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Research article

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Effects of low-dose, short-duration periods of asymmetric radiation on colony formation of C6 glioma cell cultures

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Abstract

Background/Aim: Previous studies on fractionation in radiation therapy have been mainly based on applying equal doses over at least 6 h. The main purpose of fractionation is to increase normal tissue tolerance rather than tumor sensitivity. Thus, one can apply higher doses to the tumor. In contrast, new molecular studies indicate that high and low doses of radiation act by different mechanisms. This study was conducted to investigate the radiobiological effect of asymmetrical radiation doses.
Methods: This is an experimental study done *in vitro* with a G6 glioma cell line to investigate the responses when C6 glioma cells are irradiated with single doses of 30 and 230 cGy using an orthovoltage therapy device or doses split into 30 and 200 and 115 and 115 cGy within periods of 15 and 30 min. A total of 5×10^3 cells were transferred to polyethylene culture flasks for colony formation. A cluster containing more than 30 cells was considered a new colony.
Results: A single dose of 230 cGy caused a 56.8% reduction in colony formation. However, when 230 cGy was divided over 15- and 30-min periods in fractions of 30 and 200 cGy, colony formation was significantly reduced compared to the control group (68.13% and 52.64%, $P = 0.030$, respectively). This effect continued when the radiation dose was divided into equal fractions (115 and 115 cGy) with periods of 15 and 30 min (42.60%, $P = 0.021$ and 20.77%, $P = 0.008$, respectively).
Conclusion: According to these results, (i) short interval (15 and 30 min) fractionation significantly reduces colony formation compared to a single equal dose; and (ii) the protective mechanisms activated in cell response probably vary at different radiation doses and different fractions.

Keywords: C6 glioma cells, Fractionation, Radiation, Interval, Low dose

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Introduction

Radiation therapy is one of the main modalities used in cancer treatment. Shortly after the discovery of ionizing radiation, it began to be used to treat many diseases [1]. Although radioactive irradiation was quickly introduced to clinical application, its mechanism of action is not fully understood even today, and the evolution of treatment protocols and doses is empirical [2]. Soon, it was recognized that splitting the dose into parts (fractionation) did not harm tumor control while increasing the tolerance of normal tissues, and the concept of fractionated treatment was born [3].

As generally accepted, (i) cells that divide rapidly are more affected than those that divide more slowly. (ii) Dividing the dose into daily fractions ensures normal tissue tolerance, and (iii) the maximum dose that could be applied depends on dose-limiting tissues. As a further attempt, the procedures of hyperfractionization (daily dose divided into two or three equal loads) and continuous hyperfractionization (no interval at weekends) were tested for this purpose. However, they did not provide the expected benefit and remained experimental in daily treatment practice [4].

Studies that aim to optimize the therapeutic ratio of radiation therapy are based on the principle of applying multiple doses in a day within a minimum interval of 4 h. However, emerging data in molecular biology demonstrated that cellular response to irradiation in the first minutes differs from those after hours. There are numerous studies on the effects of low-dose irradiation on biological and gene expression [5]. Studies revealed a protective priming effect in low doses of radiation on the cell if a second dose is given after hours. A study on human lymphoblasts indicated that a 5 cGy low dose irradiation, followed by a standard dose of 2 Gy, upregulates genes for protein synthesis while genes for metabolism are inactivated [6]. Research indicated that irradiations activate early stress genes and large molecular panels confirm that many more genes are involved than expected [7, 8]. As a result of molecular mechanisms, an adaptive response emerges in the cells, which differs in radiation sensitivity [9, 10]. Mechanisms activated on low-dose radiation trigger apoptosis, while conventional doses abolish this effect with further molecular rearrangements [11, 12].

Most of these studies were conducted considering the genetic pattern of molecular expression in a time interval of at least 3 or 4 h between fractions. Almost all molecular mechanisms are activated within minutes and return to the initial level within hours [13]. For instance, low-dose priming irradiation causes cell proliferation and generally reduces radiation response [14]. Almost all studies divided the daily dose into equal fractions at least 6 h apart. The effect of a large fraction after a first low dose can provide a biological additive gain. This study investigates irradiation with priming doses given in asymmetric fractions in short periods on colony formation in C6 glioma model cells.

Materials and methods

Cell culture

A C6 glioma cell lineage (American Cultural Collections) was used in the study. C6 glioma cell colony formation is a well-defined cell culture model that has been successfully used in radiobiology due to its adhesion to culture flasks, which allows colony counting. Briefly, cells were incubated at 37°C in a 95% O_2, 5% CO2 condition containing 10% fetal bovine serum; 1% L-glutamine and 1% essential amino acid, supported by 10,000 units of penicillin and 10 mg/ml of streptomycin solution DMEM (Sigma Chemicals Co., In St Louis, Missouri). During the experimental phase, the cells were treated with trypsin-EDTA, separated from the culture environment, washed after being turned into a single cell suspension, and re-suspended in a full nutrition medium. A total of 500,000 cells were transferred to polyethylene culture flasks with areas of 25 cm^2 and 5 ml volume in an attempt for colony formation. The passages were checked twice per week using an inverted microscope [15]. These steps were carried out at the Department of Histology and Embryology of Istanbul University, Istanbul Medical Faculty.

