

RESEARCH ARTICLES

TOTAL MALONDIALDEHYDE LEVELS AND SUPEROXIDE DISMUTASE ACTIVITIES IN THE SERA OF GUINEA PIGS AFTER WHOLE-BODY IRRADIATION

İYONİZE RADYASYON UYGULANAN KOBAYLARDA SERUM TOTAL MALONDİALDEHİT DÜZEYLERİ VE SÜPEROKSİT DİSMUTAZ AKTİVİTELERİ

Öznur MERTOĞLU, M.D., Ayşe BİLGİHAN, M.D., Yıldız GÜNEY, M.D.,
Gürsel BİBEROĞLU*, M.D., Muharrem POLAT**, M.D.

Gazi University, Faculty of Medicine, Departments of Biochemistry and Pediatrics*, Ankara, Turkey

Ankara Oncology Hospital, Department of Radiation Oncology**, Ankara, Turkey

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ABSTRACT

Purpose: The aim of this study was to investigate the acute effects of whole-body irradiation in doses of 8 Gray (Gy) and 15 Gy, to malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities in the sera of guinea pigs, 24 hours after irradiation. **Methods:** Guinea pigs were irradiated at doses of 8 Gy (n=10) and 15 Gy (n=10). Twenty-four hours after a single whole-body irradiation, serum total MDA levels were measured by high pressure liquid chromatography (HPLC) and SOD activities were measured using spectrophotometric techniques. These parameters were also evaluated in nonirradiated guinea pigs (n=10). **Results:** Serum total MDA levels were significantly lower ($p<0.05$) in the 8 Gy irradiated group than in the control group. However, with 15 Gy irradiation, MDA levels were not significant ($p>0.05$) with respect to the control group. In both of the irradiated groups, SOD activities did not change ($p>0.05$) in comparison with the control group. **Conclusion:** The results suggest that serum MDA levels are a useful parameter for monitoring the acute effects of irradiation since they vary depending on irradiation dose.

Key Words: Malondialdehyde, Superoxide Dismutase, Whole-Body Irradiation.

INTRODUCTION

In living organisms, radiation is known to produce various oxygen species, such as the hydroxyl radical (OH.), superoxide radical ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2), and cause

ÖZET

Amaç: Bu çalışmada, kobayların 8 Gray (Gy) ve 15 Gy dozlarında, tüm vücut ışınlanmalarından 24 saat sonra, ışınlamanın seruma yansıyan akut etkilerini gözlemlemek için total malondialdehit (MDA) düzeyleri ve süperoksit dismutaz (SOD) aktivitelerinin ölçülmesi amaçlanmıştır. **Gereç ve Yöntem:** Kobaylar 8 Gy (n=10) ve 15 Gy (n=10) dozlarında, tek doz, tüm vücut ışınlanmasına maruz bırakılmışlardır. Işınlamadan 24 saat sonra kan örnekleri alınmış ve bu örneklerden elde edilen serumlarda total MDA düzeyleri yüksek basınçlı sıvı kromatografisi (HPLC) yöntemiyle ve SOD aktiviteleri ise spektrofotometrik olarak ölçülmüştür. Aynı ölçümler ışınlama uygulanmayan kobaylarda da (n=10) yapılmıştır. **Sonuçlar:** 8 Gy dozla ışınlanan kobay grubunda, serum total MDA düzeyleri kontrole göre anlamlı ($p<0,05$) bir düşüş göstermiştir. Fakat, 15 Gy dozdaki MDA düzeyleri ve her iki dozla ışınlanan gruplardaki serum SOD aktiviteleri kontrole karşılaştırıldığında anlamlı bir fark ($p>0,05$) izlenmemiştir. **Yorum:** Bu çalışma sonucunda, serum MDA düzeylerinin doza bağlı olarak değişmesi, ışınlamanın akut etkisini izlemek açısından serum MDA düzeylerinin seçilebileceğini göstermektedir.

