Cancer Chemother Pharmacol (2008) 61:15–21
DOI 10.1007/s00280-007-0439-2

ORIGINAL ARTICLE

Modification of multidrug resistance of tumor cells by ionizing radiation

Yuri N. Korystov · Vera V. Shaposhnikova · Antonina F. Korystova · Maksim O. Emel'yanov · Ludmila N. Kublik

Received: 7 November 2006 / Accepted: 13 February 2007 / Published online: 14 March 2007

Abstract
Purpose The effect of ionizing radiation on multidrug resistance (MDR) of human larynx cancer HEp-2 cells has been investigated. We studied the dependence of the radiation effect on radiation dose, time after irradiation and cell density.
Methods MDR was determined from an increase in cell sensitivity to daunorubicin, taxol and vincristine by the inhibitors of multidrug resistance cyclosporin A and avermectin B_1, and from the suppression by cyclosporin A of the transport of rhodamine 123 out of the cells. The cells were irradiated with X-ray beams (dose rate 1.12 Gy min^{-1}) at room temperature.
Results It was shown that, at 8 and 16 h after irradiation with doses up to 4 Gy, the multidrug resistance of cells increases, and at 24 h it decreases to the control level. The effect was maximal by 16 h after irradiation with a dose of 1 Gy. Both, the contribution of active transport to the rate of rhodamine 123 efflux from cells and their resistance to vincristine, increased. The effect of irradiation on multidrug resistance of HEp-2 cells depended on the density of cells on the substrate, being maximal at a density of 80,000–100,000 cm^{-2}.
Conclusion The irradiation-induced changes in the MDR of tumor cells should be taken into account when combining radiotherapy with chemotherapy. It was assumed that the dependence of multidrug resistance of HEp-2 cells on radiation dose and cell density is determined by changes in the amount of reactive oxygen species in the cells.

Keywords Multidrug resistance · Rhodamine 123 · Tumor cells · Radiation

Y. N. Korystov (✉) · V. V. Shaposhnikova · A. F. Korystova · M. O. Emel'yanov · L. N. Kublik
Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow region 142290, Russia
e-mail: ykorystov@rambler.ru

Introduction

Multidrug resistance (MDR) of tumor cells, which is caused by the expression of transport proteins removing antitumor preparations from the cells, is one of the main factors that hinder the successful chemotherapy of tumors. In the case of drug-resistant tumors, it would be expedient to use other therapeutic methods, in particular, radiotherapy or radiotherapy in combination with chemotherapy. For the effective combined use of radiation and antitumor preparations, it is necessary to know how radiation affects the MDR of tumors. At present, two papers devoted to the effect of radiation on the expression of mRNA of the MRP (multidrug resistance protein) gene in human leukemia cells [1] and the expression of ABC-1 transport protein [2] in human glioma cells have been reported. In both studies, irradiation was shown to increase the expression. There is also substantial evidence that indirectly indicates that radiation can affect the MDR of tumor cells caused by transport proteins. The expression and activity of these proteins are affected by oxidative stress [3, 4], and depend on protein kinase C [5–7] and the transcription factors, NFkB and c-Jun [5, 6, 8, 9]. It is also known that ionizing radiation induces an increase in the amount of reactive oxygen species (ROS) in cells (oxidative stress) [10] and transcription factors [11–13] and enhances the activity of protein kinase C [14, 15].

In the above-cited studies on the effect of radiation on MDR of tumor cells, it was shown that irradiation increases

the amount of mRNA and transport proteins, whereas the effect of radiation on their activity and the resistance of cells to toxic agents have not been investigated. In the present work, we studied the effect of radiation on the rate of efflux of the substrate of transport protein rhodamine 123 from human larynx cancer cells HEp-2 and on the resistance of cells to vincristine.

Materials and methods

Chemicals, medium, buffers and reagents

The antitumor preparations were vincristine (Gedeon Richter, Hungary), doxorubicin and taxol (both Sigma, United States). The inhibitors of transport proteins were cyclosporin A (Sigma, USA) and avermectin B_1 (Farmbiomed, Russia). Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium, fetal calf serum (FCS), gentamicin, Hank's balanced salt solutions (HBSS), crystal violet, SDS and digitonin were purchased from Sigma, USA. Rhodamine 123 was from ICN, USA. Daunorubicin was dissolved in HBSS; vincristine was dissolved in physiological solution supplemented with 0.9% benzyl alcohol; other agents were dissolved in ethanol. Ethanol at the maximum concentration used (0.1%) did not affect cell survival; it was $103 \pm 3\%$.

