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Comparison of DSB effects of the beta particles of iodine-131 and 6 MV X-ray at a dose of 2 Gy in the presence of 2-Methoxyestradiol, IUdR, and TPT in glioblastoma spheroids

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ABSTRACT

Glioblastoma is one of the lethal brain tumors and one of the resistant tumors against radiotherapy. Multiple treatment methods and different types of radiation and Radiosensitizers drugs have been combined to optimize the efficacy of radiotherapy. Radiosensitizers are employed to reinforce tumor cell killing and have much fewer effects on the normal tissue. Inducing DNA double strand break in tumoral cells is a major goal of radiation sensitivity. In this study, the level of DNA double strand break in glioblastoma spheroids irradiated by 2 Gy beta particles of iodine-131 and 6 MV X-rays in the presence of 2-Methoxyestradiol (2ME2), iodo-deoxy-uridine (IUdR) and Topotecan (TPT) was measured using the PicoGreen method.

Spheroids of the U87MG cell line were cultured to reach a 300 μm diameter. In the phase one of the study, the spheroids were treated in four groups individually, including 2 Gy of iodine-131, TPT+iodine-131, IUdR +iodine-131, IUdR+2ME2+iodine-131. In the next phase, the cells were treated with 2 Gy of 6 MV X-ray, TPT +6 MV X-ray, IUdR+6 MV X-ray, TPT+IUdR+6 MV X-ray. DSB lesions were measured by the Pico Green assay. The amount of DSB lesions in groups irradiated with iodine-131 individually was greater than the group irradiated with 6 MV X-ray ($p < 0.05$). DNA double strand breaks became more significant in combination with TPT. However, the amount of DSBs in the two independent groups of TPT+IUdR+2ME2+iodine-131 and TPT +IUdR+2ME2+6 MV X-ray was approximately in the same range ($P > 0.05$). The level of DNA double strand breaks in cells irradiated with Iodine-131 was higher than cells irradiated with 6 MV X-ray at the same dose and Topotecan had a positive effect on inducing the damage. The role of 2ME2+IUdR in increasing the damage caused by beta particles of iodine-131 was not significant. Iodine-131 could lead to major DSB damage than 6 MV X-ray at the same dose due to its cross fire effect and spatial distribution of energy in different angels. This study showed that a combination of chemotherapy and iodine-131 had better efficacy than radiotherapy with 6 MV X-ray in the treatment of glioblastoma.

1. Introduction

Glioblastoma is one of the most common and malignant primary brain tumors. Its high proliferation rate and its cellular invasion to peripheral brain tissues and extensive angiogenesis are characteristics of this type of brain tumor (Singh et al., 2003, 2004). The median survival of GBM patients is 15 months despite adjuvant treatments including surgery, chemotherapy, and radiotherapy (Valtonen et al., 1997). The resistance of glioblastoma against conventional treatment techniques has made researchers to use a combination of therapies in clinical and experimental researches. A combination of radiotherapy and chemotherapy has been recognized as the standard treatment of cancer. It is known that the magnitude of any sensitizing effect is significantly greater in the tumor than the critical normal tissues (Chalmers, 2012).

Iododeoxyuridine (IUdR) is a thymidine analogue which weakens the DNA and acts as a radiosensitizer by replacement in the DNA molecule. IUdR replacement in the DNA molecule depends on the cell

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cycle and synthesis phase; therefore, it has no effect on cells which are out of the cell cycle (Epstein et al., 1994; Kassis et al., 2000). 2-Methoxyestradiol (2ME2) does not allow the cells to remain in the G0 phase in hypoxic conditions through polymerization of microtubules (Kamath et al., 2006; Stathopoulos et al., 2010). Studies showed that the amount of DSB damage induced by IUdR increased in the presence of 2ME2 in GBM cells because of more absorbance of IUdR (Eyvazzadeh et al., 2015a, 2015b; Neshasteh-Riz et al., 2010). TPT as a radiosensitizer agent is one of the newest chemotherapy drugs. Poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP1) is an enzyme involved in DNA repair, and PARP1 inhibition has been shown to enhance the efficacy of low-dose radiation (Groß et al., 2001). TPT induces a significant DSB damage in glioblastoma cells by inhibition of PARP1 enzyme (McCluskey et al., 2012).

Clinically, for the treatment of a tumor, in vitro experiments in which therapeutic factors in the tissue model have the most similar response to in vivo experiments are desirable. Spheroids as a 3D cell culture model have the most similarity to the in vivo condition because of their shape and cellular environment. The cellular shape and condition can effect gene expression and cell attitude (Kim, 2005). Iodine-131 is beta and gamma emitter and its special radiation in inducing damage is the beta particles. Beta particle irradiation, in comparison with 6 MV X-ray, has advantages in the cellular damage. However, in addition to create self dose in the surrounding cells, iodine-131 transfers a cross dose to adjacent and distant cells which increases DNA strand breaks. Therefore, if tumoral cells obtain less self-dose, it may be compensated by absorbing the cross dose (Neshasteh-Riz et al., 2013). So, the goal of this study was to determine and compare DNA lesions of the chemotherapy agent TPT and radio-sensitizer drug in the presence of beta particles of iodine-131 or 6 MV X-ray on glioblastoma spheroids. DNA lesions were assessed by the PicoGreen assay, which is a simple, rapid, and quantitative assay using the fluorescent probe Pico Green (Schröder et al., 2006).

