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Inhibition of Recovery from Potentially Lethal Damage by Chemicals in Chinese Hamster Cells is Realized through the Production of Irreversible Damage

Jin Kyu Kim*, Ludmila N. Komarova[1], Marianna D. Tkhabisimova[1] and Vladislav G. Petin[1]

Korea Atomic Energy Research Institute, Jeongeup, 580-185 Korea;
[1]Biophysical Laboratory, Medical Radiological Research Center, 249036 Obninsk, Kaluga Region, Russia

Abstract - The inhibition of cell recovery might be proceeded via either the damage of the mechanism of the recovery itself or via the formation of irreversible damage which could not be repaired at all. Both these processes may take place at the same time. Any of these possibilities would result in a decrease in both the rate and extent of cell recovery. To distinguish them, a quantitative approach describing the process of recovery as a decrease in the effective radiation dose was applied to experimental data on the recovery from potentially lethal damage in Chinese hamster cells exposed to X-rays alone or combined with various chemicals (pyruvate, novobiocin, lactate, nalidixic acid, 3-aminobenzamide). For these particular cases, it is concluded that the recovery process itself is not damaged and the inhibition of the recovery is entirely due to the enhanced yield of the irreversibly damaged cells.

Key words : recovery, irreversible damage, chemical, radiation, Chinese hamster cell

INTRODUCTION

It is believed that DNA repair is one of the most important factors in determining cellular sensitivity to ionizing radiation and some other cytotoxic agents (Alper 1979). Many tumours are known to become resistant to ionizing radiation due to the increased efficiency of DNA repair (Burt *et al.* 1991). Therefore, the impairment of the cell ability to recover from radiation damage would be of great relevance in cancer treatment. A lot of chemicals are known to enhance the inactivation effect of ionizing radiation on various cellular systems. It seems generally accepted now that the enhancing effects may be due to both direct drug toxicity and to the enhancement of the cellular radiosensitivity (Streffer and Müller 1984; Hill and Bellamy 1984). It is assumed that drug radiosensitization may be brought about by the inhibition of repair on a cellular level including the recovery from potentially lethal radiation damage. This type of recovery was demonstrated in the cells of various origins and may play a role in the treatment of tumours with ionizing radiation (Weichselbaum 1986). The inhibition of cell recovery (Kumar *et al.* 1985a, b; Little *et al.* 1989; Utsumi *et al.* 1990) and DNA double strand breaks repair (Boothman *et al.* 1989; Yang *et al.* 1995; Takahashi *et al.* 2000) by chemicals is expressed both as a retarded recovery rate and a lesser extent of recovery. It is obvious that these observations may be caused by the following reasons: (i) the damage or inhibition of the recovery process itself, (ii) the increase in the portion of irreversible damage, (iii) both of these reasons. In our recent publications it was shown that the inhibition of the recovery from potentially lethal damage in yeast cells exposed to hyperthermia and ionizing radiation (Petin and Kim 2004) or hyperthermia and UV light (Kim *et al.* 2004) was realized only through the enhanced yield of the irrever-

* Corresponding author: Jin Kyu Kim, Tel. 063-570-3130, Fax. 063-570-3139, E-mail. jkkim@kaeri.re.kr

sible damage whereas the recovery capacity itself was not damaged or impaired. Although there are considerable interests in combining ionizing radiation with chemicals in order to improve tumor treatment, there are few reports in the literature on a separate estimation of the chemicals influence on the process of recovery itself and the production of irreversible damage. Therefore, it would be of particular interest biophysically to implement such a study. In this paper, a quantitative approach describing the recovery of a cell from potentially lethal damage as a decrease in the effective dose will be used to estimate separately the probability of recovery per unit time and the fraction of irreversible damage. As an example, this approach will be applied to experimental data published by others (Kumar *et al.* 1985a, b) and related with the inhibition of recovery from potentially lethal damage by chemicals (pyruvate, novobiocin, lactate, nalidixic acid and 3-aminobenzamide) in Chinese hamster V79 cells.

