RESEARCH ARTICLE

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Quantification of the Most Effective X-Ray Dosage for Inhibiting Invasion of Glioma Cell Line

Elham Ghasemi[1], Zeynab Zamanzade[1], Fatemeh Seif[2], Mahdieh Mondanizadeh[3]*

Abstract

Background: One key feature that distinguishes cancerous cell populations from their normal counterparts is a heightened tendency towards uncontrolled growth and invasive activity. Therapeutic techniques like radiotherapy can impact the viability and invasive behavior of cancer cells by modifying the structure of DNA and inducing programmed cell death. **Methods:** This research is an experimental study and involved a comprehensive investigation into the effects of 6MV X-ray radiotherapy on various absorbed doses (ranging from 4 to 10 Gy by steps 2Gy) on toxicity, migration, and colony formation in C6 glioblastoma cellular cultures. **Results:** Our detailed analysis revealed that the cytotoxic responses increased in a dose-dependent manner, while there was a significant decrease in both the ability to migrate and form colonies in the C6 cell line. **Conclusions:** This thorough examination provides new insights into the way malignant cell populations respond to ionizing radiation in a dose-dependent manner within a clinical setting. By directly impacting cellular functions and causing disturbances, this occurrence leads to a slowing down of disease progression.

Keywords: Glioma- C6 cells- MTT Assay- X-ray radiotherapy

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Introduction

Glioma is a type of abnormal cell growth that develops from glial cells in the central nervous system, mainly in the brain and spinal cord [1, 2]. While gliomas can occur in people of different ages, they are more common in adults [3]. Symptoms of glioma include persistent headaches, changes in cognitive and neurological functions, seizures, and difficulties with motor skills [2, 4]. Analysis of population-based data from the United States reveals that gliomas constitute approximately 27% of the total incidence of brain tumors [5].

The therapeutic modalities available for glioma encompass surgical intervention, radiation therapy, chemotherapy, targeted therapy, immunotherapy, and supportive care [6, 7]. Among the available treatment modalities, radiation therapy has emerged as a prominent and esteemed therapeutic approach for glioma [8]. It is characterized by its remarkable safety profile and substantial therapeutic effectiveness. This approach utilizes ionizing radiation delivered through high-energy beams to elicit cytotoxic effects or impede the proliferation of malignant glioma cells [9]. The therapeutic application of X-ray radiation in glioma treatment involves the utilization of high-energy X-rays to selectively target and eradicate neoplastic cells within the brain [10]. radiotherapy denotes the precise energy level of the radiation beam employed in this modality. External beam radiation therapy, facilitated by a linear accelerator apparatus, is the customary method employed for administering this treatment [11]. The primary objective is to deliver accurate and controlled doses of radiation to the tumor, minimizing adverse effects on adjacent healthy tissues, and thereby facilitating tumor reduction and preventing recurrence [12]. X-ray radiation investigations have yielded valuable insights into the molecular and cellular mechanisms underlying DNA damage and repair, as well as the apoptotic pathways activated in response to varying doses of X-ray irradiation [13]. These studies have provided evidence of elevated levels of reactive oxygen species (ROS), DNA double-strand breaks (DSB), cellular apoptosis, alterations in G2/M phase distribution, and suppression of cellular proliferation subsequent to irradiation [14, 15]. However, following radiation exposure, there is an increase in the expression of proteins associated with apoptosis, such as Bcl-2-associated X protein (Bax), caspase-9, and BH3 interacting domain death agonist (Bid) [16, 17]. On the other hand, the MTT assay is a widely used colorimetric technique for assessing cell metabolic activity, particularly in studies of cell viability and cytotoxicity. This method relies on the reduction of the yellow tetrazolium salt, MTT (3-[18]-2,5-diphenyl tetrazolium bromide), into purple

*[1]Student Research Committee, Arak University of Medical Sciences, Arak, Iran. [2]School of Allied Medical Sciences, Department of Radiotherapy, Arak University of Medical Sciences and Khansari Hospital, Arak, Iran. [3]Department of Biotechnology and Molecular Medicine, School of Medicine, Arak University of Medical Sciences, Arak, Iran. *For Correspondence: m_mondanizadeh@yahoo.com. Elham Ghasemi and Zeynab Zamanzade have equal countribution in this study.*

formazan crystals by viable cells, which reflects their metabolic activity [18].

In this research, we used 6MV X-ray radiation as a treatment method to address C6 cells. We assessed the effect of radiotherapy on the proliferation of C6 cells through MTT assays, colony formation assays, and cell migration assays.

