Journal of Pharmacological Reviews and Reports

Extended Abstract
Vol. 2, Iss. 4
2019

Pharma Sci -12C-Beam Induces More Chromosomal Damage In Chemo-Radio-Resistant Cells Than 16O-Beam- Rupak Pathak- University of Arkansas for Medical Sciences

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Introduction

Resistance to chemotherapeutic drug and/or therapeutic radiation continues to be a major obstacle for cancer treatment. Chemo-resistance may develop via increasing drug inactivation and/or efflux, enhancing DNA repair ability, inhibiting cell death, and promoting epithelial-mesenchymal transition (EMT). On the other hand, radio-resistant cancer stem cells may develop as a result of conventional radiotherapy with low-LET photons (X- and gamma-rays) because of EMT . Therefore, development of an alternative therapeutic strategy is imperative to kill tumor cells effectively, especially those which have developed chemo-radio resistance.

As opposed to low-LET photons, particle radiations or high-LET radiations are more effective in cell killing because of their higher relative biological effectiveness (RBE). This higher RBE of particle radiation is thought to be the result of various factors, such as 1) deposition of more energy per unit distance, 2) generation of unrepairable DNA damage 3) formation of complex chromosomal aberrations (CAs), and 4) similar cell-killing efficiency irrespective of cell cycle phases or cellular oxygen content. Moreover, compared to low-LET radiations, high-LET radiations deposit energy more precisely, thus significantly reduces the risk of normal tissue toxicity surrounding the tumor. All these beneficial properties indicate that high-LET radiation could be a better option as opposed to gamma- or X-rays in cancer treatment. However, it is first necessary to determine how the quality of various high-LET radiations influences the genotoxicity.

Ionizing radiation (IR) exerts genotoxic effects by inducing DNA double strand breaks (DSBs). Inappropriate rejoining of DSBs results in CAs, which subsequently may lead to induction of apoptosis. We demonstrated that a strong correlation exists between cell death and the level of CAs/apoptosis after exposure to IR. Importantly, nature of DNA damage, which depends on quality of radiation, influences the spectrum, yield, and complexity of CAs. For example, in contrary to low-LET radiations, high-LET radiations generate “dense” ionization events leading to “clustered” DNA damage. These clustered damages are more prone to induce complex CAs. However, no study was undertaken to determine the effects of various high-LET radiations on CAs.

In this article we examine the spectrum of CAs in M5 cells, a chemotherapeutic drug (methotrexate) as well as to gammarays resistant cell strain, after exposure to various doses of ^{12}C-beam (LET = 295 keV/μm) and ^{16}O-beam (LET = 625 keV/ μm) at different time intervals. We observed frequency and pattern of CAs depends on radiation quality and post-irradiation times.

Materials and Methods

Cell line and cell culture

M5 cell strain was used in this study. The origin and characteristics of M5 have been previously described. Cells were cultured in Eagle MEM medium (HiMedia, India); supplemented with 10% heat inactivated membrane sterilized Fetal

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

Bovine Serum (Biological Industries, Israel), and 1% antibiotic, Gentamicin (Roche Diagnostics, GmbH).

Irradiation

Irradiation procedure has been described elsewhere. Briefly, 20 h prior to radiation exposure, 0.6×10^6 cells were seeded in each of the specially fabricated Petri-dish like structure, composed of a steel ring measuring 24 mm in diameter with a 6 µ-thick polypropylene sheet. The confluent cell monolayer was exposed to ^{12}C- and ^{16}O-beam at Inter University Accelerator Centre (New Delhi, India) using a 15UD Pelletron accelerator as previously described. Cells were exposed to various doses of ^{12}C-beam (0, 1.18, 2.36, and 4.73 Gy) and ^{16}O-beam (0, 2.46, 4.91, and 9.83 Gy), which correspond to identical particle fluence (0, 1×10^6 , 5×10^6 , and 1×10^7 particle/cm2). The beam energy was calculated using the Monte Carlo Code TRIM. Homogeneity of the beam was measured with a surface barrier detector fitted with a collimator (aperture area 0.886 mm^2). Immediately after irradiation, cells were briefly trypsinized, transferred to normal tissue culture Petri-dishes and incubated for 24 h and 48 h before harvest.

Dosimetry

Dosimetry has been previously described. Briefly, a silicon surface barrier detector was used to count the number of particles and also to measure the beam energy. Fluences was calculated from the relation of the detector count and aperture area of the collimator. Dose (Gy) was calculated from the quantities of fluence as follows:

Dose [Gray] = $1.6 \text{ X } 10^{-9}$ X (dE / dx) [keV / µm] X F [p / cm^2] X 1/ ρ [cm^3/gm]

Where dE/dx = LET, ρ = density of stopping material (in this case, the cell is considered as water equivalent, therefore the value of ρ is 1) and F = the particle fluence.

Preparation, staining and scoring technique of metaphase chromosomes

Chromosomes were arrested at metaphase with 2 h Colcemid treatment (0.1 µg/mL). The cells were then trypsinized, washed twice with PBS, treated hypotonic solution (0.56% KCl, 20 min at 37°C) and fixed in methanol:acetic acid (3:1) fixative. Finally, cell suspension was dropped onto a chilled, pre-cleaned glass microscope slides. The slides were stained in 4% Giemsa in PBS (pH 7.0). 100 metaphase spreads were scored under 1000x magnification. Metaphase spreads were analyzed for chromosome- type aberrations—including chromosome-type breaks, double-minutes, dicentrics, and rings and chromatid-type aberrations including chromatid-type breaks and chromatid-type exchanges. Special considerations were made for ring and dicentric chromosomes. For instance, when a dicentric or ring chromosome was scored, one acentric fragment was subtracted from the number of fragments found in the cell.

