RADIOSENSITIVITY OF A431, CHO, AND SK-BR-3 CELL LINES TO LOW-INTENSITY BETA RADIATION FROM A SR-90+Y-90 MIXED SOURCE

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Abstract. Currently, radionuclide therapy of tumors using sources of alpha and beta radiation is actively developing. However, the radiosensitivity of tumor cells has been studied mainly using acute gamma radiation. In this regard, studies aimed at determining the ranges of radiosensitivity of cells of various origins in relation to beta-emission radionuclides are gaining relevance. The study was carried out on A431, CHO and SK-BR-3 cell lines using beta-emission sealed sources Sr-90+Y-90. Cell viability was assessed *via* MTT-assay. Dose dependences were obtained for irradiating cells with a beta source: the LD_{50} range was from 17 to 19 Gy, and LD_{37} was from 24 to 36 Gy. It was shown that at the same dose of radiation, the percentage of viable cells relative to the control of 72 hours after irradiation is significantly less than after 24 hours. The revealed LD_{50} values for tumor cells under chronic beta-irradiation are higher than with acute gamma-irradiation, which should be considered when selecting doses during the development of potential radiopharmaceutical treatment. Decreased cell viability in response to beta radiation is due to both cytotoxic and cytostatic manifestations.

Keywords: radionuclide therapy, Sr-90+Y-90, cell culture, radiosensitivity, cancer, beta radiation.

List of Abbreviations

Cs-137 – cesium-137
DMSO – dimethyl sulfoxide
DNA – deoxyribonucleic acid
Gy – Gray
I-123/I-131 – iodine-123/ iodine-131
LD37 – dose at which survival was 37% of control
LD50 – dose at which survival was 50% of control
LET – Linear energy transfer
MBq – megaBecquerel
MeV – megaelectron Volt
MTT-reagent – 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide
MTT-assay – microtiter assay, colorimetric assay for assessing cell metabolic activity
Re-188 – renium-188
Sr-90 – strontium-90
Tcm-99 – metastable nuclear isomer of technetium-99
Y-90 – yttrium-90

Introduction

Cancer treatment includes various methods such as surgery, chemotherapy, photodynamic therapy, radiation therapy, targeted and immunotherapy. Among the above methods, radiation therapy is one of the main approaches and is second in prevalence only to chemotherapy: according to data provided by a number of national councils and committees related to health care (Lievens et al., 2017; Ringborg et al., 2003), about 40% of cancer patients are receiving radiation therapy in one form or another. At the same time, along with classical methods of external radiation therapy using gamma sources, methods of brachytherapy and radionuclide therapy using sources of alpha or beta radiation are becoming increasingly widespread (Deng et al., 2017; Kodina & Krasikova, 2014). High efficiency, and in some cases lack of alternatives to the usage of radionuclides for the treatment of malignant neoplasms, have ensured the steady and rapid development of nuclear medicine in recent years. Currently, targeted radiopharmaceuticals and new treatment approaches are being actively developed in order to ensure the selectivity of action on the tumor focus and at the same time minimize the negative effect on healthy organs and tissues to reduce the total radiation burden on the body

(Chernov et al., 2017; Gudkov et al., 2016; Guryev et al., 2018). Selective accumulation of radionuclides in a tumor is achieved by using antibodies (Gudkov et al., 2015) or alternative proteins that recognize target molecules on tumor cells' surface (Guryev et al., 2018).

Despite the expanding range of possibilities of using beta emitters in diagnostics and treatment of oncological diseases, the overwhelming majority of studies on radiosensitivity at the cellular level were performed using external acute gamma irradiation with a high dose rate. At the same time, studies of radiosensitivity using the chronic effects of beta radiation constitute only a small part of the total volume of radiosensitivity studies. In general, studies are carried out on cell line cultures in order to establish the ranges of radiosensitivity of cells and to understand the mechanisms of the development of a response to radiation exposure with the subsequent possibility of modifying radiosensitivity (Freudenberg et al., 2012; Maucksch et al., 2018).

