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THE EFFECTS OF IRRADIATION WITH ^{106}RU AND ^{125}I ON THE GROWTH AND METASTASES OF EXPERIMENTAL MELANOMA*

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The effect of two different kinds of ionizing radiation: β-rays (^{106}Ru, 3.54 MeV) and α-rays (^{125}I, 35.4 keV) on melanoma implanted into the hamsters eye were investigated. Tumors growing in the anterior chamber were irradiated with 3 and 6 Gy in four equal fractions at 24 hours intervals. The short-time irradiation effects (primary tumor growth) and long-time effects (metastases) were examined in the experiments. ^{125}I was more effective in both, delaying of the tumor growth and decreasing the metastatic spread in comparison with ^{106}Ru. It was observed that in the case of low doses of β-irradiation the mass of metastases increased in comparison with control (untreated animals). The factors affecting different biological effectiveness of two tested radiation emitters are briefly discussed.

INTRODUCTION

Radiotherapy of ocular melanoma with several kinds of plaques e.g. ^{106}Ru, ^{125}I are used routinely in clinical practice (Starzycka, 1998). Regression of primary tumors is often achieved, but metastases still cause potential danger for the life. In the animal model we can observe either short (inhibition of the growth of the radiation treated tumor) or long-time effects (metastases) (Urbanska, Romanowska-Dixon, Elas, Pajak, Paziewski, Bryk, Kukielczak, Slominski, Zygulska-Mach & Lukiewicz, 2000). The strategy of radiotherapy must take into consideration the complicated nature of interactions between the used radiation, the tumor and the organism as a whole. Some of the factors engaged in particular steps of radiation response of the irradiated tumors are presented in the Fig. 1. The schema shows some of the known physicochemically (Riley, 1994) and biologically important processes (Szumiel, 1998) responsible for the complete effect observable in our experimental animal model (Urbanska et al, 2000). We compare the influence of two kinds of radiation emitters of similar dose rates on short and long-time biological effects.

MATERIAL AND METHODS

Animals

Male and female Syrian hamsters were obtained from the breeding facility at the Medical Academy of Silesia (Katowice, Poland). A total of 40 hamsters weighing between 80 and 120 g were used in the experiments. They were kept under standard condition (12-hours light cycle, rodent chow and water *ad libitum*). The experimental protocols used in the study were reviewed and approved by the University Committee for Bioethics of Experiments with Animals (Jagiellonian University: permission #280/96 and #384/99).

Tumors

The experiments described in this paper were performed using Bomirski Hamster Melanoma (BHM); Ma melanotic subline (BHM Ma) (Bomirski, Slominski & Bigda, 1988) implanted into the anterior chamber of hamster's eye (Romanowska, Kukielczak, Bryk, Mirkiewicz-Sieradzka, Heitzmann & Lukiewicz, 1995).

Tumor implantation and growth in the hamster's eye

Method of implantation was described previously (Romanowska *et al.*, 1995; Urbanska *et al.*, 2000). Briefly, freshly excised pieces of tumors were implanted into the anterior chamber of ham-

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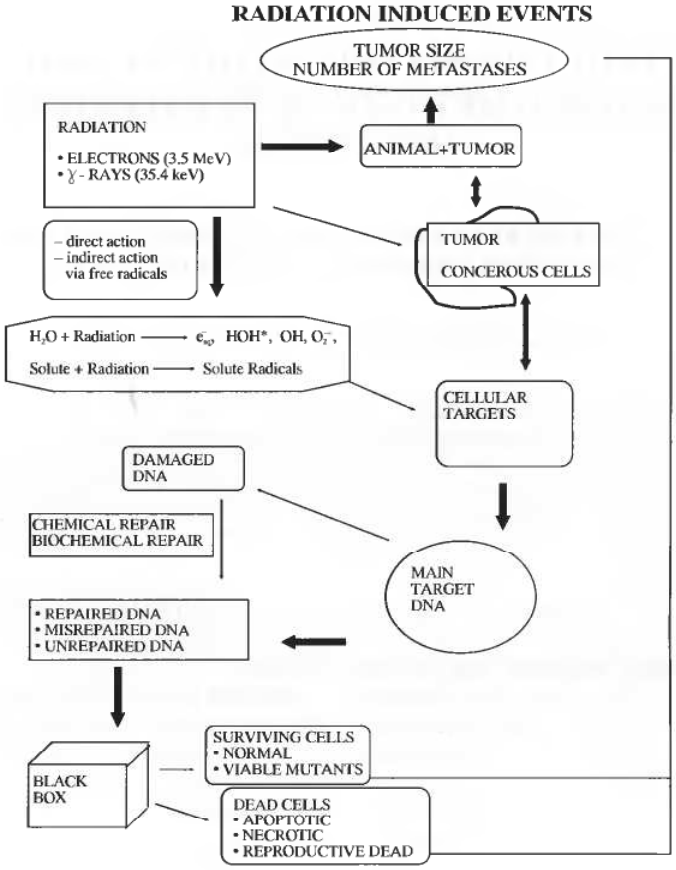

