Study of HeLa Cells Clone Survival after X-ray Irradiation in the Presence of Cisplatin

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Abstract. Radiation therapy in the presence of heavy elements nuclei ($Z > 53$) is widely developed these days. The presence of such nuclei in cancer cells results in the local increase of energy release from primary photon beam thus increasing relative biological efficiency. In this paper we present the preliminary results of the cell survival study while irradiating cells by X-Ray photon beam in the presence of cisplatin (Pt, $Z = 78$). The preliminary results show the decrease of the cell survival in the presence of both radiation and cisplatin.

INTRODUCTION

The research in the modification of radiation therapy methods using the so called dose supplementary agents introduced into a tumor is being carried out in many countries in order to increase the relative biological effectiveness of the therapy. These agents are any atoms, nuclei or nanoparticles that result in the increase of energy release in the tumor, thus increasing the therapy efficiency. For example, boron-10 is used for neutron-capture therapy. The agents that contain heavy nuclei ($Z > 53$) can be used for the increase of the energy release under the primary photon beam irradiation. The technologies based on the dose supplementary agents are also called binary technologies [1, 2].

One of the first studies of the modification of the radiation therapy course in the presence of a chemotherapeutic drug was conducted at the end of the 20th century. These studies demonstrated a high clinical response rate compared with the standard radiation therapy without drug administration [3–5]. The results of that research served to further develop the binary radiation therapy and facilitated possibility for modifying the standard therapy methods.

In the case of the primary photon beam the binary technologies are sometime called “photon-capture therapy” by analogy with “neutron-capture therapy” [6, 7]. The interaction of the primary photon beam and nuclei of heavy elements results in generating a large number of secondary characteristic X-rays and low-energy Auger electrons (Fig. 1a). This secondary low-energy (short range) radiation ionizes nearby atoms and leads to the occurrence of series of highly active radicals, which causes the destruction of the macromolecules of DNA and RNA, proteins, cells, and other structures.

If any dose supplementary agent is introduced into the tumor tissue, the process may significantly increase the likelihood of death of the tumor cells (Fig. 1b). It was shown [8–12] that with the introduction of heavier elements with an atomic number $Z \geq 53$ into the tissue the energy release increases. The most frequently used elements are radiosensitizers, chemotherapeutic agents, and nanoparticles of platinum and gold [13–19].

In this work we present the preliminary results of our studying the binary radiation technologies in the form of irradiation of cells by X-rays in the presence of cisplatin (Pt, $Z = 78$). HeLa cells clonogenic survival was studied after the irradiation by different doses.

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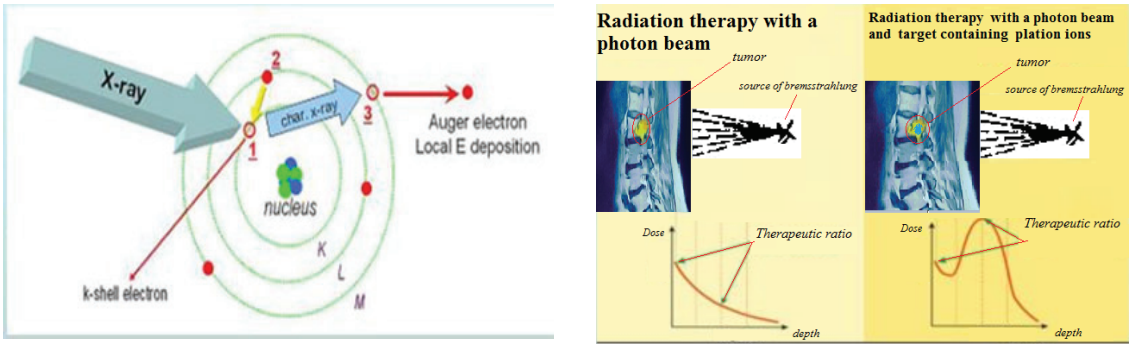

(a) (b)

FIGURE 1. The basic principles of photon-capture radiation therapy

MATERIALS AND METHODS

Since the processes of photoelectric effect and Compton scattering effect are most probable in the orthovoltage X-ray range, the studies were conducted on the X-ray tube. The important part of this research is the use of an X-ray tube, which generates orthovoltage photons with a sufficiently high dose rate. The Tomsk Regional Oncology Center uses X-ray tube Xstrahl300 (Fig. 2a) for routine radiation therapy of skin cancer. The tube that allows generation of bremsstrahlung in the range from 180 kV was used.

The field with a transverse size of 10×10 cm^2 was used during the experiment. The spectrum was modified by the low-energy part absorbers, which are standard for the system.

