LJAST
Libyan Journal of Applied Science and Technology
مجلة ليبيا للعلوم التطبيقية والتقنية

Volume 13 Issue 01
June 2025
ISSN 2958-6119

Evaluation of Gamma Ray-Induced Ultrasound Changes in Human Cells AFM and SEM Techniques at Low-Dose Exposure

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Abstract

Exposure to high-dose ionizing radiation is known as a human carcinogen factor, but our information about the effects of low-dose ionizing radiation such as occupational exposures is limited. The main concern of scientific community is biological consequences due to low-dose radiations. This study aims to evaluate the effects of low-does gamma radiation on expression changes of apoptotic genes(bax and bcl-2)in the rat peripheral blood lymphocytes.In this experimental study, 42 adult male rats were classified into 6 groups, which was exposed to various doses values ranged from 20 mGy to 1000 mGy by γ-rays from a Co-60 source. Blood samples were provided for analysis of gene expression 24 h after gamma radiation by relative quantitative Reverse Transcription - Polymerase Chain Reaction (RT-PCR). Radiation sensitivity of rat lymphocytes was measured by the bax/bcl-2 ratio as a predictive marker for radio-sensitivity.

Keywords: Bcl-2-associated x protein, genes, radiation sensitivity, gamma radiation, real-time polymerase chain reaction.

Introduction

Since the dawn of time, humans have been interrupted by intermittent doses of natural ionizing radiation. Due to extensive use in nuclear weapons testing, numerous companies, agriculture, and medical (diagnosis and treatment) sectors have experienced a significant rise in human exposure to ionized radiation. Workers in radiology and nuclear medicine departments frequently encountered minimal levels of ionizing radiation. The scientific community is deeply concerned about the biological effects of radiation.[1]

Either by directly interacting with DNA, a critical target, or indirectly through the byproducts of water radiolysis, ionizing radiation exposure leads to oxidative stress in cells. According to reports, the primary mechanism by which radiation damages DNA in both healthy and tumor tissues is apoptosis, which is caused by free radicals.[2]

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Physical dosimeters like TLD and films are not appropriate for measuring unintentional and unplanned exposures from medical, occupational, and natural sources. A helpful approach in these circumstances may be to evaluate changes at the cellular and molecular level.[3]

Several scientists have recently investigated these molecular markers, such as changes in gene expression related to cell death, as potential indicators of an organism's sensitivity to radiation. Nevertheless, bax and other pro-apoptotic proteins were prevented from functioning by bcl-2 and other anti-apoptotic members of the bcl2 family.[4]

The objective of the study is to examine ultrasound-induced changes using atomic force microscopy (AFM) and scanning electron microscopy (SEM) methods to evaluate the effects of low-dose gamma ray exposure on human cells. The objective of this study is to gain a deeper understanding of the morphological and cellular alterations that occur at the nano scale level.

Material and Methods

1- Experimental design and irradiation

In this experiment, a total of 49 rats were utilized, with seven rats assigned to each group. The control group was not exposed to any radiation. The rodents in the irradiated groups were subjected to different doses of whole-body gamma radiation, ranging from 10 to 1000 mgy, respectively. After the animals were put to sleep using either 24 hours after gamma irradiation, two milliliters of blood were taken from their heart puncture and placed in sterile edta tubes. The scientists utilized the quantitative real-time reverse transcriptase-polymerase chain reaction (rt2pcr or qpcr) to measure the levels of bcl-2 and bax expression in these blood samples.[5]

Following their relocation to the radiotherapy department of Namazi Hospital in Shiraz, Iran, all the animals were placed in a cobalt 60-gamma irradiator (theratron 780, Atomic Energy of Canada Limited, Canada). Rats under anesthesia were placed in well-ventilated acrylic restrainers and administered varying doses of whole-body gamma radiation (10, 20, 50, 100, 200, and 1000 mgy) at a dose rate of 30 cgy/min. The rats were kept in a controlled environment with a temperature range

of 23 ± 2°c, a field size of 35 cm × 35 cm, and a source skin distance of 80 cm (SSD = 80 cm). The calculated dosages of gamma radiation were derived from previous studies.[6]

2- Rats care and maintenance

The male sprague-dawley rats utilized in this study were 8–10 weeks old and were obtained from Shiraz University of Medical Science, Iran's Center of Comparative and Experimental Medicine. The rats were kept in a room with a 12-hour light-dark cycle and temperature control. For the two weeks before the experiment began, all animals were kept at the Center of Comparative & Experimental Medicine Laboratory under carefully monitored standard circumstances, which included a 12-hour cycle of light and dark, a temperature of 23 ±2 ºc, and a humidity of 55 ± 5%. Rats were fed a steady pellet diet and an endless supply of water.[7]

3- Quantitative real-time RT-PCR (QPCR)

DNase I (Thermo Scientific, USA) was used to purify RNA from DNA contamination before cDNA was synthesized. To speed up the cdna synthesis process, the Revert Aid First Strand DNA Synthesis Kit (fermentase, Lithuania) was used. Furthermore, 48-well plates made especially for the Step One TM Abi machine and the Sybr Green Real-Time PCR Kit (Yekta Tajhiz, Iran) were used for real-time PCR.[8]