Materials and Methods

Cell culture

The present study is experimental. The C6 glioblastoma cancer cell line (Pasteur Institute, Tehran, Iran) was employed as the experimental model for the conducted protocols. The cell line was cultured under precisely regulated laboratory conditions, maintaining a constant temperature of 37°C and a CO_2 concentration of 5% to ensure standardized and optimal growth conditions. Ham`s.F-12K (Gibco) served as the culture medium for cell growth. To optimize the culture conditions, the medium was supplemented with 2.5% fetal bovine serum (FBS, BioWhatter, MD), 15% horse serum (HS) (Sigma, St. Louis, MO), as well as 1% streptomycin (Sigma, St. Louis, MO).

Cell 6MV X-ray irradiation

The cellular model was subjected to 6MV X-ray irradiation produced by an Elekta Versa_HD Linac, SAD technique (SAD=100cm, 1.5 cm slabs for build-up and SSD=98.5 cm). The physics calculations were done to necessary absorbed dose (ranging from 4 to 10 Gy by steps 2Gy) delivered to cells (Dose rate 400MU/min). The irradiation procedure was implemented within the specialized facility located at the Radiotherapy Department of Arak Khansari Hospital. To ensure precise dosimetry, a comprehensive physical evaluation of the radiation dose was carried out before irradiation session, employing a pinpoint ionization chamber in PTW slab phantom.

Cell viability and proliferation assay

The cytotoxicity of radiotherapy with 6MV X-rays against C6 cancer cells was assessed through the implementation of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay. Initially, a cell seeding density of 3×10^3 cells was established in 96-well plates and allowed to adhere overnight at a temperature of 37°C. Subsequently, the cells were exposed to various absorbed doses (ranging from 4 to 10 Gy by steps 2Gy) of 6MV X-rays [19]. The cells were then incubated under controlled conditions at 37°C in an environment containing 5% CO_2 for 72 hours. Afterward, the cells were treated with 10µl of MTT solution, followed by an additional incubation at 37°C for 6 hours. Subsequently, the MTT solution was discarded, and 100µl of dimethyl sulfoxide (DMSO) was introduced to dissolve the formazan crystals that had formed in viable cells. Finally, the absorbance of each sample was measured at a wavelength of 570 nm using an ELISA plate reader. The relative cell viability was determined by calculating the ratio of the optical density (OD) of the test sample to the OD of the control sample, multiplied by 100. The average value was obtained from three independent measurements.

Colony Formation assay

To evaluate the colony-forming potential of C6 cells following exposure to 6MV X-rays, a colony assay was conducted. In brief, a total of 300 cells were seeded in triplicate within a 12-well plate. After an incubation period of 24 hours at a temperature of 37°C with 5% CO_2, the cells were treated with radiation of 6MV X-rays. Subsequently, the cells were further incubated for 7 days under conditions of 37°C with 5% CO_2 to form colonies. To facilitate staining and quantitative analysis of the resulting cell colonies, the supernatant was aspirated, and the cells were subjected to two washes with phosphate-buffered saline (PBS). Afterwards, a fixative solution containing a mixture of methanol and acetic acid in a 1:7 ratio was applied to the wells at room temperature for 20 minutes to secure the fixation of the colonies. The fixative solution was subsequently discarded, and the colonies were stained with a 0.5% crystal violet dye for 20 minutes at room temperature. Following the staining process, the dye was removed, and the wells were washed four times with distilled water. Subsequently, the wells were left to air-dry at room temperature, and the number of formed colonies was assessed through visual counting.

Cell migration

The C6 cell line was cultured at a density of 10^4 cells/well. Following a 24-hour incubation period at a controlled temperature of 37°C and an atmosphere of 5% CO_2, the cells were subjected to 6MV X-ray radiation. Subsequently, a scratch was created on the bottom of the plates using a 100 µl tip. To investigate cell migration, time-lapse microscopy was employed, utilizing an inverted light microscope equipped with a 5X objective lens. Phase contrast images were captured continuously for 24 hours (0- 72 h). To analyze cell movement, a manual cell tracking approach was implemented using ImageJ software.

Statistical analysis

The statistical analysis was performed utilizing GraphPad Prism 9 software. The experimental procedures were replicated independently at least three times, and the outcomes were reported as the mean ± standard deviation (M±SD). Analysis of variance (ANOVA), accompanied by suitable post hoc tests, was employed to compare the groups and determine the significance of the observed differences. A significance level of $p<0.05$ was chosen as the critical threshold for statistical significance. For measurement and quantification, ImageJ software was employed.

