Cell Recovery after Combined Action of Ionizing Radiation and Chemicals

Jin Kyu Kim [a*], Changhyun Roh [a], Tae Ho Ryu [a], Ludmila N. Komarova [b], Vladislav G. Petin [b]

[a] *Korea Atomic Energy Research Institute, Jeongeup 580-185, Korea*
[b] *Medical Radiological Research Center, Obninsk 209036, Russia*
[*] Corresponding author: jkkim@kaeri.re.kr

1. Introduction

Damage repair in malignant cells would be problematic in sterilization of microorganisms and treatment of cancer, as well. The inhibition of cell recovery [1] and DNA single and double strand breaks repair [2] by chemicals is expressed both as a deceleration of recovery rate and a lesser extent of recovery. Three possibilities are involved in the inhibition of cell recovery: (1) impairment of the recovery process itself, (2) increased irreversible damage, and (3) simultaneous exert of the two. There have been fee publications regarding these problems. The aim of this study was to determine which of these points are involved in the inhibition of cell recovery. In this study, a quantitative approach describing cell recovery from potentially lethal damage as a decrease in the effective dose was used.

2. Materials and Methods

Experimental data published by others [1, 2] have been used in this study. To determine the time course of the inhibition of recovery from potentially lethal damage, immediately after X-irradiation the stationary phase of V79 Chinese hamster cell were incubated with pyruvate, lactate or novobiocin during 6, 12, and 24 h before they were plated without chemicals to determine their survival by colony-forming ability. Chemicals were added following irradiation in order to estimate their ability to inhibit cell recovery.

The method for an estimation of the yeast cell recovery parameters has already been described in our publication [3]. During recovery, much of the primary radiation damage is eliminated, resulting is increased cell survival. This can be considered to be a reduction in the initial dose D_1 to a certain effective dose $D_{eff}(t)$ that is proportional to the mean amount of residual damage, both reparable and irreversible, after recovery for t hours. It has been shown that the decrease in the $D_{eff}(t)$ with the recovery time t could be fitted by an equation of the form

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

where K is an irreversible component of radiation damage and β is the recovery constant that characterizes the probability of cell recovery per unit time. In other words, the recovery constant is equal to the fraction of radiation damage recovering per unit time. This equation was used previously to fit the recovery kinetics of various biological organisms irradiated with ionizing radiation alone and was seldom used for combined treatments of chemicals and ionizing radiation [5]. Irreversible component K may be expressed as a fraction of the initial irradiation dose by

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

where $D_{eff}(plat)$ is determined when the recovery curve reaches a plateau. Then the function

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

reflects the relative part of the initial radiation dose or the primary radiation damage, both repairable and irreversible, which has not been repaired during t hours of repair. In other words, $K(t)$ represents the fraction of unrepaired damage. During the recovery process, the number of repairable damage diminishes resulting in the reduction of $K(t)$. The minimal value of $K(t)$ is just the irreversible component K (Eq. (2)). Combining equations (1) and (2), one can deduce

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

The right part of this Equation indicates the relative part of the repairable damage that has not been repaired yet after t hours of reparation. Let us designate

$$A(t) = e^{-\beta\cdot t}, \quad (5)$$

then

$$\beta = -\left[\ln A(t)\right]/t. \quad (6)$$

Thus, knowing the survival and recovery curves after different conditions of combined action of ionizing radiation and chemicals, one can calculate the corresponding values of $D_{eff}(t)$ and $D_{eff}(plat)$. It enables to calculate the fraction of unrepaired damage $K(t)$ in the dependence of repair time t (Eq. (3)) and to evaluate the irreversible component K (Eq. (2)). Having calculated the fraction of repairable damage $A(t)$ (Eq. (5)) in the dependence of repair time t, one can estimate the recovery constant β (Eq. (6)).

3. Results

Fig. 1 shows survival (A) and recovery (B) curves of stationary phase cells of Chinese hamster V79 cells irradiated (300 kV X-rays, dose rate being 1.25 Gy/min) and recovered without chemicals. Repair of potentially lethal damage during liquid holding recovery (LHR) is reflected in an increase in the number of viable cells if the cells are kept in an innutritious condition after irradiation for a certain time before they are forced to divide on a nutrient agar [4]. Arrows indicate the initial dose D_1 as well as examples of the effective doses $D_{eff}(t)$ and $D_{eff}(plat)$ estimation. Both these curves were obtained by the averaging of six dose-effect and four time-effect curves published by other authors [1, 2]. In these papers kinetics of recovery from potentially lethal radiation damage were published. It was showed that the survival increase due to recovery observed in the controls was gradually reduced as the chemical concentration increased, i.e. the inhibition of recovery was drug concentration dependent. Using these results and data presented in Fig. 1, we calculated the dependence of the relative fraction of unrepaired damage $K(t)$ on the duration of recovery time for the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors of cell recovery.

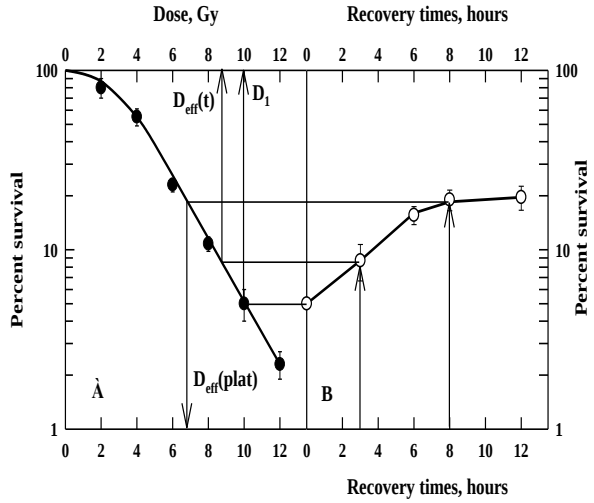

Fig. 1. Survival of Chinese hamster V79 cells in the dependence of X-ray dose (A) and the duration of recovery from potentially lethal damage (B). Cells were X-irradiated and recovered without chemicals. Arrows indicate examples of the initial dose D_1 as well as the effective doses $D_{eff}(t)$ and $D_{eff}(plat)$ determination.

Untreated cells subjected to post-irradiation recovery showed an appreciable decrease in $K(t)$ whereas this effect became less visible as the chemical concentration increased. It seems that the inhibition of recovery depends on drug concentration and this effect is expressed in a great extent with 20 mM of pyruvate and lactate and 20 μM of novobiocin. For instance, the limited values of irreversible component K are equal to 0.60, 0.75, and 0.92 for cells recovering from radiation damage without drug and in the presence of pyruvate. Qualitatively similar results were obtained for other chemicals. The obvious increase in the irreversible component with drug concentration should certainly lead to a decrease in the recovery rate because of the decrease in a number of cells capable of recovery.

4. Conclusions

The results of this paper indicate to the opportunity to search agents, selectively or simultaneously acting on the probability of recovery and the yield of irreversible radiation damage. The results obtained in this study may have a practical use, rather than being concerned only with theoretical position. The recognition that specific inhibitors of recovery may exist, such as an inhibitor of recovery process itself and that resulting in the increased yield of irreversible damage, would provide both a possibility to analyze the mechanism of drug and ionizing radiation interaction from this point of view and an expectation that useful regimens in cancer research may be devised to make use of these inhibitors.

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