Anahtar Kelimeler: Malondialdehit, Süperoksit Dismutaz, Tüm Vücut Işınlaması.

various types of tissue damage due to successive free radical reactions (1). This indirect effect of radiation is used during radiotherapy. Though radiotherapy is one clinical means by which tumors can be treated, many biochemical complications, such as damage to cellular DNA

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and membrane structures arise that may also affect surrounding normal tissues as a result of radiation treatment. Radiation induced oxygen radicals attack membrane phospholipids and cholesterol esters and cause lipid peroxidation (2). In the degradation of cell membranes, reactive oxygen species react with the double-bonds of polyunsaturated fatty acids (PUFAS) to yield lipid hydroperoxides. After the breakdown of such hydroperoxides a wide variety of aldehydes can be formed. These aldehydes are relatively stable. The measurement of carbonyl secondary products and their detection is useful as an indicator of the extent of tissue damage (3). However, organisms have protective systems to reactive oxygen species, like endogenous antioxidant enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) constitute primary enzymatic defense system. Among these, SOD catalyzes the dismutation of $O_2^{\cdot-}$ radicals to H_2O_2 (4, 5).

It is known that SOD activities in living bodies fall, depending on the concentration of active oxygen species generated by high dose irradiation (6). However, in low dose irradiation, SOD activities might increase (7). Antioxidant concentrations in plasma have been reported to decrease following whole body irradiation in humans and rats (8-11). However, there are reports that indicate almost no change in SOD activity even after high dose irradiation (4, 12).

In this study, we exposed a group of guinea pigs to whole body irradiation at doses of 8 Gray (Gy) and 15 Gy and examined total malondialdehyde (MDA) levels and SOD activity in the serum.

MATERIALS AND METHODS

Animal Models: Thirty guinea pigs of both sexes, each weighing approximately 350 g, were used during the experiment. The protocol was reviewed and approved by the Animal Care Committee of Gazi University Medical School. During the experiment, there were 3 groups (control group and 2 irradiated groups) each consisting of 10 animals. The guinea pigs were irradiated one at a time using a cobalt60 source (General Electric, ALCYON2, French production, 1994) at Ankara Oncology Hospital, Department of Radiation Oncology. The whole body of each guinea pig was evenly irradiated at

single doses of 8 Gy and 15 Gy using the source axis distance (SAD) (80 cm) technique. During irradiation, the field size was approximately 17x15 cm and the depth of each animal was adjusted to 3-3.5 cm. All groups were anesthetized by an intramuscular injection of ketamine HCl (2.5mg/kg, ketalar®, Eczacıbaşı, Türkiye). Twenty-four hours after irradiation, intracardiac blood was obtained. Serum was separated and kept at -80 °C.

Chemicals: Chloroform, acetic acid, acetonitrile (HPLC grade), sodium hydroxide, hydrochloric acid, sulfuric acid, sodium carbonate and cupric chloride were purchased from MERCK, while xanthine oxidase, 1,1,3,3-tetraoxypropane (TEP), EDTA, nitroblue-tetrazolium and 2,4 dinitrophenylhydrazine were obtained from SIGMA.

ASSAYS

Total Malondialdehyde Measurement: Total MDA levels were measured in serum by Pilz's method (3) using high pressure liquid chromatography (HPLC, Waters 486, USA). The MDA levels were measured in serum after derivatization with 2,4-dinitrophenylhydrazine. Derivatization was accomplished in 10 minutes at room temperature and subsequently chromatographed by HPLC on a reversed phase 4 μ m C18 column with ultraviolet detection at 310 nm. The results were expressed as nmol/mL serum.

Superoxide Dismutase Activity: Serum SOD activity was measured by the method of Yi-Sun (13). The SOD activities of serum were determined by the inhibition of nitroblue tetrazolium reduction with xanthine-xanthine oxidase used as an ($O_2^{\cdot-}$) generator. The results were expressed as unit/mL serum, with 1 unit of SOD defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50 %.

Statistical Analysis: Kruskal-Wallis variance analysis and the Mann-Whitney U test were used under SPSS 10.0 for Windows. The results were expressed as mean \pm standard deviation. Significant difference was considered at $p < 0.05$.

