New Aspects of Radiotherapy in Rheumatoid Arthritis Biomarker: Radio Sensitivity Response to Acute Ionizing Radiation Induces Cell Characteristic Reprogramming and Enhanced Cytokine Release of In Vitro Activated Macrophage Cell Line (RAW 264.7)

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Research Article

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Abstract

The response of biological systems to various types of radiations have many ambiguous dimensions. Among ionizing radiations, in vitro external gamma radiation therapy has mostly studied as model to declare the biological system challenges with radiation effects.

Cell/organism exposure to gamma radiation, caused cascade of ionization events such as severe irreversible biological damages. However, the biological responses and oxidative stress related mechanisms under acute radiation conditions poorly understood in inflammatory systems. Following study tried to give a model about ionizing radiation effect on the macrophage that had a key role in inflammation mechanisms; to evaluate the impact of radiotherapy approach for inflammatory disease as rheumatoid arthritis.

To this aim, Macrophage cell line (RAW 264.7) culture, exposed to different doses of gamma radiation (0,4, 6, 8, 10 Gy). Cell viability, apoptosis, cell cycle, migration; NO and PGE2 production; expression of pro-inflammatory and apoptotic genes and cytokine secretion of macrophages was also evaluated.

The results showed that gamma treatment, at 4 Gy radiation, have slight effect on macrophage characteristics and cytokine secretion pattern. Versus, higher doses (8 and 10 Gy) increased DNA damage, expression of apoptotic genes and secretion of NO and PGE2 cytokines. 6 Gy radiation, the maximum radiation dose, show moderate nondestructive effects and inflammation process modulation.

In this study, doses higher than 6 Gy of Gamma radiation caused cell mortality. It seems that 6 Gy Gamma radiation modulate the inflammatory cascade caused by macrophage cell, as a central core of autoimmune inflammatory disease in acute dose radiation therapy.

Introduction

Immune system’s response to destructive stimuli like damaged cells, pathogens, toxins, and irradiations called inflammation, which removes harmful agents and initiates the process of healing. However, uncontrolled acute inflammation can become chronic and cause a range of chronic inflammatory disorders and pathophysiology [1–3].

Inflammation is common effector mechanisms lead to extracellular matrix remodeling, fibrosis, angiogenesis, oxidative stress and tissue damage in different organs[4]. Acute and chronic inflammation responses in the pancreas, kidney, heart, brain, intestinal tract, reproductive system, liver and lung cause inflammatory disease such as atherosclerosis, arthritis, inflammatory bowel disease, kidney and lung disease[5, 4].

IL-6, TNF-α and IL-1β are Inflammatory cytokines and biomarkers used as inflammatory disease prognosis, diagnosis biomarkers and therapeutic decisions [6–10]. Monocytes, macrophages and other cells are responsible for local responses to infection and tissue damage. Although, Monocytes can be
differentiated into macrophages and dendritic cells and attract to damage site via chemotaxis. Most disorders including asthma, cancer, diabetes, atherosclerosis, chronic inflammatory disease, degenerative and autoimmune diseases related to alternations of inflammation mediated immune cell [11–13].

According to extensive uses of ionizing irradiation in medication, low doses or acute ionizing irradiation is new approach that is widely under study and investigation. Although, its therapeutic effects is not clearly obvious, its harmful biological effects can't be neglected [14]. It shown that radiation can cause inflammatory response and has effects on expression of ZEBRA protein and stress mediators (glucocorticoid) in Epstein - Barr virus (EBV). EBV reactivation induced by 2 and 4Gy gamma irradiation caused the combination of glucocorticoid and gamma radiation that play a key role in the inflammatory response [15].

