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Combined Action of Ionizing Radiation and Chemical Inhibitors on Cell Recovery : Quantitative Estimation

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Abstract

The purpose of this work was to determine whether the synergistic interaction of ionizing radiation and some chemicals in Chinese hamster cells was related with inhibition of the liquid holding recovery (LHR) or with the production of irreversible damages which could not be repaired. Using the proposed mathematical model describing the process of the LHR and experimental data published by others it was demonstrated that the recovery constant, i.e. the probability of the recovery per time unit, was independent while the irreversible component was increased with drug concentration. It is concluded on this basis that the LHR process itself is not damaged after the combined action of ionizing radiation and chemical inhibitors of recovery, and that the mechanism of their action may be related with the enhanced yield of irreversible damages.

Key Words : recovery, synergism, mathematical model, the probability of recovery, irreversible component

1. Introduction

Many 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[1]. It is assumed that drug radiosensitization may be displayed by an inhibition of repair on the cellular level including recovery from potentially lethal

radiation damage, which was demonstrated by holding cells under suboptimal conditions between radiation exposure and plating[2]. This type of recovery is known as the liquid holding recovery (LHR) and may play a role in the treatment of tumours with ionizing radiation[3]. Repair inhibition is usually described as retarded repair rates. The retardation of the recovery rate after combined action of ionizing radiation and chemicals may be related to the following reasons: (i) the damage or inhibition of the recovery process on its own, (ii) the increase in the portion of irreversible damage, or (iii) both of these issues. It is of interest to estimate quantitatively the role of each of these reasons. To realize this purpose, a mathematical model of the LHR will be proposed and the data obtained by others[4] for Chinese hamster cells will be used to determine the influence of various drugs (pyruvate, lactate, nalidixic acid and novobiocin) on the parameters describing the recovery process. These chemicals were considered as inhibitors of recovery[4-6].

2. Mathematical model of the LHR

During the LHR process the survival of irradiated cells is increased, i.e. a decrease in the effectiveness of the initial dose D_1 takes place. Then for any recovery time t a certain survival $S(t)$ and the corresponding effective dose $D_{eff}(t)$ can be indicated. An example is shown in Figure 1 indicated by an arrow. The ratio

$$K(t) = D_{eff}(t) / D_1 \qquad (1)$$

shows the relative part of radiation dose or radiation damage which has not been repaired for t hours of recovery. If t is sufficiently large (for mammalian cells it is about 24 hours), the recovery curves reach a plateau when the capability of cells to recover is saturated or exhausted. For this moment, we can write

$$K = K(plateau) = D_{eff}(plateau) / D_1. \quad (2)$$

In this expression, $D_{eff}(plateau)$ - the effective dose corresponding the plateau of the recovery curve. The ratio $D_{eff}(plateau) / D_1$ can be considered as an irreversible component of radiation damage that can not be repaired. It was demonstrated [1,7] that the decrease in the effective dose $D_{eff}(t)$ with the recovery time t can be presented as follows

$$D_{eff}(t) = D_1[K + (1 - K)\, e^{-\beta t}], \quad (3)$$

where D_1 is the initial radiation dose, K is an irreversible component of radiation damage expressed in fractions of the given dose and described by Eqn. 2, e is the basis of the natural logarithm, and β is the recovery constant characterizing the probability of recovery of radiation damage per time unit. In other words, the recovery constant is equal to a fraction of radiation damage recovering per time unit. It follows from Eqn. (3) that to describe quantitatively the process of the LHR one needs to know two parameters – the irreversible component K and the recovery constant β. Taking into account Eqns. (2) and (3), it can be easily shown that

$$e^{-\beta t} = [D_{eff}(t) - D_{eff}(plateau)] / [D_1 - D_{eff}(plateau)]. \quad (4)$$

Designating the right part of this Equation as $A(t)$, we have

$$\beta = -[lnA(t)] / t. \quad (5)$$

Thus, knowing the survival and recovery curves after ionizing radiation applied alone or combined with various chemicals, one can calculate $D_{eff}(t)$ and $D_{eff}(plateau)$. It allows using Equation (1) to draw $K(t)$ in dependence of recovery

time t and using Equation (2) to calculate the irreversible component K. Having calculated the dependence of $lnA(t)$ on recovery time t, one can evaluate, using Equation (5), the recovery constant β.

