Quantitative estimation of recovery parameters after combined action of ionizing radiation and chemical agents

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ABSTRACT

Background: Treatment of ionizing radiation combined with chemical agents can enhance the inactivation of cells. The aim of this study was to determine the parameters involved in the inhibition of cell recovery. ***Materials and Methods***: A mathematical model describing the process of recovery as a decrease in the effective radiation dose was used. The model includes two parameters, recovery constant and irreversible component. Both parameters were estimated quantitatively by using experimental survival and recovery data reported by others. ***Results***: The inhibition of cell recovery might be done 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 could take place at the same time. Another mechanism was the higher probability to produce primary radiation damage without changing the recovery process. ***Conclusions***: The results indicate the opportunity to search agents, selectively or simultaneously acting on the probability of recovery and the yield of irreversible radiation damage. The results obtained may have a practical use, rather than being concerned only with theoretical position.

Keywords: *Radiotherapy, Chemotherapy, cell recovery, mathematical model*

INTRODUCTION

The combined action of ionizing radiation with various chemical agents is often used to enhance the inactivation effect of tumor cells in cancer treatment. The enhancing effect seems to be accountable by direct chemical toxicity, cell recovery inhibition or the higher probability to produce primary radiation damage without changing the recovery process [1–4]. Repair of DNA, as reflected in rejoining of strand breaks, may be of relevance to the cell recovery from sub lethal and potentially lethal damage [2, 5]. Preferential impairment of DNA repair in malignant cells would be of great relevance in improving cancer treatment. The inhibition of cell recovery [2, 3, 6, 7] and DNA single and double strand breaks repair [8–11] by chemicals is expressed both as a deceleration of recovery rate and a lesser extent of recovery. It is obvious that the inhibition of cell recovery may be caused by the following reasons: (i) the damage or impairment of the recovery process itself, (ii) the increase in the portion of irreversible damage, and (iii) both these reasons may took place simultaneously.

There are only a limited number of publications concerning these problems. In our earlier publications it was shown that the inhibition of the recovery from potentially lethal damage in yeast cells exposed to hyperthermia and ionizing radiation [12] or hyperthermia and UV light [13] was realized only through the enhanced yield of the irreversible damage whereas the recovery capacity itself was not damaged or impaired. Similar data were obtained for cultured mammalian cells [14]. It would be of interest to find out whether or not this conclusion can be justified for various mammalian cells and chemicals used in combination with ionizing radiation. In this paper, a quantitative approach describing cell recovery from potentially lethal damage as a decrease in the effective dose will be used to estimate separately the probability of recovery per time unit and the fraction of irreversible damage which cannot be repaired at all after combined action of ionizing radiation with different chemical agents.

MATERIALS AND METHODS

Experimental procedures

Experimental data published by others [2–4, 6, 7, 15] have been used in this study. The details of cell culture, irradiation, chemicals used and recovery can be found in these papers. 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 of V79 Chinese hamster cells 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 [2, 3].

The inhibition of the repair of potentially lethal damage by novobiocin at nontoxic concentrations was obtained with V79 Chinese hamster cells; clone V79-B310H [7]. After the overnight growth of initially single cells to yield groups of cells, cells were X-irradiated when they were in active growth. Following irradiation, growth medium was returned to the dishes with or without novobiocin. If the inhibitor had been added, the medium was removed at the appropriate time, the dishes were rinsed, and fresh medium was returned to them. After incubation for 7–10 days at 37ºC for colony formation, colonies were stained with methylene blue, dried, and counted for the determination of surviving fraction.

Differential response of human and rodent cell lines to chemical inhibition of the repair of potentially lethal damage was investigated by Little *et al.* [6]. They have examined the effects of several classes of metabolic inhibitors on the repair of potentially lethal damage. The following cell lines were used in this investigation: human squamous cell carcinoma SCC-61, human diploid fibroblast cell strain AG1522, Chinese Hamster Ovary (CHO) cells, and mouse C3H 10T1/2 cells. Cells were incubated with the various inhibitors (3-aminobenzamide, hydroxyurea, 9-β-D-arabinofuranosyladenine–ara-A, 1-β-D-arabinofuranosylcytosine – ara-C) and their concentrations beginning 18–24 h prior to X-irradiation. Survival was determined by a standard colony forming ability.

