Chemical Inhibition of Cell Recovery after Irradiation with Sparsely and Densely Ionizing Radiation

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Abstract - The dependence of cell survival on exposure dose and the duration of the liquid-holding recovery (LHR) was obtained for diploid yeast cells irradiated with ionizing radiation of different linear energy transfer (LET) and recovering from radiation damage without and with various concentrations of cisplatin - the most widely used anticancer drug. The ability of yeast cells to recover from radiation damage was less effective after cell exposure to high-LET radiation, when cells were irradiated without drug. The increase in cisplatin concentration resulted in the disappearance of this difference whereas the fraction of irreversible damage was permanently enlarged independently of radiation quality. The probability of cell recovery was shown to be constant for various conditions of irradiation and recovery. A new mechanism of cisplatin action was suggested according with which the inhibition of cell recovery after exposure to ionizing radiations was completely explained by the production of irreversible damage.

Key words : Yeast cells, Gamma-rays, Alpha-particles, Chemical inhibitor, Cell recovery

INTRODUCTION

The problem of biological action of high-LET radiation is now very important not only for radiation industry but also for radiation therapy, sterilization, aerospace flights and ecology problems newly arisen after Chernobyl and Fukushima reactor accidents. Actually, densely ionizing radiations are responsible for a half of natural radiation dose (Hall and Giaccia 2012). Secondly, it is promising to use densely ionizing radiation in the cancer treatment because of high values of the relative biological effectiveness (RBE), as well as due to the suppression of the cell ability to recover from sub-lethal and potentially lethal damage (Dasu and Toma-Dasu 2008; Dale *et al*. 2009; Jones *et al*. 2011). While considerable knowledge about repair processes in cells exposed to ionizing radiations has been gained during past decades, the situation is far less clear for the combined action of ionizing radiations of different linear energy transfer (LET) with medical drugs applied for inhibition of cell ability to recover from radiation damage (Raleigh and Haas-Kogan 2013). It is known that the chemical inhibition of this process is related with inhibition of cell recovery, which is displayed by decreased repair rates at molecular and cellular levels (Kim *et al*. 2005, 2007). It

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may be inferred that the mechanism of cell recovery inhibition by drugs can be attributed to either the damage of the recovery process itself or to the increase in the portion of irreversible damage that could not be repairable at all and reduces or prevents further recovery to occur. In the last case, the process of recovery may be either also damaged or stayed unchanged. However, the data distinguishing these possibilities are lacking in literature. It would be of interest to estimate quantitatively the role each of these possibilities. Although the combination of ionizing radiation with drugs is of considerable current interest, there have been no reports in the literature on a quantitative estimation of each of these reasons after exposure to high-LET radiation (Hall and Giaccia 2012). Moreover, there are little comparative investigations of cell recovery inhibition after exposure to low- and high-LET radiation (Petin and Kim 2005; Petin *et al*. 2014; Evstratova *et al*. 2016). Cisplatin is used in this paper as an example of chemical compound that inhibits the recovery process. This drug is one of the most widely used anticancer drugs (Dasari and Tchounwou 2014). DNA damaged by the drug has proved to be a valuable tool for exploring the details of the nucleotide excision repair pathway (Zamble and Lippard 1995; Modig *et al*. 2013).

Yeast cells were chosen as a test object in this study because of some reasons. First, radiobiological responses of yeast cells are qualitatively identical to those of cultured mammalian cells (Petin and Kapultcevich 2014). Second, their recovery has been well studied both on cellular and molecular levels (Petin and Kim 2016). Furthermore, the ability of eukaryotic cells to recover from radiation damage was first discovered in experiments with yeasts (Korogodin 1958; Patrick *et al*. 1964). And at last, a quantitative approach describing the liquid holding recovery (LHR) of yeast cells was successfully applied for combined action of ionizing radiation with various chemical agents on cultured mammalian cells (Petin *et al*. 2013; Petin and Kim 2016). This approach enables the estimation of the probability of recovery per unit time and the fraction of irreversible damage. Therefore, we studied the anticancer drug cisplatin and their impact on the ability of yeast cells to recover after cell exposure to radiations with different LET. The LHR is often considered to be an indicator of other dark repairs. Getting a better insight into the relationship between radiation quality and cell recovery from potential lethal radiation damage is of interest both from a fundamental standpoint and with respect to practical applications of densely ionizing radiation in tumour therapy.