Results

6MV X-ray cytotoxicity

The MTT assay was used to investigate the effects of varying doses of 6MV X-ray radiation on C6 cells, and the results indicate that an IC_{50} value of 8 Gy for 6MV

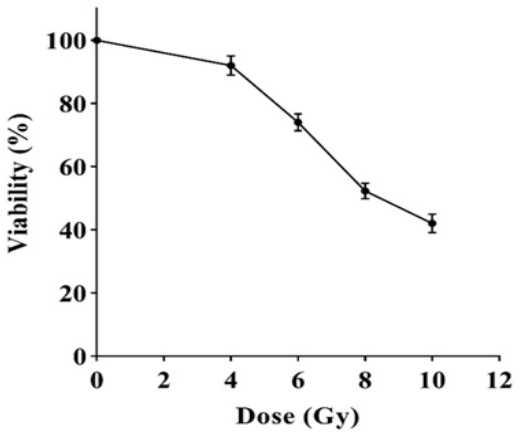

Figure 1. Dose-Response Curve Used to Generate IC_{50} for 6MV X-ray Irradiation.

X-ray has been determined. These findings are depicted in Figure 1, which visually illustrates the relationship between different doses of 6MV X-ray radiation and their impact on cell cytotoxicity.

Cell migration

After creating a wound on C6 cell cultures and exposing them to 6MV X-rays, the cancerous cells showed coordinated efforts to heal the injured area with the help of signalling molecules and cytokines that promote cell growth. Interestingly, the groups treated with a 6MV X-ray dosage of IC50 (8Gy) exhibited a significant decrease in both cell movement and replication compared to the control group. The initial size of the wound at the start of the study (0 hour) was determined as a comparison criterion, and changes in distance from this baseline were measured using Image J software, as shown in Figure 2.

Colony formation

The study was conducted to evaluate how 6MV X-ray irradiation affects the ability of C6 cells to form colonies. The findings depicted in Figure 3 offer concrete proof

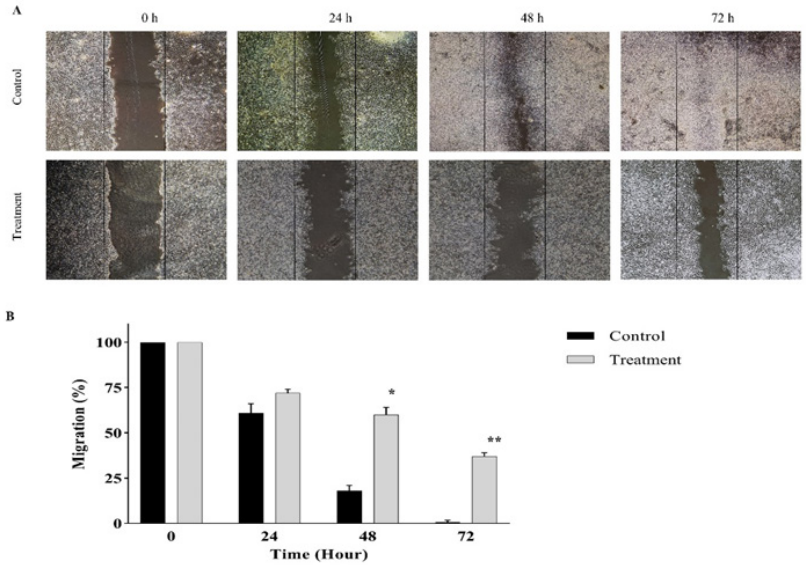

Figure 2. Cell Migration Assay (A) Representative images from migration assay of C6 cells treated with 6MV X-ray, demonstrated that cell invasion into the cell-free region is reduced in comparison to the control group. (B) Summary graph showing typical migration rates by C6 cells exposed to 6MV X-ray (mean ± SD (n = 3). *P < 0.05 and **P < 0.01 compared with 0 h).

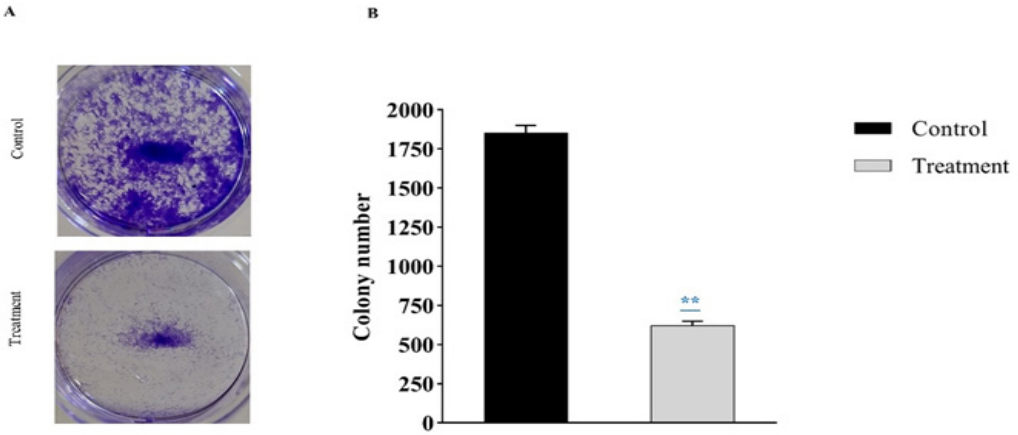

Figure 3. Colony Formation Assay. (A) The staining of the colony formation with crystal violet and the resulting image, which is representative of the colony formation in C6 cells treated with 6MV X-ray. (B) The number of colonies was counted. Data are presented as mean ± SD (n = 3). **P < 0.05.