RESULTS

We examined the generation of serum MDA as an indicator of lipid peroxidation in the whole

body of guinea pigs. Twenty-four hours after irradiation, serum MDA levels were decreased significantly following the 8 Gy dose ($p < 0.05$). On the other hand, serum MDA levels were not significant at 15 Gy with respect to control group levels ($p > 0.05$) (Table 1).

Table 1. The total MDA levels (nmol/mL) in the sera of guinea pigs 24 hours after irradiation.

Dose of Irradiation	MDA Level (nmol/mL)
8 Gy group (n=10)	1.68 ± 0.34 *
15 Gy group (n=10)	3.09 ± 0.37 **
Control group (n=10)	2.84 ± 0.39

* $p < 0.05$ significant with respect to the control group

** $p > 0.05$ not significant with respect to the control group

Since SOD is an antioxidative enzyme, we examined serum SOD activities resulting from irradiation (Table 2). In both of the irradiated groups, SOD activities did not change ($p > 0.05$) in the serum with respect to the control group 24 hours after irradiation.

Table 2. SOD activities (U/mL) in the sera of guinea pigs 24 hours after irradiation.

Dose of Irradiation	SOD Activity (U /mL)
8 Gy group (n=10)	11.05 ± 1.80**
15 Gy group (n=10)	10.44 ± 1.10 **
Control group (n=10)	11.05 ± 1.29

** $p > 0.05$ not significant with respect to the control group.

DISCUSSION

This study has explored the acute effects of whole-body single irradiation in serum with 8 Gy and 15 Gy doses 24 hours after exposure. It has been known for a long time that the extent of tissue damage is correlated with the production of carbonyl secondary products such as MDA and usually its measurement is evaluated by the thiobarbituric acid (TBA) test, which is the favored assay because of its simplicity. This is true despite the fact that the TBA test is nonspecific for MDA since various non-lipid related materials are TBA positive, such as carbohydrates, several aminoacids, deoxyribose, bile pigments and sialic acids. Furthermore, the TBA test shows varying results when applied to human serum samples. In our data, the specificity of the assay was improved by using an HPLC method (3).

At 8 Gy irradiation, the observed decrease in serum MDA levels could show that the oxidative damage due to irradiation did not reach a level in the tissues that allows for the release of lipid peroxidation products into the blood. In different studies, it has been reported that lipid peroxidation products increased in the spleen and liver 2-7 days after irradiation (14,15) and MDA levels decreased in the plasma on the second day after 8 Gy whole body irradiation (16). Our findings are in agreement with these reports. An alternative mechanism that could explain this decrease is that the MDA is released from tissues into the plasma and trapped from the plasma by the kidneys and spleen (16).

The level of irradiation is important for lipid peroxidation levels in tissues (14). At a 15 Gy dose, MDA levels were observed to be near those of the controls. This could be explained by the effect of the increased dose on lipid peroxidation.

Furthermore, almost no changes in SOD activities occurred, even after high dose irradiation. Our data confirm those of studies that found no significant differences in SOD activities in liver homogenates after whole body irradiation at doses of 7.5 Gy and 15 Gy (4). The results suggest that 24 hours after irradiation, SOD activities in serum were not affected by these experimental conditions. The effects of irradiation on SOD activities have been reported by several investigators (4,12). Their results were dependent on experimental conditions such as irradiation dose or period after irradiation. These studies demonstrated that low dose irradiation induced the activity of SOD, while high dose irradiation did not.

Our observations seem to suggest that when monitoring the acute effects of irradiation, serum MDA levels could be useful, since total MDA levels vary depending on the irradiation dose; however, further investigations are needed to clarify such details.

Correspondence to: Öznur MERTOĞLU, M.D.
Bülbülderesi Cad. 49/11
Küçükesat
06660 ANKARA - TÜRKİYE
Phone : 312- 433 57 64
e-mail: oznur732000@yahoo.com

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