Cell, cell culture and cell survival determination

Human larynx cancer HEp-2 cells (Collection of cell cultures, Institute of Cytology, St. Petersburg) in DMEM supplemented with 10% FCS and 40 μg ml^{-1} of gentamycin were grown attached to the substrate in 96 well plates (ICN, USA), with 0.1 ml of medium in each well at a concentration of 100,000 ml^{-1}. After 24 h incubation at 37°C in an atmosphere of 5% CO_2, the agents being examined were added and incubation was continued for an additional 2 days. In experiments in which the effect of radiation on MDR of cells was determined by the criterion of survival, the amount of cells per well was greater, 0.1 ml at a concentration of 140,000 ml^{-1}, since the effect of radiation on MDR depends on the concentration of cells on the support (see Results). In experiments on the effect of irradiation with a dose of 1 Gy, the cells were seeded at 11 h and irradiated at 17 h; 16 h after the exposure, they were treated for 30 min at 37°C with vincristine. The cells were irradiated with X-ray beams from an RUT-250-15-1 X-ray facility (dose rate 1.12 Gy min^{-1}, power 200 kV, current strength 20 mA, 1 mm Al filters and 1 mm Cu filters, focus distance 37 cm) at room temperature. After the treatment, the cells were washed thoroughly with the medium and incubated for 2 days at 37°C in an atmosphere of 5% CO_2. After the termination of incubation, the cells were stained with 0.02% crystal violet in 20% ethanol for 10 min, then the dye was removed, the wells were washed with water and a 0.1% SDS solution was added to extract the dye from the cells. The optical density of the extracted dye was determined at 570 nm on a Multiscan Plus spectrofluorimeter. The background optical density was determined in stained wells, which contained the medium and serum without cells. The photometric crystal violet assay was linear, from 1,000 to 30,000 cells in the well. The survival of cells was determined by the formula: $N_o/N_k = (D_o - D)/(D_k - D) \times 100\%$, where D_o, D_k and D are the optical densities in the experiment, control and the background density, respectively, and N_o/N_k is the cell survival, i.e., the amount of live cells in the experiment relative to the control. The distribution of cells with respect to cell cycle phases was determined by measuring the content of DNA on a one-channel flow-through cytometer. The device provides a resolution of 2–4% with the rate of cell counting at 10^2 cell s^{-1}. The cells were fixed in a 70% ethanol solution and stained with the fluorescent dye Hoechst 33258 (Serva, Germany).

Determination of rhodamine 123 transport

The activity of transport proteins that provide MDR was determined from the rate of efflux of rhodamine 123 [16, 17]. The cells were seeded in DMEM, supplemented with 10% FCS and gentamycin (40 μg ml^{-1}), in 25-ml plastic culture flasks; the medium volume in each flask was 6 ml. Prior to seeding, the flask was made to stand on the narrow side and two glass plates (50 × 9 mm, 2 mm thick) were placed into it. The density of cells seeded on the plates varied from 20,000 to 300,000 cm^{-2}. The cells were irradiated on an X-ray facility, 16 h prior to the determination of the transport rate of rhodamine 123. After a 24-h incubation in a CO_2 incubator at 37°C, the cells were washed with RPMI 1640 and loaded for 60 min at 37°C with rhodamine 123, 0.5 μg ml^{-1} (1.3 μM) in RPMI 1640 containing 5% fetal calf serum. After incubation, the cells were washed, from the dye, three times (3 × 10 min) with a cold (2°C) HBSS supplemented with 1% FCS. After washing, the plates with the cells were stored in HBSS, supplemented with 0.5% serum, on ice till measurements. The plate with the cells was placed on a cuvette of an MF44 Perkin-Elmer spectrophotometer containing HBSS with 0.5% FCS (3 ml of volume) at 37°C, and the content of rhodamine 123 in the medium was determined at intervals of 2 s under continuous stirring. The wavelengths of excitation and emission were 488 and 520 nm, respectively. After several minutes of efflux of the dye, the inhibitor of transport proteins, cyclosporin A, 0.3–1 μg ml^{-1} (0.24–0.8 μM), was added into the cuvette to determine the coefficient R of the inhibition of active transport of rhodamine 123, which character-

izes the MDR [16]. To determine the total amount of rhodamine 123 in the cells, at the end of the experiment, the cells were made permeable by adding 0.02% digitonin. The determination rate constants for normal (k) and inhibited (k_i) rhodamine efflux from cells on one plate reduce the error in the determination of their ratio, which is necessary for calculating the coefficient R ($R = k/k_i$).

