The biochemical changes in rats' blood serum levels exposed to different gamma radiation doses

Mohamed Anwar K Abdelhalim¹ and Sherif A. Abdelmottaleb Moussa²,³

¹Department of Physics and Astronomy, College of Science, King Saud University, Saudi Arabia.
²Department of Physics, College of Science, Al-Imam Muhammad Ibn Saud Islamic University, P. O. Box 90950, Riyadh, 11623, Saudi Arabia.
³Biophysics Group, Biochemistry Department, Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Giza, Egypt.

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This study aimed to address the different gamma radiations doses effect on the liver and kidney function of rats: in vivo. A total of 60 healthy male Wistar-Kyoto rats were whole body gamma irradiated with Co 60 source with 0.883 cG/sec dose rate at the beginning of the experiment. The rats were randomly divided into 4 gamma irradiation groups (25, 50, 75 and 100 Gy) The serum levels of activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), UREA, creatinine (CREA) and uric acid (UAC) were measured using automated biochemical analyzer. The ALT, GGT, ALP values significantly decreased with the different gamma radiation doses compared with the control. The AST and UREA values significantly decreased after irradiation with 25 and 50 Gy gamma radiation doses compared with the control while it significantly increased with 75 and 100 Gy gamma radiation doses. The levels of CREA values decreased with no significant manner after the irradiation with gamma radiation doses compared with the control. The levels of UAC values significantly increased with 50, 75 and 100 Gy gamma radiation doses. The serum ALT and AST levels are common markers for hepatic toxicity: A lower amount of ALP indicates liver problems. The decreased CREA and the increased UAC levels might indicate development of nephritis and renal dysfunction. The excess UAC might be converted to crystals depositing in the tiny tubes of the kidney and causing acute kidney damage. It is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation, and after the onset of oxidative stress, antioxidant treatment should be applied to delay or prevent the progression of damage.

Key words: Gamma radiation, different radiation doses, liver function, kidney function, rats.

INTRODUCTION

Serious problems are generally encountered after acute exposure to high doses of ionizing radiations (Yousri et al., 1991). Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction (Berroud et al., 1996). Exposure of mammals to ionizing radiations, leads to the development of a complex, dose-dependent series of changes, including injury to different organs, causing changes in the structure and function of cellular components, and resulting in tissue damage and death. Thus, radiation-induced damage might result in adverse health effects within hours to weeks or delayed effects observable many
months after exposure (Vijayalaxmi et al., 2004). Recently, oxidative stress is possibly involved in the pathology of some diseases and other inborn errors of lipid and protein metabolism (Onody et al., 2003). Oxidative stress with subsequent production of reactive oxygen species (ROS) has been postulated as one of the mechanisms of radiation toxicity (Finkel and Holbrook, 2000). It was also reported that ionizing radiation affected liver and kidney functions (El-Kashef et al., 1986; Roushdy et al., 1984).

Radiation damage, is to a large extent, caused by the overproduction of ROS, including superoxide anion ($O_2^{-*}$), hydroxyl radical (•OH), and hydrogen peroxide ($H_2O_2$), that decrease the levels of antioxidants, resulting in oxidative stress and cellular damage. ROS cause damage by reacting with cellular macromolecules such as nucleotides in nucleic acids, polyunsaturated fatty acids found in cellular membranes, and sulfhydryl bonds in proteins. If this damage is irreparable, then injury, mutagenesis, carcinogenesis, accelerated senescence, and cell death can occur (Spitz et al., 2004).

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It became apparent that radiation can penetrate the living cells and deposit within them in random fashion, leading to radiation damage. The blood chemistry measures the levels of chemicals, enzymes, and organic waste products that are normally found in the blood. It was of particular interest to investigate the toxicity associated with oxidative stress and the damage to rats induced by gamma-radiation exposure. In medicine, there is a limited knowledge on the levels of chemicals, enzymes, and organic waste products that are normally found in the blood after irradiating with different gamma radiation doses. Thus, it is of particular interest to investigate the mechanisms of radiation toxicity (Finkel and Holbrook, 2000). Ionizing radiations interact with biological systems through free radicals generated by water radiolysis. This indirect action plays an important role in the induction of oxidative stress leading to cellular damage and organ dysfunction (Berroud et al., 1996).

Materials and Methods

Chemicals

All reagents were of the highest purity available. All chemicals for biochemical analysis were purchased from Sigma Chemical Co.