Irradiation procedure

An orthovoltage teletherapy device (Stabilipan) was used for radiation exposure [16]. Before the experiment, a preliminary study was conducted to determine the radiation sensitivity of the C6 glioma cell line to standardize the dose. Cells were irradiated with a single fraction of 50, 100, 200, 400, and 800 cGy to determine the LD50 dose in colony formation. The results were plotted on a graph, and the dose that inhibits colony formation by 50% was extrapolated from the logarithmic scale, thus reaching a dose of 230 cGy. This dose was divided into two fractions as low and conventional doses of 30 and 200 cGy. In contrast to the 6-h exposure interval in classical hyperfractionation, the time between fractions was shortened to 15 and 30 min. In this way, we aimed to investigate the biological effect of a low priming dose followed by a conventional fraction size at short intervals.

Determination of colony-forming

Culture flasks were transferred to the Institute of Oncology of Istanbul University in thick styrofoam containers to avoid temperature differences. Culture flasks were placed in the center of the 20 × 20 cm field, and radiation was applied. Care was taken to ensure that all culture dishes were homogeneously affected by temperature change. After irradiation, containers were rapidly transferred to the Department of Histology and Embryology of the Istanbul Medical Faculty. A cell count per mm^3 was performed by applying trypsin to the cell culture within an hour. One thousand cells from each sample were transferred to the new culture medium and incubated for 7 days. Flasks were evaluated under an inverted microscope; a cluster containing more than 30 cells was considered and counted as a new colony.

Statistical analysis

The experiment results were compared between groups using ANOVA with the Bonferroni test. Differences were considered significant at *P*-values less than 0.05. All results are expressed as mean SEM calculated from triplicate data.

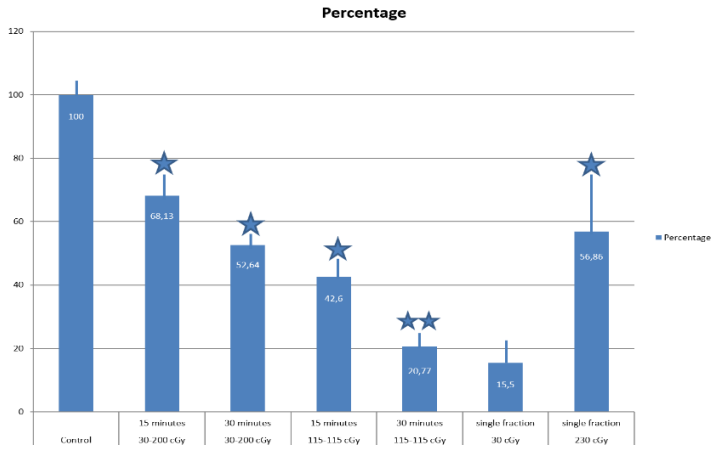
Results

The different irradiation procedures on C6 flioma cell lineage data are shown in Table 1. Single-dose 30 cGy irradiation reduced colony formation by 15.50%, but there was no significant difference compared to the control group. In contrast, a single dose of 230 cGy caused a decrease in colony formation close to the calculated value in the preliminary study (56.8%). However, when 230 cGy was divided into 30 and 200 cGy fractions over 15- and 30-min periods, it significantly reduced colony formation compared to the control group. (68.13% and 52.64%, P = 0.038, respectively). This effect was also detected when the radiation dose was divided into equal fractions (115 and 115 cGy) with periods of 15 and 30 min (42.60%, P = 0.021 and 20.77%, P < 0.01, respectively; Figure 1).

Table 1: Data of different irradiation procedures on the C6 flioma cell lineage.

Execution	Control	30-200 cGy 15 min	30-200 cGy 30 min	115-115 cGy 15 min	115-115 cGy 30 min	30 cGy single fraction	230 cGy single fraction
mean	568	387	299	242	118	480	323
(SD)	(20.97)	(62.63)	(20.42)	(24.84)	(3.84)	(35.42)	(156.37)
Percentage	100	68.13	52.64	42.60	20.77	15.50	56.86
P-value		0.030	0.038	0.021	0.008	0.0913	0.0363

Figure 1: Effects of different dose and fraction periods on colony formation percent inhibition in C6 glioma cells.