Statistical analysis

All experiments were carried out in no less than three replicates. In the figures, arithmetic means and standard errors of means are given. Statistical significance was considered for P value < 0.05. The exponential and linear curve fitting and statistical analysis were performed using OriginPro 7 graphic software.

Results

Human larynx cancer HEp-2 cells possess MDR, which is due to the efflux of toxic agents. This is evidenced by an increase in their sensitivity to various antitumor drugs (daunorubicin, taxol and vincristine) in the presence of the inhibitors of transport proteins, cyclosporin A and avermectin B_1 (data not shown). The concentrations at which cell survival decreased by 50% (IC_{50}) were 300, 27 and 13.5 nM for daunorubicin, taxol and vincristine, respectively. The inhibitors by themselves at the concentrations used do not affect cell survival [18]. Cyclosporin A decreases IC_{50} value of daunorubicin 6.7 times and that of taxol 11 times. Avermectin B_1 decreases the IC_{50} of vincristine 3.3 times. Cyclosporin A and avermectins also decrease the rate of efflux of rhodamine 123 from these cells [18].

Changes in the expression of mRNA of the genes of transport proteins and of the proteins themselves in the cells were observed at 2–4 and 24 h after the exposure to radiation [1, 2]. Correspondingly, changes in the efflux of substrates of transport proteins from the cells and in the resistance of cells to toxic agents should occur at the same time or somewhat later. We studied the effect of radiation on the MDR of HEp-2 cells at 8, 16 and 24 h after exposure to a dose of 1 Gy (Fig. 1, Table 1). Typical curves of decrease in cell fluorescence versus time in control and after irradiation are shown in Fig. 1. The efflux of rhodamine 123 in irradiated cells increases in the absence of the inhibitor and decreases in the presence of it. This suggests that irradiation not only enhances the active efflux of rhodamine 123, but also suppresses the passive outflow. The dose of 1 Gy increases the R value at 8 and 16 h, with a maximum effect being observed at 16 h (Table 1). At 24 h after irradiation, the R value decreases to the control level. The changes in cell distribution with respect to the cell cycle phases in control and after irradiation with a dose of 1 Gy are presented in Table 2. In control, the percentage of cells in the G1 phase increases and that in the S phase decreases with increasing time after seeding (80,000 cm^{-2}). This means that, at this cell density, cells move toward the stationary growth phase. The irradiation with 1 Gy did not

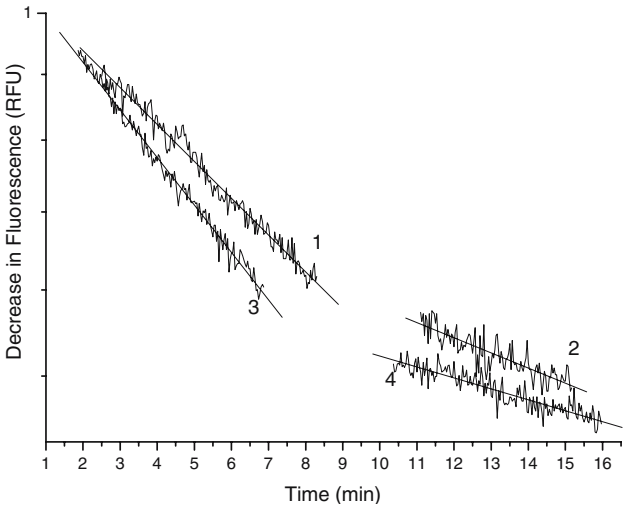

Fig. 1 The efflux rhodamine 123 from HEp-2 cells in control (*1*, *2*) and 16 h after irradiation with the dose 1 Gy (*3*, *4*). *1*, *3* without and *2*, *4* with cyclosporin A, 1 μg ml^{-1}

Table 1 Dependence of MDR (R), k and k_i of HEp-2 cells on the time after irradiation with a dose of 1 Gy