Animals

Male Wistar rats weighing 150 to 200 g, were used in the present experiments. Experimental animals were housed in cages with free access to drinking water and diet and were maintained in the animal care facility throughout the duration of the experiment. The animals were kept at 20 to 25°C with the 12 h light/dark cycle. Animal husbandry and experimentation were consistent with the Public Health Guide for the Care and Use of Laboratory Animals and in accordance with protocols approved by King Saud University Local Experimental Animal Ethics Care and Use Committee.

Experimental design

A total of 60 healthy male Wistar-Kyoto rats weighing 250 g were used in this study. All rats were whole body gamma irradiated with Co 60 source. The animals were randomly divided into groups, 4 gamma-irradiation treated rat groups (The 1st group was irradiated with 25 Gy gamma radiation ($n = 10$); the 2nd group was irradiated with 50 Gy gamma radiation ($n = 10$); the 3rd group was irradiated with 75 Gy gamma radiation ($n = 10$); the 4th group was irradiated with 100 Gy gamma radiation ($n = 10$) and one control group (NG: $n = 20$)). The 4 gamma-irradiation treated rat groups were maintained on standard laboratory rodent diet pellets and were housed in humidity and temperature-controlled ventilated cages for a period of 24 h day/night cycle. The irradiation process was carried out at Research Center, King Saud University using Co 60 source with dose rate 0.9 ($\mu$G/s) at the beginning of the experiment.

Serum biochemical analysis

Biochemical analysis was performed 24 h after the irradiation with different gamma radiation doses. The rats were anesthetized by inhalation of 5% isoflurane until muscular tonus relaxed. After sacrificing the animals, 2 ml of blood samples were collected and placed in chilled non-heparinized tubes, centrifuged at 3000 rpm for 10 min at 4°C. The serum was frozen at -20°C for biochemical measurements. The serum levels of the activity of ALT, AST, ALP, GGT, urea, CREA and UAC were measured using automated biochemical analyzer (Type 7170, Hitachi).

Statistical analysis

The results of this study were expressed as mean ± standard error (Mean ± SE). To assess the significance of the differences between the control group and the four gamma-irradiated rat groups (25, 50, 75 and 100 Gy), a statistical analysis was performed using one-way analysis of variance (ANOVA) for repeated measurements with the significance assessed at the 5% confidence level.

RESULTS AND DISCUSSION

The ALT values significantly decreased with the different gamma radiation doses for rat blood serum when compared with the control (Figure 1). This study suggests that the liver might be damaged with irradiation with different gamma radiation doses. The liver enzyme ALT rearranges the building blocks of proteins.

The AST values significantly decreased after irradiation with 25 and 50 Gy gamma radiation doses when compared with the control, while it significantly increased with 75 and 100 Gy gamma radiation doses (Figure 2).

The serum ALT and AST levels are common markers for hepatic toxicity; levels of these proteins were rapidly
increased when the liver is damaged by any cause, including hepatitis or hepatic cirrhosis. Transaminases play an important role in protein and amino acid metabolism. They are found in the cells of almost all body tissues and when diseases or injuries affected these tissues, they are released into blood stream (Kaplan, 1986).

Some investigators have reported significant elevation in the activity of liver enzymes (ALT and AST) and kidney function tests (CREA and UREA) after gamma-irradiation (Makhlouf and Makhlouf, 2012; El-Deeb et al., 2006; Bhatia and Manda, 2004), while other investigators have reported the opposite (Hanan et al., 2007). The increase or decrease in the activity of liver enzymes and kidney function parameters might indicate occurrence of liver and kidney injury. Therefore, it is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation. It is hypothesized that after the onset of oxidative stress in the tissue, antioxidant treatment should delay or prevent the progression of that damage (Halliwell and Whiteman, 2004).

A single irradiation dose of 6 Gy caused hepatic and renal damage manifested biochemically as an elevation in levels of ALT and AST as well as an increase in blood UREA. The rats' significantly countered radiation induced
biochemical disorder: liver enzymes and kidney function analysis, as well as, cholesterol level in the serum (Ibrahim, 2013). The increase in the serum aminotransferase activities could be due to liver damage induced by free radicals generated after radiation exposure (Jirtle et al., 1990).