In 2010, researchers reported that exposure to ionizing radiation showed a dose dependent increase in the expression of IL-6 at 20 hours, while immediately after radiation the release of IL-8 decreased compared to control group but elevated over the time, particularly at 0.5 Gy radiation dose [16]. As reported previously, 0.5 Gy gamma irradiation upregulated the MKP-1 and suppressed TNF-α production by P38 MAPK inactivation in mice macrophage cell lines[17]. Moreover, another researches illustrated that low dose of gamma radiation increase the expression and activation of Nrf2 in macrophage RAW264.7 cell line [18]. In 2018, enhancement of INF/IL4 in lymphocytes of BALB/c spleen by low dose of local gamma radiation, suggests that acute ionizing radiation may be a good choice in radiotherapy and can attenuate inflammatory disease. The aim of this study, evaluation of effect of different dose of gamma radiation (4, 6, 8, 10 Gy) on cell viability, apoptosis, cell cycle, migration and motility of RAW264.7 macrophage cells. In addition, cytokine production, NO and PGE2 production as inflammation mediators, expression and upregulation of pro-inflammatory and apoptotic genes were be investigated to take a complete comprehensive model of acute gamma radiation on the macrophage cell line as the main mediator of all inflammatory responses.

**Material And Methods**

**Cell Culture**

Macrophage cell line RAW 264.7 obtained from National Cell Bank of Iran (NCBI). Cells were cultured in Dulbecco’s Modified Eagle medium (DMEM) (GIBCO) supplemented with 10% fetal bovine serum (FBS) (GIBCO, Life Technologies, Ghent, Belgium), 100 U/mL of penicillin and 100 µg/mL of streptomycin. The cells grown until reached 70–80% confluence in the culture flaks (Fig. 1a). The cells passaged and plated at 1:3 or 1:2 dilutions every 2–3 days using 0.25% trypsin and 1mM EDTA (Invitrogen LT, Merelbeke, Belgium). The cells frozen in 90% DMEM with 10% DMSO (Merck, Darmstadt, Germany) in liquid nitrogen.

**Cell viability screening by MTT assay**

RAW 264.7 macrophage cells seeded in 96-well plates (1×10⁴ cells/well) and irradiated in the Radiotherapy Department of Imam Hospital at room temperature, using a Co-60 gamma-ray source
(Source Canada, Theratron 780 E) with a dose-rate of 0.4 Gy/min, at a distance of 1 m from the source. This study aimed at investigating the effect of gamma radiation with 4, 6, 8 and 10 Gy doses. In control group, cells received no radiation.

Cell viability measured by MTT (3-(4,5-Dimethyl-2-thiazoly)-2,5- diphenyl-2H-tetrazolium bromide) assays, 24h after radiation, as described previously [19]. The percentage of cell viability was calculated and compared to control (100% of viability).

Apoptosis process assay by Flow cytometry

RAW 264.7 macrophage cells seeded in 6-well plates (16 ×10^4 cells/well) and irradiated with at least to 4, 6, 8 and 10 Gy doses. Other irradiation conditions done as previous assay. Control group, received no radiation. 24 h after treatment, cells collected in 500 µl Binding Buffer (Annexin V-FITC/PI apoptosis detection kit, Jiangsu Keygen Biotechnology). Annexin V fluorescein isothiocyanate and propidium iodide (FITC; 5 µl) added to cell suspension and incubated for 15 min at room temperature in the dark. The cells analyzed with FACSCalibur flow cytometer with the BD CellQuest™ Pro Analysis system (BD Biosciences, USA).

Cell cycle analysis by Flow cytometry

After collection the cells seeded in 6-well plates, the cell pellet washed twice with PBS buffer and fixed in a cold 70% EtOH solution at 4°C for 24 h. Fixed samples washed with PBS and stained with a 1 ml propidium iodide cocktail (50 µg/ml PI + 20 µl RNase A + 1 µl triton x-100) for 30 min at room temperature. Afterwards, the samples subjected to cell cycle description by flow cytometry (BD Biosciences, USA). Cells in G0/G1, S, and G2/M-phase determined by filtering for doublets and aggregates.