Time after irradiation (h)	8	16	24
1 Gy	$k = 0.051 \pm 0.004$	$k = 0.056 \pm 0.004$	$k = 0.046 \pm 0.003$
	$k_i = 0.016 \pm 0.001$	$k_i = 0.013 \pm 0.001$	$k_i = 0.018 \pm 0.002$
	$R = 3.2 \pm 0.3$	$R = 4.3 \pm 0.1$	$R = 2.5 \pm 0.1$
Control	$k = 0.045 \pm 0.004$	$k = 0.044 \pm 0.003$	$k = 0.043 \pm 0.003$
	$k_i = 0.018 \pm 0.001$	$k_i = 0.018 \pm 0.001$	$k_i = 0.017 \pm 0.002$
	$R = 2.5 \pm 0.1$	$R = 2.4 \pm 0.1$	$R = 2.5 \pm 0.1$

k and k_i are rate constants of rhodamine 123 efflux without and with inhibitor accordingly; $R = k/k_i$

The density of cells on the plates: 80,000 cm^{-2} ($d = 50$), cyclosporin A concentration: 1 μg ml^{-1} . The number of experiments was four and more

Table 2 Time dependence of changes in the cell cycle phase distribution of HEp-2 cells after sham irradiation (control) and after irradiation with a dose of 1 Gy

Experimental conditions	Percent of cells		
	G1	S	G2 + M
Control, 4 h	41 ± 0.8	42 ± 0.4	17 ± 0.4
Control, 8 h	44 ± 0.4	32 ± 0.4	24 ± 0.8
Control, 16 h	59 ± 0.8	23 ± 1	18 ± 0.8
Control, 24 h	61 ± 0.8	22 ± 1.2	17 ± 0.8
1 Gy, 4 h	36 ± 0.2	46 ± 0.4	18 ± 1.2
1 Gy, 8 h	25 ± 0.4	35 ± 1.6	40 ± 1.6
1 Gy, 16 h	63 ± 1.2	17 ± 1	20 ± 0.3
1 Gy, 24 h	61 ± 2	22 ± 2	17 ± 0.4

The number of experiments was three in all variants

lead to a delay of cells in the G1 phase, which points to the mutation of p53 gene in HEp-2 cells [19]; however, in the G2 phase, a significant delay is evident at 8 h after irradiation. In the S phase, a marked decrease in the percentage of cells after irradiation is observed at 16 h. The cell cycle distribution of irradiated HEp-2 cells returns to the control level by 24 h after irradiation.

The dependences of the radiation effect on MDR on dose and cell density were studied at 16 h after irradiation, when the effect was maximal. Figure 2 shows the dependence of the coefficient of inhibition of rhodamine 123 efflux from cells, by cyclosporin A (1 μg ml^{-1}), on the radiation dose. As it follows from the figure, *R* increases as dose increases to 1 Gy; further increase in dose leads to a decrease in *R*. At a dose of 4 Gy, the *R* value does not differ from the control value. At a dose of maximum effect, 1 Gy, the *R* value increases 1.6 times.

The effect of radiation on MDR depends on the density of cells on the plate, reaching a maximum at 80,000–100,000 cm^{-2} (Fig. 3). It is evident from Fig. 3 that the *R* value for control cells does not depend on the density of the cells on the plate, which is consistent with the theoretical predictions [16]. The results shown in Fig. 3 were obtained at a lower concentration of cyclosporin A (0.3 μg ml^{-1}); however, the *R* value at a dose of 1 Gy also increases relative to the control (1.6 times), as it happens at a concentration of cyclosporin A of 1 μg ml^{-1}. Figure 3 shows the dependence of *R* on the density of cells seeded on the plate, when the number of cells was determined by direct counting with a hemocytometer. The number of cells on each separate plate can also be determined from the amount of rhodamine 123 accumulated on the plate, which is measured after the treatment of cells with digitonin (*d*). The dependence of *d* on the density of cells is shown in Fig. 4. The dependence is strictly linear up to a density of 150,000 cm^{-2}, and the point for a density of 270,000 cm^{-2} deviates from the linear dependence, probably due to the substances that leave the cells after their treatment with digitonin. Figure 5 shows the dependence of the effect of irradiation at a dose of 1 Gy on *d*. It is also evident from the figure that the effect of irradiation on *R* depends on the number of cells. The *R* value reaches a maximum at *d* equal to 50–60, which also corresponds to the density of cells on the plate of 80,000–100,000 cm^{-2} (Fig. 4).

Thus, 16 h after irradiation of cells with doses less than 4 Gy, the *R* value increases. A maximum effect is observed at a dose of 1 Gy and with a density of cells on the plate of 80,000–100,000 cm^{-2}.