The GGT values significantly decreased with the different gamma radiation doses for rat blood serum when compared with the control (Figure 3). A significant increase in serum GGT activity after exposure of rats to ionizing radiation has been observed (Makhlof and Makhlof, 2012). The GGT is a key enzyme in the catabolism of glutathione (GSH) (Djavaheri-Mergny et al., 2002; Lee et al., 2002). Recently, it has been reported that the extracellular cleavage of GSH by GGT induces production of ROS, suggesting that GGT plays a pro-oxidant role (Lee et al., 2004). The significant decrease observed in serum GGT activity might be due to the presence of oxidative stress and damage. The present results might indicate that GGT is considered as one of the enzymes related to oxidative stress after the exposure to gamma radiation dose. The GGT is inversely associated with antioxidants (Kilanczyk and Bryszewska, 2003).

ALP values significantly decreased with the irradiation with different gamma radiation doses for rat blood serum when compared with the control (Figure 4). ALP is processed in the liver and excreted into the digestive tract in the bile. A higher ALP levels than normal indicates liver problems.

It has been reported that a significant increase (P ≤ 0.05) in ALP activities, CREA and urea levels were recorded in the serum of irradiated rats which might result from radiation-induced cell membrane damage followed by the release of intracellular molecules to the blood stream (Kaplan, 1986).

In this study, the levels of urea values significantly decreased with the irradiation with 25 and 50 Gy gamma radiation doses when compared with the control while it significantly increased with the 75 and 100 gy gamma radiation doses (Figure 5). Blood urea nitrogen is a part of urea; the waste product that is left over from the breakdown of protein. Urea circulates in the blood until it is filtered out by the kidneys and excreted in the urine. If the kidneys are not functioning properly, there will be excess urea levels in the bloodstream.

It has been reported that irradiation of male rats increased serum urea without significant changes in serum CREA. It is known that radiation causes an increase in glutamate dehydrogenase enzyme levels, which might increase carnabonyl phosphate synthetase activity leading to an increase in urea concentration (Roushdy et al., 1984). The increased serum CREA in the irradiated group indicates development of nephritis and renal dysfunction, a result in agreement with. This result may be attributed to impairment of glomerular selective properties caused by irradiation.

The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The increased concentration of thiobarbituric acid reactive substances (TBARS) and nucleic acid in the rat liver, indicating high level of oxidative stress, markedly enhanced with increasing radiation dose (Makhlof and Makhlof, 2012); similar observations were reported on radiation-induced oxidative damage in several organs (Bhatia and Manda, 2004; Sener et al., 2003) and mitochondrial. Ionizing radiation generates ROS as a result of water radiolysis. In actively metabolizing cells, there is considerable water apart from the target macromolecules. These ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes (Cadet et al., 2004).

Lipid peroxidation such as malondialdehyde (MDA) and 4-hydroxy nonenal (4NHE) have the ability to interact with and alter macromolecules, possibly resulting in diseases (Petersen and Doorn, 2004).

Oxidative damage to proteins, as assessed by formation of carbonyl groups is a highly damaging event, and may occur in the absence of lipid peroxidation (Dean et al., 1997). Thus, modification of lipids and proteins by ROS is implicated in the etiology of radiation-induced physiological disorders and diseases. This study suggests that the energy-radiation induced by cesium-137 source, produced a significant oxidative damage in the rats after whole body exposure. It has been reported that whole-body exposure of rats to high energy radiation from Co-60 causes tissue damage in several organs, as assessed by increased lipid peroxidation 2 and 12 h after irradiation (Sener et al., 2003).

In this study, the levels of CREA values decreased with no significant manner after irradiation with 25, 50, 75 and 100 Gy gamma radiation doses when compared with the control (Figure 6). CREA is a compound that is produced by the body and excreted in the urine. Compounds that leave the body in the urine are processed by the kidney; therefore CREA may be used to monitor the kidney function.

The levels of UAC values decreased with no significant manner with irradiation with 25 Gy gamma radiation dose when compared with the control, while it significantly increased with the 50, 75 and 100 Gy gamma radiation doses (Figure 7). UAC is the end product of the digestion of certain proteins and is normally eliminated through the urine. Excess UAC may be a side effect of some cancer treatments, and may lead to a condition called tumor lysis syndrome. When excess UAC is present, it is converted to crystal. These crystals may be deposited in the tiny tubes that are part of the kidney and cause acute kidney damage, which can ultimately lead to kidney failure.