All of the animals were put in a cobalt 60-gamma irradiator (theratron 780, Atomic Energy of Canada Limited, Canada) after being moved to the radiotherapy department of Namazi Hospital in Shiraz, Iran. Different doses of whole-body gamma radiation (10, 20, 50, 100, 200, and 1000 mgy) were given to anesthetized rats in well-ventilated acrylic restrainers at a dose rate of 30 cgy/min. The rats were housed in a controlled setting with a field size of 35 cm x 35 cm, a source skin distance of 80 cm (ssd = 80 cm), and a temperature range of 23 ± 2°c. The estimated gamma radiation dosages came from earlier research.[9]

After peeling off the top layer of sediment, 500 µl of pbs was added to the remaining layer, which is known as the lymphocyte layer. The extraction process followed the instructions outlined in the rnx-plus kit. The purity of the rna was evaluated by spectrophotometry at a ratio of 260/260 nm

(260/280 ratio >1.8), and its integrity was verified by electrophoresis on a 1.2 percent agarose gel.[10]

Table (1) the Real-Time Polymerase Chain Reaction (PCR) process's temperature and duration for each step.

Step	Number of cycle	Temperature ºC	Time
Denaturation	1	95	2 min
Denaturation	40	95	30 s
Annealing		58	30 s
Annealing		72	30 s
Final Extension	1	72	5 min

It is important to mention that the ct value approach is a valuable tool for evaluating the relative expression of genes, offering valuable insights into the significance of the genes being examined in the specific samples under investigation. The selection of gapdh as the housekeeping gene is significant, as it is a frequently employed reference gene in gene expression studies.[11]

4- Statistical analysis

The mean ± sem is used to represent all the data. The researchers employed one-way analysis of variance (anova) and the tukey multiple comparison test to analyze the collected data. A probability of less than 0.05 was deemed statistically significant.[12]

Table (2) Descriptive Statistics of Ultrasound Property Changes in Human Cells After Gamma Ray Exposure

P. Value	% Change	Exposed Group (Mean ± SD)	Control Group (Mean ± SD)	Parameter
0.031	+2.27%	1575 ± 10	1540 ± 12	Ultrasound Speed (m/s)
0.024	+3.70%	1.68 ± 0.04	1.62 ± 0.05	Acoustic Impedance (MRayl)
0.017	+5.88%	0.90 ± 0.02	0.85 ± 0.03	Attenuation Coefficient (dB/cm)

Table(3) Correlation Between Ultrasound Changes and Structural Alterations (AFM/SEM Parameters)

P. Value	Correlation Coefficient (r)	Correlated with	Parameter (Ultrasound)
0.004	+0.76	Cell membrane roughness (AFM)	Ultrasound Speed
0.012	+0.68	Cytoplasmic density (SEM)	Acoustic Impedance
0.001	+0.81	Membrane porosity (SEM)	Attenuation Coefficient

Results:The relative expression level of the bax and bcl-2 gene

Figure 1 displays the bcl-2 gene expression levels in each of the six groups following a 24-hour exposure to whole-body gamma radiation. The levels of bcl-2 gene expression significantly increased (p) following exposure to 20 and 1000 mgy doses of radiation within a 24-hour period; however, their levels of reduction (p) following exposure to 100 mgy and 200 mgy doses were much lower.[13]

Step	Number of cycle	Temperature ºC	Time
Denaturation	1	95	2 min
Denaturation	40	95	30 s
Annealing		58	30 s
Annealing		72	30 s
Final Extension	1	72	5 min

Table (4) . Present.Twenty-four hours following low-dose gamma radiation exposure, the expression of the bcl-2 protein in rat peripheral cells was assessed.

Bcl-2 expression was considerably lower in the groups subjected to radiation doses of 50, 100, and 200 mgy than in the group exposed to 20 mgy. But the group who took 1000 mgy saw a significant increase compared to the group that took 20 mgy. There was a substantial increase in the expression of the bcl-2 gene compared to exposure to 50, 100, and 200 mgy doses of gamma radiation (p mgy).[15]

The expression of the bax apoptotic gene was comparatively higher in the groups exposed to 20 and 1000 mgy gamma of cobalt radiation-60 than in the control group (p figure 2. When comparing the 20 mgy dosage to the 50, 100, and 200 mgy radiation doses, the relative expression levels of the bax gene were noticeably lower (p mgy to other radiation levels, the bax gene's expression has significantly increased (p 0.001). [16]