Effects of 5'-iododeoxyuridine (IdU) on the repair of radiation induced potentially lethal damage were considered by Wang and Iliakis [15]. Experiments were carried out with CHO cells, strain 10B. Before X-irradiation cells were allowed to grow to a plateau-phase for 5 days. IdU was supplied to the culture medium at the time of culture preparation, in the appropriate amounts Cultures were resupplied with IdU in two days. The rationale for this refeeding protocol has been outlined [16]. Repair of potentially lethal damage was measured by delayed plating plateau-phase cells. To assay for colony forming ability, cells were enzymatically detached from the culture dishes, counted and plated at the appropriate numbers to generate 25–400 colonies per dish.

Estimation of the recovery parameters

The method for an estimation of the yeast cell recovery parameters has already been described

in our publication [12]. The main features of this approach can be briefly summarized as follows. During recovery, much of the primary radiation damage is eliminated, resulting is increased cell survival. This can be described as 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 [17–20] that the decrease in the $D_{eff}(t)$ with the recovery time t could be fitted by an equation of the form

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

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. Irreversible component K may be expressed as a fraction of the initial irradiation dose by

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

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

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

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 (equation 2). The expression

$$A(t) = e^{-\beta\cdot t} \qquad (4)$$

reflects the relative part of the repairable damage that has not been repaired yet after t hours of reparation.

Combining equations [1–3], one can deduce

$$\beta = -\frac{1}{t}\cdot\left[\ln\frac{D_{eff}(t) - D_{eff}(plat)}{D_1(t) - D_{eff}(plat)}\right] \qquad (5)$$

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 (equation 3) and to evaluate the irreversible component K (equation 2). Having calculated the fraction of repairable damage $A(t)$ (equation 4) in the dependence of repair time t, one can estimate the recovery constant β (equation 5).

The described approach was used previously to fit the recovery kinetics of various biological organisms irradiated with ionizing radiation alone [17–20] or combined with hyperthermia [12, 13]. In the last papers, hyperthermia affected the irreversible component of potentially lethal damage, but did not influence the rate of recovery. This mathematical approach was seldom used for combined treatments of chemicals and ionizing radiation [21]. In this paper we present the results of applying this methodology to the extensive results of the combined action of ionizing radiation and chemical radiosensitizers.

RESULTS

Figure 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. Both these curves were obtained by the averaging of six dose-effect and four time-effect curves published by other authors [2, 3]. Arrows indicate the initial dose D_1 as well as examples of the effective doses $D_{eff}(t)$ and $D_{eff}(plat)$ estimation. In these papers [2, 3] 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 figure 1, we calculated the dependence of the relative fraction of unrepaired damage $K(t)$ on the duration of recovery time (equation 3) for the Chinese hamster V79 cells recovering after

irradiation without chemicals and in the presence of various chemical inhibitors of cell recovery. The results are shown in figure 2A, B, and C. One can see that 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 appears 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 mM 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 10 and 20 mM pyruvate, respectively (figure 2A). Qualitatively similar results were obtained for other chemicals (figure 2B, C). 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.

In this regard, it is of interest to clarify whether or not the observable deceleration of the recovery rate could completely explained by the increase in the irreversible component, we estimated the probability of recovery for various conditions of recovery. The experimental data [2, 3] make it possible to calculate the function *A(t)* (equation 4) in the dependence of the recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals and in the presence of various chemical inhibitors. The results are shown in figure 2D, E, and F. One can see that this function declines exponentially with the recovery time independently of whether the recovery took place without chemicals or with the increasing concentration of various drugs. Using equation 5 and the results shown in figure 2D, E, and F, we calculated the recovery constant β for all recovery conditions. It turned out that in all cases the recovery constant was independent of recovery conditions (β = 0.14 ± 0.01 hr^{-1}) whereas the irreversible component was gradually enhanced as the chemical concentration increased. This value of β means that 14 percent of recoverable damage is recovered every hour. It is worth to note that a part of these results has been published preliminary [21]. Particularly, it has been shown that the recovery constant after combined action of ionizing radiation with nalidixic acid and 3-aminobenzamide was equal to 0.15 hr^{-1} independently of irradiation and recovery condition, while the irreversible component *K*