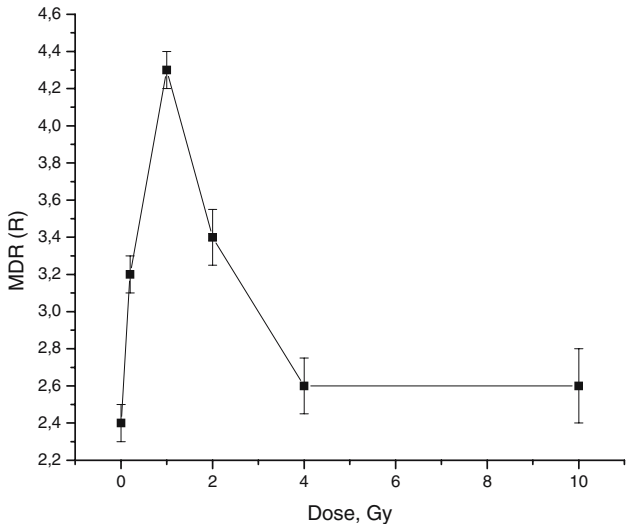

Fig. 2 Effect of irradiation on MDR (*R*) of HEp-2 cells 16 h after irradiation. The average density of cells on the plates, 70,000 cm^{-2} (*d* = 40). Cyclosporin A concentration, 1 μg ml^{-1}

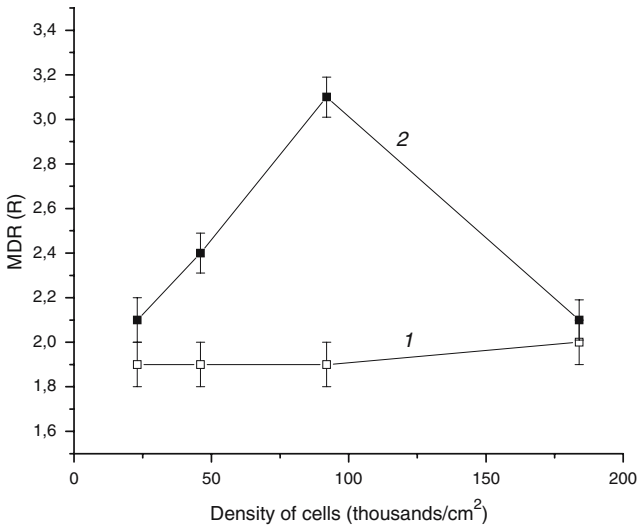

Fig. 3 Dependence of MDR (*R*) of HEp-2 cells on the density of cells on plates in control (*1*) and 16 h after irradiation with 1 Gy (*2*). Cyclosporin A concentration, 0.3 μg ml^{-1}

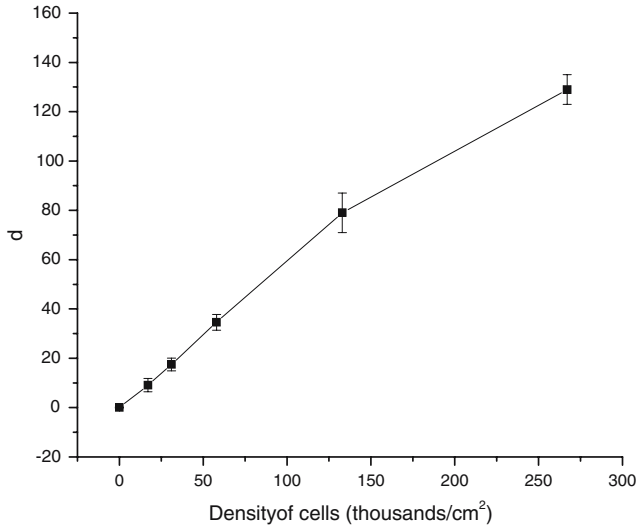

Fig. 4 Dependence of the amount of rhodamine 123 in cells (*d*) on the density of cells on the plates

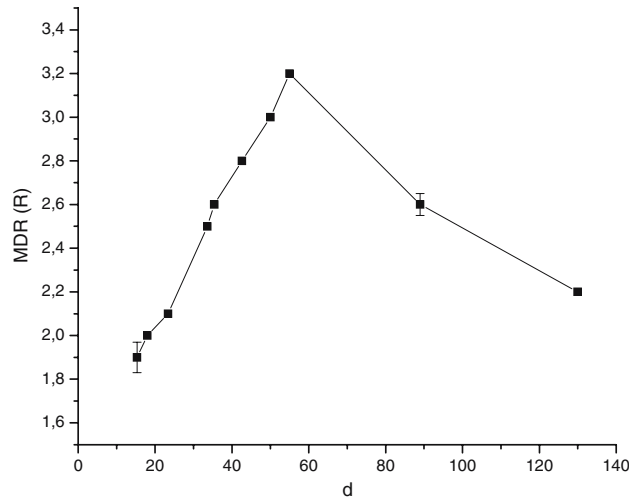

Fig. 5 Dependence of MDR (*R*) HEp-2 cells on the number of cells on the plates, i.e., the content of rhodamine 123 in the cells (*d*) 16 h after irradiation with 1 Gy. Cyclosporin A concentration, 0.3 μg ml^{-1}