The radiosensitivity of cells depends on many factors, namely, on the type of radiation (α, β or γ), the dose rate, and the nature of the effect (chronic, acute). Despite the formal equivalence of the absorbed and equivalent doses for beta and gamma radiation, experiments have repeatedly shown differences in radiosensitivity when exposed to photonic and corpuscular ionizing radiation (Wendisch et al., 2010; Maucksch et al., 2018; Freudenberg et al., 2012). In addition, along with the type of radiation, the source's power significantly influences the experimentally determined value of the radiosensitivity of both cell cultures and organisms (Li Sha et al., 2016; Pomp et al., 1999).

In this work, we determined the ranges of radiosensitivity of cell lines of various origins: A431 – human epidermoid carcinoma cells, CHO – immortalized Chinese hamster ovary cell line, SK-BR-3 – human breast adenocarcinoma cells; LD50 and LD37 are determined. The difference between the cell culture's viability was shown when evaluating the effect 24 and 72 hours after irradiation.

Materials and methods

Cell culture. Cell lines used in the work: A431 – human epidermoid carcinoma cells; CHO – immortalized Chinese hamster ovary cell line (INC RAS); SK-BR-3 – human breast adenocarcinoma cells (ATCC, USA).

The cells were cultured in 25 cm^2 culture flasks (Corning, United States) at 37 °C and 5% CO2 atmosphere. DMEM (HyClone, USA) for CHO and A431, Mc'Coys (HyClone, USA) for SK-BR-3, respectively, with the addition of 10% fetal bovine serum (HyClone, USA) and 2 mM L-glutamine in the case of DMEM medium (PanEko, Russia). To remove cells from the culture flask, trypsin: EDTA solution (1:1) (PanEko, Russia) was used, and 10 mM phosphate-buffered saline (PBS) was used to wash off the cells.

Growth curves. To determine cell cultures' main growth parameters, growth curves were preliminarily recorded (Freshney, 2018). For this, cells were planted in 96-well plates at a concentration of 500 to 10,000 cells per well and incubated for 5 days at 37 °C and 5% CO_2 atmosphere. Cells in the wells were counted every 24 hours using a hemocytometer. The obtained values were used to determine the doubling period, lag and log phases using the GraphPad Prism 6 software (GraphPad Software, USA).

Irradiation. Beta-emission sealed sources Sr-90+Y-90 were used as radioactive sources. The half-life of the Sr-90 isotope is 28.8 years; the maximal decay energy is 0.5 MeV. The half-life of the Y-90 isotope is 64 hours; the maximal decay energy is 2.3 MeV (Audi et al., 2017) (Figure 1). In the experiments, we used sealed sources with an activity of 1 MBq on a polypropylene wafer with a working zone of 10 mm in diameter and 3 MBq on a steel wafer with a working zone of 24 mm in diameter (STC Amplitude, Russia). The dose rates of the sources were 0.5 Gy/h and 1.5 Gy/h, respectively.

Before irradiation, cells were seeded on 96-well culture plates (Corning, United States) in the amount of 500–2000 cells per well. Twelve hours later, after the cells were attached to the

bottom of the plate, the cells were exposed with a beta-emission sealed of Sr-90+Y-90, which was placed above and/or under the well of the plate (Figure 1). The radiation dose was varied by the power of the source (a combination of several sources and the use of screens of various thicknesses). The incubation time with the source was 24 hours. Experimental (irradiated) and control wells were located at a distance of two wells with 200 µl of water (16.7 mm) (Figure 1). After the irradiation, the growth medium was replaced with a fresh one in the control and experimental wells.