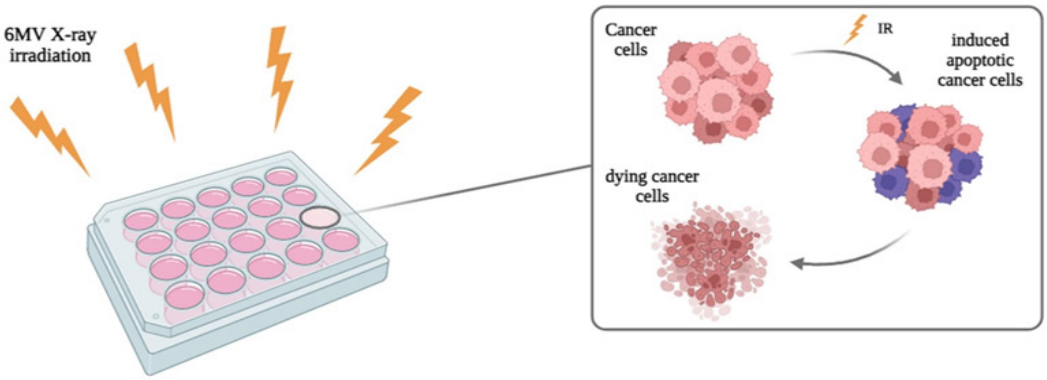

Figure 4. Cell 6MV X-ray Irradiation

that the treatment groups exhibited a reduced capacity for colony formation. Specifically, the groups that received the IC50 dosage of 6MV X-ray (8 Gy) showed a significant decrease in the number of colonies compared to the control group.

Discussion

The uncontrolled growth of cancerous cells triggers the start of tumor formation and the spread into nearby tissues, resulting in the formation of metastases, a process seen in many different types of cancers such as glioblastoma, breast cancer, pancreatic cancer, and others [20, 21]. The invasion and spreading of cancer cells cause changes in the shape of cells, deformation, and disturbance of communication between cells, leading to their movement to healthy tissues and the creation of new groups of cells at these locations [22].

The results show that the C6 cell line, which originated from cancerous rat cells, exhibits a significant rise in its rate of growth and a reduced level of communication between cells [23, 24]. These traits allow the cells to spread through the blood to normal tissues, where they form colonies and produce new groups of cells [25, 26]. We initiated the study by conducting focused research on particular cellular processes, namely cell cytotoxicity, migra tion and colony formation, with a primary focus on C6 malignant tumor cells [27]. Subsequently, we proceeded to functionally assess and analyze the effects of 6MV X-ray radiation on these specific cellular activities Figure 4.

The study findings revealed that the phenotypic and functional properties of the cells exposed to various doses of 6MV X-ray radiation exhibited a dose-dependent alteration. Specifically, higher radiation doses resulted in increased cell-killing effects, as well as decreased ability for cell movement and formation of colonies compared to the control group. Furthermore, the results obtained from the cytotoxicity assay demonstrated that the C6 cell line, which has a high rate of growth, required an extended period for incubation with MTT to achieve the best results in this study, with an optimal duration of 6 hours. Moreover, the results indicated that the characteristics and functional properties of the cells exposed to different doses of 6MV X-ray radiation were altered in a manner that depended on the dosage.

In Conclusion, the findings demonstrate that 6MV X-ray irradiation mitigates the metastatic capacity of tumor cells, highlighting the important role of radiotherapy in treating cancer. This treatment method effectively slows down cell division and promotes programmed cell death in cancer cells, making a significant contribution to the overall management of cancer.

Author Contribution Statement

E.G., Z.Z., F.S., M.M.; Investigation, Validation, Formal analysis, Data curation, Software and Methodology. F.S., M.M.; Resources. E.G., Z.Z., M.M.; Visualization. E.G., Z.Z., M.M.; Writing - Review & Editing. E.G., Z.Z., M.M.; Writing - Original Draft. M.M.; Project administration and Supervision. All authors read and approved the final manuscript.

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