The Effects of ^{201}Tl Myocardial Perfusion Scintigraphy Studies on Oxidative Damage in Patients

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ABSTRACT

*The aim of this study was to investigate gamma radiation-induced oxidative damage in erythrocytes after ^{201}Tl myocardial perfusion scintigraphy. Twenty patients (8 women and 12 men) who performed ^{201}Tl myocardial perfusion scintigraphy were included in this study. The blood samples were taken from patients just before, 1 hour after and three hours after injection of the radiopharmaceutical. Malondialdehyde (MDA) and antioxidant enzymes such as glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) levels were measured to evaluate the gamma radiation induced oxidative damage. The enzyme activities of SOD, GPX and CAT were decreased 1 hour after (*p *= 0.042,* p *= 0. 697 and* p *= 0.653 respectively) and 3 hours after (*p *= 0.003,* p *= 0. 573 and* p *= 0.002 respectively) injection of the radiopharmaceutical. Malondialdehyde levels were increased 1 hour after (*p *= 0.10) and 3 hours after (*p *= 0.47) injection of the radiopharmaceutical. In this study, we found that radiation due to ^{201}Tl myocardial perfusion scintigraphy decreased the erythrocyte antioxidant levels and increased MDA levels.*

Efectos de los Estudios de Escintigrafía de Perfusión Miocárdica ^{201}Tl Sobre el Daño Oxidativo en los Pacientes

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RESUMEN

*El objetivo de este estudio fue investigar el daño oxidativo inducido por radiaciones gamma, sobre los eritrocitos luego de realizada una escintigrafía de perfusión miocárdica ^{201}Tl. Veinte pacientes (8 mujeres y 12 hombres) a quienes se les realizó escintigrafía por perfusión miocárdica ^{201}Tl, fueron incluidos en este estudio. Las muestras de sangre fueron tomadas de los pacientes antes, 1 hora más tarde, y tres horas después de inyectar el radiofármaco. Se midieron los niveles del malondialdehido (MDA) y las enzimas antioxidantes tales como la glutationa peroxidasa (GPX), superóxido dismutasa (SOD), y la catalasa (CAT), a fin de evaluar el daño oxidativo inducido por la radiación gamma. Las actividades de las enzimas SOD, GPX y CAT fueron disminuidas 1 horas después (*p *= 0.042,* p *= 0. 697 y* p *= 0.653 respectivamente) y tres horas (*p *= 0.003,* p *= 0. 573 y* p *= 0.002 respectivamente) tras la inyección del radiofármaco. Los niveles de malondialdehido fueron aumentados 1 hora después (*p *= 0.10) y tres horas después (*p *= 0.47) de la inyección del radiofármaco. En este estudio, hallamos que la radiación a causa de la escintigrafía de perfusión miocárdica ^{201}Tl disminuyó los niveles antioxidantes del eritrocito y aumentó los niveles de MDA.*

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INTRODUCTION

The biological targets of radiation are the cells. Radiation produces lethal effects on cells, particularly during division and delays the onset of mitosis. Eventually, cell death or the loss of reproductive capacity occurs. Radiation directly affects the DNA molecule of the cell nucleus and results in

chromosomal abnormalities. Indirect effects of radiation occur via free radicals which are highly reactive (1–2). Free radicals are oxygen metabolites, which cause damage by oxidizing and reducing structures in the local environment (3). The key reactive species are superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (4). Ionizing radiation produces the first two as its immediate agents of cellular damage (5). Hydroxyl radicals are generated by ionizing radiation either directly by oxidation of water or indirectly by the formation of secondary partially reactive oxygen species [ROS] (6).

The process of lipid peroxidation is one of oxidative conversion of polyunsaturated fatty acids to native aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal by a well-studied, biologically relevant free radical reaction. Malondialdehyde itself, because of its high cytotoxicity and its inhibitory action on protective enzymes, has been suggested to act as a tumour promoter and a co-carcinogen (7). Malondialdhyde, an end product of lipid peroxidation, has been used as an index of oxidative damage (8). The aim of this study was to investigate gamma radiation-induced oxidative damage in erythrocytes after 201 Tl myocardial perfusion scintigraphy.

SUBJECTS AND METHODS

The study was approved by the ethics committee of the Suleyman Demirel University Hospital. Malondialdehyde and several antioxidant enzymes such as glutathione (GPX), superoxide dismutase (SOD) and catalase (CAT) were measured to evaluate gamma radiation induced oxidative damage.