MATERIAL AND METHODS

1. Experimental procedures

Experimental data published by others (Kumar *et al.* 1985a, b) have been used for this study. Nevertheless some important points should be mentioned. To determine the time course of the inhibition of recovery from potentially lethal damage, immediately after X-irradiation the stationary phase Chinese hamster cells were incubated with 0, 10, 20 mM of pyruvate, lactate or 3-aminobenzamide, 0, 5, 10, 20 μM of novobiocin or nalidixic acid during 6, 12, and 24 h before they were plated without chemicals to determine their survival by colony-forming ability. Besides, cells were treated with 20 mM of pyruvate and lactate and 20 μM of novobiocin and nalidixic acid starting 1 h after irradiation for 6, 12, and 24 h and then replated for colony formation without chemicals. Chemicals were added following irradiation in order to limit their effect to the recovery period only. For details of protocol see the initially published papers (Kumar *et al.* 1985a, b).

2. Estimation of the recovery parameters

During the recovery process the survival of the irradiated cell increases, which means the decrease in the effectiveness of the initial dose D_1 takes place. Then a certain survival $S(t)$ and the corresponding effective dose $D_{eff}(t)$ can be indicated following the recovery during t hours. In other words, the effective dose is equal to the dose in which the cells should initially be irradiated to attain $S(t)$. It was demonstrated (Korogodin *et al.* 1968; Korogodin 1993) that during the recovery of yeast cells from potentially lethal radiation damage, the decrease in the effective dose could be described by an equation of the form

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

where D_1 is the initial radiation dose; e is the basis of the natural logarithm, β is the recovery constant that characterizes the probability of recovery from radiation damage per unit time, and K is an irreversible component of the radiation damage expressed as a fraction of the initial irradiation dose by

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

where $K(\infty)$ and $D_{eff}(\infty)$ are determined for mammalian cells at $t = 24$ h when the recovery curves reach a plateau (conditionally $t = \infty$) and the capability of the cells to recover is saturated or exhausted. The ratio $D_{eff}(\infty)/D_1$ can be considered as an irreversible component of the radiation damage. The cells are believed to be incapable to recover from this part of damage even if they are incubated for a long time at the conditions promoting the recovery. Then the change of this component during the recovery

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

reflects the relative part of the initial radiation dose or the primary radiation damage, both reparable and irreversible, which has not been repaired during t hours of recovery. Taking into account Eqs. (1) and (2), we have

$$e^{-\beta t} = [D_{eff}(t) - D_{eff}(\infty)]/[D_1 - D_{eff}(\infty)] \tag{4}$$

The right part of this Equation reflects the relative part of the reparable damage that has not been repaired after t hours of recovery. Designating this part through $A(t)$, we can write

$$\beta = -[lnA(t)]/t \tag{5}$$

Thus, by knowing the dependence of the cell survival on a radiation dose and the recovery pattern after exposure to ionizing radiation applied alone or combined with various

chemicals, one can calculate $D_{eff}(t)$ and $D_{eff}(\infty)$. It enables us to calculate $K(t)$ in the dependency of the recovery time t (Eq. 3) and evaluate the irreversible component K (Eq. 2). Having calculated the dependency of $lnA(t)$ on the recovery time t, one can estimate the recovery constant β (Eq. 5). It is worth noting that very similar approach was used before to describe the biological effects after whole body gamma irradiation (Davidson 1957), the recovery of yeast cells irradiated with ionizing radiation (Korogodin *et al.* 1968; Korogodin 1993) or exposed to heat combined with ionizing radiation (Petin and Kim 2004) and UV light (Kim *et al.* 2004). So far its applicability to mammalian cells irradiated alone or combined with chemicals has never been tested.