In vitro migration assay

For this assay, 4×10^4 cell/well seeded onto 24-well plates. After overnight incubation at 37°C and 5% CO2, cells starved for 24 h. A scratch then made on the monolayer using a sterile 200 ll-pipette tip. The monolayer was rinsed three times with and placed in DMEM with 1% FBS followed by irradiation (4, 6, 8 and 10 Gy). Reduction in the scratch areas monitored by inverse light microscope (ECLIPS 80i with WG filter, Nikon, Tokyo, Japan) equipped with digital camera. The images were taken at the interval time of 0, 24, and 48 h [20]. The percentage of migration calculated based on the reduction in scratch area measured at the specific time as described below:

\[
\text{Migration} = \frac{\text{Scratch area at } 0 \text{ h} - \text{Scratch area at specific time}}{\text{Scratch area at } 0 \text{ h}} \times 100
\]

= ((Scratch area at 0 h - Scratch area at specific time)/ Scratch area at 0 h)*100

Three-dimensional movement assay
Trans-well migration assay performed to measure three-dimensional movement of the cells under treatment condition. The cells (4×10^4 cell/well) were plated in DMEM supplemented with 1% FBS in the upper chamber of 8 µm pore, 24-well trans-wells plate (SPL life science Co., Gyeonggi-do, Republic of Korea). The cells allowed to adhere for 2 h then exposed to irradiation (4, 6, 8 and 10 Gy). After 24 h rest in the incubator, the polycarbonate membrane removed. The cells that migrated through pores to lower surface rinsed with PBS, fixed in 100% methanol and stained with ethanol-based crystal violet solution. Relative to control migrated cells shown as the means of the cell numbers in fifteen randomly selected vision.

Stimulation inflammatory pathways of Raw264.7 Cells with LPS

Lipopolysaccharide (LPS) (1 µg / ml) used to stimulate and activate inflammatory pathways in Raw264.7 Macrophage cells. The cells incubated for 18 h with LPS at 37 °C, 5% CO2 and 95% humidity.

Cytokine releases assay by ELISA

Cytokines secreted from the cells to the media, determined by ELISA kits (BD Biosciences, San Jose, CA, USA), according to the manufacturer's instructions. Briefly, RAW264.7 macrophage cells (2×10^4 cells/well) cultured in a 48-well plate with DMEM containing 10% FBS, incubated at 37°C and 5% CO2 incubator. The content level of IL-1β, IL-6 and TNF-α in culture media evaluated at 450 nm in a BioTek ELx808 microplate reader (control, LPS and irradiated with 4, 6, 8 and 10 Gy groups). The absorbance values converted to concentrations of IL-1β, IL-6, and TNF-α (pg/mL) using standard curves prepared with serial dilutions of recombinant IL-1β, IL-6, and TNF-α standard protein.

NO and PGE2 production

After 24 h cell rest from the irradiation (4, 6, 8 and 10 Gy) cultured cells in the 6 well plates (16 × 10^4 cell/mL), the quantity of nitrite as indicator of NO release to the culture medium measured. The level of nitrite - stable metabolite of NO - measured using Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid). Briefly, 100 µL of cell culture medium mixed with 100 µL of Griess reagent. Subsequently, the mixture incubated at room temperature for 10 min then the absorbance measured at 540 nm by microplate reader. Fresh culture medium used as a blank in every experiment. Quantity of nitrite determined using sodium nitrite standard curve. The concentration of PGE2 in cell culture media determined using a PGE2 assay ELISA kit (Cayman Chemicals, Ann Arbor, MI, USA) following the manufacturer's instructions.

