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Serum AOPP, selenium and vitamin E levels after irradiation

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ABSTRACT

Proteins are susceptible to free radical damage. We measured advanced oxidation protein products (AOPP), selenium and vitamin E levels in serum after whole body irradiation in doses of 8 Gy and 15 Gy in guinea pigs. After 24 hours from irradiation, the serum AOPP levels were increased significantly at 15 Gy dose with respect to the control levels ($p<0.05$). In both of the irradiated groups, Vitamin E and selenium levels did not change ($p>0.05$) in serum with respect to the control group, after 24 hours from irradiation. We found that gamma-irradiation caused dose dependent effect on AOPP levels. This result may be related to the fact that a high dose of ionizing irradiation cause excessive oxidative stress while antioxidants such as vitamin E and selenium were not affected after 24 hours from irradiation. [Turk J Cancer 2006;36(1):19-22].

KEY WORDS:

Advanced oxidation protein products (AOPP), ionizing irradiation, selenium, vitamin E

INTRODUCTION

Radiation injury to living cells is, to large extent, due to oxidative stress (1). However, irradiation also damages non-target cell or tissue. Free radicals and reactive oxygen species (ROS) react with DNA, lipids, proteins, carbohydrates and damage them. Nevertheless, organisms have protective systems against free radical reactions such as antioxidants and antioxidative enzymes. Antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase and non-enzymatic antioxidants such as vitamin E and selenium offer protection against ionizing radiation induced oxidants (2,3).

Biochemical effects of free radicals also include oxidative modification of proteins (4) yet protein oxidation has not been extensively studied in clinical settings until recent years for lack of easily accessible methods to detect protein damage.

The aim of this study was to evaluate a novel spectrophotometric assay, which allows detecting advanced oxidation protein products (AOPP) and antioxidants like selenium and vitamin E in serum after whole body irradiation at the doses of 8 Gy and 15 Gy in guinea pigs.

MATERIALS AND METHODS

Animal Models

Guinea pigs weighting approximately 350 g were used in this study. The guinea pigs were divided into three groups

each consisting of 8 animals. Group 1: Control; group 2: Irradiated with a dose of 8 Gy (single dose, whole body); group 3: Irradiated with a dose of 15 Gy (single dose, whole body). Irradiation was carried out using a ^{60}Co source. All animal procedures were carried out according to the rules of local Ethics Committee.

The animals in group 2 were exposed to a dose of 8 Gy irradiation ^{60}Co, source axe distance (SAD 80 cm) to whole body following ketamine hydrochloride anesthesia. The guinea pigs in group 3 were applied to a dose of 15 Gy irradiation to the whole body following ketamine HCl anesthesia (2,5 mg/kg, Ketalar®, Eczacıbaşı, Türkiye). After 24 hours from irradiation, intracardiac blood was obtained. Serum was separated and kept at -80° C until the subsequent assays.

Assays

Determination of AOPP

Determination of AOPP was based on spectrophotometric detection according to Witko-Sarsat et al. (5). 200 µl of blood serum diluted 1:5 with PBS, 200 µl of chloramin T (0-100 µmol/l) for calibration and 200µl of PBS as blank were applied. 10 µl of 1.16 M KI and 20 µl of acetic acid were added and absorbance at 340 nm was measured immediately. Concentration of AOPP is expressed in chloramine units (µmol/L).

Selenium Assay

The mixture of $HNO_3/HClO_4$ is used to mineralize the samples. As is known the inclusion of $HClO_4$ in the digestive process is essential for complete decomposition of the organic matrix and the conversion of organoselenium to Se(VI).

After digesting process, selenium determination has been carried out using the hydride generation-atomic absorption spectrometry (HG-AAS) technique (Unicam model 939 atomic absorption spectrometer) (6). The results have been expressed as ng/ml of serum.

Vitamin E assay

The serum vitamin E levels were measured by using a fluorometric method (7). In this method, Vitamin E is extracted from serum into hexane after precipitation of proteins with ethanol. The fluorescence of the hexane layer is measured at 340 nm after excitation at 295 nm. The results were evaluated by using a calibration curve and expressed as mg/L serum.

Statistical Analysis

Kruskal Wallis variance analysis and Mann-Whitney U test were used by the SPSS 10.0 for windows. Results were expressed as mean ± standard deviation. Significant difference was accepted at $p<0.05$.

RESULTS

We examined the AOPP levels as an indicator of protein oxidation in serum of the guinea pigs. After 24 hours from irradiation, the serum AOPP levels were increased significantly at 15 Gy dose with respect to the control levels ($p<0.05$). In both of the irradiated groups, Vitamin E and selenium levels did not change ($p>0.05$) in serum compared with the control group, after 24 hours from irradiation (Table 1).