Fig. 1 General schema of radiation-induced events during tumor radiotherapy.

ster's eye. Animals were anesthetized with i.p. injection of Vetbutal (Biowet, Pulawy, Poland). The anterior chamber was observed every day after implantation. As soon as the tumors became visible (6-10 days after implantation), the animal was classified to one of the experimental groups.

Eyeballs were enucleated when the anterior chamber was completely filled with the tumor. Tumor-bearing animals were sacrificed on day 48 after implantation. The organs (regional lymph nodes, lungs, heart, kidney, liver, spleen) were sectioned and the presence of metastases was estimated.

The radiosensitivity of tumors was evaluated by comparing the time needed for a tumor to fill out (T_E) the anterior chamber (short-time effect). The long-time effects were evaluated on the basis of metastatic spread by comparing the weight of lungs with metastases and lungs without metastases.

Irradiation procedure

Tumors were irradiated at a dose of 3 and 6 Gy with a ^{106}Ru plaque (model Rue.EBB, 11.8 Mbq - October, 1995; BEBIG GmbH, Germany) at 0.017 Gy/min and ^{125}I plaque at 0.012 Gy/min. The dose was applied in four equal fractions at 24 hours intervals.

All radiation exposures were preceded by injections of Vetbutal (36 mg/kg body weight).

Time of irradiation varied from 62 to 125 min. ^{106}Ru emitted electrons have the energy of 3.54 MeV and ^{125}I is a source of α-rays with energy of 35.4 keV (Fig. 2).

RESULTS

Short-time effects of radiation

A dose-dependent delay in tumor growth was observed for both emitters with a higher efficacy

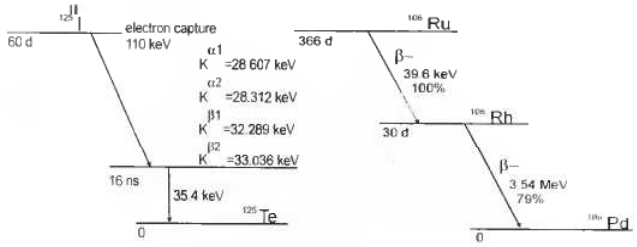

Fig. 2 Schema of splitting ^{106}Ru – β emitters and ^{125}I – α emitters.

for ^{125}I than for ^{106}Ru. The kinetics of tumor growth after fractionated therapy revealed two separate phases of growth. During the first 4-10 days (first phase) tumor growth was inhibited and in the second phase the tumor volume started to increase. The growth rate in the second phase was slower than that of untreated tumors. The effects of radiotherapy applied to hamster melanoma growing in the eye are illustrated in Fig. 3. The efficacy of the same dose of ^{125}I plaque is higher than for ^{106}Ru (1.59 and 1.37 times for 3 and 6 Gy, respectively).

Long-time effects (metastases)

Untreated Bomirski Ma melanoma implanted into the hamster eye metastasized almost exclusively to the lung (Urbanska *et al.*, 2000). Metastases were found in 100% of animals with tumors growing in the eye. The regional lymph nodes were often found to be enlarged and grey. After ^{106}Ru irradiation the size of pulmonary metastases increased for 3 and 6 Gy in comparison with untreated animals (Fig. 4), whereas decreased for the same dose in the case of ^{125}I irradiation.

DISCUSSION

Melanoma is considered to be a rather radioresistant tumor, but in the light of new data this problem is still controversial (Jenrette, 1996). It is now evident that a variety of factors must be taken into account when we evaluate the results of radiation treatment: kind and energy of radiation, fraction of hypoxic cells, density of vascularization, the degree of reoxygenation during radiation and in addition the repair of sublethal and potentially lethal radiation damage. The relationship between the pigment content of tumors and their radiosensitivity versus different kinds of radiation was also tested (Lukiewicz, 1976; Cieszka, Lazarska, Gurbiel, Tyralska & Lukiewicz, 1980; Lukiewicz, Pilas, Nowicka, Cieszka & Gurbiel, 1980; Urbanska *et al.*, 2000). It seems that melanin makes the tumors more resistant to low LET radiation.