Quantitative analysis of gene expression

Total RNA extracted from each one of the groups (control, LPS and irradiated with 4, 6, 8 and 10 Gy groups) using RNeasy Mini Kit (Qiagen, USA) according to the manufacturer’s recommendations. Quantity and quality of extracted RNA determined by UV spectrophotometry (Eppendorff, Germany). cDNA synthesis performed from 500 ng DNase-treated RNA samples with a Quantitect Reverse
Transcription Kit, using oligo (dT) primers. The specific primers used for PCR reactions (Table 1). q-PCR reaction performed using Master Mix and cyber green in an Applied Biosystems, StepOne™ thermal cycler (Applied Biosystems, USA). PCR program started with an initial melting cycle for 5 min at 95°C to activate the polymerase, followed by 40 cycles of melting (30 Sec at 95°C), annealing (30 Sec at 58°C) and extension (30 Sec at 72°C). The quality of the PCR reactions confirmed by melting curve analyses. Efficiency of primers determined for each gene using a standard curve (logarithmic dilution series of cDNA of each sample). The reference gene (GAPDH) and target genes amplified in the same run. The target genes normalized by reference gene (that had equal expression in all runs) and expressed relatively to calibrator.

## Results

Radiation causes changes in the viability

The effect of radiation viability of macrophage cell line investigated by MTT assay. After 24 h cell rest following exposure to 4, 6, 8 and 10 Gy of gamma-radiation, the cytotoxicity of each dose of radiation assayed. The results show that viability of RAW 264.7-treated cells not affected by radiation dose treatment less than 10 Gy. In this dose of radiation the viability significantly increased (p < 0.05) (Fig. 2b).

Radiation increase apoptosis in Raw264.7 cell line

The rate of apoptosis percent in the radiation treated macrophage cells (the lower right quadrant) increased in all treated groups significantly (Fig. 2a). Therefore, at versus the percent of live cells significantly decreased in all treated groups except 4 Gy radiation received group. High dose exposed groups (8 and 10 Gy) showed increase in apoptosis percent (Fig. 2b).

Gamma radiation induces G2/M arrest in macrophage cell line

Influence of Gamma radiation on the cell cycle progression and distribution, were analyzed using flow cytometry. The results indicated that arrest of the cells in G2/GM phase increased in all treatment groups. This inhibition was greater in the 8 and 10 Gy groups (Fig. 2c,d).

In vitro migration assay

Evaluation of the effect of gamma radiation on migration potential of macrophages assayed. Scratch method mimics some extent migration cells movements in vivo. The monolayer culture of Raw264.7 treated with different dose of gamma radiation showed a significant lower potential rate of migration compared to control group after 0, 24 and 48h time duration. (Figs. 3a,b). That means gamma radiation reduced the migration potential of macrophage cells.

Gamma radiation increases cell motility in Raw264.7 cells
Another important characteristic of gamma radiation biological effects involved in its effect on the cell ability to migrate. In order to minimize cell division, cells pass starvation time by supplementation of the culture media just with 1% FBS. Trans-well migration assay illustrated the three-dimensional movement. Macrophage cells were cultured in the upper chamber of 8 µm pore size trans-wells and allowed to adhere for 2 h and irradiated. The cells incubated for 24 h and migrated to the bottom of the trans-well filter. At the end time of rest, the migrated cells counted. The results showed that gamma radiation significantly increases the ability to migrate and mobility of the cells (Fig. 3c).

Inhibitory effect of gamma radiation on pro-inflammatory cytokines

In order to determine whether gamma radiation might also have effects on modulation of the pro-inflammatory cytokines in RAW264.7 macrophage cells, the pro-inflammatory cytokines, such as of IL-1β, IL-6 and TNF-α, determined by cytokine detection ELISA kits. As shown in Fig. 6a, b and c, the treatment of RAW264.7 macrophage cells with LPS, 4, 6, 8 and 10 Gy irradiation significantly increased the IL-1β, IL-6 and TNF-α secretion to the cell culture media. The induction effect of the cytokines by 10 Gy radiation were significantly greater than other groups. These findings strongly suggest that radiation is able to modulate inflammatory action by inducing the release of pro-inflammatory cytokines (Fig. 4a,b,c).

NO and PGE2 production

Under LPS treatment as model agent for elicitation of inflammatory pathway in the macrophage cell, gamma radiation (4, 6, 8 and 10 Gy) caused nitrite oxide release as inflammation signal mediator from the treated cells in the culture media, were determined using the Griess reaction method assay. Gamma radiation significantly induce NO production in a dose-dependent manner (Fig. 8a). Increase dose of gamma irradiation led to elevation of NO release to the media.

