Effects of Low-dose Gamma Radiation on Expression of Apoptotic Genes in Rat Peripheral Blood Lymphocyte

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ABSTRACT

Background: 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.

Objective: This study aims to evaluate the effects of low-dose γ-radiation on expression changes of apoptotic genes (bax and bcl-2) in the rat peripheral blood lymphocytes.

Material and Methods: 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.

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: These results suggest that changes in the bax/bcl-2 ratio especially in radiation workers, as a key factor in apoptosis, can be considered as a biological marker in low-dose gamma radiation.

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Keywords

Gamma Radiation; Real-Time Polymerase Chain Reaction; Bcl-2-Associated X Protein; Genes, P53; Radiation Sensitivity

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Introduction

Since the beginning of existence, humans have been exposed to low-dose natural ionizing radiation. Humans exposure by widespread use of ionizing radiation in a variety of fields such as medicine (diagnosis and therapy), industry, agriculture and nuclear weapons

tests has been growing rapidly [1, 2]. Medical radiation workers in nuclear medicine and radiology centers have been introduced as chronically exposed society of low-dose ionizing radiation. Although high-dose radiation is known to be a human carcinogenic factor inducing various biological effects, the biological effects of low-dose ionizing radiation such as occupational radiation have not been adequately known yet, and the main concern of scientific community is the biological consequences of the radiation [3]. Physical dosimeters such as TLDs, films as well as other devices and methods in accidental and unplanned exposures from natural, medical and occupational resources are not suitable. In such cases, evaluating changes in cellular and molecular level can be a good technique [4-7].

Cellular exposure to ionizing radiation leads to oxidative stress through direct interactions of radiation with DNA, as critical target, or via products of water radiolysis [8, 9]. It has been reported that the main mechanism of radiation damage (tumoral and normal tissues) through DNA damage is apoptosis or cell death caused by free radicals [10, 11].

In recent years, many researchers have considered these molecular endpoints, such as changes in the expression of genes involved in apoptosis as biological markers of radiation sensitivity [12-18].

P53 gene seems to play a vital role in initiating radiation-induced apoptosis, especially at the onset of apoptosis. Up-regulation of bax gene and its transfer from cytoplasm to mitochondria lead to activation of p53. On the other hand, the anti-apoptotic members of the bcl2 family, such as bcl-2, counteracted the actions of bax and other members of the pro-apoptotic proteins. Therefore, the bax/bcl2 ratio can be considered as a radio-sensitivity marker [19, 20].

The aim of this study was to evaluate the changes in expression of apoptotic genes (bax, bcl-2 and bax/bcl-2 ratio) in rat peripheral blood lymphocytes following low-dose gamma radiation.

Material and Methods

Rats care and maintenance

In this experimental study, 8-10 week male Sprague-Dawley rats were purchased from the Center of Comparative & Experimental medicine, Shiraz university of Medical science, Shiraz, Iran. All animals were kept under controlled standard conditions of temperature (23 ±2 ºC), humidity (55 ± 5%), with a 12-h light and 12-h dark cycle at the center of comparative & experimental medicine laboratory for two weeks before the start of experiment. Rats were fed based on the standard pellet diet and water was given ad libitum. All experiments were performed in accordance with the guideline of the Ethics Committee of Shiraz University of Medical Sciences.

Experimental design and irradiation

All animals were transferred to cobalt 60-gamma irradiator (Theratron 780, Atomic energy of Canada limited, Canada) facility in the department of radiotherapy, Namazi hospital, Shiraz, Iran. Anesthetized rats were placed in well-ventilated acrylic restrainers and exposed to whole-body gamma radiation of different doses (10, 20, 50, 100, 200 and 1000 mGy) at dose rate of 30 cGy/min with a source skin distance of 80 cm (SSD = 80 cm) and fixed field size of 35 cm × 35 cm at room temperature (23 ± 2 ºC). The doses of gamma radiation selected were based on previous studies [12, 19].

This experiment was performed using 49 rats with 7 rats in each group. Control group did not receive irradiation. The rats in irradiated groups were exposed to whole-body gamma radiation with doses of 10, 20, 50, 100, 200 and 1000 mGy, respectively. 24 h after exposure to gamma irradiation, all the animals were anesthetized with ether, and the blood samples (2 ml from each animal) were collected from the

heart puncture in EDTA sterile tubes. These blood samples were used for measurement of bcl-2 and bax expression levels using quantitative real-time reverse transcriptase- polymerase chain reaction (RT^2PCR or QPCR).

