

## THE EFFECT OF IONIZING RADIATION ON THE ANTIOXIDANT CAPACITY OF THE HUMAN BLOOD

GABRIELLA BOGNÁR, GABRIELLA MÉSZÁROS AND GYORGY J. KÖTELES

Frédéric Joliot-Curie National Research Institute for Radiobiology and Radiohygiene, József Fodor National Center for Public Health, Budapest, Hungary

**ABSTRACT:** The total antioxidant capacity, activities of antioxidant enzymes like glutathione peroxidase (GPx) and superoxide dismutase (SOD) and the effect of ionizing radiation on these antioxidants in human blood were measured by the RANDOX assays. The different doses of ionizing radiation caused dose-dependent decreases of antioxidant activities. The total antioxidant status (TAS) value of plasma linearly decreased up to 1 Gy dose. At higher dose (2 Gy), no additional decrease of TAS value could be detected. Radiation-induced decrease of the antioxidant enzymes GPx and SOD were linear up to 1 Gy dose. Between 1 and 2 Gy doses, a further mild decrease could be detected.

**KEY WORDS:** Antioxidant capacity, GPx, SOD, human plasma, ionising radiation

### INTRODUCTION

Free radicals have been implicated in over one hundred human and animal diseases. In some conditions they play a significant role in the development of diseases. Each free radical formed in the body can initiate a series of oxidative chain reactions. Free radicals disappear from the body following reactions with other free radicals or, what is more important, due to the actions of the antioxidant system. Monitoring of antioxidant levels in individuals may be useful in the diagnosis of diseases, searching for therapy, and investigating disease processes (Miller et al., 1993).

In radiation biology, the developments of radiation effects are modified by antioxidants (El-Nahas et al., 1993; Sarma and Kesavan, 1993; Kunugita et al., 1997; Weiss et al., 2000). The reactive oxygen species play an important role in the action of ionizing radiation. The first *in vivo* studies on protection by antioxidants against ionizing radiation were performed half a century ago (Patt et al., 1949). In radiation protection the investigations on the role of antioxidant enzymes in the resistance against ionizing radiation have primary importance (Bognár et al., 1997; Sun et al., 1998; Köteles et al., 1999).

*Corresponding author: Gabriella Bognár*

*Frédéric Joliot Curie NRIR, József Fodor National Center for Public Health*

*Anna u. 5*

*H-1221 Budapest, Hungary*

*Tel/Fax: (+36) 1 226 0026, (+36) 1 226-6974, (+36) 1 226 6531*

*E-mail: bognar@hp.osski.hu*

Abbreviations: GPx = glutathione peroxidase  
SOD = superoxide dismutase  
TAS = total antioxidant status

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*Corresponding author: Gabriella Bognár*

*Frédéric Joliot Curie NRIR, József Fodor National Center for Public Health*

*Anna u. 5*

*H-1221 Budapest, Hungary*

*Tel/Fax: (+36) 1 226 0026, (+36) 1 226-6974, (+36) 1 226 6531*

*E-mail: bognar@hp.osski.hu*

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In the present work we have investigated the effects of different doses of ionizing radiation on the antioxidant capacity of the human blood and on the activities of antioxidant enzymes: glutathione peroxidase (GPx) and superoxide dismutase (SOD), *in vitro*.

## MATERIALS AND METHODS

Human blood samples obtained from healthy male persons (aged 20–25) were taken with lithium-heparin anticoagulant. The X-irradiation of whole blood was performed under the following conditions: 200 kV, 20 mA, 1 mm Cu filter, 60 cm SSD, 0,287 Gy  $\text{min}^{-1}$  dose rate, room temperature, 5 ml volume in Falcon plastic flasks.

The activities of antioxidant enzymes, GPx and SOD of haemolysate of heparinized whole blood, and the total antioxidant status (TAS) of plasma were determined using the colorimetric tests of RANDOX Laboratories Ltd. (United Kingdom).

Immediately after irradiation the human blood was centrifuged for 20 minutes at 1500 rpm. The activity of TAS was determined from heparinized plasma. In the reaction the ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with a peroxidase (metmyoglobin) and  $\text{H}_2\text{O}_2$  to produce the radical cation  $\text{ABTS}^+$ . This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration.

The concentration of GPx enzyme was determined from haemolysate of heparinized whole blood. In this method, GPx catalyses the oxidation of glutathione by cumene hydroperoxidase. In the presence of glutathione reductase and NADPH, the oxidised glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to  $\text{NADP}^+$ . The decrease in absorbance at 340 nm is measured.