Thus, the purposes of this study were as follows: (1) to compare the effectiveness of yeast cell recovery inhibition by cisplatin after cell exposure to radiations with different LET; (2) to evaluate whether the change in the extent and the rate of recovery is proceeding through the alteration repairable and irreversible portions of the damage inflicted; and (3) to answer the question whether or not high-LET radiation affects the recovery capacity *per se* or it only produces a higher level of severe irreversible damage that cannot be repaired at all.

MATERIALS AND METHODS

The yeast *Saccharomyces cerevisiae* (wild-type diploid strain XS800), in stationary phase of growth was used for these experiments. Cells from the same stock solutions were irradiated with ^{60}Co γ-rays (23 Gy min^{-1}) and with ^{239}Pu α-particles (20 Gy min^{-1}). The LET of the particles reaching the cell monolayer was estimated to be about 120 keV μm^{-1}. Just at this LET value the maximum in RBE-LET relationship was observed for most eukaryotic and some prokaryotic unicellular organisms. The small range of the α-particles necessitated the use of a monolayer of the yeast cells for α-irradiation: 0.02 ml of the cell suspension was placed on the surface of a non-nutrient agar, and the water from this drop of suspension was evaporated. Immediately after irradiation, a part of the samples was plated on nutrient agar plates for the assay of colony-forming ability. Other parts of the irradiated cell suspension with and without various concentration of cisplatin were placed into conditions suitable for the LHR (nonnutriet media, 30°C) and their colony-forming ability was determined as a function of the incubation time in recovery conditions. The survival response was determined by colony counts at the end of 5-7 days of incubation at 30°C. Each data point represents average survival for three to six Petri dishes, each containing 50-200 clones. Experimental points at all figures have errors of 2% or less, that is, approximately the size of the points. Dose-effect curves have been drawn by visually fitting the experimental points. Dosimetry, irradiation and other details have been already published (Petin and Kim 2005, 2016).

Procedure of quantitative estimation of the recovery parameters have been described in detail (Kim *et al*. 2005, 2007). It is worth to mention here the basic steps. The pro-

cess of LHR may be considered as a reduction of the initial dose D_1 to a certain effective dose $D_{eff}(t)$ after a recovery during t hours. The decrease in the effective dose $D_{eff}(t)$ with the recovery time t may be fitted by an equation of the form

$$D_{eff}(t) = D_1[K + (1 - K) \cdot e^{-\beta \cdot t}], \quad (1)$$

where β is the recovery constant that characterizes the probability of the recovery per unit time. The fraction of radiation damage K is an irreversible component of radiation damage, which can be determined as

$$K = D_{eff}(\infty)/D_1, \quad (2)$$

where $D_{eff}(\infty)$ is the effective dose corresponding to the plateau of the recovery curve. Then the function

$$K(t) = D_{eff}(t)/D_1 \quad (3)$$

reflects the relative part of unrepaired damage, both repairable and irreversible, which has not been repaired during t hours. Combining equations (1) and (2), one can deduce

$$A(t) = e^{-\beta \cdot t} = \frac{D_{eff}(t) - D_{eff}(\infty)}{D_1 - D_{eff}(\infty)}. \quad (4)$$

In biological terms, $A(t)$ reflects the relative part of the reparable damage that has not been repaired after t hours of recovery. It follows from this equation that the recovery constant β may be presented as

$$\beta = -[\ln A(t)]/t \quad (5)$$

Thus, knowing the survival and recovery curves after cell exposure to low- and high-LET radiation, one can calculate the corresponding values of $D_{eff}(t)$, $D_{eff}(\infty)$, K, $A(t)$, and β.

RESULTS

Fig. 1A shows the survival curves of diploid yeast cells of *Saccharomyces cerevisiae* (strain XS800) irradiated with graded doses of γ-rays without cisplatin. The recovery kinetic patterns we observed are shown in Fig. 1B. A comparison in the ability of yeast cells to undergo LHR carried out at equal levels of survival (i.e. at equal amounts of lethal lesions). When samples were plated at different times after treatment, the number of viable cells increased as a function of time, reaching a plateau after about 3 days. Cells were recovered during postirradiation period at various cisplatin concentrations. The presented data suggest that both the rate and the extent of recovery decreased with increase in cisplatin concentration at which the recovery occurred. The cisplatin concentration of 0.02 mg ml^{-1} completely inhibited the LHR.