Quantitative real-time RT-PCR (QPCR)

According to the standard protocol, lymphocyte isolation from each of the blood samples was performed by Ficoll Lymphodex (Innotrain, Germany). Blood was diluted 1:3 with Phosphate-Buffered Saline (PBS) and carefully layered onto the Ficoll in the ratio of 2:1 (Blood + PBS: Ficoll). The blood was centrifuged at room temperature at 3000 RPM for 20 min. The layer was removed and centrifuged at 1400 RPM for 10 min after three times washed with PBS. The supernatant layer was removed and 500 µL of PBS was added to the sediment layer (Lymphocyte layer). RNA extraction was performed according to the protocol of RNX-Plus Kit. RNA purity was measured by spectrophotometry at 260/260 nm ratio (260/280 ratio >1.8) and integrity was confirmed by electrophoresis on a 1.2% agarose gel. The DNase I (Thermo Science, USA) was used before cDNA synthesis to remove pollution of RNA with DNA. The synthesis of cDNA was done by the protocol of Revert Aid First Strand cDNA synthesis kit (Fermentase, Lithuania). Moreover, RT PCR was performed with SYBR Green Real Time PCR kit (Yekta tajhiz, Iran) using the plates of 48 wells specialized for Step One ™ ABI machine. Number of cycles and temperature conditions for Real-time PCR were listed in the Table 1.

The comparative CT (threshold cycle) value method was used to measure the relative expression of the genes of interest in each of the samples. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene [21].

Statistical analysis

All results are expressed as mean ± SEM. The data were analyzed using One-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. A value of $p < 0.05$ was considered statistically significant.

Results

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

Figure 1 shows the levels of bcl-2 gene expression in the six studied groups after 24 h following exposure of whole-body gamma radiation. Exposure to 20 mGy and 1000 mGy doses in 24 h after irradiation in comparison with control group resulted in a significant increase of bcl-2 gene expression levels ($p < 0.001$); although the expression of this gene significantly decreased in 100 mGy and 200 mGy doses, ($p < 0.001$ and $p < 0.01$, respectively).

In the groups exposed to radiation 50, 100 and 200 mGy in comparison with the group exposed to 20 mGy, a significant decrease in

Table 1: The time and temperature conditions in the various steps of Real-Time Polymerase Chain Reaction (PCR).

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

expression of Bcl-2 was observed. In contrast, there was a significant increase in the 1000 mGy group compared to the 20 mGy group ($p < 0.001$). Bcl-2 gene expression in 1000 mGy exposure showed a significant increase in comparison with exposure to 50, 100 and 200 mGy doses of gamma radiation ($p < 0.001$).

According to Figure 2, in exposed group to irradiation of 20 and 1000 mGy gamma of cobalt radiation-60 in comparison with the control group, a relative increase of bax apoptosis gene expression was seen ($P < 0.05$ and $p <$

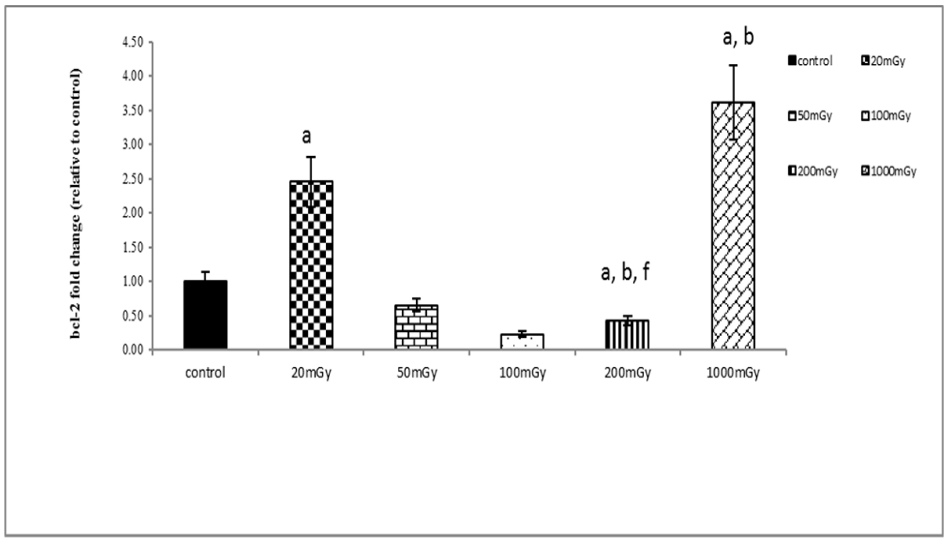

Figure 1: Effect of low-dose gamma radiation on bcl-2 expression in rat's peripheral lymphocytes 24 h after exposure. Vertical bars represent mean ± SEM (standard error of mean), n=7 for each group. [a] $p < 0.05$ when compared to the control group, [b] $p < 0.05$ when compared to 20 mGy, [f] $p < 0.05$ when compared to 1000 mGy.