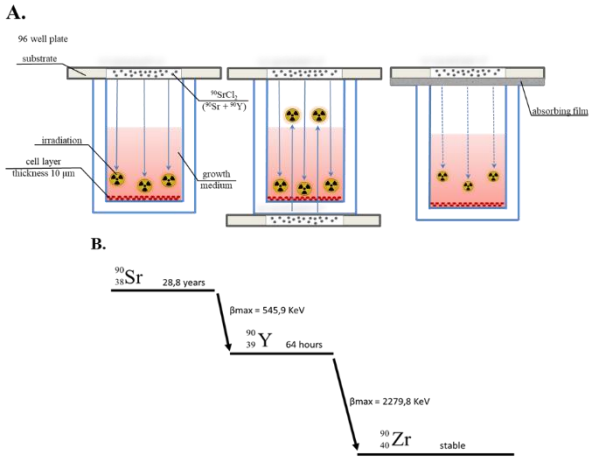

Fig. 1. Scheme of cell irradiation (A) and decay (B) of Sr-90+Y-90. Sealed sources with an activity of 1 MBq on a polypropylene substrate with a working area of 10 mm in diameter and 3 MBq on a steel substrate with a working area of 24 mm in diameter. Cells (attached to the bottom of the plate) were exposed to a source positioned above and / or below the well of the plate. The radiation dose was varied by the power of the source (by combining several sources and using screens of different thicknesses)

Cell survival (*MTT-assay*). To determine the range of radiosensitivity of the studied cell lines, the viability of cells after exposure to the beta source of Sr-Y-90 was assessed by the microtitration test to analyze metabolic activity (MTT-assay). Cell viability was assessed 24 and 72 hours after the sources were removed. The cells were incubated with MTT reagent in medium (0.5 mg/ml) for 4 hours; the formed formazan was dissolved in DMSO and photometrized at a wavelength of 570 nm using an EMax Plus Microplate spectrophotometer (Thermo Fisher Scientific, USA). The relative viability of cells was calculated in relation to the control – non-irradiated cells – and expressed as a percentage.

The calculation of LD_{37} and LD_{50} (the radiation dose leading to a decrease in the relative cell viability to 50% and 37% relative to the control, respectively) was performed using the GraphPad Prism 6 software (GraphPad Software, USA) by nonlinear regression using a four-parameter dose-effect model.

Results

Analysis of the main growth parameters, such as lag-, log-phase, doubling period and reaching a plateau, for the studied cell cultures showed that the most intensive growth of A431 and CHO cultures persists for 4 days with a characteristic doubling time of about 20–25 hours (26 hours for A431 and 19 hours for CHO, Table 1). The exponential growth phase in the SK-BR-3 cell line lasted up to 5 days; the doubling period was 37 hours. It should be noted that the obtained values of the growth parameters of cells under the used conditions of cultivation are consistent with the literature data, for example, the doubling period of A431 according to the literature is 24 hours (Bonner et al., 2009), CHO – 20 hours, SK-BR-3 is about 30 hours (according to the international collection of cell cultures ATCC Cell Lines, www.atcc.org).

Based on the results of assessing the viability of cells 24 and 72 hours after irradiation at a dose of 12 Gy, it can be seen that the viability of cells at an interval of 72 hours after irradiation is significantly lower than at a 24-hour interval: 60% and ~ 80%, respectively (p-value < 0.001, Mann-Whitney U-test). Thus, we see that the effect of radiation on cells becomes more pronounced with an increase in the elapsed time from the moment of irradiation. The observed effect may be due to the fact that in 72 hours the cells undergo several cycles of division, so during this time not only cytotoxic, but also cytostatic manifestations of the action

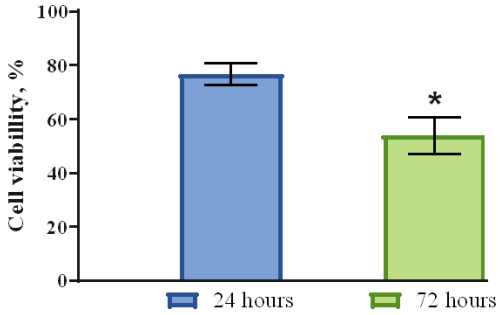

Fig. 2. The percentage of surviving cells relative to the control for the A431 cell line when irradiated with a beta-emission sealed sources of Sr-90+Y-90 at a dose of 12 Gy, estimated 24 and 72 hours after irradiation. Error bars are represented by standard error of the mean (SEM). Statistical differences Mann – Whitney U-test: * – P-value < 0.001

Table 1

Growth cycle parameter values

Cell line	Doubling period (95% confidence interval), h	Lag-phase, h	Log-phase, h	Plateau, h
A431	26 (24,9; 26,9)	~12	12-96	120
CHO	19,3 (18,1; 20,6)	~12	12-96	120
SK-BR-3	37,1 (33,4; 41,6)	~12	12-120	144

of radiation can be recorded. Subsequently, the survival rate was determined only 72 hours after the end of irradiation.