RESULTS

Fig. 1 shows the survival (curve 1) and recovery (curve 2) curves of the stationary phase cells of Chinese hamster V79 cells irradiated (300 kV X-rays, dose rate being 1.25 Gy min^{-1}) and recovered without chemical treatments. Arrows indicate an example of the effective dose estimation. Both these curves were obtained by averaging six dose-effect and four time-effect curves published by other authors (Kumar *et al.* 1985a, b). Kinetics of the recovery from potentially lethal radiation damage showed (Kumar *et al.* 1985a, b) that the survival increase due to recovery observed in the controls was gradually reduced as the chemical

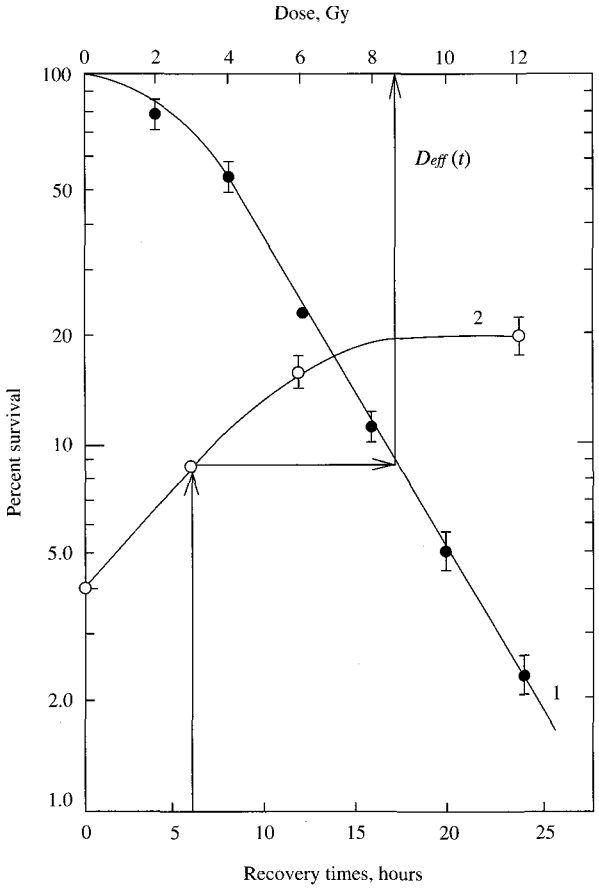

Fig. 1. Survival of Chinese hamster V79 cells in the dependence of X-ray dose (curve 1) and the duration of recovery from potentially lethal damage (curve 2). Cells were irradiated and recovered without chemicals. The results were obtained by averaging of six dose-effect and four time-effect curves published by other authors (Keifer *et al.* 1985a, b). Arrows indicate an example of the initial dose D_1 and the effective dose $D_{eff}(t)$ determination. *Points*, mean; *bars*, SE.

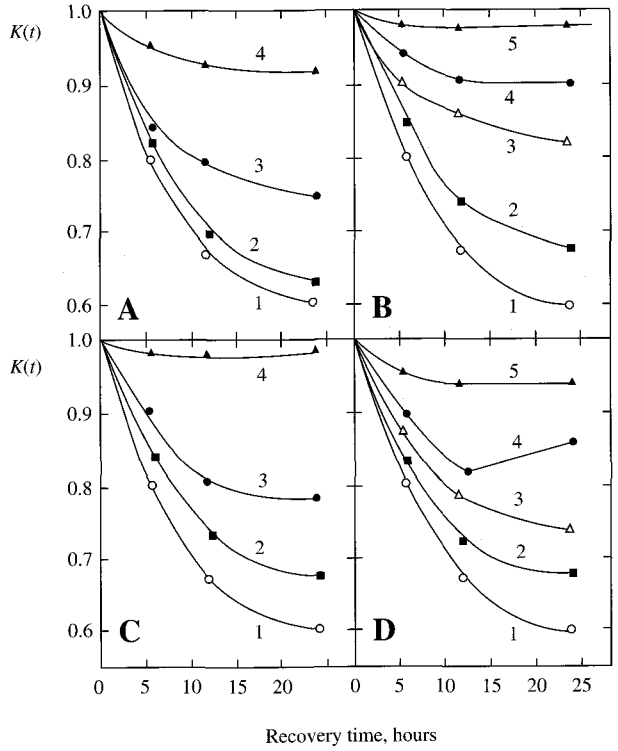

Fig. 2. The dependence of the relative fraction of irreversible damage $K(t) = D_{eff}(t)/D_1$ on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (curves 1) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 20 mM 1 h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). B, novobiocin: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5). C, lactate: 20 mM 1 h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). D, nalidixic acid: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5).