Table 1
AOPP, vitamin E and Se levels of all groups

	AOPP (µmol/L)	Vit E (mg/L)	Selenium (ng/ml)
Controls	56.00±27.18	4.68±1.03	7.75±2.00
8 Gy	59.65±18.64	4.98±0.73	7.53±0.83
15 Gy	97.97±37.53*	4.48±0.62	7.32±0.60

AOPP: advanced oxidation protein products; Vit E: vitamin E; Se: selenium
All results are expressed as mean ± SD
** p <0.05 compared with controls*

DISCUSSION

Radiation is known to produce various active oxygens in biological systems such as hydroxyl radical, superoxide and hydrogen peroxide, and cause various types of tissue damage due to successive free radical reactions (8). ROS, initiate lipid peroxidation, protein oxidation and DNA damage leading to carcinogenesis and cell death if the antioxidant potential is insufficient. Proteins are among the main targets of oxidation in plasma (9). In vivo studies of radiation damage have been performed by invasive methods such as the measurement of lethal dose (e.g. LD50) and tissue damage. These in vivo methods only show the final results of free radical reactions. Therefore, a noninvasive in vivo method to measure free radical reactions is necessary to investigate the mechanism of radiation damage in the whole body (8). Oxidative modifications of proteins are good oxidative stress markers, with much better stability than lipids (10). Several forms of protein oxidation can occur, including the formation of protein carbonyls or the formation of cross-linking molecules by oxidation of sulfhydryl groups or advanced oxidation protein products (AOPP) (11). AOPP are defined as dityrosine containing cross-linked protein products (12). This definition is important since it excludes protein aggregates that form as a result of disulphide links following a suitable oxidative stress. Therefore, the presence of AOPP may be a good marker of oxidative stress (12).

In the present study, we examined the acute effects of whole-body single irradiation in serum with the doses of 8 Gy and 15 Gy, after 24 hours from the exposure. We found that ionizing irradiation caused dose dependent effect on AOPP levels. After 24 hours from irradiation, the serum AOPP levels were increased significantly at 15 Gy dose with respect to the control group ($p<0.05$). This result may be related to the fact that a high dose of ionizing irradiation cause excessive oxidative stress. Similarly Umegaki and Icheikawa (13) demonstrated that whole-body irradiation increased the levels of lipid peroxides in the bone marrow in dose dependent manner.

Vitamin E plays an important protective role against radiation induced peroxidation of polyunsaturated fatty acids in vitro, and erythrocyte damage in vivo (14,15). It has been shown that systemic and topical application of vitamin E reduced ultraviolet-induced lipid peroxidation and skin damage, and prevents the incidence of skin cancer (16). In this way, changes in the vitamin E content of the body seem to affect the extent to which damages are induced by irradiation (17). Because of this, in this study we evaluated serum vitamin E levels after 24 hours from whole-body single irradiation with the doses of 8 Gy and 15 Gy. Levels of vitamin E in serum did not significantly change in both of the irradiated groups after 24 hours from irradiation ($p>0.05$). Similarly, Umegaki and Ichikawa (13) observed that serum vitamin E levels were not changed up to 10 Gy of irradiation. Feurgard et al. (18) applied 4 Gy whole-body irradiation to the rats. Four days after irradiation, plasma vitamin E levels were decreased and demonstrating that irradiation reduces antioxidant stores markedly, this was contrary to our results.

Selenium (Se) is an antioxidant trace element and shows a close relation in its chemical properties to sulfur (S). A physiologic role for Se has been established when it has been discovered that it is an essential structural component of glutathione peroxidase (19). This has been resulted in a broader concept of the role of Se in biologic systems, which also acts as a cellular antioxidant (20). Turkoglu et al. (21) suggested that antioxidant treatments prior to irradiation especially selenium plus vitamin E treatment, may have some protective effects against irradiation-induced intestinal injury.

In the present study, there was no significant difference between the selenium levels in control and irradiated groups after 24 hours.

Guney et al. (22) applied 8 and 15 Gy single whole body ionizing irradiation dose to each guinea pig and all the animals were euthanized after 24 hours. Liver selenium levels were examined in unirradiated, 8 and 15 Gy irradiated groups. They suggested that liver selenium levels were not found to be significantly changed after irradiation.

In the current study, the animals were sacrificed 24 hours after irradiation thus the period might not be enough to influence serum selenium and vitamin E levels.

To our knowledge, we are the first to demonstrate the relationship between AOPP levels and a high dose of ionizing irradiation. In conclusion, a high dose of ionizing irradiation is speculated to be associated with increased serum AOPP levels while antioxidants such as vitamin E and selenium levels are not affected in the early period.

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