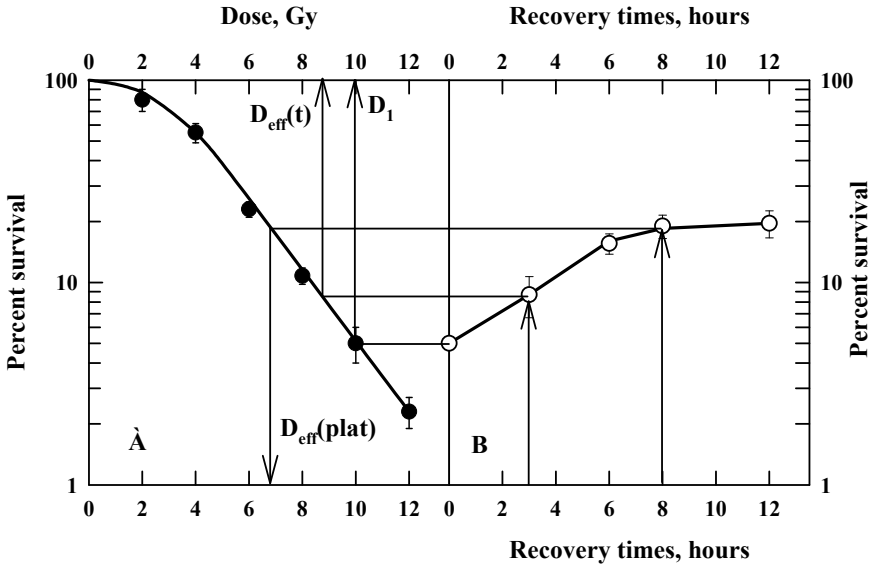

Figure 1. Survival of Chinese hamster V79 cells in the dependence of X-ray dose (A) and the duration of recovery from potentially lethal damage (B). Cells were X-irradiated and recovered without chemicals. Arrows indicate examples of the initial dose D_1 as well as the effective doses $D_{eff}(t)$ and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

ranges from 0,60 for X-irradiation alone to 0.90–0.95 for the highest chemical concentration used in experiments. Thus, it can be inferred from the above findings that (i) the irreversible component of radiation damage was gradually enhanced as the chemicals concentration increased and (ii) the recovery constant was independent of whether the process of recovery happened with or without chemicals sensitizing the radiation effect.

Very similar results were obtained with V79 Chinese hamster cells, clone V79-B310H, Chinese hamster ovary cells (CHO), and SCC-61 human tumor cells. The inhibition of the increase in the survival of X-irradiated (1250 cGy) V79 cells by the topoisomerase II inhibitor novobiocin was published by Utsumi *et al.* [7]. Novobiocin was used at concentrations that do not interfere with cell proliferation. The effect of incubation of X-irradiated (900 cGy) CHO cells with 5 mM 3-aminobenzamide on potentially lethal damage was published by Little *et al.* [6]. These authors also published data for SCC-61 human tumor cells recovering after irradiation without chemicals and in the presence of chemical inhibitors of cell recovery: 5 mM 3-aminobenzamide, 1 mM ara-C, 5 мМ hydroxyurea, and 10 mM 3-aminobenzamide. Using the eauations developed above, the relative fraction of unrepaired damage *K(t)* (equation 3) and the relative part of the repairable damage *A(t)* (equation 4) were assessed on the duration of recovery time (figure 3). It is evident from these data that the irreversible component *K* is slightly increased from 0.76 to 0.88 for V79 cells and from 0.83 to 0.9 for CHO cells after irradiation without and with repair inhibitors – novobiocin and 3-aminibenzamid,