In another hand, another inflammation signal mediator, PGE2 assay using a PGE2 detection ELISA kit, show Gamma radiation significantly enhanced PGE2 production. Therefore, the results demonstrated a positive liner correlation between dose of irradiation and release of biochemical inflammation mediators (Fig. 5a, b).

Gamma radiation increases apoptotic genes expression

The results showed that exposure of macrophages to 6, 8, and 10 Gy gamma radiation caused increased BAX and p53 expression as pro-apoptotic genes, wherever the expression of Bcl-2 (anti apoptotic) found to be down-regulated at 10 Gy (Fig. 6a). The results also indicate Bcl2 expression was not affected by radiation show that low dose (4 Gy).

Inflammatory genes were up-regulated by gamma radiation

The expression of TNFα, IL-6, IL-1β iNOS genes according to qPCR analysis results show significant increase in both radiation exposed groups and LPS model group. The expression of inflammatory genes were slightly higher in 4 Gy than control but there is no statistical difference (Fig. 6b).
Application of Ionizing radiation in cancer therapy has improved over the last few years. It has been shown that low dose of radiation is more effective than daily conventional doses of 1–2 Gy in cancer therapy. Several studies illustrated that ionizing radiation could cause diverse effects on cell biology, so it seems to be so far way to have clear perspective about dose dependent effects of radiation on the biological systems. Low dose ionizing radiation could improve physiological functions. As reported in some studies, low dose gamma radiation have the ability to be used in some special diseases such as allergies and can ameliorate them. However all of these studies performed under certain exposure conditions and cell lines or animal models [21].