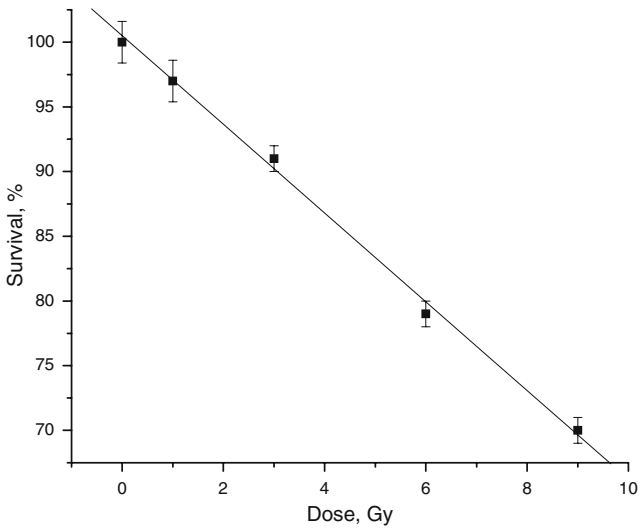

Fig. 6 Effect of radiation on survival of HEp-2 cells

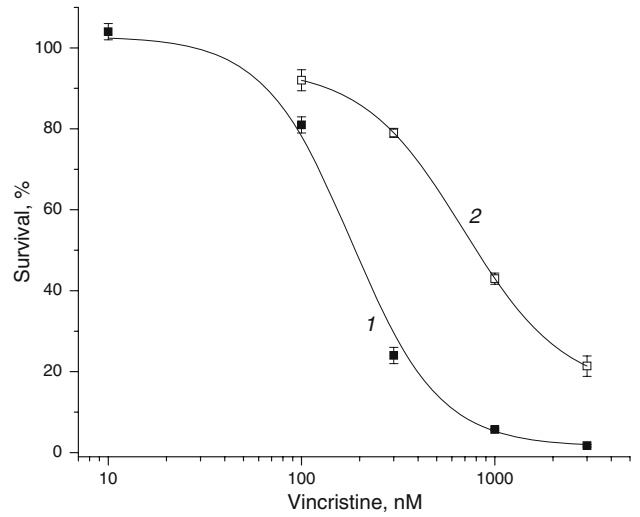

Fig. 7 Effect of vincristine on the survival of (*1*) HEp-2 cells and (*2*) Hep-2 cells irradiated with a dose of 1 Gy, 16 h prior to treatment with vincristine; duration of treatment, 30 min. IC_{50} was 190 nM (*1*) and 800 nM (*2*)

Irradiation affects not only the efflux of rhodamine 123 from cells, but also the sensitivity of cells to vincristine. Before studying the effect of vincristine on the survival of irradiated cells, it was necessary to estimate the effect of radiation itself. Figure 6 gives the dose dependence of cell survival. Irradiation with a dose of 1 Gy decreases the survival only by 3% compared with the control. The poor radiotoxicity (3%) after 1 Gy can be explained as due to the early time after irradiation exposure (2 days only). At such an early time after irradiation, cell cycle delay rather than cell death would be the reason for reduced cell numbers. Figure 7 shows the effect of a short-term (30 min) treatment of cells with vincristine on the survival of control and irradiated (1 Gy) cells. The cells were treated with the agent, 16 h after exposure to radiation. In these experiments, 14,000 cells per well were seeded, which corresponds to a density of 70,000 cm^{-2}, as in experiments presented in Fig. 2. The IC_{50} value for vincristine under these conditions is equal to 190 nM in control cells and 800 nM for cells at 16 h after irradiation with a dose of 1 Gy. Thus, at 16 h after irradiation with a dose of 1 Gy, not only the rate of rhodamine 123 efflux out of cells, but also their resistance to vincristine (4.2 times) increases.