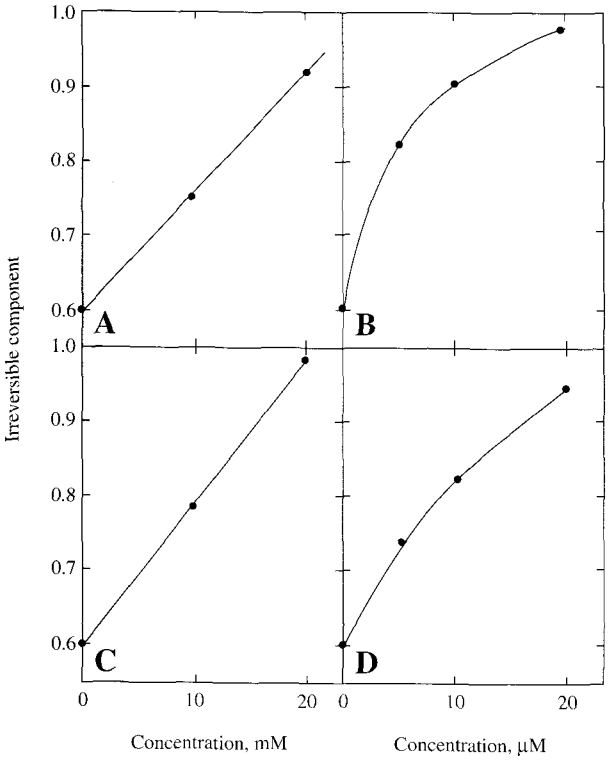

Fig. 3. Irreversible component $K(\infty)$ of radiation damage in Chinese hamster V79 cells recovering from potentially lethal damage in the presence of various concentrations of chemicals: pyruvate (A), novobiocin (B), lactate (C), and nalidixic acid (D).