Direct or indirect cell exposure to Ionizing radiation induced various biologic pathway alteration, as frequent enhancement of micro nucleation, apoptosis, mutations, DNA strand breaks, alternation of regulatory proteins activity and enzymes, oncogenic transformation and reduction of colonogenic efficiency [22]. Up to 10Gy whole body Gamma radiation in animal models caused acute induction of inflammatory system and cytokines expression burst [23]. The systemic effect of low dose radiation on immune system is still obscure. Some studies reported that low dose radiation induce immune system, while another studies show suppressive effect on the immune system[14]. In fact, further studies needed to clarify the molecular events included in this progress. Low dose of gamma radiation has various effects on living cells, enhance the cell survival, oxidative stress resistance, improve immune system function and stimulation of cell growth. In another hand, studies show that Gamma radiation caused suppression or modulation of immune system mediators, so could assigned as valuable therapeutic method in outoimmuninc or chronic inflammatory diseases. As reported, Exposure to 0.5 Gy gamma radiation promote natural killer (NK) cells activity, elevation of glutathione level and prevent tumor growth factors. It suppressed pro-inflammatory cytokines from peritoneal macrophages and demonstrate strong potential anti-inflammatory effects [17]. 0.5 Gy gamma radiation unregulated MKP-1, activation of P38 MAPK consequence then suppression of TNF-α in mouse macrophages cell lines [17]. Interestingly, recent studies revealed that low dose of gamma irradiation in collagen-induced arthritis (CIA) decreased the antibody and pro-inflammatory cytokines release, versus the induction of regulatory T cells count, thereby caused attenuation of disease symptom [24]. Beside, another study show that in rheumatoid arthritis (RA), human T lymphocytes were resistant to apoptosis induced by 0.5 Gy gamma radiation. It found that the apoptosis gene expression in had a different pattern in the RA patients [25]. In this study, 6, 8 and 10 Gy doses of gamma radiation caused elevation of apoptotic cells and decrease of alive cells (Fig. 2). According to previous studies, low doses of gamma radiation (less than 50 mGy) enhance some cell mechanisms like DNA repair and apoptosis reduction led to elevation of cell protective efficiency [14]. Our data confirmed that low dose (4 Gy) group caused no significant apoptosis, while enhanced considerably when radiation increased especially in high doses such as 8 and 10 Gy. Previous studies, show that there is a positive correlation between the Fas gene expression and gamma irradiation (4 and 10 Gy) in malignant lymphoma cells [21]. Ionizing radiation induced signaling pathways such as P53 and Ataxia telangiectasia mutated kinase (ATM) involved in DNA damage repair [17]. Although, pre-apoptotic
protein (BAX) gene expression was down-regulated, while anti-apoptotic protein (Bcl2) gene upregulated under low dose of gamma ionization [26]. As Radiation can activate the check points of cell cycle which arrest the cells at G1/S, S, G2/M stages [21], G2/M checkpoint is the final step before the cells enter to mitosis, it had reported that this checkpoint can be activated as a response to DNA damage. In this study, It was found that in all treated groups, arrested cells in G2/GM phase increased and was remarkable at 8 and 10 Gy treated groups (Fig. 4). It seems increasing of gamma radiation, enhanced DNA damage subsequently elevate the number of cells, which arrested in G2/M. Align to previous results that reported sub-low dose radiation (2.5 Gy) caused cell accumulation in G2 phase. In this radiation doses, after the treatment, cells entered mitosis or entered to apoptosis progress [27]. It is important to note that ionizing radiation can also lead to secondary malignancy when used for cancer therapy, especially when the cells remain alive and some of these cells can turn into metastatic or invasive form. It had reported that ionizing radiation elevated a motile form or metastatic form of cancer cells in vitro by hyper-activation of the TGF-β signaling pathway in human carcinoma cells [28]. Low dose ionize radiation through knockdown of Nr4a2, suppressed mast cell migration and subsequently, PI3K and Btk signaling pathways inhibited. It was found that low dose of radiation could chemotactic cytokine down regulate MCP-1 (monocyte chemoattractant protein 1) gene expression mediated by Nr4a2 in mast cells and surpassed cell migration [29]. This study reviled that, macrophage exposure to gamma radiation reduced Anti-inflammatory mediator’s profile. It could suggested that pro-inflammatory like macrophages maybe contribute to the impression of local radiotherapy. Pro-inflammatory macrophages produced cytokines such as IL-1B, IL-6, and TNF-α and expressed some genes like CD80, CD86, HLA-DR, CCR7 and NFKβ involved in the cell cycle regulation. On the other hand, anti-inflammatory macrophages induce anti-inflammatory cytokines such as IL-10 and TGF-β and protein expression of CD163 and MRC [30]. The study illustrated that ionizing radiation can regulate macrophages and change their phenotype to the pro-inflammatory form. So it could be suggested that regulation of inflammatory responses under gamma radiation exposure derived by promoting the production and release of pro-inflammatory cytokines (Fig. 5a,b,c).

According to previous studies, it found that gamma irradiation could enhance NO production in RAW264.7 cells led to DNA damage through induction of nuclear factor pathway (NF)-Kβ[31]. Recent study too show that NO and PGE2 biosynthesis enhanced in a dose dependent manner after gamma radiation treatment (Fig. 5d,e). As it is clear iNOS gene was upregulated in radiation and LPS groups. Determination of genes stimulated by gamma radiation in Raw264.7 cells show that the gene expression of pro-inflammatory cytokines TNFα, IL-6, IL-1β and iNOS substantially increased in radiation treated groups as LPS induced model. The expression of pro-inflammatory genes were slightly higher in 4 Gy compared to control group but there is no statistical difference (Fig. 6b).

