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E. B. Burlakova, V. N. Erokhin, G. E. Zaikov

THE DEVELOPMENT OF MALIGNANT TUMORS AND LOW-LEVEL IRRADIATION

Keywords: malignant tumors, irradiation.

The AKR mice of age 3-4 months were exposed to ^{137}Cs γ-irradiation with doses of 1.2-2.4 cGy for 2-4 days at a dose-rate of 0.6 cGy/day. S-shaped curve of survivals (percent of live animals-age) for the control (intact animals) and experimental (exposed) groups were obtained. The curves were approximated by the Hompertz function. The rate of development of the malignant process was determined by the modal age (the inflection point on the curve of survivals) when the death rate in a population reaches a maximum (mode). The irradiation of animals produced a promoting effect (approximately by a factor of 1.3) on the development of malignant process. The average and maximum of lifespans decreased approximately by 20 and 120 days, respectively.

Ключевые слова: злокачественные опухоли, облучение.

Мышей высокораковой линии АКР подвергали γ-облучению в дозах 1,2-2,4 сГр при мощности дозы 0,6 сГр в сутки. Получены S-образные кривые выживаемости (процент живых животных – возраст) для контрольной (интактные животные) и экспериментальных (подвергнутые облучению) групп. Эти кривые аппроксимировались функцией Гомпертца. Скорость развития злокачественного процесса оценивалась по так называемому модальному возрасту (точка перегиба на кривой выживаемости), когда скорость смертности в популяции достигает максимума (моды). Облучение животных ускоряет развитие злокачественного процесса (приблизительно в 1,3 раза). Средняя и максимальная продолжительность жизни уменьшаются (соответственно на 20 и 120 дней).

At present, effects of low-level irradiation on living organisms became topical in the context of a scope of environmental problems. Investigation of the regular trends of low- and ultralow-dose irradiation impact is of special importance. According to the studies carried out at the Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, (Burlakova, 1994) a high-dose irradiation impact on biological objects may be comparable with the effect produced by doses that are several orders of magnitude lower. In this case, the dose—effect dependence of irradiation is non-monotonic and nonlinear. These results are of practical importance, since they may be a factor for a choice of models for assessment of radiation risks and working out countermeasures. In the radiation medicine, a linear dose dependence is used in evaluating the risks of irradiation-induced malignant tumors. This choice of a model is claimed to answer the human purposes: to avoid an underestimation of the risk. Nevertheless, some radiobiologists believe that although low-dose irradiation below a certain (threshold) level produces some effect on biological objects but the effect is not harmful. However, comprehensive studies of the action of low doses of physical and physicochemical agents showed that this view is erroneous and alarming (Burlakova *et al*., 1996).

The aim of this work is to evaluate the effect of low-dose γ-irradiation on occurrence and development of malignant tumors --- one of the serious long-term biological aftereffects of radiation. As a tumor-growth model, we chose leucosis, because it develops spontaneously (it is important) in 65—90% AKR line mice of age of 6—11 months (see *Laboratory Animals Lines for Medicobiological Studies,* 1983). This line of mice with a high cancer frequency (instead of that with a low cancer-frequency, which is usually the subject of most studies) was chosen for comprehensive studying the generation (the tumor-producing transformation of cells) and development of malignant tumors induced by low doses of radiation.

It should be noted that origination, clinical symptoms, and pathological and morphological parameters of spontaneous leucosis in mice are similar to that of leucosis in man (Kassirskii, 1964; Bergoltz and Rumyantsev, 1966). A detailed study of the kinetics of development of leucosis by a number of indices was carried out by Belich and Erokhin (1972). The disease is characterized by leucotransformation of thymus cells induced by a leucosis virus in mice. For a certain age of the animals, this transformation is associated with changes in a number of physicochemical parameters of healthy cells (the rate of formation of active oxygen forms, activity of antioxidizing enzymes, antiradical and antioxidizing activity of lipids, properties of plasmatic membranes of cells --- composition, viscosity, etc.), which makes it possible to use these parameters to determine the time and dose of irradiation of the animals (Burlakova and Molochkina, 1973; Zhizhina *et al*.; Vartanyan *et al*., 2000). The maximum sensitivity of the animals to irradiation was determined by these parameters for the age of 3—4 months. These data and results of preliminary studies of biochemical parameters of organs and tissues of intact AKR line mice carried out during the generation of spontaneous leucosis made it possible to choose conditions (doses and time) of the most effective irradiation of the mice.