Discussion

Discussion

This study indicates that fractionated equal doses of radiation with short periods of 15 and 30 min reduce the statistically significant ability of cells to create colonies more than the administration of the total dose in a single fraction. Dividing the dose into two doses over a short period, even if the fraction sizes are different, causes more colony inhibition than the application of the same dose in a single fraction. This effect persists when the dose is given in two loads of 115 and 115 cGy. These results differ from previous studies using longer fraction intervals [17, 18]. It can be argued that the colony-forming inhibition effects of irradiation depend not only on the dose but also on the function of the period between the fractions.

In conventional radiotherapy schedules, hyperfractionation aims to increase normal tissue tolerance. It is generally accepted that treatment periods should be at least 6 h. However, another reason to prefer 6-h periods is that this is likely the maximum feasible period in clinical practice. In daily treatment procedures, the so-called “set-up” (laying the patient in treatment position) takes 80% of the total treatment duration before irradiation. Thus, after positioning, most devices complete radiation application within seconds. For this reason, previous studies, even experimental, have been based on intervals of at least 4 h [19]. However, these results indicate that 15- and 30-min fraction intervals can provide a further advantage in colony-forming ability.

Recently published research has revealed that multiple mechanisms modulate cell response to radiation in terms of temporal aspect [20]. The radiation exposure initiates a stress response that becomes active within seconds [21]. In contrast, the effects of this acute response are short-lived. If programmed cell death does not occur, it has no impact on cell survival [22]. The radiation dose that leads to programmed cell death is much lower than the doses used in clinical practice. Thus, increasing doses abolishes the priming effect of low-dose irradiation.

For this reason, it is important to evaluate whether the biological effects of a conventional fraction will provide an additional therapeutic advantage if applied split by minutes (asymmetric low-high) without changing the total dose [23]. This study demonstrated that a low priming dose followed by a standard dose divided into two asymmetric fractions reduced the colony-forming property of C6 cells. A possible explanation of this phenomenon is molecular changes triggered after low priming dose administration. For instance, it has been demonstrated that even a low dose of 0–22 cGy in human A549 lung adenocarcinoma, T98G glioma, and MCF7 breast carcinoma cell lineages has an inhibitory effect on the p53 gene expression [24]. In a further study, it has been shown that when radiation was administered at a low dose rate (72–168 h) on HeLa Hep2 cells, the expression of early response genes was induced [25]. Another explanation for the additive suppression of colony formation by short-period radiation is the synchronization of cell cycles. Thus, within minutes after the first irradiation, cells go to the synchronous division stage, reinforcing the effect of the following second dose [26, 27]. Previous studies have confirmed that conventional single fraction significantly reduces colony formation [28]. In accordance with our study, if 230 cGy doses are divided into two equal fractions, the additive effect differs. Furthermore, extending the interval between fractions from 15 min to 30 min strengthens the effect. However, this research is a preliminary experimental study. It could not cover the exact limit of the optimal dose-time interval, and its clinical interpretation may be completely controversial.

Conclusions

This result supports other studies that have used initial irradiation with a low priming dose, followed by a conventional dose, effectively reducing colony-forming ability in the C6 glioma cell line. A limitation of this study is that no other molecular markers were used besides fractionation. The C6 glioma cell line is an appropriate model for evaluating the effects of radiation therapy. In contrast, unlike the general approach, asymmetric fractions were applied within short periods in this study. However, since the study aimed to test a hitherto untested approach, it confirms that dividing the dose into parts produces a separate effect.

