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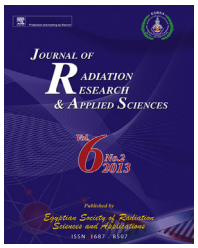

Effect of low dose gamma rays on certain essential metals and oxidative stress in different rat organs

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ABSTRACT

The present work aimed to determine the effect of low dose (0.03 Gy) γ-rays on certain essential metals namely Fe, Cu, Zn and Ca levels in various tissues (liver, kidney, testis, spleen, intestine, heart and brain) in rats. Also, lipid peroxidation as malondialdehyde (MDA) and metallothionein (MT) levels were measured in liver, kidney and testis. Rats were exposed to total dose of 0.03 Gy γ-rays along 12 h at a low dose rate (2.5 mGy/h). The results obtained manifested elevation in all essential metals studied in liver. All organs showed elevated Ca levels except that of brain with non-significant change in intestine. Decrease in Fe levels were observed in kidney, testis, intestine, heart and brain tissues with insignificant change in spleen tissue. Elevation in MDA in liver and kidney tissues was recorded and an inhibition in MT in liver, kidney and testis tissues was demonstrated. In conclusion, the previous essential metals tested are very sensitive to low dose (0.03 Gy) γ-rays. Such dose can induce alterations in the oxidative stress parameters under investigation.

1. Introduction

The biological consequences of exposure to ionizing radiation are mediated by a series of physical, chemical, biochemical, and cellular responses initiated after the deposition of radiation energy in the medium. The energy associated with ionizing radiation is significantly greater than the bond energies of many molecules and can cause homolytic bond scission and the generation of secondary electrons. The time scale of the initial steps of energy deposition and molecular bond scission is on the order of 10^{-13} s (Soule et al., 2007). Since water is the main constituent of cellular matter, it is primarily the ionization of water that results in the production of secondary species with high reactivity and short life times (10^{-10}–10^{-9} s) such as the OH radical, aquatic electrons, or hydrogen atoms which is the secondary species that mediate the chemical reactions that damage biologically important molecules as the cell membrane, enzymes and other proteins, and DNA that are possible critical targets in radiation-induced cytotoxicity. However, it is thought that DNA is the crucial target which, when damaged, can result in both cell death and genetic alterations (Soule et al., 2007). Exposure of healthy tissues to low doses ionizing radiation results in induced NFκB and SOD activity and transcriptional activation of NFκB-signal

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transduction/target molecules (Veeraraghavan, Natarajan, Herman, & Aravindan, 2011). Consequently these reactions extend to alter the physiological processes that depend on trace metals such as Fe, Cu, Zn and Ca and lead to their accumulation or release from the body. These transition metals have numerous biological roles as both structural and catalytic cofactors for proteins (Andreini, Bertini, Cavallaro, Holliday, & Thornton, 2008) and fulfill various essential biological functions and are thus vital for all living organisms (Bohic et al., 2011). Also there is strict requirement for transition metals in a variety of cellular processes, such as immune function (Wintergerst, Maggini, & Hornig, 2007) and neuronal function (Zheng & Monnot, 2011).

Therefore the present work aimed to investigate the effect of very low dose (0.03 Gy) γ-rays at very low dose rate (2.5 mGy/h) on certain essential metals, lipid peroxidation and metallothionein levels.

2. Material and methods

2.1. Animals

Male albino Wistar rats weighing 120–150 g were kept in plastic cages and were allowed free access to water and normal pellet diet and were maintained under controlled conditions of humidity, temperature and a diurnal environment of light and dark. Animals were acclimatized to laboratory before starting the experiment and during the irradiation period. All animal procedure were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the "Guide for the care and use of laboratory animals" published by the US National Institutes of Health (NIH publication No 85-23, 1996). Galvanized cylindrical metal cages of double walls at 32 and 48 cm diameter × 22 cm height, especially designed for this experiment. Rats were kept between the two walls of the cage which the middle axis of the cage was approximately at 20 cm from the source, taking in consideration the width between the two walls is 8 cm.

2.2. Irradiation procedure

Whole body γ-irradiation of animals was performed using low dose rate ^{137}Cesium source of 2.5 mGy/h belonging to National Centre for Nuclear Safety and Radiation Control (NCNSRC), Atomic Energy Authority, Nasr City, Cairo, Egypt.