Materials and methods

In this work, about 200 AKR female mice were used. The mice were provided by the Scientific Research Laboratory of Experimental and Biological Models, Russian Academy of Medical Sciences.

The animals of the age of 3 to 4 months were γ-irradiated (^{137}Cs) at the State Scientific Center: Institute of Biophysics (doses 1.2—2.4 cGy, dose-rate 0.6 cGy/day, period of irradiation 2—4 days). In view of the

fact that a stress (similar to irradiation) promotes the generation of active oxygen species, control animals were subjected to the transportation (required to irradiate the experimental animals) together with the animals of the experimental group.

Leucosis was diagnosed post mortem in the pathological anatomy studies of thymus and spleen of dead animals. We determined the life-spans, leucoses frequency in the control and experimental groups (the effect of irradiation), and changes in thymus and spleen.

On the basis of the life-span data, corresponding curves of survival (share of survived animals --- age) were constructed. A quantitative analysis of the curves involved a non-linear approximation of the Hompertz function

$$S(t) = \exp[(-h_o/\gamma)(e^{\gamma t} - 1)]$$

where *S(t)* is the share of survived animals at the age *t;* γ and h_0 are the function parameters (Konradov and Kutyrkin, 1988). The rate of the process depends on the parameter γ.

For a numerical approximation, the Gauss--Newton non-linear least squares method was used. In the calculations, including statistical ones, the MATLAB mathematical package was used.

Results and discussion

Figure 1 shows the data on the effect of irradiation of AKR female mice; a corresponding approximation of these data by the Hompertz function is shown in Fig. 2. Curve *1* shows the survival rate (the share of survived animals depending on the age) for mice irradiated with doses of 1.2—2.4 cGy; curve 2 shows the survival rate for intact animals.

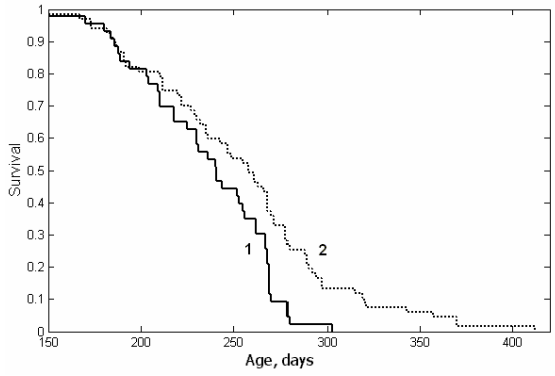

Fig. 1 - Curves of survival rates of AKR female mice (1) exposed to irradiation with doses 1.2—2.4 cGy and (2) controls

As seen from Figs 1 and 2, the irradiation of mice with the above doses results in a confident (confidence level $p < 0.05$) 1.3-fold acceleration of the leucosis process (the relationship between the maximum death rate from leucosis in the experiment and control).

The Hompertz function approximation made it possible to evaluate quantitatively the rate of development of a leucosis process (Fig. 2). The so-called modal age (marked with a dark square in Fig. 2) was calculated:

$$\tau_{mod} = (\ln\gamma - \ln(h_o))/\gamma.$$

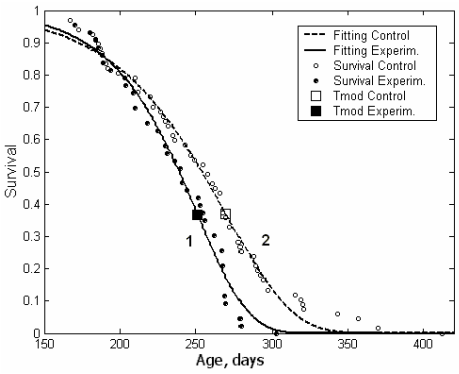

Fig. 2 - Curves of survival rates approximated with the Hompertz function: in (1) experiment and (2) control

This is the age at which the function of survival yields a maximum slope (the infliction point of the curve) and the death rate of the population reaches a maximum (mode). The lower the modal age, the higher the rate of development of the leucosis process; the difference between these values

$$\Delta\tau_{mod} = \tau_{mod}^{contr} - \tau_{mod}^{exp}$$

in the control and experiment may characterize the effect quantitatively. Table 1 shows that irradiation produces a significant promoting effect ($\Delta\tau_{mod}$ = 18.7 days).