Gamma rays act either directly or by secondary reactions to produce biochemical lesions that initiate series of physiological symptoms. Ionizing radiation is known to induce oxidative stress through the generation of ROS resulting in imbalance of the prooxidant and antioxidant
activities, ultimately resulting in cell death (Srinivasan et al., 2006). Numerous attempts have been made to investigate different means for controlling and protection from radiation hazards using chemical, physical and biological means.

This study suggests that additional experiments will be performed taking into consideration the oxidative stress through the generation of ROS such as hydrogen peroxide and oxygen free radicals; resulting in imbalance of the prooxidant and antioxidant activities, ultimately leading

Figure 3. The levels of gamma glutamyltransferase (GGT) versus the different gamma radiation doses for rat blood serum. *Means are significantly different (P<0.05).

Figure 4. The levels of alkaline phosphatase (ALP) versus the different gamma radiation doses for rat blood serum. *Means are significantly different (P<0.05).
leading to cell death. Investigation of the different means of controlling and protection from radiation hazards is through the use of chemical, physical and biological means through the prior administration of radioprotection agent to the irradiation with different gamma radiation doses preventing the oxidative stress.

Significant increase in the levels of serum lipid profile and low density lipoprotein (LDL) are demonstrated post radiation exposure of rats, possibly as a result of liver injury. These changes are in agreement with previous studies (Feurgard et al., 1999). This indicates that ionizing-radiation-induced oxidative stress which might alter hepatic lipid metabolism and serum lipoproteins. It seems that there is an association between radiation-induced oxidative stress and elevated levels of lipid fractions and LDL (Onody et al., 2003). This association is
similarly observed in other conditions characterized by increased oxidative stress (El-Missiry et al., 2004; Zwiriska-Korczala et al., 2003). Therefore, this study suggests that additional experiments will be performed taking into consideration the levels of LDL, high density lipoprotein (HDL), lipid peroxidation through MDA, GSH content, catalase (CAT) activity and DNA content.

Conclusions

Some investigators have reported significant elevation in the activity of liver enzymes after gamma-irradiation, while other investigators have reported the opposite. ALT, AST and GGT levels significantly decreased with the different gamma radiation doses in rat blood serum when compared with the control. Serum ALT and AST levels are common markers for hepatic toxicity: levels of these proteins are rapidly increased when the liver is damaged by any cause, including hepatitis or hepatic cirrhosis. The extracellular cleavage of GSH by GGT might induce ROS, suggesting that GGT plays a pro-oxidant role, and GGT might be considered as one of the enzymes related to oxidative stress after exposure to gamma radiation dose and it is inversely associated with antioxidants. The increase or decrease in the activity of liver enzymes and kidney function parameters might indicate liver and kidney damage. It is proposed that oxidative stress is linked to the organ damage following exposure to ionizing radiation, and it is hypothesized that after the onset of oxidative stress in the tissue, antioxidant treatment should be applied to delay or prevent the progression of that damage. Elevation and reduction in ALP, CREA and urea levels might result from radiation-induced cell membrane damage followed by the release of intracellular molecules to the blood stream. The decrease in ALP might be attributed to a transitory reduction in the release of alkaline phosphatase to the enzymatic circulation by rapidly metabolizing cells and/or injury to the intestinal mucosa after irradiation. The changes in urea might indicate kidneys failure. The changes in glutamate dehydrogenase enzyme levels might be attributed to carbamoyl phosphate synthases activity leading to changes in urea concentration. The changes in serum CREA in the irradiated rats group might indicate nephritis and renal dysfunction due to impairment of glomerular selective properties. The major forms of cellular damage induced by radiation are DNA damage, lipid peroxidation, and protein oxidation. The increased concentration of TBARS and nucleic acid in the rat liver, indicates high level of oxidative stress, which markedly enhanced with increasing radiation dose; similar observations were reported on radiation-induced oxidative damage in several organs and mitochondrial.

The modification of lipids and proteins by ROS might be implicated in the etiology of radiation-induced physiological disorders and diseases. Excess of UAC level might be converted to crystals, depositing in the tiny tubes of the kidney and causing acute kidney damage, leading to kidney failure. The ionizing radiation might induce oxidative stress through the generation of ROS, resulting in imbalance of the prooxidant and antioxidant activities, resulting in cell death, altering hepatic lipid metabolism and serum lipoproteins, and producing biochemical lesions that initiate series of physiological symptoms.

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REFERENCES