The radiosensitivity values were compared to each other for several cell lines of different origins: A431 – human epidermoid carcinoma cells; CHO – immortalized Chinese hamster ovary cell line; SK-BR-3 – human breast adenocarcinoma cells. The studied cell lines form the following sequence in terms of radiosensitivity: SK-BR-3≤CHO≤A431 (Figure 3, Table 2). It can be noted that the rapidly dividing tumor cell line A431 (doubling period of 26 hours) showed greater radiosensitivity than the slowly dividing tumor line SK-BR-3 (doubling period 37 hours), which is consistent with the idea of an increase in the radiosensitivity of cells in the interphase of the cell cycle. Despite the high rate of division (doubling period of 19 hours), the CHO cell line took an intermediate position. This phenomenon may be related to the origin of cell culture; it is known that the DNA repair systems in rodent cells differ from those in human cells, which explains the resistance to radiation of many rodents, including the Chinese hamster (Kudryashov, 2004).

Figure 4 represents microscopic photographs of irradiated (A) and non-irradiated (B) A431 cells 24 and 72 h after Sr-Y-90 irradiation at a dose of 36 Gy. Non-irradiated cells retain their typical morphology, namely attachment and spreading on the substrate, forming cell-cell contacts regardless of the observation time. 72 h after cell seeding, cell confluence increases, which indicates active cell division. On the contrary, some of the irradiated cells show signs of apoptotic death (cell detachment from the substrate and obtaining of the round shape, as well as blebbing or «boiling» of the cell membrane) already 24 h after the end of the irradiation, yet the majority of cells in the field of view retain their usual morphological state of attached epithelial cells. Massive cell death is observed 72 h after irradiation and is presumably expressed in a large number of apoptotic cell residues with the formation of apoptotic bodies. Along with cell death, the formation of «giant» cells can be noted. Such cells are viable but are unable to divide.

Discussion

The severity of cells' response to the action of ionizing radiation, depending on the elapsed time from the moment of irradiation, has been repeatedly highlighted in the literature (Meijer et al., 2005). Our experiments also demons-

trated a significant difference in the recorded viability of the epidermoid carcinoma culture 24 and 72 hours after exposure to a beta source at the same dose. The observed effect may be due to the fact that radiation damage to cells can manifest itself both in the form of a cytotoxic effect – a violation of the structure of the main macromolecules and membrane organelles of the cell – and in the form of a cytostatic action caused by blocking the cell cycle or mitosis (Zölzer et al., 2014). It is known that cells that cannot overcome the radiation block of mitosis and continue the cell cycle undergo mitotic death within 2–3 doubling cycles (Xiaopeng et al., 2019). Considering that the period of doubling of A431 cells we established is about 26 hours, the cells should go through 2-3 doubling cycles in 52–78 hours. Thus, the measurement of cell survival after 72-hour irradiation seems to be the most reasonable, since it allows registering the action of cytostatic effects. At the same time, cultures of this age do not leave the phase of logarithmic growth; for the studied cultures, it is about 100-120 hours from the moment of planting (Figure 2, Table 1).

The obtained data are in line with the morphological state of the cells: the irradiated cells exhibit rare, pronounced signs of incipient cell death (cell detachment from the substrate and obtaining of the round shape and blebbing of the membrane) 24 h after exposure. In turn, a large number of dead cells, apoptotic bodies and rare giant cells are observed in the same cell culture 72 h after irradiation, which confirms the need to assess the delayed response to radiation exposure (Figure 4).

The reported LD_{50} values under conditions of chronic exposure to external beta emitters for all cell lines turned out to be significantly higher than those using acute external photonic irradiation at a high dose rate published by other research groups (Table 2). The radiosensitivity in a number of studied cell lines was distributed in the following sequence: SK-BR-3 ≤ CHO ≤ A431.