In the present experiments we asked which of two sources of radiation was more effective for melanoma tumor in light of the short and long-time effects. We compared β and α-emitters with similar geometry, almost the same dose rate (0.017 and 0.012 Gy/min.) but different energy: 3.5 MeV and 35.4 keV for ruthenium and iodine, respectively. The results are quantitatively different in both estimated biological effects. ^{125}I is more effective with the same doses in comparison with ^{106}Ru.

There are some differences in these two kinds of radiation: γ-radiation has a greater penetration depth and its energy is attenuated more homogeneously in the tissue whereas β-radiation penetrates the tissue less effectively and the distribution of energy is non-homogenous.

Considering these fundamental physical facts

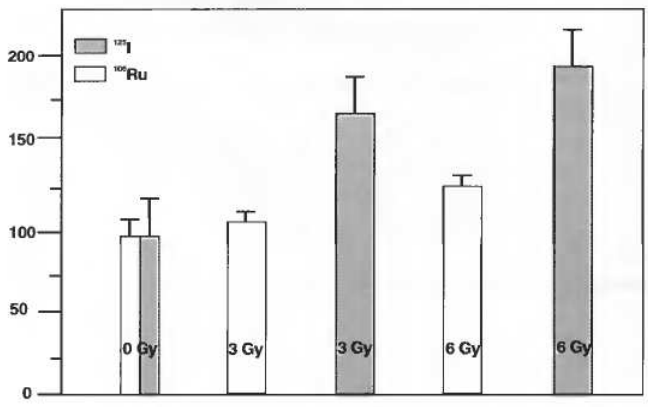

Fig. 3 TE - time needed for tumor to completely fill the anterior chamber of the hamster's eye. After radiotherapy this time is longer (expressed as % of control) than for untreated tumor (control - 100%). 5-8 animals were in one experimental group.

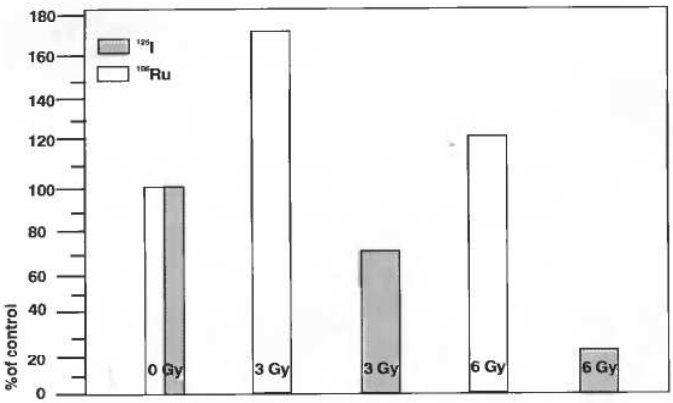

Fig. 4. Mass of the lung metastases in the untreated animals is shown as 100% (control). For animal treated with different doses of irradiation the mass of lung metastases is changed. A tendency to increase the mass of lung metastases in the case of ^{106}Ru for 3 and 6 Gy and to decrease in the case of ^{125}I irradiation for the same doses (3 and 6 Gy) was observed.

and their influence on radiation-induced oxidative stress, we can claim that the tumor cells are in different oxidative conditions in each of the above-mentioned cases, which leads to a decrease in the proliferation ability (40% for iodine irradiation in comparison with only 10% for ruthenium irradiation with the same dose of 3 Gy). Difference between β and γ irradiation is almost the same for two tested doses (3 and 6 Gy).

For a long-time biological effect the difference between two tested emitters was even more significant: β-irradiation increased the mass of metastases in comparison with control (untreated animals) whereas the same dose (3 Gy) of γ-rays inhibited the mass of metastases by 30%. This could be explained by the damage of blood vessels in the eye. For the higher dose (6 Gy) this effect is even more pronounced (for β-irradiation the mass of metastases is still higher than in control animals whereas for γ-rays is reduced by more than 80%). In the light of this observation we conclude that the application of excessively low doses of radiation could be risky because the ability to form metastases may sometimes increase under such conditions.

Our discussion on the role of various factors in the observed effect can occasionally be of speculative nature, since many factors are engaged in the interaction of radiation with tumors and only suitable mathematical models to be elaborated in future will be able to answer several questions which were formulated in the preceding parts of this paper.

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