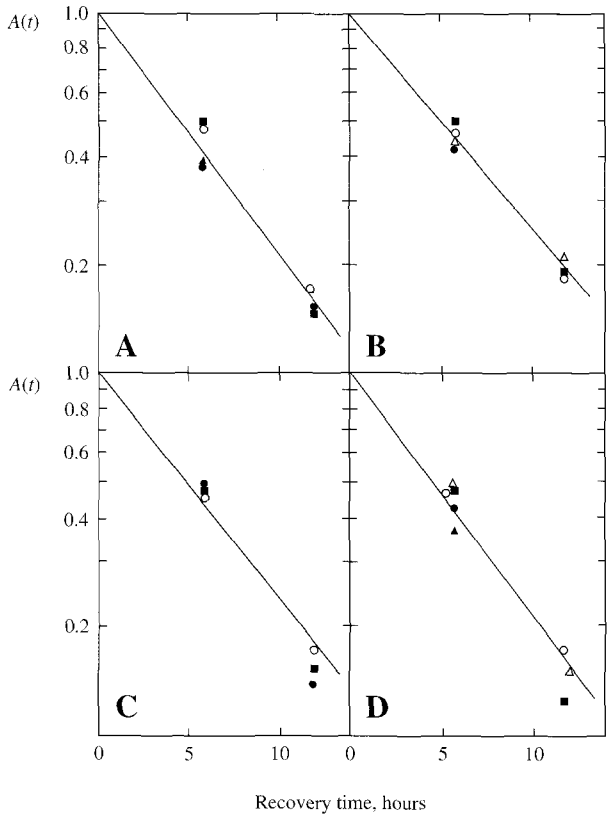

Fig. 4. The dependence of $A(t) = [D_{eff}(t) - D_{eff}(\infty)]/[D_1 - D_{eff}(\infty)]$ on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (open circles) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 20 mM 1 h after irradiation (closed squares), 10 and 20 mM immediately after irradiation (closed circles and triangles, respectively). B, novobiocin: 20 μM 1h after irradiation (closed squares), 5, 10 and 20 μM immediately after irradiation (open triangles, closed circles and triangles, respectively). C, lactate: 20 mM 1 h after irradiation (closed squares), 10 and 20 mM immediately after irradiation (closed circles and triangles, respectively). D, nalidixic acid: 20 μM 1 h after irradiation (closed squares), 5, 10 and 20 μM immediately after irradiation (open triangles, closed circles and triangles, respectively).

concentration increased, i.e. the inhibition of the recovery was drug concentration dependent. Using these results and the data presented in Fig. 1, we calculated (Eq. 3) the dependency of the relative fraction of the irreversible damage $K(t) = D_{eff}(t)/D_1$ on the duration of the recovery time of the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors of the cell recovery. The results are shown in Fig. 2. It can be noted that the untreated cells subjected to post-irradiation recovery showed an appreciable decrease in $K(t)$ whereas this effect became gradually worse as the chemical concentration increased. It appears that the inhibition of the recovery depends on the drug concentration and is almost complete with 20 mM of pyruvate and lactate and 20 μM of novobiocin and nalidixic acid. For instance, the limited values of $K(t)$, i.e. the values of the irreversible component $K = K(\infty)$, are equal to 0.60, 0.75, and 0.92 for the cells recovering from radiation damage without a drug and in the presence of 10 and 20 mM pyruvate, respectively.

Qualitatively similar results were obtained for other chemicals. Fig. 3 shows the calculated irreversible component of radiation damage, $K(\infty)$, in Chinese hamster V79 cells recovering from potentially lethal damage with and without various inhibitors of recovery. 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 the number of cells capable of recovery.

To clarify whether or not this decrease could explain the observable deceleration of the recovery rate, we estimated

Table 1. Radiobiological parameters of Chinese hamster cells recovery

Chemicals	Conditions of recovery	Irreversible component κ	Recovery constant β, hr^{-1}
Without chemicals	Without chemicals	0.60	0.15
Pyruvate	20 mM 1 h after irradiation	0.63	0.16
	10 mM immediately after irradiation	0.75	0.16
	20 mM immediately after irradiation	0.92	0.16
Novobiocin	20 μM 1 h after irradiation	0.68	0.14
	5 μM immediately after irradiation	0.82	0.14
	10 μM immediately after irradiation	0.90	0.14
	20 μM immediately after irradiation	0.98	–
Lactate	20 mM 1 h after irradiation	0.67	0.14
	10 mM immediately after irradiation	0.78	0.14
	20 mM immediately after irradiation	0.99	–
Nalidixic acid	20 μM 1 h after irradiation	0.68	0.15
	5 μM immediately after irradiation	0.74	0.15
	10 μM immediately after irradiation	0.82	0.15
	20 μM immediately after irradiation	0.94	–
3-aminobenzamide	20 mM 3 h after irradiation	0.68	0.15
	10 mM 1 h before +after irradiation	0.71	0.15
	20 mM 1 h before +after irradiation	0.95	–