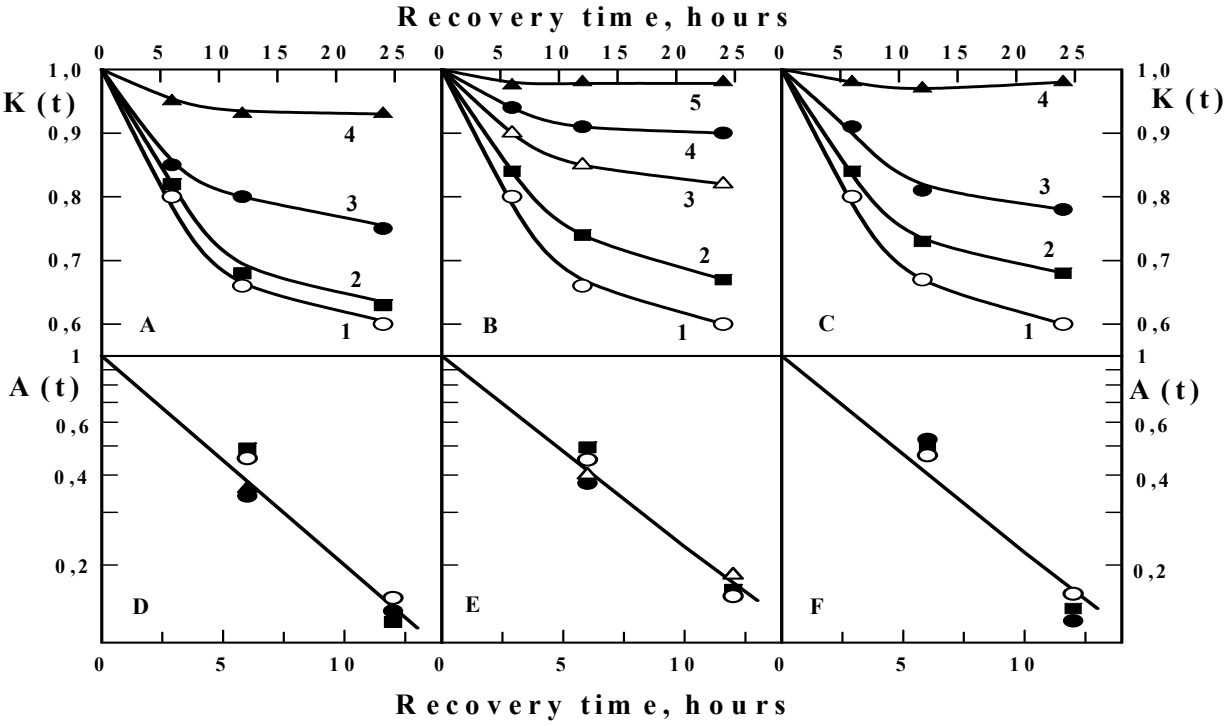

Figure 2. The dependence of the relative fraction of unrepaired damage *K(t)* (A, B, and C) and the relative fraction if repairable damage *A(t)* (D, E, and F) on the duration of recovery time of Chinese hamster V79 cells recovering after irradiation without chemicals (curves 1, open circles) and in the presence of chemical inhibitors of cell recovery. A, D – pyruvate: 20 mM 1h after irradiation (curve 2, closed squares), 10 mM (curve 3, closed circles) and 20 mM (curve 4, closed triangles) immediately after irradiation B, E – novobiocin: 20 mM 1h after irradiation (curve 2, closed squares), 5, 10 and 20 mM immediately after irradiation (curve 3, open triangles, curve 4, closed circles, and curve 5, closed triangles respectively). C, F – lactate: 20 mM 1h after irradiation (curve 2, closed squares), 10 and 20 mM immediately after irradiation (curve 3, closed circles, and curve 4, closed triangles, resectively). Here and in other Figures *K(t)* curves were fitted to the data points by eye and for *A(t)* by the least-squares method.

respectively. The similar results were obtained for SCC-61 human tumor cells. the irreversible component K was estimated to be of 0.54, 0.61, 0.68, and 0.82 for ionizing radiation applied alone and combined with ara-C, hydroxyurea, and 3-aminobenzamide respectively. It means that all these chemicals resulted to an increased yield of irreversible damage in comparison with cell exposure and recovery without chemical inhibitors of recovery.

Having used the data depicted in figure 3D, E, and F, we estimated the recovery constant β (equation 5). The values of this parameter were calculated to be independent on the conditions of recovery and equal to 0.03 and 0.01 min^{-1} for V79 and CHO cells. For SCC-61 human tumor cells recovering after irradiation without chemicals β = 0.004 min^{-1}. The corresponding values of the recovery constant β were estimated to be of 0.005, 0.004, and 0.003 min^{-1} in the presence of chemical ara-C, hydroxyurea, and 10 мМ 3-aminobenzamide, respectively. It is worth to note that the authors [6] analyzed recovery kinetics for SCC-61 human tumor cells rather rarely so we estimated the recovery constant only due to the single experimental point (figure 3D). So it is not excluded that the recovery constant variations obtained in our calculations are simple statistical spread (β = 0.004 ± 0.0005 min^{-1}). Hence, the observations described in figures 2 and 3 support the conclusion that all chemicals investigated doesn't influence the repair process itself and chemical inhibition of the recovery of potentially lethal damage can be completely explained by the increase in the formation of irreversible damage The major point to be inferred from data presented is that the basic effect of the chemicals tested at a

