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The cytotoxicity of high-linear energy transfer radiation is reinforced by oxaliplatin in human glioblastoma cells

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Abstract

The combination of high-linear energy transfer (LET) radiation with chemotherapeutic agents may offer new perspectives in cancer treatment. We therefore assessed the consequences of a treatment in which U-87 human glioblastoma cells were irradiated with p(65) + Be neutrons in the presence of oxaliplatin, a third generation platinum anticancer drug having higher apoptosis-inducing activity than cisplatin. Cell survival, apoptosis, cell cycle progression as well as p21 and p53 protein expressions were analyzed. Results show that an enhanced cytotoxic effect was obtained when the two treatments were combined and that, unlike what we previously observed with cisplatin, this was not due to a reinforcement of apoptosis. Altogether, our results also indicate the potential of oxaliplatin for use in association with high-LET radiation against tumors refractory to conventional photon radiotherapy.

Keywords: Oxaliplatin; Apoptosis; Glioblastoma; Fast neutrons; High-LET radiation

1. Introduction

The simultaneous exposure of malignant cells to ionizing radiation (IR) and anticancer drugs is a new efficient approach to reinforce the overall cytotoxicity of these two types of therapeutic agents. Consequently, this approach has found clinical applications for treating certain types of solid tumors [1,2]. So far, however, only a small number of drugs have been utilized in such therapeutic protocols. Among them, cisplatin has been shown to be remarkably effective [3]. Other platinum derivatives have also been successfully associated with IR, including carboplatin [4] and heptaplatin [5]. Oxaliplatin, a third generation platinum anticancer analog, is currently used for patients with colorectal cancers [6]. It exhibits a safer toxicity profile than

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cisplatin and, in some clinical situations, remains effective towards cisplatin-resistant tumors [7]. The association of oxaliplatin and radiation has been recently reported to delay the growth of human mammary tumors transplanted in athymic mice [8].

High-linear energy transfer (LET) radiations, on the other hand, offer decisive advantages over low-LET ones to cure cancers that are unresponsive to conventional radiotherapy. This mainly reflects the fact that they induce complex lesions in DNA, which are far less repairable and more cytotoxic than those initiated by photons [9]. Therefore, high-LET radiations (essentially carbon ions) are currently used against radioresistant cancers in a few hadrontherapy facilities around the world [10].

We previously reported that the cytotoxicity of fast neutrons against two types of tumor cell lines was strongly enhanced by cisplatin [11,12]. The purpose of the present study was to evaluate the capacity of oxaliplatin to affect the growth and the survival of U-87 glioblastoma cells when associated to such high-LET particles. Since this platinum compound possesses a higher capacity than cisplatin to induce apoptosis [7]. we assumed that a markedly increased cytotoxicity might result from its combination with high-LET radiation.

2. Materials and methods

2.1. Cell culture and reagents

The human glioblastoma U-87 cell line was purchased from the American Type Culture Collection (ATCC). Cells were grown in Dulbecco's modified Eagles's medium (Sigma, Saint-Quentin Fallavier, France) supplemented with 10% fetal bovine serum (Invitrogen, Cergy Pontoise, France), 1 mM sodium pyruvate, 1 mM non-essential amino-acids and 50 μg gentamycin/ml (Sigma). Cultures were maintained in a humid atmosphere of 5% CO_2. Cell detachment and disaggregation was obtained by a 5-min incubation at 37 °C with a solution of trypsin-EDTA (Sigma). Stock solution of oxaliplatin (1 mg/ml, Synthélabo-Aventis, France) were prepared in bidistilled water and stored at −20 °C.

2.2. Irradiation procedures

Asynchronous, exponentially growing cells were exposed at room temperature to p(65) + Be neutrons produced by the accelerator CYCLONE at the Cyclotron Research Center (CRC) of Louvain-la Neuve (Belgium). Cells were contained in 25 cm^2 flasks filled with 10 ml culture medium, or, in some experiments, in 96-flat bottomed wells microplates (Falcon F-3072). Dose rate was 0.2 Gy/min. Doses usually ranged from 2 to 8 Gy. Each experiment was repeated at least three times.

2.3. Treatment schedule

Oxaliplatin was added to the culture 1 h before the irradiation at concentrations varying between 1.5 and 25 μM, and was maintained in the culture medium 24 h afterwards. It was then washed out and replaced with fresh medium. Such a protocol was established in order to allow oxaliplatin to begin its interaction with the cells before the irradiation, without modifying their number. At different times post-irradiation, cells were trypsinized, counted and seeded into 12-well plates (Falcon F-3043), as detailed below.

2.4. Proliferation and clonogenic survival assays

Cells survival was determined using the 3-(4,5-dimethylthiazol-2,5-diphenyltetrazolium) (MTT) assay. Twenty-four hours following the irradiation, cells were trypsinized, washed, and their concentrations adjusted to 10^4 cells/ml. They were seeded on a 96-well microplate (Falcon F-3072) and incubated for 4 or 7 days. Culture medium was removed and replaced with fresh medium containing 10 μl of MTT solution (5 mg/ml). Following a 4-h incubation, 100 μl of DMSO was added to each well to dissolve the formazan crystals. Absorption was determined at a wavelength of 570 nm with a reference of 630 nm, using a MRX (Dynatech) plate reader. The mean of six replicate wells was calculated and values were expressed in % of control, untreated and unirradiated cells. Cell numbers were also measured daily after irradiation using a Coulter Counter. Each experiment was repeated at least three times. For the determination of clonogenicity, between 10^2 and 10^6 cells were seeded into 6-wells microplates (Falcon F-3046). Colonies were allowed to form for 15 days. Plates were then washed with distilled water and air-dried. Colonies were stained with 1% crystal violet in methanol and the number of those containing more than 50 cells was determined using a Gel Doc 2000 (Bio-Rad) image analyzer. The survival fraction was determined according to the formula: number of colonies measured/number of cells seeded × plating efficiency of the control (untreated, unirradiated group).

2.5. Detection of apoptotic cells

Apoptotic cells were quantified according to Nicoletti et al. [13]. Briefly, U-87 cells (5×10^5) were fixed in cold 70% ethanol for 1 h. Then they were washed in phosphate-buffered saline, pH 7.2 (PBS) and resuspended in 100 μl of PBS containing 25 μg of RNase A, 2 mM EDTA and 10 μg of propidium iodide (Sigma, Saint-Quentin Fallavier, France). After incubation in the dark at 37 °C for