The comparison of the obtained results with the literature data showed that the LD_{50} value approximately doubles in the transition from acute photonic to chronic corpuscular irradiation for the studied cell lines. Different types of

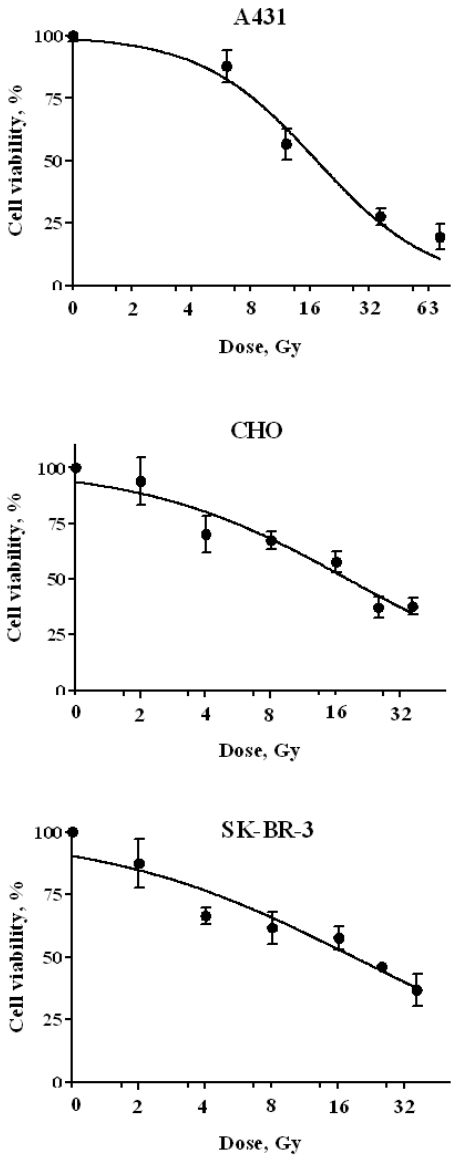

Fig. 3. Percentage of viable cells relative to control for different cell lines depending on the absorbed dose when irradiated with a beta-emission sealed sources of Sr-90+Y-90. Error bars are represented by standard error of the mean (SEM)

irradiation can explain differences in LD_{50} values. It is shown that radiosensitivity depends on the type of radiation and the power of the source. For example, Wendisch et al., 2010, reported a fivefold difference in survival when using the Tc-99m gamma emitter compared to the I-131 beta emitter in the presence of perchlorate. For the human epidermoid carcinoma cell line A431, the LD_{50} under irradiation with

Table 2

Radiosensitivity of different cell lines

Cell line	Experimental data			Literature data		
	LD_{50}, Gy	95% confidence interval LD_{50}, Gy	LD_{37}, Gy	LD_{50}, Gy	Irradiation type and mode	Source
A431	17	(13; 23)	24	4	Co-60, acute gamma radiation, 23 Gy/hour	(Kraxnera et al., 2001; Sandro, 2006)
				6	Tc-99m	(Maucksch et al.,2018)
				8	Re-188	(Maucksch et al.,2018)
CHO	18	(13; 25)	32	10	Co-60, acute gamma radiation, 76 Gy/hour	(Roos et al., 2002)
SK-BR-3	19	(13; 27)	36	9,5	acute, X-ray radiation, 6MV, 39 Gy/hour	(Hai-Tao et al., 2010)