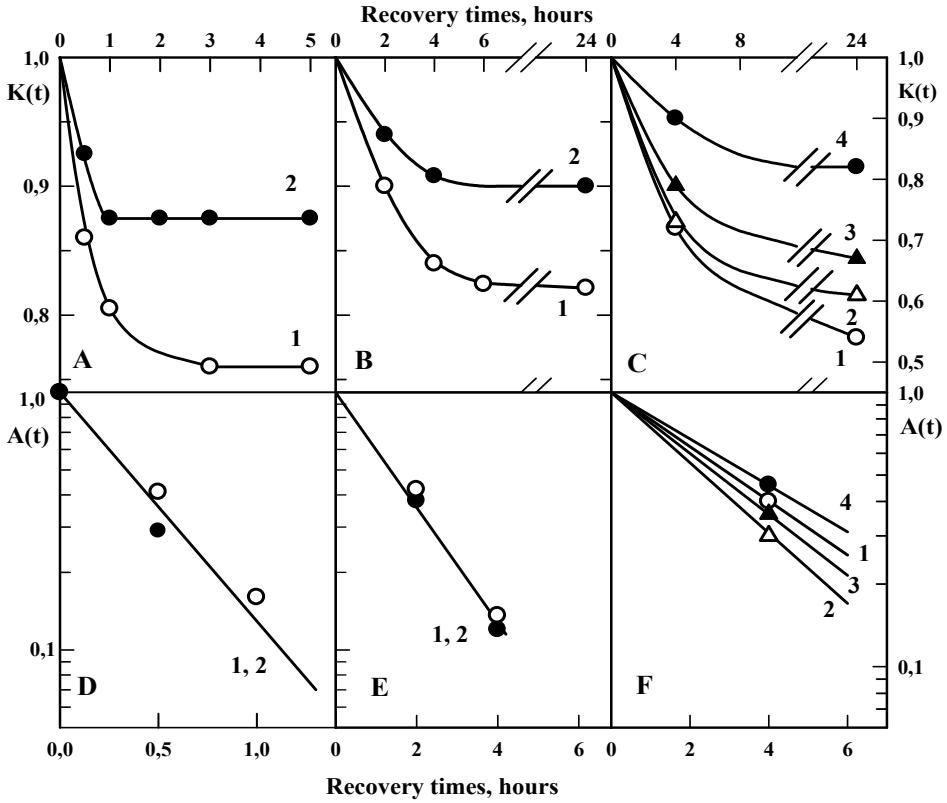

Figure 3. The dependence of the relative fraction of unrepaired damage *K(t)* (A, B, C) and the relative fraction if repairable damage *A(t)* (D, E, F) on the duration of recovery time of Chinese hamster V79-B310H cells (A, D), CHO cells (B, E) and SCC-61 human tumor cells (C, F) recovering after irradiation without chemicals (curves 1, open circles) and in the presence of various chemical inhibitors of cell recovery. A, D – novobiocin in nontoxic concentration (curve 2, closed circles). B, E – 5 mM 3-aminobenzamide (curve 2, closed circles). C, F – 1 mM ara-C (curves 2), 5 mM hydroxyurea (curves 3), and 10 mM 3-aminobenzamide (curves 4).

concentration sufficient to inhibit recovery from potentially lethal damage appears to be the reduction in the number of cells capable to recover owing to the increase in the irreversibly damaged cells. It seems plausible that the enhanced yield of irreversible damage may be formed due to the synergistic interaction of damages produced by ionizing radiation and chemicals. This suggestion put forward in our previous papers [12, 22].