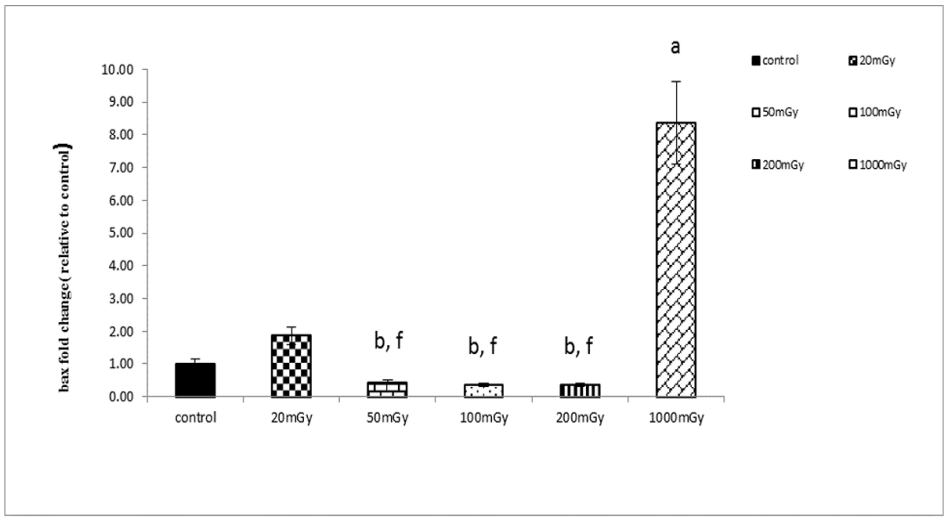

Figure 2: Effect of low-dose gamma radiation on bax expression in rat's peripheral lymphocytes 24 h after exposure. Vertical bars represent mean ± SEM (standard error of mean), n=7 for each group. [a] $p < 0.05$ when compared to the control group, [b] $p < 0.05$ when compared to 20 mGy, [f] $p < 0.05$ when compared to 1000 mGy

0.001 respectively). The relative expression levels of bax gene in 50, 100 and 200 mGy doses radiation have shown a significant decrease compared to the 20 mGy dose ($p < 0.001$). In 1000 mGy radiation dose, the relative expression of BAX gene has shown a significant increase compared with other radiation doses ($p < 0.001$).

Evaluation of bax/bcl-2 ratio

In Figure 3, the bax/bcl-2 ratio was shown in six groups under study subsequent to 24 h exposure to cobalt gamma-60. Although the bax/bcl-2 ratio in the mGy 50 group has had a significant reduction in comparison with the control group ($p < 0.05$), this ratio has showed a significant increase in the groups exposed to 100 and 1000 mGy ($p < 0.001$).

Exposure to 100 and 1000 mGy resulted in a significant increase of this ratio compared with 20 mGy ($p < 0.001$). In groups 100 and 1000 mGy, the ratio of bax/bcl-2 had a significant increase in comparison to the 50 mGy group ($p < 0.001$). This ratio decreased significantly in the 200 mGy radiation group compared to the 100 mGy radiation group ($p < 0.001$). However, the ratio of bax/bcl-2 had a significant increase in mice of the 1000 mGy radiation group compared with the 100 mGy ($p < 0.001$).

Discussion

Today, in addition to exposure to natural radiation, the widespread application of ionizing radiation in various fields of human knowledge leads to human exposure inevitably [22-24]. The researchers have investigated the effects of exposure (early and late) to low dose of ionizing radiation (LDIR) on human health in recent decades [25-27]. These studies have shown that tissue, cell, and molecular level responses are different after exposure to low doses of ionizing radiation (LDIR) compared with high dose of ionizing radiation (HDIR) [28]. Unlike physical dosimeters, the use of biomarkers in various scenarios such as medical imaging, occupationanal, and spatial radiation can be appropriate [29, 30]. Lymphocytes are known to be the most sensitive cells that are easily affected by apoptosis in patients undergoing radiotherapy and public [31, 32].