Statistical Analysis

Except for 48 h after ^{16}O-irradiation, all the data of CAs were fitted with a linear equation ($Y = \alpha D$). Standard errors on the frequencies were calculated by $\sqrt{a}/A$, where ‘a’ is the number under consideration and ‘A’ is the total number of cells analyzed.

Discussion

Quantification of CAs is an important parameter to better understand the radiobiological effects of high-LET radiation. High- LET radiation-induced complex DSBs has a direct bearing on cellular repair and proliferation kinetics. We demonstrated that high-LET 7Li-, ^{12}C-, and ^{16}O-beam can kill chemo-radio-resistant M5 cells more effectively

than 60Co gamma-rays and that cell killing is strongly correlated with the frequency of CAs. Therefore, a comparative study of CAs after exposure to various high-LET radiations will help to understand how the physical qualities of radiation influence the structural abnormality of chromosomes. This knowledge will be crucial to strategize treatment planning.

In general, the current study demonstrated that ^{12}C-beam causes linear dose-dependent increase in total CAs at 24 h and 48 h. However, after ^{16}O-irradiation, linear dose-dependent increase was only observed at 24 h, not at 48 h. At 48 h, the frequency of total CAs increased linearly up to 4.91 Gy, then steeply declined at 9.83 Gy after ^{16}O-irradiation. This decline may be due to the elimination of heavily damaged cells from the population. We observed similar decline at 48 when V79 cells were exposed to 9.83 Gy of ^{16}O-beam. The slope values of dose-response curves, 0.38 ± 0.01 and 0.29 ± 0.01 for ^{12}C- and ^{16}O-beam at 24 h, respectively, clearly indicate ^{12}C-beam induces more chromosomal damage than ^{16}O-beam. These data suggests that particle radiation with relatively lower LET value may induce more chromosomal damage. This validates previous findings of Tenhumberg et al, who noticed ^{12}C-ions with a relatively lower LET value (153.3 keV/μm) was more effective than Ni-ions (2455 keV/μm) in inducing cytogenetic damage in human fibroblast cells. Thus, it is evident that a strong correlation exists between LET values and aberration yield.

We also noticed a substantial decline in total CAs in M5 cells with the increase in post-irradiation incubation time, which corroborates our previous observations with V79 cells. However, the rate of decline varies depending on the aberration types. For example, the decline in chromatid-type breaks was considerably less than the chromosome-type breaks. Approximately 46% and 94% decrease in chromatid-and chromosome-type breaks, respectively, was observed at 48 h after exposure to 4.73 Gy of 12Cbeam. Similar trend was also noticed after 9.83 Gy of ^{16}O-irradiation. Interestingly, at 24 h, ^{12}C-beam induced more chromosometype breaks than dicentrics, while ^{16}O-irradiation showed complete opposite trend. The frequency of chromatid-type exchange was substantially higher than chromatid-type breaks for both radiation types. The ratio of chromatid-type exchange to chromatid break at 24 h was considerably higher after ^{16}O- than ^{12}C-irradiation. These data suggests that radiation quality strongly influence aberration spectrum.

Both ^{12}C- and ^{16}O-beams induce more chromosome- than chromatid-type aberrations, which suggest that the confluent M5 cells were in G1 phase during irradiation. This could be due to contact inhibition of cell proliferation. Irradiation of cells in G0/ G1 phase is also known to favor chromosome-type aberrations. Interestingly, we observed chromosome- and chromatid-type aberrations in the same metaphase spread, which is contrary to the general belief and suggests that these chromatid-type aberrations are induced in pre-DNA synthetic stage. This could be the result of alkali-labile sites or single strand DNA breaks induced in G1 cells after high-LET radiation; since substantial number of single strand breaks remain as “unrepaired lesions” and lead to formation of chromatid-type aberrations. Ritter et al. also reported presence of both chromosome- and chromatid-type aberrations in the same metaphase spread of V79 cells exposed to high-LET Ar-ions (LET = 1840 keV/μm) in G1 phase. Presence of both chromosome- and chromatid-type aberration after high-LET radiation, unlike low-LET gamma or X-rays, is expected to increase the overall aberration burden, leading to cell-cycle perturbations and mitotic delay, and ultimately enhanced mitotic death.

This work is partly presented at World Congress on Advances in Pharmaceutical Sciences

Conclusion

Resistance to chemotherapeutic drugs and therapeutic radiation are the major limiting factors for successful cancer treatment. Methotrexate is still employed to treat certain types of human malignancies and radiation is used for treating half of the cancer patients, which may make the tumor cells eventually become resistant to therapy. Using a methotrexate plus radiation resistant cell line (M5), current study clearly demonstrated that quality of high-LET radiation determine the extent and spectrum of chromosomal damage in a time-dependent manner and ^{12}C-beam is more effective in inducing CAs than ^{16}O-beam. These findings will certainly help to design the strategy to treat cancer patient with high-LET radiation.

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