It would be of interest to search for chemical agents increasing cell radiosensitivity by inhibition of cell recovery both by the increase in the irreversible component and due to the decrease in the recovery constant. A typical example is human diploid fibroblast cells (strain AG1522) exposed to ionizing radiation alone and in the presence of 5 mM 3-aminobenzamide, 0.12 mM ara-A, and 5 mM hydroxyurea. The results were published by Little *et al.* [6]. These data also enable assessment the relative fraction of unrepaired damage *K(t)* (equation 3) and the relative part of the repairable damage *A(t)* (equation 4) in the dependence of recovery time (figure 4A, C). On this basis we calculated the dependence of irreversible component *K*, i.e. the lowest value of *K(t)*, and the recovery constant β (equation 5) on the condition of irradiation and recovery. Here again we observed an increase of *K* from 0.5 for exposure with ionizing radiation alone to 0.59–0.61 for all chemical agents tested. The recovery constant β was equal to 0.005 min^{-1} after ionizing radiation applied alone or combined with 3-aminobenzamide and ara-A, while hydroxyurea resulted in a decrease of this parameter to 0.0036 min^{-1}. It means that hydroxyurea had an influence to the process of recovery itself.

The results presented in figure 4B, D provide an additional confirmation of this conclusion.

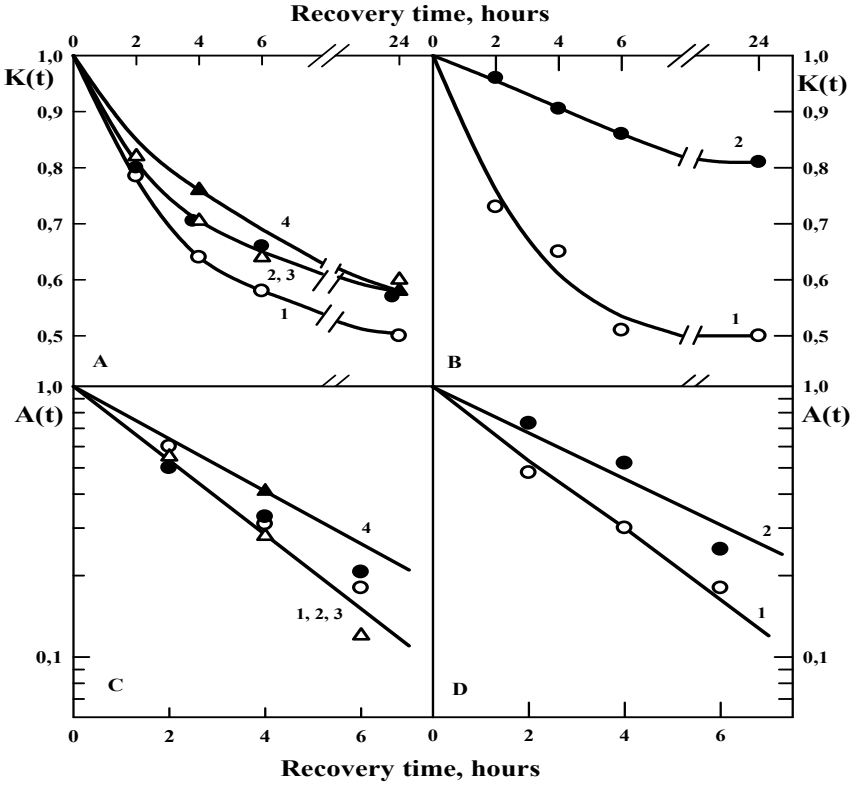

Figure 4. The dependence of the relative fraction of unrepaired damage *K(t)* (A, B) and the relative fraction of repairable damage *A (t)* (C, D) on the duration of recovery time of human diploid fibroblast cell strain AG1522 (A, C) and mouse cells line C3H10T1/2 (B, D), recovering after irradiation without chemicals (curves 1, open circles) and in the presence of various chemical inhibitors of cell recovery. A, C – 5 mM 3-aminobenzamide (curves 2, closed circles), 0.12 mM ara-A (curves 3, open triangles), 5 mM hydroxyurea (curves 4, closed triangles). B, D – 5 mM 3-aminobenzamide (curves 2).

Original data for our calculation have been published by others [6]. Taking these data into account, we estimated (as was already mentioned) the dependence of the relative fraction of unrepaired damage *K(t)* and the relative fraction if repairable damage *A(t)* on the duration of recovery time of mouse cells, line C3H10T1/2 recovering after irradiation without chemicals and in the presence of 5 mM 3-aminobenzamide. It is evident that both the irreversible component *K* and the recovery constant β for mouse cells depend on the condition of irradiation and recovery. Here again the irreversible component *K* ranges from 0.5 for exposure with ionizing radiation alone to 0.81 for postirradiation recovery with 5 mM 3-aminobenzamide. The recovery constant β was calculated to be of 0.005 min^{-1} after ionizing radiation applied alone while the combined action of ionizing radiation and 3-aminobenzamide resulted in a considerable decrease in this value – 0,003 min^{-1}. Hence, the results presented in figure 4 reveal that some inhibitors can simultaneously act both through the production of a greater part of irreversible damage and through a certain disturbance of cell repair.

