Abstract

Boron Neutron Capture Therapy (BNCT) is a type of hadrontherapy based on the ability to capture thermal neutrons on the ^{10}B isotope nucleus and on decay reactions. This reaction leads to the decay of ^{10}B nucleus into high-energy alpha particle and ^{7}Li which remain within the cancer cells. BNCT is a bimodal method what means that firstly, ^{10}B compound (BPA or BSH) have to be administered to the patient and later on, irradiation is performed with neutron radiation. The effectiveness of the therapy depends on the uniformity of ^{10}B compound distribution inside cancer cells. In presented research, the sensitizing effect of gold nanoparticles (GNPs) on breast cancer cell lines of triple-negative subtype MDA-MB-231 and luminal subtype MCF-7 was studied in the case of BNCT. The main aim of the research work was to investigate the possibility of increasing the effectiveness of boron-neutron therapy using gold nanoparticles in triple-negative and luminal breast cancer cells.

The research was carried out at the National Center for Nuclear Research in Świerk, where the MARIA research reactor was used as the source of mixed radiation beam. The mixed radiation consisted of 50% gamma radiation and 50% of neutron radiation. Two breast cancer cell lines MDA-MB-231 and MCF-7 were used for the experiment. GNPs were added to cells in two sizes: 50 nm and 100 nm, and 4-hour and 24-hour incubation with cells was supplemented. Borophenylalanine (BPA) was used as a carrier of the ^{10}B isotope for the studies.

MTT cytotoxicity assay was performed to optimize the research in terms of selecting the appropriate concentrations of GNPs and BPA. The experiment showed that the tested concentrations of both GNPs and BPA for 4-hour incubation time of cells with gold nanoparticles, did not show statistically significant differences between individual trials. In the case of 24-hour incubation with GNPs, all test samples were statistically significant, with metabolic activity increasing in MDA-MB-231 and decreasing in MCF-7. This means that each of the proposed concentrations would be suitable for conducting research on BNCT on breast cancer cells. GNPs were added at a concentration of 8 μl per 1 ml of medium and the concentration of BPA was 30 $mg \cdot ml^{-1}$. In order to analyze the penetration of nanoparticles into cells, the SSC coefficient was examined. The test showed that GNPs 100 nm penetrate better in combination with BPA after 24 h incubation with MDA-MB-231 cells. On the other hand, GNPs 50 nm penetrate better in MCF-7 cells after 24 h incubation.

In order to examine the effect of GNPs in combination with BNCT on breast cancer cells, an analysis of cell cycle progression, cell survival, improper repair of DNA damage was performed by examining the frequency of micronuclei, the level of DSBs in cells based on the fluorescence intensity of γ-H2AX repair foci and the level of cell apoptosis. The obtained research results were diverse and were also influenced by the type of treated breast cancer cells. In the cell cycle, an increased percentage of cells in the G_2 phase of the cycle was observed in both cell lines after the application of radiation alone, while in MDA-MB-231 cell line, the block of cells in the G_2/M phase was at a higher level. Additional treatment of cells with GNPs and BPA increased the level of cells in the G_2 phase of the cycle. This result indicates that the DNA of the cells was seriously damaged by the interaction of GNPs with BPA and with mixed radiation, which caused dysfunction of cell cycle checkpoints. MDA-MB-231 triple-negative breast cancer cells showed greater damage after such treatment than luminal type cells - MCF-7. In the case of both cell lines, GNPs 100 nm with 24h incubation in combination with BPA increased the effectiveness of the therapy the most. This suggests that larger nanoparticles more effectively affect the level of DNA damage in interaction with BPA and radiation.

In both cell lines, cell survivability significantly decreased after irradiation. It was observed that GNPs increased this effect contributing to an even lower survival fraction in the experimental trials. This means that nanoparticles maximize the level of damage leading to the degradation of cells with more extensive DNA strand breaks. In MDA-MB-231 cells, the best efficiency was obtained in the trials irradiated with BPA with added GNPs 100 nm and GNPs 50 nm with 24h incubation, and in MCF-7 with GNPs 50 nm with 24h incubation, the same as in MCF-7 cells. Analysis of the apoptosis level showed a decrease in the percentage of cells in early apoptosis and late apoptosis and necrosis in the non-irradiated test samples with gold nanoparticles and with BPA. A decrease in the population of late apoptotic and necrotic cells was observed, especially in the trial non-irradiated with GNPs 50 nm with 24h incubation in combination with BPA. In the case of MDA-MB-231 a similar correlation was observed after the use of 100 nm GNPs with 24-hour incubation with BPA. The obtained results show the need for additional studies to more thoroughly analyze the effect of GNPs on triple-negative and luminal breast cancer cells in terms of apoptosis. Analysis of the frequency of micronuclei showed significant differences between the experimental trials. The synergistic effect of radiation with BPA and GNPs increases the frequency of micronuclei, which indicates a higher level of genotoxic damages to the cells. GNPs 100 nm with 24h incubation had the greatest effect on MN in MDA-MB-231 cells, while in MCF-7 it was GNPs 50 nm with 4h and 24h incubation. The micronucleus assay showed an increased level of incorrectly repaired DNA damage in both cell lines. These results are also confirmed by the analysis of the γH2AX test, in which a high level of fluorescence intensity from repair foci was noticeable after 24h. Radiation plays a key role in the induction of DNA damage, and in combination with BPA and GNPs this effect is increased. In MDA-MB-231 cells, a better effect was obtained after 24-hour incubation with 100nm GNPs, and in MCF-7 both GNPs 50 nm and GNPs 100 nm with 24h incubation. Boron-neutron therapy is a promising method of hadrontherapy for the cancer treatment. Researches conducted around the world allows for its further development to increase its effectiveness and refine every detail of its action. Each of the performed studies on neutron radiation sources, new boron-10 carriers or on the treatment of various types of cancers brings scientists closer to achieving the highest possible effectiveness of the therapy. The results obtained from the research presented in this work provide important information on BNCT. They can lead to the development of boron-neutron therapy for the breast cancer treatment with an additional increase in the effectiveness. Moreover, they show the complexity of the process that takes place during BNCT and its varied impact depending on the type of cancer.

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