Finally, current study, suggests that Raw264.7 cells as marker key cells in inflammatory disease exposed to gamma radiation (0, 4, 6, 8, 10 Gy), higher dose radiation damaged DNA of the cells cells were arrested at G2/M phase. If the cells could not repair the damage (this effect is common in higher dose), apoptosis genes such as P53 and BAX were upregulated. In fact, there are bidirectional effect
between P53 and BAX genes, P53 regulates BAX and BAX is involved in P53 apoptosis mechanism. Therefore, increased expression of both, lets the cells with DNA damage entered to apoptotic pathway. Versus, Bcl\textsubscript{2} (which is a kind of anti-apoptotic gene) down regulated aligned to this process. Moreover, pro-inflammatory cytokines (IL1B, IL6 and TNF-\textalpha), NO and PGE2 production enhanced during DNA damage and apoptosis steps. NO burst is closely linked to excessive activation or production of different inflammatory cytokines like TNF-\textalpha, IL6 and IL1B [31]. Although, high dose of radiation, caused most of cells entered to apoptosis phase and the number of viable cells reduced, the current study reviled that, these results were not considerable at 4 Gy radiation. Interestingly, high gamma radiation could increase cell motility and migration and this effect could not be neglected, especially when it is used in cancer therapy because may turn cells into metastatic form.

**Conclusion**

In these days, gamma radiation extensively used as a therapeutic choice in medicine. The therapeutic use of low dose of gamma radiation as could be beneficial in cancer therapy and suppression/modulation of immune responses in chronic inflammatory disease, have different doubtful aspects should cleared carefully. The results of this study show that 4 Gy gamma radiation had no destructive effect on macrophage cell line, although higher doses derived the cells to the apoptosis progress caused by irreversible damages in cellular and molecular levels.

**Declarations**

**Conflict of Interest Statement**

There is no any conflict of interest.

**Role of the funding source**

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**Ethical approval statement**

We confirm that the manuscript has not been submitted or previously published in whole or in part elsewhere. The manuscript is not currently being considered for publication in another journal. In addition, no data have been fabricated or manipulated (including images) to support our conclusions and no data, text, or theories by others are presented as if they were the author's own ("plagiarism"). All authors, whose names appear on the submission, have been personally and actively involved in substantive work leading to the manuscript have, and all agreed to submit the manuscript to this journal.

**Informed consent**

Formal consent is not required for this type of study.
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Figures

Figure 1

(a) RAW264.7 cells were cultured in DMEM medium (×10). (b) MTT assay for Raw264.7 line with different gamma dose: change in viability percent with increasing irradiation dose
Figure 2

Flow cytometry results for apoptosis assay and cell cycle distribution. (a) Dot plot of apoptosis assays by Annexin V and PI method in cells treated with gamma radiation after 24 h. Q4; live cells, Q3; early apoptosis cells, Q2; late apoptosis cells, Q1; necrosis cells. (b) Flow cytometry histogram of different treatment (c). Gamma radiation reduces live cell percent and arrest cell in G2/M phase. Radiation reduces live cell percent and increase in apoptotic population (d). *P<0.01, **P<0.01, ****P<0.0001 vs. control group.
Figure 3

Gamma radiation stimulates motility of macrophage cells (a) Representative micrographs of scratch areas 24 and 48 h. (b) Migration graph of different treated groups. (c) Relative to control the fold increases of migrating cells are shown as the means of the cell numbers in fifteen randomly selected vision. The error bars indicate the SD. **P<0.01, ***P<0.001 vs. control group.
Figure 4

Effect of LPS and gamma radiation (4, 6, 8 and 10 Gy) on pro-inflammatory cytokine production in RAW264.7 macrophage cells. (a) TNF-α, (b) IL-1β and (c) IL-6. The results are expressed as mean ± SD. (n = 3). *p ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001 vs. control group.

Figure 5

(a) NO production of LPS and gamma radiation (4, 6, 8 and 10 Gy) groups was analyzed by a Griess reagent assay. (b) PGE2 production of LPS and gamma radiation (4, 6, 8 and 10 Gy) groups was analyzed using a PGE2 detection ELISA kit. The results are expressed as mean ± SD. (n = 3). *p ≤ 0.05; **p ≤ 0.01, ***p ≤ 0.001 vs. control group.
Figure 6

Real-time quantitative analysis of the fold change of apoptotic (a) and inflammatory (b) genes after irradiation (relative to control). Values expressed as mean ± SD of three independent samples. *P<0.01, **P<0.01, ****P<0.0001

Supplementary Files

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