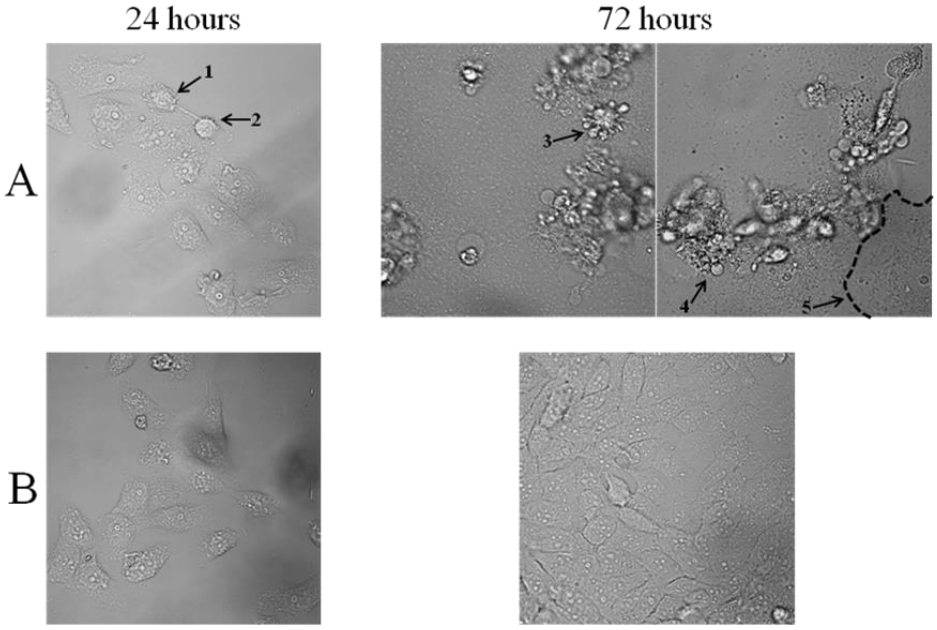

Fig. 4. Microscopic photographs of irradiated (A) and non-irradiated (B) A431 cells 24 and 72 h after Sr-Y-90 irradiation. Arrows indicate morphological changes induced by irradiation: 1 – blebbing; 2 – obtaining round shape; 3 – apoptosis; 4 – the remains of dead cells; 5 – giant cell

a gamma emitter and an electron source Tc-99m and a beta emitter Re-188 was 6 and 8 Gy, respectively (Maucksch et al., 2018). In another study comparing the radiotoxicity of the isotopes Tc-99m and I-123 with external X-ray irradiation (200 keV), the LD37 dose values for a rat thyroid culture PCCl3 are given at 3.5 Gy for Tc-99m and 3.8 Gy for beta-emitter I-123 (with the LD37 for external irradiation of 2.6 Gy).

In the work (Li Sha et al., 2016), different ranges of radiosensitivity were shown when using sources of different powers by the example of proton irradiation with different LET

(25 keV/μm and 10 keV/μm) on the A431 cell line, LD50 was 1.5 and 3 Gy, respectively. The effect of source dose rate has also been shown for the culture of human epithelial carcinoma of the urinary bladder RT112 (Pomp et al., 1999). The LD50 under irradiation with a Cs-137 γ-emitter was 7 Gy, in the case of a low dose rate (4.25 Gy/h) and 5 Gy at a high dose rate (63 Gy/h). One of the possible factors causing such a difference is the duration of the life cycle of a cell or a whole organism (Sazykina & Kryshev, 2016). This difference proves the importance of determining the ranges of radiosensitivity for each type of source with different dose rates.

Conclusions

During the study using an external sealed source of beta radiation, the LD50 and LD37 values were determined for three cell cultures of different origins: A431, CHO, SK-BR-3. The LD50 was 17 Gy, 18 Gy, and 19 Gy, respectively. LD37 was 24 Gy, 32 Gy and 36 Gy. Differences in the radiosensitivity of A431 and SK-BR-3 tumor cell lines correspond to the rate of cell division; the highest resistance is shown for cells of a slowly dividing culture. The difference between the number of viable cells 24 and 72 hours after irradiation was shown: an increase in the observation time after irradiation led to a decrease in the viability of the culture, which suggests the role of cytostatic effects in the development of the response to irradiation.

The obtained results of the radiosensitivity of cell lines of various origins and their comparison with the literature data showed a significant effect of the type and mode of irradiation. Thus, under chronic irradiation with low-intensity corpuscular radiation, the experimentally recorded LD50 value is approximately 2 times higher than that for acute photonic irradiation. Thus, the assessment of the radiosensitivity of tumor cells under conditions of the use of chronic low-intensity beta-irradiation, in particular, in the development of radionuclide therapy schemes, cannot be based on the values of radiosensitivity under conditions of acute gamma-irradiation with a high dose rate and must be experimentally confirmed.

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