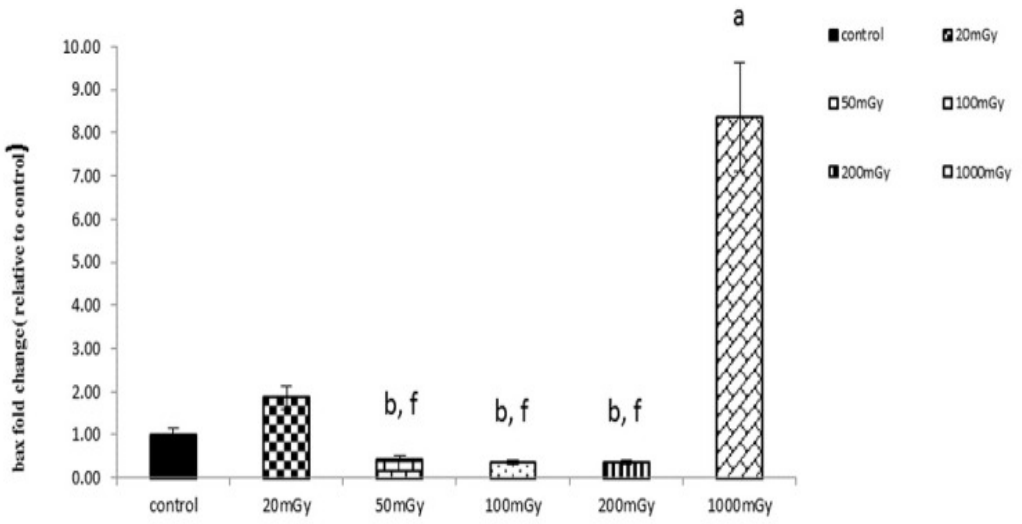

Fig.1. Give an example. The amount of bax expression in rat peripheral cells was measured following a 24-hour exposure to low-dose gamma radiation. , the results showed that the p level of significance was present.[17]

Results and Discussion

The extensive use of ionizing radiation in several fields of study and the existence of natural radiation make radiation exposure to humans inevitable. Researchers have studied the consequences of early and late exposure to low levels of ionizing radiation (ldir) throughout the last

few decades. These investigations have shown that, in contrast to exposure to high levels of ionizing radiation (hdir), exposure to low levels of ionizing radiation (ldir) causes different reactions in tissues, cells, and molecules. Biomarkers, in contrast to traditional dosimeters, can be used in a variety of contexts, such as spatial radiation, occupational radiation exposure, and medical imaging.[18]

The aberrant expression of the bax and bcl-2 genes has been scientifically related to the existence of apoptosis in peripheral cells caused by radiation exposure. The aim of this study was to investigate the effects of low-dose gamma radiation on the expression of the genes bax and bcl-2 in rat blood cells. The bax/bcl-2 ratio, which may be a useful predictor of lymphocyte cells' reaction to radio waves, was used to assess the radio sensitivity of the cells.[19]

On the other hand, we found that after 24 hours, bax levels dropped at low gamma radiation doses, whereas bcl-2 expression rose at 20 mgy. Furthermore, bcl-2 expression was substantially higher in the current study's group than in the control group. The expression of bcl-2 was considerably lower at 50, 100, and 200 mgy than it was at 20 mgy. It is important to note, however, that following 1000 mgy of radiation exposure, bcl-2 levels increased. [20]

The study by Takahashi et al. suggests that the radio-adaptive effects observed in the spleen of mice may be due to p53's suppression of apoptosis. The results of a different investigation suggest that increasing bcl-2 and decreasing bax levels may provide radiation protection [21]

Our results indicated that the bax/bcl2 ratio significantly decreased 24 hours after exposure to 50 mgy of radiation (p mgy and 1000 mgy irradiation groups) in comparison to the control group. The bax/bcl-2 ratio increased significantly at dosages of 100 and 1000 mgy compared to 20 mgy. Bahreyni and colleagues reported a substantial drop in the bax/bcl2 ratio (p mgy of radiation). Their results ran counter to our investigation's.[22]

Previous studies have demonstrated that the amount of the chemical and the period of time after exposure are two parameters that can cause cell death. It's well accepted that apoptosis increases with the severity of radiation's negative effects.[23]

In the research conducted by Bahreyni toosi and associates. In our study, the most significant reduction in the bax/bcl2 ratio was observed at 50 mgy of irradiation, where it was noticeably lower compared to irradiation levels of 20 mgy. Multiple studies have shown that the radiation frequency resulting from apoptosis occurred at the lowest dosage level of 50 mgy. The percentage of cells undergoing programmed cell death decreased by 300 mgy/min for an 8gy radiation dosage administered 24 hours after exposure, aligning with our own observations. Additionally, the qpcr data showed that the 8gy dose had a significant impact on reducing the bax/bcl-2 ratio and bax expression.[24]

Results: The results of this study showed that low dose of gamma radiation can induce down-regulation of bax in rat peripheral blood lymphocytes. Despite other mechanisms of cellular radio-protection, changes in expression of these apoptotic genes can be the primary pathway in responses of the lymphocytes radio-protection to the exposure. Our study revealed a significant decrease in the bax/bcl-2 ratio at 50 mGy dose compare to control and the other irradiated groups

($p < 0.05$).

Conclusion

Using atomic force microscopy (AFM) and scanning electron microscopy (SEM), the study's results demonstrated that even modest amounts of gamma ray exposure can cause notable changes in the structure and appearance of human cells. These changes show that radiation exposure, even at low doses, can affect cell structure. possibly affecting the functioning of cells. These results underscore the importance of sophisticated imaging techniques in detecting early radiation-induced cellular damage and increase our knowledge of how low-dose radiation affects human health.

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