On the basis of the obtained parameters of approximation, the average life-span (LS_{theor}) was calculated by the formula

$$LS_{theor} = (\ln\gamma - \ln(h_o) - 0.577)/\gamma$$

(see Table 1). For comparison, the experimental average life-span calculated by the standard formula is also shown in Table 1: it is evident that the experimental promoting effect of irradiation on a leucosis process resulted in a decrease in the average and maximum life-spans (almost by 20 and by 120 days, respectively). In addition, note an increase in the percentage of leucosis cases after a single γ-irradiation with doses of 1.1—2.4 cGy: 80% and 82% in the control and experiment, respectively ($p < 0.01$; in some experiments, differences were more pronounced). Experimental studies of the effect of irradiation on development of leucoses in male mice (the data are not presented here) showed that the percentage of irradiation-induced leucoses increased in this case (from 64% in the control to 77% in the experiment).

One of the main features of the effect of low-dose irradiation is the dependence of the effect on a dose and a dose-rate; the dependence is not only qualitative but also quantitative. Some effects manifest themselves within narrow dose and dose-rate ranges. Such a situation was evident when a comparison was made between our data and those obtained by Japanese scientists (Ishii *et al.*, 1996) who processed their results

Table 1 - Dynamic parameters of development of spontaneous leucoses in AKR female mice in the control and after exposure to low-level irradiation with doses of 1.2—2.4 cGy

Parameters of curves of survival	Experiment	Control
h_0	$[0.77\pm2.299]\times10^{-5}$ confidence interval $0.77\pm2.299]\times10^{-5}$	4.585×10^{-5}, 95% confidence interval $[3.551\pm5.885]\times10^{-5}$
γ	0.0296, 95% confidence interval [0.0278±0.0325]	0.0229, 95% confidence interval [0.0218±0.0240]
Modal age, days	251.3	270.0
$\tau_{mod\ con} - \tau_{mod\ exp}$	18.7	
Average life-span (theor.), days	232.2	244.8
Average life-span (exp.), days	235.4±7.2	254.4±10.7

with the same statistical methods that we used in our studies. In the work by Ishii *et al*. (1996), AKR mice were subjected to fractionated chronic irradiation with doses of 5 and 15 cGy three and 2 times a week, respectively, for 40 weeks (the cumulative doses received by the animals were 600 and 1200 cGy, respectively). In this case, the effect was opposite: there was observed an increase in the average and maximum life-spans of the animals---leucosis-carriers (by 20—30 and 140 days, respectively); the percentage of leucoses incidence decreased. Thus, a comparison between our results and the data obtained by the Japanese scientists showed that experimental low-level irradiation with a varying irradiation mode may yield opposite results.

Conclusion

The effect of low-level irradiation (doses 1.2—2.4 cGy, dose-rate 0.6 cGy/day) on the incidence rate and development of spontaneous leucoses in AKR mice was studied. It was shown that irradiation results in an increase in the leucoses incidence rate, acceleration of the death rate among animals — leucosis-carriers, and shortening of the average and maximum life-spans of sick animals.

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© E. B. Burlakova - д-р биол. наук, проф., зам. дир. Института биохимической физики им. Н.М.Эмануэля РАН (Emanuel Institute of Biochemical Physics, Russian Academy of Sciences); **V. N. Erokhin -** канд. биол. наук, ст. науч. сотр. Института биохимической физики им. Н.М.Эмануэля РАН (Emanuel Institute of Biochemical Physics, Russian Academy of Sciences); **G. E. Zaikov -** д-р хим. наук, проф. каф. технологии пластических масс КНИТУ, ov_stoyanov@mail.ru.