The clonogenic survival was analyzed in HeLa cell line that was cultured in Dulbecco's Modified Eagle's Medium (DMEM) (PanEco) at 37°C, 5% CO_2. The cells were grown in T-25 culture flasks (TPP) up to 70–80% confluency. The cells were incubated with 0.3 μM cisplatin for 4 hours and irradiated on an X-ray tube Xstrahl300 (Fig. 2c) with 2, 4, 6 and 8 Gy (Fig. 2d). The cells were transported on ice, washed with fresh medium and seeded in 6-well plates (TPP). The number of colonies was assessed 2 weeks after the irradiation. The experiment was conducted in three repeats.

RESULTS AND DISCUSSION

As a result of the study the "dose-effect" (survival of tumors cells) dependencies were obtained. Table 1 presents the main results of the survival cells in the presence of cisplatin in tumor cells and in the absence of the drug.

Based on the obtained data, the analysis of the comparison of the clonogenic survival of the cancer cells between the samples containing cisplatin (Cis3) and in the absence of the drug (Cis0) was carried out. Figure 3 show that the presence of cisplatin has a significant effect on the survival of the cancer cells.

(a) (b) (c) (d)

FIGURE 2. Irradiating cell samples on X-ray tube Xsrtahl300: (a) system Xstrahl300, (b) samples of cancer HeLa cells, (c) geometry of cells irradiation, (d) samples of cancer cells 2 weeks after irradiation

TABLE 1. Clonogenic survival of HeLa cells after irradiation. Cis0—clonogenic survival after irradiation without cisplatin; Cis3—clonogenic survival after incubation with 0.3 μM cisplatin for 4 hours before irradiation; Cis0/Cis3—the ratio of clonogenic survival without cisplatin and samples with it.

Dose, Gy	Cis0, %	Cis3, %	Cis0/Cis3
0	100.0	40.1	2.5
2	117.8	17.3	6.8
4	41.1	7.9	5.2
6	11.9	4.1	2.9
8	3.6	1.2	2.9

The concentration of cisplatin equal to 0.3 μM caused a decrease in clonal survival by about 60% without irradiation. Also, a significant decrease in cell survival after the incubation with cisplatin versus cells non-exposed to cisplatin was observed also after the irradiation. At a dose of 2 Gy, the clonogenic survival was reduced in the cells, incubated with cisplatin, by 6.8 fold, and at a dose of 4 Gy this ratio was 5.2. With a further increase of dose at 6 and 8 Gy, a decrease in clonogenic survival by 2.9 times was observed in the cells, incubated with cisplatin.

CONCLUSION

The results of the research show that the presence of cisplatin in HeLa cells had a significant effect on the clonogenic survival. Such preliminary results look promising for the implementation of radiotherapy together with cisplatin. A combined course of chemo- and radiotherapy is already used in the clinical practice; however, the systems and methods of delivered dose planning do not take this treatment modality into account. This fact makes it complicated to predict the efficiency of the combined chemo- and radiation therapy.

The results presented are the very first step into the implementation of the joint therapy into the clinical practice in the modern level when the efficiency of the treatment can be predicted for every person based on individuals' cancer secularities. For the highest efficiency of the combined treatment the optimal concentration of chemical drug, optimal time between chemotherapy course and irradiation as well as number of fractions and delivered dose per fraction should be known.

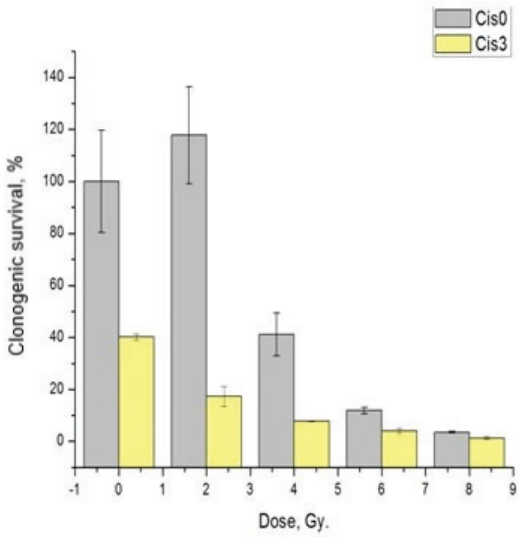

FIGURE 3. Clonogenic survival of HeLa cells after irradiation with (Cis3) and without (Cis0) incubation with 0.3 μM cisplatin

CONFLICTS OF INTEREST

All authors have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations.

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