Ten patients (8 women and 12 men) who had ^{201}TI myocardial perfusion scintigraphy were included in the study. The median age was 54 ± 9 (range 37–72) years. The blood samples were taken from patients before, 1 hour after and three hours after injection of the 92.5 MBq ^{201}TI (Monrol-Turkey).

Oxidative stress studies in erythrocytes

The MDA concentrations as an indicator of lipid peroxidation and antioxidant enzymes such as GPX, SOD and CAT were used to evaluate gamma radiation induced oxidative damage. Blood samples were otained in EDTA. The tubes were centrifuged at 3000 g for 3 minutes. Erythrocyte pellets were obtained immediately from the EDTA blood by centrifuging at 3000 g for 3 minutes. Plasma and buffy coat were removed and the erythrocytes were washed three times in 5 ml of cold 0.9% NaCl solution. Erythrocytes were haemolysed by adding cold distilled water. Haemolysate samples were stored at -80° and all measurements were made within 2 months. The erythrocytes were thawed and the levels of MDA and the activities of SOD, GPX and CAT were assessed.

MDA was determined by the double heating method of Draper and Hadley (9).

The measurement of SOD was based on the principle in which xanthine reacts with xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye (10).

The determination of GPX activity was based on the method described by Paglia and Valentina (11). CAT activity was measured according to the method of Aebi (12, 13).

An autoanalyzer, Abbott Aeroset, was used to determine the activities of SOD and GPX, and the spectrophotometer, Shimadzu UV-1601 (Japan), was used to estimate MDA and CAT. Details of the experimental arrangements have been described (13).

Statistical Analysis

Data were analysed using the statistical package SPSS for Windows (Ref. 9.05, SPSS Inc, Chigago, IL.). Results were expressed as mean ± SD. Statistical significance was set at the 0.05 levels. Differences within the same group were tested by repeated measures of ANOVA since they are all time-dependent data.

RESULTS

Results are tabulated in Table 1. The enzyme activities of SOD, GPX and CAT were decreased 1 hour after (p = 0.042, p = 0. 697 and p = 0.653 respectively) and 3 hours after (p = 0.003, p = 0. 573 and p = 0.002 respectively) injection of the

Table 1: MDA and antioxidant levels (Ort ± Sd)

	MDA (nmol/mgHb)	SOD (U/g Hb)	GPX (U/g Hb)	CAT (k/g Hb)
Before radiopharmaceutic	5143 ± 2339	2051 16 ± 438 53	88 216 ± 18 592	54 80 ± 12 93
1 hour after radiopharmaceutic	5512 ± 1874[a]	1555 51 ± 183 57[a]	73 110 ± 19 346	4057 ± 1619[b]
3 hours after radiopharmaceutic	7251 ± 941[ab]	1744 60 ± 254 69	60 091 ± 32 596	4477 ± 1470[b]

[a] $p < 0.05$ compared to before radiopharmaceutical group.
[b] $p < 0.05$ compared to 1 hour after radiopharmaceutical group.

radiopharmaceutical. MDA levels were increased 1 hour after (p = 0.10) and 3 hours after (p = 0.47) injection of the radiopharmaceutical. The MDA levels and enzyme activities of SOD, GPX and CAT are shown in Figures 1–4 respectively.

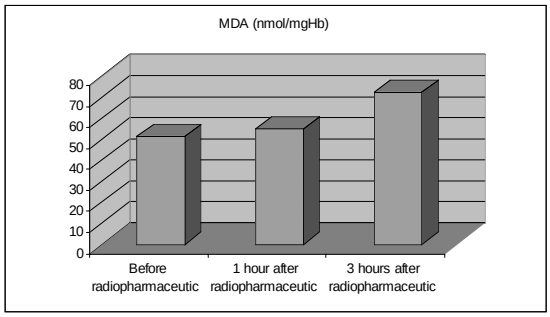

Fig. 1: MDA levels.

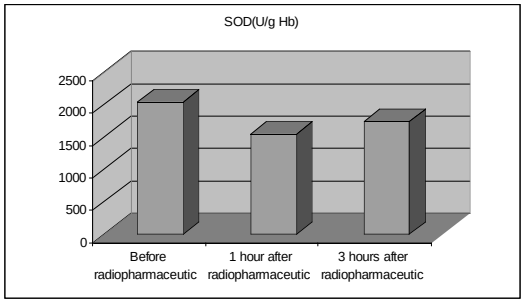

Fig. 2: The enzyme activities of SOD.