2.3. Experimental study

Animals were divided into two groups, control group (non irradiated animals) and irradiated group. Animals were exposed to total dose of 0.03 Gy γ-irradiations along 12 h at a low dose rate (2.5 mGy/h). At the end of the irradiation process (12 h), eight rats from each group were anesthetized with diethyl ether and sacrificed during 1 h after irradiation. The liver, kidney, testis, spleen, intestine, heart and brain tissues were excised rapidly washed in deionized water, wiped dry with a filter paper and weighed for the assessment of the essential metals concentration. Liver, kidney and testis tissues were washed in saline for biochemical analysis.

2.4. Trace metals analysis

Fe, Cu, Zn & Ca concentrations were measured in the liver, kidney, testis, spleen, intestine, heart and brain tissues. For the digestion process using Milestone MLS-1200 Mega, High Performance Microwave Digestor Unit (Italy). 0.5 to 1 g of each organ was put in special vessels with 6 ml nitric acid and 1 ml hydrogen peroxide. After complete digestion, samples were diluted to suitable levels for metals analysis by UNICAM 939 Atomic Absorption Spectrophotometer (AAS) (England).

2.5. Lipid peroxidation determination

Lipid peroxidation levels were ascertained by the formation of MDA levels. Samples were weighed and perfuse in saline, rapidly removed and homogenized in four volumes of 0.25 M sucrose, then centrifuged at 3000 rpm for 15 min at 4 °C (Sardar, Chakraborty, & Chatterjee, 1996). In 10 ml centrifuge tube, 0.5 ml of supernatant taken with 2.5 ml of 20% tirchloroacetic acid, then 1 ml of 0.67% thiobarbituric acid was added, shaken and warmed for 30 min in a boiling water bath followed by rapid cooling. A 4 ml of n-butyl-alcohol was added and shaken, then centrifuged at 3000 rpm for 10 min at 4 °C. MDA content was colorimetrically determined in the resultant n-butyl-alcohol layer at 535 nm using the standard 10 nmol of MDA bis-diethylacetal (1,1,3,3-tetra-ethoxy propane) (Yoshioka, Kawada, Shimada, & Mori, 1979).

2.6. Metallothionein determination

MT levels were determined by Ag-saturation hemolysate method. Samples were weighed and perfuse in saline, rapidly removed and homogenized in four volumes 0.25 M sucrose, then centrifuged at 5000 rpm for 20 min at 4 °C, 0.05 ml of aliquot of the resulting supernatant fraction was incubated with 0.5 ml of 20 ppm Ag for 10 min at 20 °C to saturate the metal-binding sites of MT. The resulting Ag-MT was incubated in 0.5 ml 0.5 M glycine-NaOH buffer (freshly prepared) at pH = 8.5 for 5 min. Excess Ag will remove by addition of 0.1 ml rat RBCs hemolysate (Irato et al., 1996; Onosaka & Cherian, 1982), to the assay tube, shaken, followed by heat treatment in boiling water bath for 5 min to precipitate Ag-bond hemoglobin and other proteins, except MT which is heat stable. Denatured proteins were removed by centrifugation at 3000 rpm for 5 min. The hemolysate/heat/centrifugation (hem treatment) were repeated three times to ensure the removal of unbound metal Ag (Bienengräber, Forderkunz, Klein, & Summer, 1995; Scheuhammer & Cherian, 1986). The amount of Ag^{+} in the final supernatant fraction was estimated by using UNICAM 939 Atomic Absorption Spectrophotometer (AAS), England, where it is proportional to the amount of MT present (Irato et al., 1996). Blood of control rat was collected by heart puncture in heparinized tube. A 20 ml of 1.15% KCl was added to 10 ml blood, mix well and centrifuged at 3000 rpm for 5 min at 10 °C. The pellet containing the RBCs was suspended in 30 ml 1.15% KCl and centrifuged. The washing and centrifugation steps were repeated twice, then the washed RBCs were suspended in 20 ml freshly prepared 30 mM Tris–HCl buffer at pH = 8.0, kept at room temperature for 10 min for hemolysis. The membrane fraction was removed by