The concentration of SOD enzyme was determined from haemolysate of heparinized whole blood. The role of SOD is to accelerate the dismutation of the toxic superoxide radical ( $\text{O}_2^-$ ) – produced during oxidative energy processes – to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyl-tetrazolium chloride to form a red formazan dye. The SOD activity is then measured at 505 nm by the degree of inhibition of this reaction.

## RESULTS AND DISCUSSION

Radiation-induced alterations of the antioxidant enzymes, GPx and SOD have been investigated.

The activities of GPx and SOD were determined after X-irradiation of human blood with various doses (0.3 – 0.5 – 0.7 – 1.0 – 2.0 Gy).

In the two experiments we used blood samples from two different persons. The concentrations of enzymes were measured from haemolysate of heparinized whole blood, immediately after irradiation.

TABLE 1. Effect of ionizing radiation on the antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD)

Dose (Gy)	GPx (U/l)		Dose (Gy)	SOD (U/l)	
	I	II		I	II
0	7966	7286	0	192	145
0.3	5794	4414	0.3	179	131
0.5	5655	4028	0.5	172	124
0.7	5311	3828	0.7	158	119
1.0	4966	3448	1.0	147	109
2.0	4623	2862	2.0	134	101

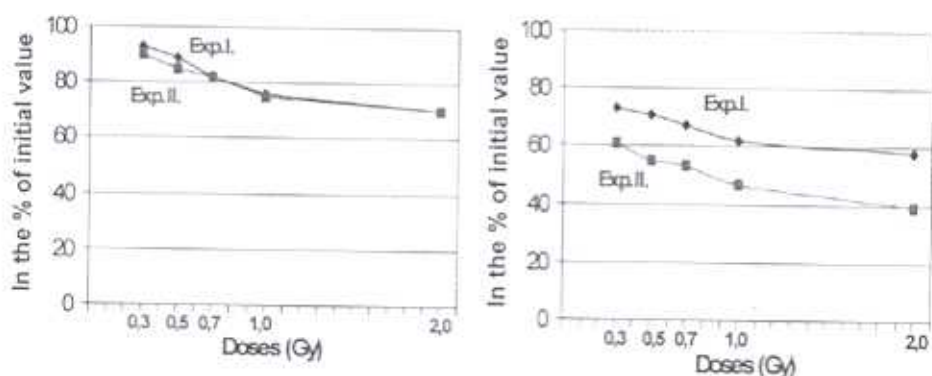


Fig. 1. Effects of ionizing radiation on the antioxidant enzymes, glutathione peroxidase (GPx) and superoxide dismutase (SOD) (determination from 2 persons' blood samples).

As it is to be seen in *Table 1* and *Fig. 1*, the activities of GPx and SOD decreased linearly upon the effects of various doses of ionizing radiation till 1 Gy. Between 1 and 2 Gy doses, however, only further mild decreases could be detected. The human blood retained 40–60% of its antioxidant enzymes, GPx and SOD concentrations.

Glutathione peroxidase is a selenium-dependent enzyme. At low concentrations, there is a correlation between GPx levels and selenium status. Low levels of selenium and hence of GPx have been implicated in several diseases. Examples include some form of cancer (Combs et al., 1997; Greeder and Milner, 1980), cardiovascular diseases (Salonen et al., 1982), and cataracts (Fecondo and Augusteyn, 1983).

Superoxide dismutase is an enzyme the function of which is to increase the dismutation rate of superoxide anion. This enzyme is thought to protect aerobic cells against oxygen toxicity. Determination of level of SOD in human blood may be useful in the diagnosis of several diseases and as a research tool for assessing new therapies. Changes in SOD levels can also be used to identify sepsis patients at risk

from developing adult respiratory distress syndrome (Leff et al., 1993). Differences in SOD levels between normal and malignant cells may form the basis of a diagnostic test for cancer (Oberley and Buettner, 1979).

### Effect of ionizing radiation on the total antioxidant capacity of human plasma, in vitro

The antioxidant system has many components. A deficiency in any of these components can cause a reduction in the overall antioxidant status of an individual. Reduction in total antioxidant status has been implicated in several disease states, such as cancer, heart disease, rheumatoid arthritis, diabetes, and age-related conditions. Analysis of antioxidant status, therefore, allows antioxidant or dietary supplementation to patients at risk of disease, and may be used to optimize their treatment.