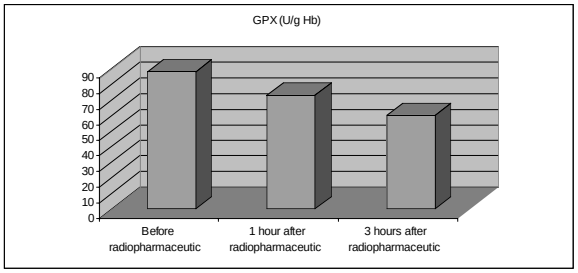

Fig. 3: The enzyme activities of GPX.

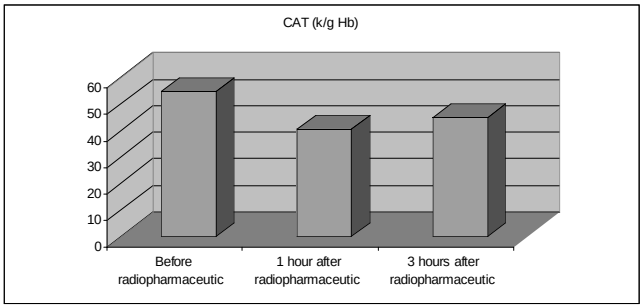

Fig. 4: The enzyme activities of CAT.

DISCUSSION

Ionizing radiation causes harmful effects through the generation of free radicals (14). Radiation is a known producer of ROS. When water, the most abundant intra and extracellular material, is exposed to ionizing radiation, decomposition occurs through which a variety of ROS, such as the superoxide radical , hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) are generated. These ROS formed in cells contribute to radiation injury in cells. Although all respiring cells are equipped with protective enzymes such as SOD and CAT or GPX, increased oxidative stress in cells that stem from ionizing radiation may overwhelm the protective systems, leading to cell injury (15). SOD converts superoxide anion radical to H_2O_2, thus decreasing the amount of and the formation of peroxynitrite anion ($ONOO^-$), a highly destructive product of the interaction between O_2 and nitric oxide (7).

Sabitha and Shyamaladevi (16) demonstrated that activities of erythrocyte SOD, CAT and GPX enzymes were significantly lower after radiotherapy than before radiotherapy in their study. This suggests that ionizing radiation causes enzyme deficiencies, arising as a result of enormous production of free radicals in the system. Lee *et al* (17) reported that SOD was an important anti-oxidant protein in the protection of yeast cells against ionizing radiation.

Lipid peroxide accumulation is believed to be a cause of cell membrane damage. Malondialdehyde is an index of lipid peroxidation. Its level increases in tissue after radiation-induced tissue injury (18). Green *et al* (19) and Buyukokuroglu *et al (*2) reported that ionizing radiation increased the level of MDA. In agreement with these results, we found that gamma radiation increases erythrocyte MDA levels. Nikishkin *et al* (20) reported that levels of enzymatic and non-enzymatic antioxidants decrease after irradiation. SOD and GPX each play a role in the antioxidant defence system but their response to radiation is unclear. Green *et al* (21) found that radiation did not significantly affect GPX activities in the long term while Kaya *et al* (22) reported that GPX activities were not decreased significantly after irradiation compared with sham controls. Our results demonstrate that the activities of GPX are not different in the before and after radiopharmaceutical groups. These results confirm those of Kaya *et al* (22) and Reiter (23).

The enzyme activities of CAT were decreased 1 hour after ($p > 0.05$) and 3 hours after injection of the radiopharmaceutical ($p < 0.05$). The enzyme activities of SOD were found decreased in the 1st hour samples ($p < 0.05$) and 3rd hour samples ($p < 0.05$). Malondialdehyde levels were increased 1 hour after ($p < 0.05$) and 3 hours after ($p < 0.05$) injection of the radiopharmaceutical.

In conclusion, it is known that ionizing radiation affects cells by increasing the levels of free radicals. On the other hand, the effects of nuclear medicine studies on free radicals are not clear. In this study, we found that radiation due to ^{201}TI myocardial perfusion scintigraphy applications

decreased the erythrocyte antioxidant levels and increased MDA levels.

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