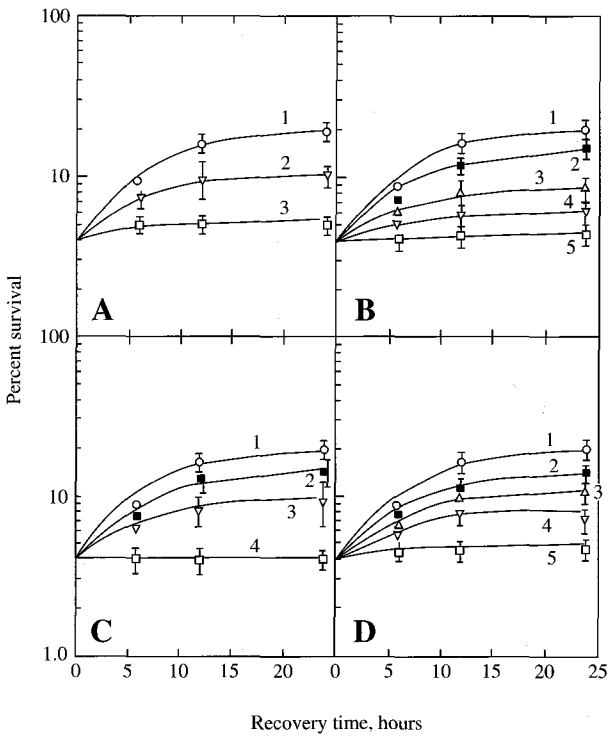

Fig. 5. Survival of Chinese hamster V79 cells in dependence of the duration of recovery from potentially lethal damage recovering after irradiation without chemicals (curves 1) and in the presence of chemical inhibitors of cell recovery. A, pyruvate: 10 and 20 mM immediately after irradiation (curves 2 and 3). B, novobiocin: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5). C, lactate: 20 mM 1 h after irradiation (curve 2), 10 and 20 mM immediately after irradiation (curves 3 and 4). D, nalidixic acid: 20 μM 1 h after irradiation (curve 2), 5, 10 and 20 μM immediately after irradiation (curves 3, 4 and 5). Experimental points were taken from (Keifer *et al.*, 1985a). Solid lines were calculated in accordance with Eq. 1 and parameters of the recovery estimated in this study.

the probability of recovery for various conditions of the recovery. The experimental data make it possible to calculate the function $A(t) = [D_{eff}(t) - D_{eff}(\infty)]/[D_1 - D_{eff}(\infty)]$ in the dependency of the recovery time of the Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors. The results are shown in Fig. 4. One can see that this function declines exponentially with the recovery time independently of whether or not the recovery took place without chemicals or with an increasing concentration of various drugs. By taking Eq. (5) and the results shown in Fig. 4, we calculated the recovery constant β for all the recovery conditions which had been tested. The total set of parameters describing the recovery of the Chinese hamster cells under different postirradiation conditions are summarized in Table 1. This Table also includes the parameters describing the recovery of the Chinese hamster cells in the presence of different concentrations of 3-aminobenzamide estimated on the basis of the experimental data published by others (Kumar *et al.* 1985b) but not presented here in Figures. It can be seen that in all the cases the recovery constant was independent of the recovery conditions ($\beta = 0.15 \pm 0.01$ $hour^{-1}$) whereas the irreversible component was gradually enhanced as the chemical concentration increased.

To illustrate the correctness of the approach used here,

Fig. 5 exhibits the survival of the Chinese hamster V79 cells in dependency of the duration of recovery from potentially lethal damage recovering after irradiation without chemicals and in the presence of some chemical inhibitors of the cell recovery. Experimental points were taken from (Kumar *et al.* 1985a). Solid lines were calculated in the following way. The effective dose $D_{eff}(t)$ was computed in accordance with Eq. 1 and the parameters of the recovery obtained in this study (Table 1). Then the corresponding survival $S(t)$ was estimated using the example shown in Fig. 1 by the arrow. One can see a close fit of the predicted curves to the experimental data. Thereby the applicability of the mathematical approach to describe the recovery from potentially lethal damage was demonstrated.