Discussion

We have shown that the MDR of HEp-2 cells increases at 8 and 16 h after irradiation with doses up to 4 Gy, which

manifests itself as an increase in the contribution of active transport to the rate of rhodamine 123 efflux from the cells and in resistance of the cells to vincristine. The effect of irradiation on MDR is maximal by 16 h after irradiation with a dose of 1 Gy at a cell density of 80,000–100,000 cm^{-2} on the support. At 24 h after irradiation, the MDR decreases to the control level. The irradiation-induced changes in the MDR of tumor cells should be taken into account when combining radiotherapy with chemotherapy.

The *R* value is determined by a relative contribution of active transport to the efflux of an agent from the cells. An increase in active transport or a decrease in passive outflow of the agent can increase this relative contribution. Both possibilities are realized in irradiated HEp-2 cells (Fig. 1). The fall in the rate of passive rhodamine 123 efflux evidences a decrease in membrane permeability. Thus, the radiation-induced increase in MDR of HEp-2 cells is caused by the activation of transport and a decrease in cell membrane permeability. The changes in IC_{50} of vincristine after 1 Gy irradiation of cells differ from the change in rhodamine 123 transport. This discrepancy may be accounted for by the fact that the changes in active transport or passive permeability for vincristine and rhodamine 123 are different.

Trog et al. [2] reported that the expression of the ABC-1 transport protein in human glioma cells depends on cell density. They showed that the expression of the transport protein increases with cell growth and the concentration of cells in culture. Irradiation of cells (2 Gy) enhances the expression of the protein, the effect of irradiation being dependent on the growth phase of the culture. Irradiation increases the expression of the protein in cells more than 20 times on day 4 after seeding (logarithmic growth phase) and only 1.5 times on day 9 after seeding, when they are in the stationary phase and their density on the support is high. The authors did not discuss the possible mechanism of the effect and note that their results are only first evidence for the dependence of the expression of the transport protein on intercellular interactions in glioma cells. Irradiation of HEp-2 cells at a dose of 1 Gy increases the percent of cells in the G2 phase at 8 h and decreases the percent of cells in the S phase at 16 h (Table 2). The cell cycle distribution of HEp-2 cells irradiated with a dose of 1 Gy returns to the control level by 24 h after irradiation, and the *R* value decreases to control level also (Table 1). These data suggest that there is a relationship between the cell cycle distribution and the *R* value; however, other data are in conflict with this suggestion. First, the cell cycle distribution depends on cell density, whereas the *R* value in the control does not (Fig. 3). Second, the cell cycle distribution in the control changes with time after seeding, whereas the *R* value does not (Tables 1, 2). It is known that the effect of vincristine is maximal in G2/M. At 16 h after 1 Gy, the portion of cells in G2/M increases by 2%. The increase in the amount of cells in G2/M must enhance the cell sensitivity to vincristine (opposite to the registered effect). The change in the distribution of cells in the cycle after irradiation can somewhat contribute to changes in cell sensitivity to vincristine, but this contribution may be low in accordance with small changes in the cell cycle distribution.

When discussing the results on the dependence of MDR of tumor cells on radiation dose and cell density, it should be noted that both parameters affect the amount of ROS in cells. As dose increases, the generation of ROS by cells increases [10], and as the density of cells on the support increases, their oxygenation and hence the formation of ROS due to oxygen uptake during respiration decrease [20]. In the present study, we found an optimum for the effect of irradiation on MDR for both dose and cell density. This may indicate that the MDR of HEp-2 cells and glioma cells increases at the intermediate (optimal) level of ROS. The expression of another transporter (not MRP and not ABC-1 from glioma cells), P-glycoprotein, also depends on the amount of ROS; however, the dependence is of a different type: as ROS generation decreases, the expression increases [3, 4, 21, 22]. The data reported in the literature indicate that the MDR of tumor cells can be regulated by oxidative stress in different ways, depending on the type of the transport protein. The results on the effect of irradiation on the MDR of HEp-2 cells obtained in the present study can also be explained by radiation-induced changes in the amount of ROS.

Acknowledgments This work was supported by the Russian Foundation for Basic Research (project no. 05-04-48840).