References

1. Bernier J, Hall EJ, Giaccia A. Radiation oncology: a century of achievements. Nat Rev Cancer. 2004 Nov 17;(9):737-47.
2. Holsti LR. Development of clinical radiotherapy since 1896. Acta Oncol. 1995 Jul 08;34(8):995-1003.
3. Moulder JE, Seymour C. Radiation fractionation: the search for isoeffect relationships and mechanisms. Int J Radiat Biol. 2018 Oct 02;94(8):743-51.
4. Yan W, Khan MK, Wu X, et al. Spatially fractionated radiation therapy: History, present and the future. Clin Transl Radiat Oncol. 2019 Oct 22;20:30-8.
5. Yin E, Nelson DO, Coleman MA, Peterson LE, Wyrobek AJ. Gene expression changes in mouse brain after exposure to low-dose ionizing radiation. Int J Radiat Biol. 2003 Jul 03;79(10):759-75.
6. Coleman MA, Yin E, Peterson LE, et al. Low-dose irradiation alters the transcript profiles of human lymphoblastoid cells including genes associated with cytogenetic radioadaptive response. Radiat Res. 2005 Oct 01;164:369-82.
7. Park WY, Hwang CI, Im CN, et al. Identification of radiation-specific responses from gene expression profile. Oncogene. 2002 Dec 05;21(55):8521-8.
8. Amundson SA, Lee RA, Koch-Paiz CA, et al. Differential responses of stress genes to low dose-rate gamma irradiation. Mol Cancer Res. 2003 Apr 01 ;1(6):445-52.
9. Sasaki MS, Ejima Y, Tachibana A, et al. DNA damage response pathway in radioadaptive response. Mutat Res. 2002 Jul 25;504(1-2):101-18.
10. Tomascik-Cheeseman LM, Coleman MA, Marchetti F, et al. Differential basal expression of genes associated with stress response, damage control, and DNA repair among mouse tissues. Mutat Res. 2004 Jul 11;561(1-2):1-14.
11. Ikushima T. Chromosomal responses to ionizing radiation reminiscent of an adaptive response in cultured Chinese hamster cells. Mutat Res. 1987 Oct 01;180(2):215-21.
12. Ojima M, Ishii K, Hayashi T, Ito A. Induction of radio-adaptive response in colony formation by low dose X-ray irradiation. Physiol Chem Phys Med NMR. 2001 Mar 25;33(1):41-48.
13. Chendil D, Das A, Dey S, Mohiuddin M, Ahmed MM. Par-4, a pro-apoptotic gene, inhibits radiation-induced NF kappa B activity and Bcl-2 expression leading to induction of radiosensitivity in human prostate cancer cells PC-3. Cancer Biol Ther. 2002 Jan 07;1(2):152-60.
14. Yang G, Li W, Jiang H, et al. Low-dose radiation may be a novel approach to enhance the effectiveness of cancer therapeutics. Int J Cancer. 2016 Nov 15;139(10):2157-68.
15. Ozmen T, Oktem G, Tuna S, et al. Different doses of radiation on agar colony forming development in C6 glioma cells: Assessment by thymidine labeling index, and bromodeoxyuridine labeling index. Turkey Clinic J Med Sci 2007 May 01; 27:321-7.
16. Bilge H. Beam characteristics of kilovoltage radiotherapy unit. J BUON. 2004 Jul 01;9(3):303-6.
17. Williams MV, Denekamp J, Fowler JF. A review of alpha/beta ratios for experimental tumors: implications for clinical studies of altered fractionation. Int J Radiat Oncol Biol Phys. 1985 Jan 01;11(1):87-96.
18. Withers HR. Cell cycle redistribution as a factor in multifraction irradiation. Radiology. 1975 Jan 01;114(1):199-202.
19. Elkind MM, Sutton H. Radiation response of mammalian cells grown in culture. 1. Repair of X-ray damage in surviving Chinese hamster cells. Radiat Res. 1960 Oct 01;13:556-93.
20. Hauptmann M, Haghdoost S, Gomolka M, et al. Differential Response and Priming Dose Effect on the Proteome of Human Fibroblast and Stem Cells Induced by Exposure to Low Doses of Ionizing Radiation. Radiat Res. 2016 Mar 01;185(3):299-312.
21. Nikjoo H, Emfietzoglou D, Liamsuwan T, Taleei R, Liljequist D, Uehara S. Radiation track, DNA damage and response-a review. Rep Prog Phys. 2016 Sep 21;79(11):116601.
22. Balcer-Kubiczek EK. Apoptosis in radiation therapy: a double-edged sword. Exp Oncol. 2012 Sep 01;34(3):277-85.
23. Falcke SE, Rühle PF, Deloch L, Fietkau R, Frey B, Gaipl US. Clinically Relevant Radiation Exposure Differentially Impacts Forms of Cell Death in Human Cells of the Innate and Adaptive Immune System. Int J Mol Sci. 2018 Nov 13;19(11):3574.
24. Enns L, Bogen KT, Wizniak J, Murtha AD, Weinfeld M. Low-dose radiation hypersensitivity is associated with p53-dependent apoptosis. Mol Cancer Res. 2004 Oct 01;2(10):557-66.
25. Mirzaie-Joniani H, Eriksson D, Johansson A, et al. Apoptosis in HeLa Hep2 cells is induced by low-dose, low-dose-rate radiation. Radiat Res. 2002 Nov 01;158(5):634-40.
26. Skwarchuk MW, Wouters BG, Skarsgard LD. Substructure in the radiation survival response at low dose: asynchronous and partially synchronized V79-WNRE cells. Int J Radiat Biol. 1993 Jul 03;64(5):601-12.
27. Krueger SA, Wilson GD, Piasentin E, Joiner MC, Marples B. The effects of G2-phase enrichment and checkpoint abrogation on low-dose hyper-radiosensitivity. Int J Radiat Oncol Biol Phys. 2010 Aug 01;77(5):1509-17.
28. Grobben B, De Deyn PP, Slegers H. Rat C6 glioma as experimental model system for the study of glioblastoma growth and invasion. Cell Tissue Res. 2002 Nov 06;310(3):257-70.

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