intensity of the peroxidation of lipids (Štípek 2000). Dose dependence also cannot be excluded, as was observed in certain studies on the blood plasma of rats (Deger et al. 2003, Jin et al. 2005). Thus, we consider the possibility of a future study aimed at continuous monitoring of the MDA concentration on the radiation dose in patients in the course of fractionated radiotherapy.

The last parameter evaluated in the work presented here was the AOC activity that characterizes the plasma antioxidant capacity. The lowest values were measured in the control groups; in the other groups there were higher values, the difference being, however, not always statistically significant. Racek et al. (1999) also found a significant positive correlation between the AOC value and MDA concentration in many cases and they expressed an assumption that the higher the lipoperoxidation, the higher the AOC values (Racek 2004). There are only few works dealing with changes of the oxidation-reduction system after irradiation and a work by Deger et al. (2003) is of interest in that it described a statistically enhanced activity after the whole-body irradiation of rabbits.

In considering all the results acquired with the help of the multidimensional analysis of variance it is of interest that the dynamics of changes measured in the blood plasma is comparable between the group of patients after radiotherapy and group of employees of the Temelín Nuclear Power Plant on the one hand, and between controls and group of healthy donors whose blood was exposed to *in vitro* irradiation on the other. This result suggests the possibility that in spite of the fact that the employees did not immediately work in the nuclear power plant control zone, their radiation burden was higher than that in controls. All these employees also resided at distances up to 25 km from the nuclear power plant.

Our study demonstrated that changes concerning certain parameters of the oxidation-reduction system after the ionizing radiation action that were still described in experimental studies also occur in man. In future studies it would be desirable to consider their possible changes in the course of radiotherapy, depending on the radiation dose. Changes in employees of the nuclear power plant showing a similar nature to those in patients after radiotherapy (SOD, GSHPx, MDA), are of interest.

CONCLUSIONS

The authors studied changes of selected oxidation-reduction system parameters in the human plasma and erythrocytes on three different groups of persons in association with ionizing radiation effects (patients after radiotherapy, employees of the Temelín Nuclear Power Plant, *in vitro* irradiated blood after taking samples from healthy donors and control group) and they demonstrated changes that justify planning of further studies.

ACKNOWLEDGEMENTS

The present study was implemented within grant project IGA NJ/7461-3.

REFERENCES

Bhattathiri VN, Sreelekha TT, Sebastian P, Remani P, Chandini R, Vijayakumar T, Nair MK: Influence of plasma GSH level on acute radiation mucositis of the oral cavity. Int. J. Radiat. Oncol. Biol. Phys. 29:383–386, 1994.

Bukan N, Guney Y, Hicsonmez A, Bilgihan A: Antioxidant tolerance of kidney after irradiation. Indian J. Exp. Biol. 41:267–269, 2003.

Burlakova EB, Naidikh VI: The Effects of Low Dose Radiation. VSP, Utrecht, Boston 2004.

Butomo NV, Grebenyuk AN, Legeza VI: Basis of Medical Radiobiology (in Russian). Foliant, Sankt Peterburg 2004.

Czapski G: Nature of Oxygen Containing Radicals in Radiation Chemistry and Photochemistry of Aqueous Solutions. Annual Progress Report, September 1979 – July 1980. Technical Report, Hebrew Univ., Jerusalem 1980.

Deger Y, Dede S, Belge A, Mert N, Kahraman T, Alkan M: Effects of X-ray radiation on lipid peroxidation and antioxidant systems in rabbits treated with antioxidant compounds. Biol. Trace Elem. Res. 94:149–156, 2003.

Groen HJ, Meijer C, De Vries EG, Mulder NH: Red blood cell glutathione levels in lung cancer patients treated by radiation and continuously infused carboplatin. Anticancer Res. 16:1033–1037, 1996.

Jadhav GK, Bhanumathi P, Uma Devi P, Seetharamaiah T, Vidyasagar MS, Rao KK, Hospet CS, Salomon JG: Possible role of glutathione in predicting radiotherapy response of cervix cancer. Int. J. Radiat. Oncol. Biol. Phys. 41:3–5, 1998.

Jin LH, Liu CF, Zeng Y: Protective effects of puerarin on radiation injury of experimental rats. Zhong Xi Yi Jie He Xue Bao 3:43–45, 2005.