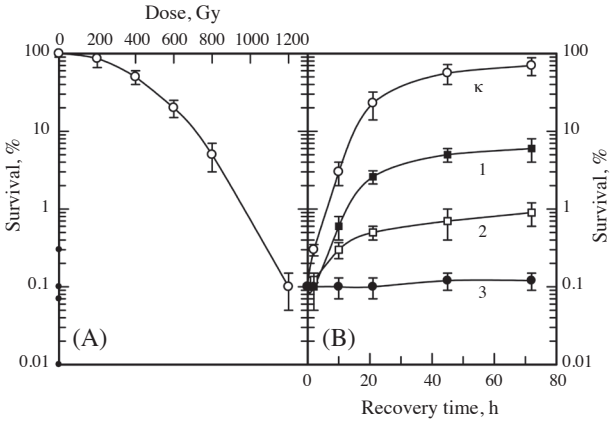

Fig. 1. Survival (A) and recovery (B) curves after irradiation with ^{60}Co γ-rays. Irradiated cells recovered without cisplatin (K) and in the presence of 0.0002 (curve 1), 0.002 (curve 2) and 0.02 (curve 3) mg ml^{-1} of cisplatin.

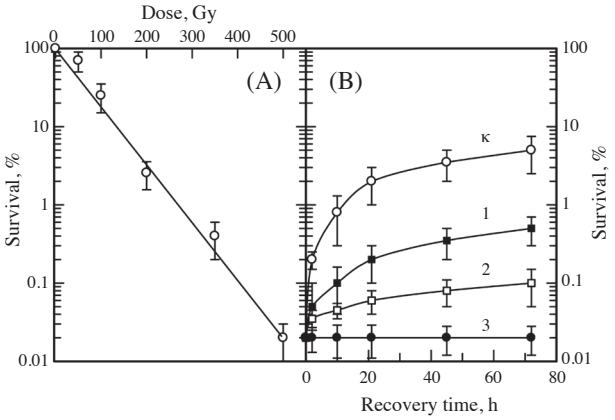

Fig. 2. Survival (A) and recovery (B) curves after irradiation with ^{239}Pu α-particles. Irradiated cells were recovered without cisplatin (K) and in the presence of 0.0002 (curve 1), 0.002 (curve 2) and 0.02 (curve 3) mg ml^{-1} of cisplatin.

Fig. 2 exhibits the results of experiments with the same diploid yeast cells exposed to α-particles (densely ionizing radiation). The dependence of cell survival on dose and the duration of recovery presents in Fig. 2A and 2B correspondingly. One can see that the volume of recovery is progressively decreased with increase in the concentration of cisplatin. Again, the cisplatin concentration of 0.02 mg ml^{-1} completely inhibits LHR. Fig. 3 presents the decrement of the relative part of the recovered radiation damage $A(t)$ as a

function of recovery time after irradiation with ^{239}Pu α-particles (panel A) and with ^{60}Co γ-rays (panel B): open circles - recovery without cisplatin, closed squares, open squares and closed circles - recovery in the presence of 0.0002, 0.002 and 0.02 mg ml^{-1} of cisplatin correspondingly.

Using the experimental results represented in Figs. 1-3 we elucidated the irreversible component (Eq. 2) and the probability of recovery per unit time (constant of recovery) (Eq. 5). The results of our computation are totalized in Table 1. From these data we can draw the following important conclusions. First of all, the irreversible component was greater for exposure to high-LET radiation in comparison with that produced by low-LET radiation when the cells were irradiated without drug whereas the increase in cisplatin concentration resulted in the disappearance of this difference for any condition of irradiation. Further, the increase in cisplatin concentration results in constant increase in the part of irreversible damage independently of radiation quality and the cisplatin concentration of 0.02 mg ml^{-1} practically completely inhibited the LHR.

One can see from the data presented in Table 1 that the recovery constant was the same after alpha- and gamma-irradiation independently of exposure conditions. The observed difference between constant recovery values was statistically insignificant after gamma- and alpha-irradiation.

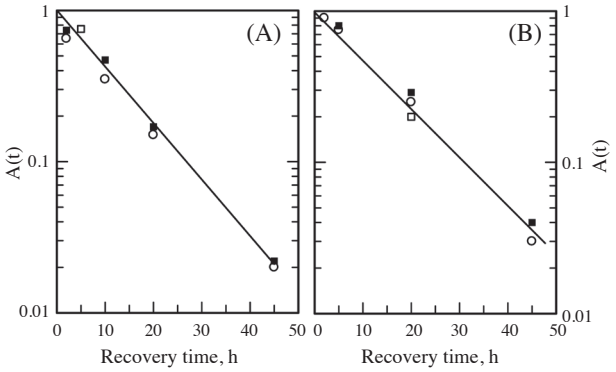

Fig. 3. The decrement of the relative part of the recovered radiation damage $A(t)$ as a function of recovery time after irradiation with ^{239}Pu α-particles (A) and ^{60}Co γ-rays (B): open circles - recovery without cisplatin, closed squares, open squares and closed circles - recovery in the presence of 0.0002, 0.002 and 0.02 mg ml^{-1} of cisplatin correspondingly.