DISCUSSION

The aim of this study was to determine whether the inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster cells (Kumar *et al.* 1985a, b) might be causally related to the impairment of the recovery capacity itself or to the production of the irreversible damage, which cannot be repaired. It was shown that the basic effect of the chemicals tested at a concentration sufficient to inhibit recovery from potentially lethal damage appeared to be a reduction in the number of cells capable of recovery owing to the increase in the irreversibly damaged cells. The findings revealed that (i) the irreversible component of radiation damage was gradually enhanced as the chemicals concentration increased and (ii) the probability of recovery was independent of whether the process of recovery happened with or without chemicals sensitizing the radiation effect. It is not excluded that the first inference may be explained by a conversion by the drugs of radiation induced repairable damage so that the enzymes could then not deal with the lesions (Hill and Bellamy 1984; Streffer and Müller 1984). It would seem probable also that chemicals could interfere with the synthesis of the requisite enzymes (Boothman *et al.* 1989; Takahashi *et al.* 2000). The second inference would imply that the same portion of the repairable damage is eliminated for a unit time independently of the recovery conditions investigated. This result strongly suggests that the analysed chemicals don't damage the repair enzymes responsible for recovery. Hence, the observed inhibition of the recovery occurred without the damage of the recovery processing *per se* but due to the decrease in the number of damage which cell is capable of recovery. Similar results have been obtained for diploid yeast cells exposed to hyperthermia and ionizing radiation (Petin and Kim 2004) or hyperthermia and UV light (Kim *et al.* 2004). It follows that some general mechaniosms of radiosensitization or the synergistic effects may underlie the interaction of heat and some chemicals with ionizing radiation. The mechanism should imply the failure of a direct interference with the repair process itself and favor a role of hyperthermia and chemicals analysed in the decreasing rate and the extent of the repair by facilitating either the production of irreversible damage or an early radiation damage fixation before the recovery processes are over or occur.

Detailed analysis of the molecular mechanisms involved is beyond the main aim of this study. However, it can be noted that many mechanisms have been discussed by different authors (Mattern and Painter 1979; Purnell and Whish 1980; Cleaver 1982; Ben-Hur and Elkind 1984; Kumar *et al.* 1985a, b; Utsumi *et al.* 1990). Only as examples, the following possibilities can be mentioned. Because of the critical role of the DNA topoisomerases in the synthesis and the conformation of DNA, and well-known information that ionizing radiation inhibits replicative DNA synthesis, there is a possibility that inhibitors of these enzymes might influence the radiation lethality (Utsumi *et al.* 1990). It can be admitted that a decrease in a quantity of these enzymes wouldn't interfere with the probability of recovery but result in a greater portion of irreversible damage. A similar situation may be realized with insufficient energy metabolism. For instance, novobiocin and nalidixic acid have been shown to inhibit DNA, RNA, and protein synthesis in several mammalian cell lines (Mattern MR and Painter 1979; Kumar *et al.* 1985a; Utsumi *et al.* 1990), their activity may also be expressed through the interference with the function of topoisomerase II in an early stage of DNA repair (Cleaver 1982; Kumar *et al.* 1985a). It was presumed that the inhibition of recovery from potentially lethal damage by lactate and pyruvate may be due to severe metabolic changes such as a decrease in the intracellular ATP concentration (Kumar *et al.* 1985a). These authors postulated that the recovery inhibition might have occurred due to the raising of the lactate and pyruvate levels com-

plicating the repair of DNA damage. This view is strengthened by the data showing that specific inhibitors of poly (ADP-R) synthesis enhance the cell killing and inhibit the DNA strand break rejoining induced by ionizing radiation (Ben-Hur and Elkind 1984). 3-Aminobenzamide has been shown to be a putative specific inhibitor of poly (ADP-R) synthetase (Purnell and Whish 1980). The results obtained by Kumar *et al.* (Kumar *et al.* 1985b) favour a possible role of the chemical in preventing repair by facilitating an early damage fixation before repair can occur, simultaneously reducing the G_2-arrest. All these observations are consistent with the results obtained in this study.

In conclusion, the results of this paper provide opportunity for searching agents, selectively or simultaneously acting on the probability of recovery and the yield of irreversible radiation damage. The recognition that specific inhibitors of recovery may exist, such as an inhibitor of the recovery process itself and that resulting in the increased yield of irreversible damage, would provide both a possibility to analyze the mechanism of a 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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