2. Experiments

2.1. Materials

Cell line: The human GBM cell line U87MG was obtained from Pasteur Institute (Tehran, Iran) and maintained in the minimum essential medium (MEM; Gibco/Invitrogen, USA) that contained 10% fetal bovine serum (FBS; GmbH/ PAA, Austria), 100 U/ml of penicillin streptomycin (GmbH/PAA, Austria) and 20 U/ml of fungizone (Gibco/ Invitrogen, USA) at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air.

2.2. Spheroid cell culture

Spheroids were cultured using the liquid overlay technique. As we described in detail in our previous study (Eyvazzadeh et al., 2015a, 2015b). in brief, a total of 5×10^5 cells were seeded into a T25 NEST coated flask with a thin layer of 1% agar (Sigma/Aldrich, Germany) with 10 ml of MEM supplemented with 10% FBS. The plates were incubated at 37 °C in a humidified atmosphere with 5% CO_2 (Memmert, Germany). For 28 days, at 72 h intervals, we measured the vertical diameters of the cells by a microscope. The measurements were performed in triplicate. Next, the cell volume was calculated according to the formula: $V = a.b^2.\frac{\pi}{6}$, where "a" is the small diameter of the, "b" is the large diameter of the cells, and "V" shows the volume of the spheroid cells. An average of nine counts was used to define each point (mean ± SEM). Half of the culture medium was replaced with a fresh medium twice a week. Then, we plotted the growth curve; in the linear area or logarithmic phase of the curve, we calculated the cell volume as follows: $v=v_0 \times e^{kt}$, where "V0" is the initial cell volume, "V" is the cell volume after time "t" and "k" shows the gradient of the logarithmic phase of the curve. After that, the volume doubling time (VDT) of the cells was determined according to the gradient of the logarithmic phase of the curve as shown in Eq. (1).

$$VDT = ln2/kt \quad (1)$$

2.3. Treatment and Irradiation

Glioblastoma spheroids were grown to reach a diameter of 300 μm, then, they were treated with 2 Gy of 6 MV X-ray and beta particle of iodine-131 and single drug and drug combinations. Samples were treated with 250 μm of 2ME2 and 1 μm of IUdR during one VDT separately; then, after 2 h incubation with 2 μm of TPT, they were irradiated with 2 Gy of 6 MV X-ray using a linear accelerator (Linac 600, GMV; Varian Medical Systems; USA), at a dose rate of 2 Gy/min field size of 35*30 cm^2 and flasks irradiated from posterior. Because the maximum dose of 6 MV X-ray occurs in the depth of 3 cm of tissue, 3 layers of 1 cm tissue equivalent material were placed under the flask to ensure electronic equilibrium. In groups irradiated with iodine-131, after treatment with IUdR and 2ME2, the cells were incubated with a solution of 10 mci iodine-131 in 0.2 M NaOH for 108 min in order to absorb the dose of 2 Gy as calculated in previous studies (Neshasteh-Riz et al., 2013; Neshasteh-Riz et al., 2012). finally, they were treated with TPT for 2 h.

2.4. Pico Green Assay

DSB damage induced in glioblastoma cells were assessed by the Pico Green assay using the protocol suggested by Schröder et al. (2006). The solution used to denature DNA was prepared as follows: The fluorescent dye stock solution was the Pico Green dsDNA quantitation reagent (solution A; Life Technology/Invitrogen, USA). Calcium – and magnesium – free phosphate buffered saline (PBS) (Ca/Mg – free PBS) contained 137 mM Nacl, 2.7 mM KCL, 4.3 mM Na_2HPO_4 and 1.5 mM KH_2PO_4 (solution B; Gibco/Invitrogen, USA). The lysing solution contained 9.0 M urea, 0.1% sodium dodecyl sulfate (SDS) and 0.2 M EDTA at pH=10 with NaOH (solution C; Sigma/Aldrich, Germany). The Lysing solution supplemented with Pico Green consisted of 10 μL of the original stock dye/ml of solution C (solution D; Life Technology/Invitrogen, USA).

In order to determine the DSB induced in GBM cells, 3 tubes with 50,000 cells/ml in each group were prepared with 300 μl of solution C and 300 μl of solution D. To lyse the cells, all groups were placed in the dark for 40 min. The amount of DSB was determined by measuring the fluorescence intensity for each group with a spectrofluorometer (Shimadzu/USA) at 485 nm excitation and 528 nm emission wavelengths.

2.5. Preparation of the calibration curve

To determine the amount of fluorescence intensity for digestion of all DNA, with reference to our previous study (Eyvazzadeh et al., 2015a, 2015b), various concentrations of DNase combined with 300 μL of the solution D and variable volumes of PBS (resulting in the final volume of 800 μL) were added to different concentrations of non-irradiated intact GBM and lysed cells (50,000 cells/ml solution C).

2.6. Statistical analysis

Statistical analysis was performed using independent-samples t-test and one-way analysis of variance (ANOVA) followed by the Scheffe test for post-hoc analysis via SPSS software version 16. P values less than 0.05 were considered significant.