DISCUSSION

Inhibitors of cell recovery from radiation damage are attaining considerable interest for the treatment of cancer (Dasari and Tchounwou 2014). From a general point of view (Hall and Giaccia 2012), the effect of high-LET radiation may be modified before or during irradiation in a lesser extent in comparison with that for low-LET radiation. In our paper, the dependence of cell survival on exposure dose and the duration of the LHR have been obtained for diploid yeast cells irradiated with ionizing radiation of different LET and recovering during postirradiation period without and with various concentration of cisplatin, widely used in clinical practice as an inhibitor of cell recovery. The major point to be inferred from the data described here is that the postirradiation inhibition of cell recovery by cisplatin may be revealed equally both after action of sparsely and densely ionizing radiation on diploid yeast cells, the simplest model of eukaryotic cells. The probability of cell recovery was shown to be constant for various conditions of recovery after irradiation with ionizing radiation of different LET. This last assertion corresponds our previous publication (Petin and Kim 2005; Evstratova *et al*. 2016), where LHR kinetics in wild-type and radiosensitive mutants of *Saccharomyces* yeast have been studied after exposure to low- and high-LET radiation. The increase in cisplatin concentration was shown to result in constant increase in the portion of irreversible damage independently of radiation quality and the cisplatin concentration of 0.02 mg ml^{-1} practically completely inhibited the LHR. Then one may believe that cisplatin inhibition of cell recovery after exposure to low-

Table 1. LHR parameters of diploid *Saccharomyces cerevisiae* yeast cells recovered after gamma and alpha irradiation without and with various concentration of cysplatin

Concentration of cisplatin (mg ml^{-1})	Irreversible component		The probability of recovery (h^{-1})	
	After gamma irradiation	After alpha irradiation	After gamma irradiation	After alpha irradiation
0	0.25 ± 0.01	0.33 ± 0.01	0.076 ± 0.005	0.078 ± 0.007
0.0002	0.66 ± 0.02	0.62 ± 0.01	0.084 ± 0.006	0.080 ± 0.005
0.002	0.80 ± 0.02	0.80 ± 0.02	0.089 ± 0.008	0.081 ± 0.009
0.02	0.98 ± 0.03	1.00 ± 0.03	–	–

and high-LET radiations may be completely explained by the production of irreversible damage from which cells are incapable to recover. Very similar results were published for cultured mammalian cells irradiated with ionizing radiation and treated by various chemical compounds before and during postirradiation LHR (Kim *et al*. 2003, 2005; Petin *et al*. 2004, 2013). On this basis one may conclude that the mechanism of inhibition of cell recovery may be general both for the simplest and the highest eukaryotes.

The new data presented in this paper have a number of fundamental and practical implications. A fundamental significance of the data described is that the results of this and other papers (Kim *et al*. 2005; Petin *et al*. 2013) ensure an opportunity for searching agents, selectively or simultaneously influence on the probability of recovery and the yield of irreversible radiation damage. The recognition that specific inhibitors of recovery may exist, such as suppressor of the recovery process itself and that resulting in the increased yield of irreversible damage, would provide both a possibility to analyze of the drugs and ionizing radiation interaction from this theoretical point of view and an expectation that useful regimens in cancer research may be devised to make use of these inhibitors. The main practical important of the results described is that there may exists effective drug suppressing cell recovery after densely ionizing radiation, and its effectiveness is comparable to its effect after applying the sparsely ionizing radiation. An important implication is also contained in the assertion that one can consider the recovery inhibition effect of cisplatin after cell exposure to α-particles as an additional advantage of densely ionizing radiation application in oncology therapy.

CONCLUSION

1. The ability of yeast cells to recover from radiation damage was less effective after cell exposure to high-LET radiation, when cells were irradiated without drug, the increase in cisplatin concentration resulted in the disappearance of this difference.
2. The increase of cisplatin concentration results in progressive increase in the fraction of irreversible damage independently of radiation quality.
3. The probability of cell recovery was shown to be constant for various conditions of recovery independently of radiation quality.
4. A new mechanism of chemical inhibitor action was suggested according with which the inhibition of cell recovery after exposure to ionizing radiations was completely explained by the production of irreversible damage.

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