It would be of interest to consider an example of chemical compounds acting through the enhanced probability to produce the primary radiation action without influence both on the recovery constant and irreversible component of radiation damage. Halogen pyrimidines are used as radiosensitizers in the treatment of poorly radioresponsive tumors [23]. The effect of 5'-iododeoxyuridne (IdU) incorporated into DNA on radiation sensitivity and cellular repair capability was published [15] for plateau-phase Chinese hamster ovary cells (strain 10B) exposed to X-rays. It was of interest to apply our methodology discussed here to estimate differentially the influence of this compound on the irreversible component and the recovery constant. Using the survival and recovery curves reconstructed and averaged on the basis of the results published by others [15, 16, 23], we calculated the relative fractions of unrepaired *K(t)* and repairable *A(t)* radiation damage in the dependence of the recovery time. The results are presented in figure 5. It is obvious that the irreversible component $K = 0.68 \pm 0.02$ independently on the condition of irradiation, i.e. whether it was occurred without or with various concentration of IdU. Having used the data depicted in figure 5B, we estimated the recovery constant β (equation 5). For cells irradiated and recovered without IdU $\beta = 0.007$ min^{-1}. This parameter was slightly changed for cells grown for 5 days in the presence of 2 μM and 8 μM IdU. The corresponding values were estimated to be of 0.006 and 0.01 min^{-1}. It indicates that in comparison with control cells the recovery constant was only slightly

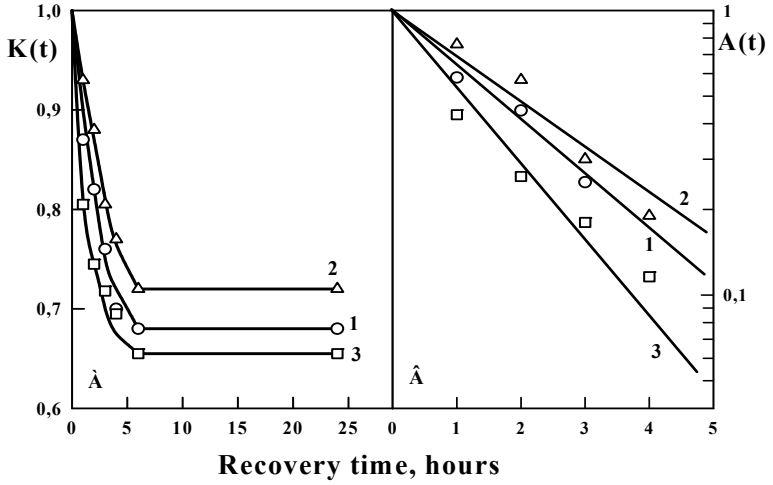

Figure 5. The dependence of the relative fraction of unrepaired damage *K(t)* (A) and the relative fraction if repairable damage *A (t)* (B) on the duration of recovery time of Chinese hamster cells, strain CHO 10B irradiated and recovered without IdU (curves 1) and for cells grown for 5 days before irradiation in the presence of 2 and 8 μM IdU (curves 2 and 3, respectively).

decreased for 2 μM IdU and even increased for 8 μM IdU. It is not excluded that these changes may reflect the possible variability of experimental data around the control value especially as the results were published from one experiment [15]. At the same time the incorporation of IdU into DNA resulted in the considerable increase in cell radiosensitivity. The dose modifying factors estimated by us as the ratio of the doses at 0.2 per cent survival in the absence and presence of the drug on the basis of the published results [15] were 1.4 and 2.4 for 2 and 8 μM IdU, respectively. Thus, it can be concluded that the considerable effect of 5'-iododeoxyuridne incorporated into DNA on radiation sensitivity of CHO cells might realized not through the increase in the irreversible component of radiation damage or through the decrease in the recovery constant. It means that the increase in cell radiosensitivity may happen through the higher probability to produce the primary radiation damage.