Kalinina EV, Novichkova MD, Charkova EM, Koppel' MA, Komisarova IA: Increase in radiation resistance of an organism after administration of metabolic drug MR-33. Radiats. Biol. Radioecol. 39:272–276, 1999.

Kandasamy SB, Kumar KS, Hartus HS: Involvement of superoxide dismutase and glutathione peroxidase in attenuation of radiation-induced hyperthermia by interleukin-1 alpha in rats. Brain Res. 606:106–110, 1993.

Kaya H, Delibas N, Serteser M, Ulukaya E, Ozkaya O: The effect of melatonin on lipid peroxidation during radiotherapy in female rats. Strahlenther. Onkol. 175:285–288, 1999.

Kojima S, Matsuki O, Nomada T, Shimura N, Kubodera A, Yamaoka K, Tanooka H, Wakasugi H, Honda Y, Honda S, Sasaki T: Localization of glutathione and induction of glutathione synthesis-related proteins in mouse brain by low doses of gamma-rays. Brain Res. 808:262–269, 1998.

Kojima S, Teshima K, Yamaoka K: Mechanisms involved in the elevation of glutathione in RAW 264.7 cells exposed to low doses of gamma-rays. Anticancer Res. 20:1589–1594, 2000.

Kumar KS, Sancho AM, Weiss JF: A Novel interaction of diethyldithiocarbamate with the glutathione/glutathione peroxidase system. Int. J. Radiat. Oncol. Biol. Phys. 8:1463–1467, 1986.

Lefevre G, Beljean-Leymarie M, Beyerle F: Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. Ann. Biol. Clin. (Paris) 56:395–418, 1998.

Marchenko MM, Kopyl'chuk GP, Grigor'eva EV: Influence of small doses of fractionated X-ray radiation on the activity of histone-specific proteases and the content of malonic dialdehyde in chromatin of the cells of rat liver. Radiats. Biol. Radioecol. 42:488–491, 2002.

Racek J, Kučera M, Holeček V, Trefil L, Pašek Z: Effects of vitamin and trace element supplements on parameters of antioxidant protection in patients with rheumatic diseases (in Czech). Klin. Biochem. Metab. 7:239–242, 1999.

Racek J: Oxidative Stress and Possibilities of its Involvement (in Czech). Galén, Praha 2004.

Russanov E, Balevska P, Leutshev S: X-irradiation effects on lipid peroxidation and superoxide dismutase and catalase activities in copper deficient and copper loaded rat liver. Acta Physiol. Pharmacol. Bulg. 5:73–78, 1979.

Štípek S: Antioxidants and Free Radicals in Health and Disease (in Czech). Grada Publishing, Praha 2000.

Steinerová A, Racek J, Holeček V: Effects of administering fenofibrate (Lipanthyl 100) and alpha-tocoferol (vitamin E) on lipid level and superoxide dismutase activity (in Czech). Klin. Biochem. Metab. 7:106–108, 1995.

Vartanyan LS, Gurevich S, Kozachenko AI, Navlech LG, Burlakova EB: Age-related peculiarities of effect of low dose ionizing radiation on blood antioxidant enzyme system

status in Chernobyl's accident liquidation participant. Adv. Gerontol. 14:48–54, 2004.

Wachowicz B, Krajewski T, Wodzinowska B, Reliszka L: Effects of gamma radiation on pig platelet function. Environ. Res. 34:1–7, 1984.

Wang Z, Zeng L, Xiao Y, Lu S, Gao X: Protective effects of green tea on mice with the irradiating damage induced by gamma-ray. Sichuan Da Xue Xue Bao Yi Xue Ban 34:303–30, 2003.

Yamaoka K, Sato EF, Utsumi K: Induction of two species of superoxide dismutase in some organs of rats by low dose X-irradiation. Physiol. Chem. Phys. Med. NMR. 26:205–214, 1994.

Yanagisawa T, Urade M, Takahashi Y, Yamamoto Y, Furuyama J: Levels of superoxide dismutases, glutathione, and poly(ADP-ribose) polymerase in radioresistant human KB carcinoma cell line. Jpn. J. Cancer Res. 88:1070–1077, 1997.

Yarmonenko SP, Vaison AA: Radiobiology of Humans and Animals (in Russian). Vysshaya shkola, Moscow 2004.