TABLE 2. Radiation-induced decrease of the total antioxidant status (TAS) value of human plasma, in vitro

Dose (Gy)	TAS mmol/l				
	Experiments				
	I	II	III	IV	V
0	1.75	2.14	2.05	2.28	1.48
0.3	1.49	1.91	1.92	2.10	1.43
0.5	1.32	1.79	1.82	1.98	1.38
0.7	1.12	1.69	1.55	1.63	1.28
1.0	1.04	1.59	1.46	1.49	1.18
2.0	1.08	1.59	1.47	1.49	1.19

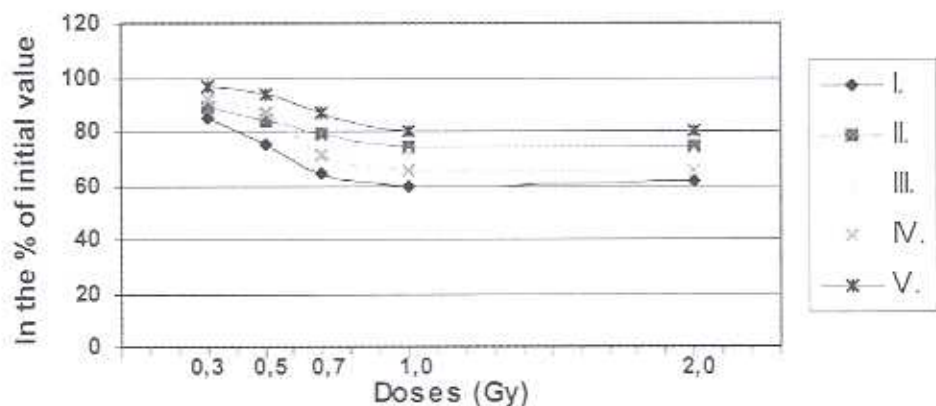


Fig. 2. Radiation-induced decrease of the total antioxidant status value of human plasma, in vitro (determination from 5 persons' blood samples).

Radiation-induced changes of the TAS value of human plasma have been investigated, following X-irradiation of human blood samples with 0.3 – 0.5 – 0.7 – 1.0 – 2.0 Gy doses of ionizing radiation.

In the five experiments we used blood samples from five different persons. After irradiation the human blood was centrifuged for 20 minutes at 1500 rpm. The activity of TAS was measured from heparinized plasma.

The TAS values decreased by the effects of various doses of ionizing radiation (Table 2, Fig. 2) linearly until 1 Gy. Between 1 and 2 Gy doses, however, no additional decrease of TAS value could be observed.

Radiation-induced decreases of the total antioxidant capacity are limited. The human blood retained 60–70% of its total antioxidant capacity, but individual differences appeared, of course. The results suggest that only an "easily available" fraction of antioxidant was reactive for the effect of ionizing radiation. The "non-available" fraction of human plasma was not reactive with the radiation-induced free radicals. This refers to the presence of antioxidant protection barriers in plasma of various radiosensitivities.

## CONCLUSIONS

The knowledge on the quantities of the antioxidants in the human blood is important as they are indicators of health status. Deficiency in individual antioxidant status has been implicated in several diseases.

The routine determinations of activities/capacities of antioxidant compounds would be of great importance in assessing individual sensitivities against oxidative effects.

The commercial availability of various kits for such determinations might also lead to population monitoring. Accordingly, our first approach was to investigate on the sensitivities of those antioxidant elements against various doses of ionizing radiation tested by the available kits. Surprisingly, linear relationships between dose and antioxidant decrease could be observed only to app. 1 Gy. Further increase of dose did not influence the respective values, although the test system still indicated their presence. These observations suggest either the limited response of antioxidant system to ionizing radiation, i.e., only 30–40% of the total activities were exhausted up to 1 Gy, or the existence of some protection system of various reactivities. It is also noticeable that the dose-response tendencies are similar in cases of TAS value where someone would suppose the decrease of low molecular weight compounds, too, and those of enzyme activities. A further problem to be answered is whether this phenomenon appears only under *in vitro* conditions or *in vivo*, too.

It is interesting that Chevion has also found that total body irradiation (TBI) causes a pronounced decrease in antioxidant capacity and an excessive increase in oxidant stress. TBI alters antioxidant homeostasis and dramatically enhances the damage inflicted on the cells (Chevion et al., 1999).