DISCUSSION

The main points which emerge from the findings pertinent to the present work can be summarized as follows. The increase in cell by chemicals may be causally related to the following reasons: (i) the impairment of the recovery capacity itself, (ii) the production of irreversible damage, which cannot be repaired at all, (iii) the higher probability to produce primary radiation damage, (iv) all or some of these issues may occur simultaneously. The aim of this study was to determine which of these points are involved in the inhibition of cell recovery. To distinguish these possibilities, a mathematical model describing the process of recovery as a decrease in the effective radiation dose was used. The model was often used previously to fit the recovery kinetics of various biological objects irradiated with ionizing radiation alone [17–20] and was seldom applied for combined treatments of chemicals and ionizing radiation [21]. This model includes two parameters – the probability of cell recovery per unit time (recovery constant) and the irreversible component, i.e. the fraction of damage which cannot be repaired at all. Both parameters can be estimated quantitatively basing on experimental survival and recovery data and allow to judge about the contribution of various processes which interfere in cell recovery process. The model was applied to experimental data published by other authors [2, 3, 6, 7, 15, 16]. It is of important to stress that the authors themselves didn't determine quantitatively the basic parameters of cell recovery. It was shown that all possibilities mentioned above could be realized for various cells and the nature of chemical compounds.

The results of this study reveal that the basic effect of the chemicals tested at a concentration sufficient to inhibit recovery from potentially lethal damage appears to be the reduction in the number of cells capable to recover owing to the increase in the irreversibly damaged cells. This effect may be interpreted as being due to the conversion by drugs of radiation induced repairable damage so that enzymes could then not deal with the lesions [1, 24]. It would seem probable also that chemicals could interfere with the synthesis of requisite enzymes [9, 11]. The independence of the recovery constant on the presence of chemical inhibitors during irradiation and/or postirradiation recovery period, observed in the most cases, would imply that the same portion of repairable damage is eliminated for unit time independently of the recovery conditions investigated. This result strongly suggests that the most analysed chemicals don't damage repair enzymes responsible for recovery. Similar results have been obtained for diploid yeast cells exposed to hyperthermia and ionizing radiation [12] or hyperthermia and UV light [13]. It follows that some general mechanism of radiosensitization or synergistic effects may underlie the interaction of heat and some chemicals with ionizing radiation. The mechanism should imply the failure of direct interference with the repair process itself and favour a role of hyperthermia and chemicals analyzed in the decreasing rate and extent of

repair by facilitating either the production of irreversible damage or early radiation damage fixation before recovery processes would be over or occur.

Some examples presented here indicate that the recovery constant can be also decreased due to the influence of chemical inhibitors to the recovery process itself. It was expressed in the decreasing of the probability of cell recovery per unit time. Hence, this observation supports the conclusion that chemical inhibition may cause the enhanced cell killing due to both interference with repair of potentially lethal damage and enhancement of expression of irreversible radiation damage.

A suitable suggestion concerning the effect of 5'-iododeoxyuridne incorporated into DNA on radiation sensitivity and cellular repair capability of CHO cells could be put forward. As it turned out, such a DNA modification resulted in a great increase of cell radiosensitivity without influence both on the recovery constant and irreversible component of radiation damage. It means that the effect of 5'-iododeoxyuridne might be expressed through the enhanced yield of primary radiation damage rather than due to the formation of irreversible damage and/or its influence on recovery process itself.

Detailed analysis of molecular mechanisms involved is beyond the main aim of this study. However, it can be noted that various mechanisms have been discussed by many authors. Only as examples, the following possibilities can be mentioned. Because of critical role of the DNA topoisomerases in the synthesis and 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 radiation lethality [7]. 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. 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 [2, 7, 25], their activity may be also expressed through the interfere with the function of topoisomerase II in an early stage of DNA repair [2, 26]. 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 [2]. These authors postulated that the recovery inhibition may be occurred due to the raising of lactate and pyruvate levels complicating the repair of DNA damage. This view is strengthened by data showing that specific inhibitors of poly (ADP-ribose) synthesis enhance cell killing and inhibit DNA strand break rejoining induced by ionizing radiation [27]. 3-Aminobenzamide has been shown to be a putative specific inhibitor of poly (ADP-R) synthetase [28]. The results obtained by Kumar *et al.* [3] favour a possible role of the chemical in preventing repair by facilitating early damage fixation before repair can occur, simultaneously reducing G2-arrest. All these observations are consistent with the results obtained in this study.

In conclusion, 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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