References

1. Harvie RM, Davie MV, Davey RA (1997) Increased MRP expression is associated with resistance to radiation, anthracyclines and etoposide in cells treated with fractionated gamma-radiation. Int J Cancer 73:164–167
2. Trog D, Moenkemann H, Haertel N, Schuller H, Golubnitschaja O (2005) Expression of ABC-1 transporter is elevated in human glioma cells under irradiation and temozolomide treatment. Amino Acids 28:213–219
3. Korystov YN, Ermakova NV, Shaposhnikova VV, Levitman MK, Kim YA, Pushkin SY (2006) Perftoran injection into p388 ascitic lympholeucosis mouse tumor decreases the number of tumor cells and their multidrug resistance. Biol Membranes 23:22–26
4. Wartenberg M, Ling FC, Muschen M, Klein F (2003) Regulation of the multidrug resistance transporter P-glycoprotein in multicellular tumor spheroids by hypoxia-inducible factor-1 and reactive oxygen species. FASEB J 17:503–505
5. Castro AF, Horton JK, Vanoye CG, Altenberg GA (1999) Mechanism of inhibition of P-glycoprotein-mediated drug transport by protein kinase C blockers. Biochem Pharmacol 58:1723–1733
6. Shtil AA (2003) Development of cellular multidrug resistance in response to exogenous stimuli. Biol Membranes 20:236–243

7. Yang JM, Chin KV, Hait WN (1995) Involvement of phospholipase C in heat-shock-induced phosphorylation of P-glycoprotein in multidrug resistant human breast cancer cells. Biochem Biophys Res Commun 210:21–30
8. Miao Z-H, Ding J (2003) Transcription factor c-Jun activation represses *mdr-1* gene expression. Cancer Res 63:4527–4532
9. Ogretmen B, Safa A (1999) Negative regulation of MDR-1 promoter activity in MCF-7, but not in multidrug resistant MCF-7/Adr cells by cross-coupled NF-kB/p65and c-Fos transcription factors and their interaction with the CAAT region. Biochemistry 38:2189–2199
10. Leach JK, Van Tuyle G, Lin P-S, Schmidt-Ullrich R, Mikkelsen RB (2001) Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res 61:3894–3901
11. Churchill JR, Chu AM, Bullon AE, Chou RH, Kim S (1991) Early induction of c-myc mRNA by ionizing radiation in human promielocytic leukemia (HL-60) cells. J Cell Biol 115:412–418
12. Lee YJ, Galoforo SS, Berns CM, Erdos G, Gupta AK, Ways DK, Corry PM (1995) Effect of ionizing radiation on AP-1 binding activity and basic fibroblast growth factor gene expression in drug-sensitive human breast carcinoma MCF-7 and multidrug-resistant MCF-7/ADR cells. J Biol Chem 270:28790–28796
13. Woloschak GE, Chang-Liu CM, Jones PS, Jones CA (1990) Modulation of gene expression in Syrian hamster embryo cells following ionizing radiation. Cancer Res 50:339–344
14. Kini CY, Giaccia AJ, Strubovici B, Brown JM (1992) Differential expression of protein kinase C_3 protein in lung cancer cell lines by ionizing radiation. Br J Cancer 66:844–849
15. Korystov YN (1995) Cross-linking of surface receptors as a trigger of cell apoptosis and proliferation. Scan Microsc 9:757–762
16. Wielinga PR, Heijn N, Westerhoff HW, Lankelma J (1998) A method for studying plasma membrane transport with intact cells using computerized fluorometry. Ann Biochem 263:221–231
17. Wielinga PR, Westerhoff HV, Lankelma J (2000) The relative importance of passive and P-glycoprotein mediated antracycline efflux from multidrug-resistant cells. Eur J Biochem 267:649–657
18. Korystov YN, Ermakova NV, Kublik LN, Levitman MK, Shaposhnikova VV, Mosin VA, Drinyaev VA, Kruglyak EB, Novik TS, Sterlina TS (2004) Avermectins inhibit multidrug resistance of tumor cells. Eur J Pharmacol 493:57–64
19. Elledge SJ (1996) Cell cycle checkpoints preventing an identity crisis. Science 274:1664–1662
20. Boag J (1969) Oxygen diffusion and oxygen depletion problems in radiobiology. Curr Top Radiat Res 5:141–195
21. Ermakova NV, Shaposhnikova VV, Levitman MK, Kim YA, Korystov YN (2004) Alternation of multidrug resistance of vincristine-resistant lympholeukosis P388 cells in the case of ascitic tumor growth. Biol Membranes 21:102–106
22. Wartenberg M, Fisher K, Hescheler J, Sauer H (2000) Redox regulation of P-glycoprotein-mediated multidrug resistance in multicellular prostate tumor spheroids. Int J Cancer 85:267–274