It has been shown that the abnormal expres-

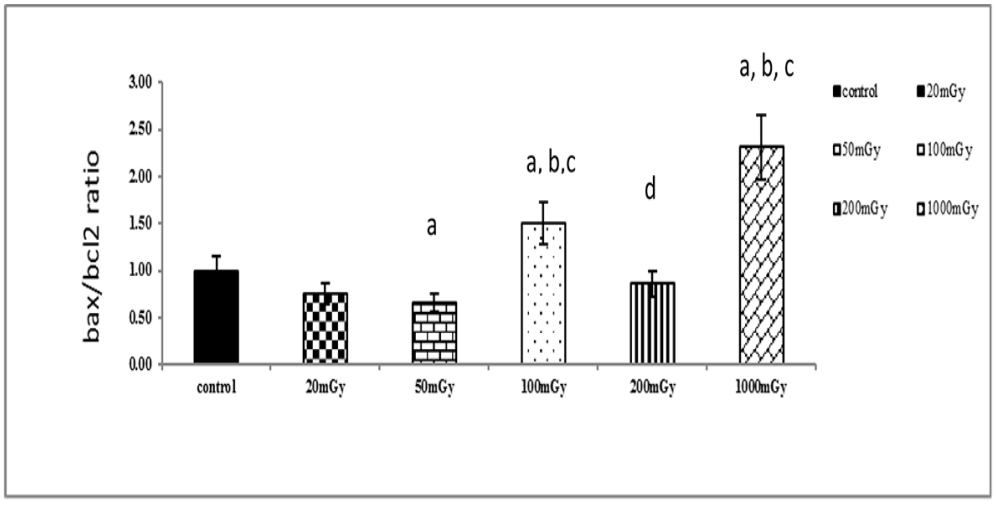

Figure 3: Effect of low-dose gamma radiation on bax expression in rat's peripheral lymphocytes 24 h after exposure. Vertical bars represent mean ± SEM (standard error of mean), n=7 for each group. [a] $p < 0.05$ when compared to the control group, [b] $p < 0.05$ when compared to 20 mGy, [c] $p < 0.05$ when compared 5o mGy, [d] $p < 0.05$ when compared to 100 mGy, [f] $p < 0.05$ when compared to 1000 mGy.

sion of bax and bcl-2 genes is closely related to radiation-induced apoptosis in peripheral lymphocytes [33]. The purpose of this study was to evaluate the effects of low-dose gamma radiation on the expression of bax and bcl-2 genes in rat peripheral blood lymphocytes. Radio-sensitivity of lymphocytes cells was measured by the bax/bcl-2 ratio (as a predictive marker for radio-sensitivity).

The results of the study carried out by Takahashi et al. showed that the radio-adaptive effects in mouse spleen may be due to a suppression of p53-mediated apoptosis [34]. Another study has also shown that the upregulation of bcl-2 and downregulation of bax can cause radiation protection responses [35].

However, the result of our study indicates that the expression of bcl-2 was found to be up-regulation at 20 mGy at 24 h after gamma radiation, and bax was down-regulation at low doses of gamma radiation at 24 h. Furthermore, in the present study, the expression of bcl-2 at 20 mGy was significantly higher than control group. bcl-2 expression significantly decreased following exposure 50, 100 and 200 mGy in compared to 20 mGy. In contrast, up-regulation of bcl-2 was indicated following irradiation 1000 mGy.

Our results demonstrated a significant decrease in bax/bcl2 ratio at 24 h following 50mGy irradiation compared to control group ($P < 0.05$). In contrast, 100 mGy and 1000 mGy irradiation groups increased significantly for the bax/bcl-2 ratio ($p < 0.05$). In addition, at doses of 100 and 1000 mGy, bax/bcl-2 ratio significantly increased compared with 20 mGy.

Bahreyni Toosi et al. have shown that the bax/bcl2 ratio significantly decreased at 24 h following irradiation 100 mGy ($P < 0.05$). Their results were not consistent with our study. In the study of Bahreyni Toosi et al. the bax/bcl2 ratio was significantly reduced in 20 mGy irradiation, but in our study, the maximum reduction in bax/bcl2 ratio at 50 mGy was observed [12]. It has been shown in several studies that the lowest dose level at which the apoptosis-induced radiation frequency was 50 mGy. These results were in line with our results [36, 37].

Our previous study showed that the percentage of apoptotic lymphocytes for 8Gy radiation dose decreased in 300 mGy/min at 24 h after exposure. In addition, based on the results of qPCR, in the dose of 8Gy, the bax expression and the ratio of bax/bcl-2 decreased significantly [17]. According to previous studies, apoptosis depends on dose value and post-exposure elapsed time. In fact, increased levels of apoptosis can explain the more damaging effects of radiation, and vice versa [4]. Our results could support the hypothesis that the radiation protection effects of low-dose radiation can be due to decrease in the bax/bcl-2 ratio leading to the increase in cell survival.

Conclusion

Based on the results obtained in this study, the low-dose gamma radiation would reduce the bax/bcl-2 ratio in irradiated rats, mainly attributed to its radio-adaptive effects.

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Authors' Contribution

Reza Fardid conceived the idea. Introduction of the paper was written by Reza Fardid and Zhila Ghorbani. Zhila Ghorbani gather data and the related literature and also help with writing of the related works. The method implementation was carried out by Reza Fardid and Zhila Ghorbani. Results and Analysis was carried out by Reza Fardid and Zhila Ghorbani. The research work was proofread and supervised by Reza Fardid. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

The Ethics Committee of Shiraz University of Medical Sciences approved the protocol of the study (Ethic cod: IR.SUMS.REC.1395.101).

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Conflict of Interest

None

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