3. Estimation of parameters describing the LHR

Fig. 1A shows survival curves of Chinese hamster V79 cells irradiated with X-rays (300 kV, dose rate being 1.25 Gy/min) alone (curve 1) and combined with treatment for 24 h after irradiation with 10 mM (curve 2) and 20 mM (curve 3) of sodium pyruvate. It is apparent there is a decrease in the shoulder width and in the final slope of the curves. This means the pyruvate acts as a sensitizing agent enhancing the effect of the ionizing radiation. Fig. 1B includes LHR recovery kinetics of irradiated cells. The recovery process occurred without chemicals (curve 1) and in the presence of 10 and 20 mM pyruvate (curves 2 and 3, respectively). One can see the survival increase due to recovery observed in the control was gradually reduced as the chemical concentration increased.

Using the results presented in Fig. 1 and Eqn. (1), we calculated the dependence of the relative fraction of irreversible radiation dose $K(t)$ on the recovery time. The results are shown in Fig. 2. These data demonstrate that the limited values of $K(t)$, i.e. the values of irreversible component $K = K(plateau)$, are equal to 0.60, 0.75, and 0.92 for cells recovering from radiation damage without chemicals and in the presence of 10 and 20 mM pyruvate, respectively. It appeared that the irreversible component of radiation damage is gradually increased with the concentration of chemical used.

The experimental data presented make it possible to calculate the function $A(t) = [D_{eff}(t) - D_{eff}(plateau)] / [D_1 - D_{eff}(plateau)]$. The results are shown in Figure 3. One can see that this function is decreased exponentially with the recovery time and does not depend on whether the recovery took place without chemicals (open circle) or with 10 mM (closed circles) or 20 mM (closed triangles) of sodium pyruvate. Taking used Equation (5) and the results shown in Fig 3, we obtained that the recovery constant β = 0.16 hour^{-1}. Therefore we can conclude that the fraction of radiation damage recovered per hour is identical for the cell recovery with or without pyruvate.

Using the results published by others [4] we calculated the irreversible component and the recovery constant for three other chemicals inhibiting the recovery process (lactate, nalidixic acid and novobiocin). The total results are summarized in Table 1, where radiobiological parameters describing the recovery of Chinese hamster cells under different postirradiation conditions are presented. It can be seen that in all cases the recovery constant was independent of recovery conditions (β = 0.16 hour^{-1}) while the irreversible component, i.e. the fraction of cells incapable of recovery, was gradually enhanced as the chemical concentration increased.

4. Conclusions

A mathematical approach was suggested to estimate quantitatively both the irreversible component of radiation damage expressed as the fraction of initial radiation dose from which cells are incapable of recovering and the recovery constant which define the probability of recovering per time unit. This approach was applied to

experimental results [4] on the combined action of ionizing radiation and chemicals inhibiting the recovery process in Chinese hamster cells.

It was found that the irreversible component of radiation damage was gradually enhanced as the chemical concentration increased while the recovery constant was independent on whether the process of recovery happened with or without chemicals sensitizing the radiation effect. It follows that the same part of radiation damage which can be repaired is eliminated for time unit independently of recovery conditions investigated. It was concluded on this basis that the deceleration of recovery rate under the combined action of ionizing radiation and chemicals takes place not because of the inhibition or damage of the recovery process on its own but due to the diminution of the amount of damage from which a cell is capable of recovering. This situation resembles the radioactive decay rate which is fluently decreased only because of the reduction in the number of nuclei capable to decay.

Thus, the mechanism of radiosensitization by chemical inhibitors of recovery in Chinese hamster cells is not related with real inhibition of the recovery process itself and may be caused by the enhanced yield of irreversible damage which was formed, for example, due to synergistic interaction [8,9] of damage produced by ionizing radiation and chemicals. The mathematical approach described here may be helpful to search chemicals selectively acting on the probability of recovery and the yield of irreversible radiation damage. It can be expected that their combination could be a perspective aid in cancer treatment.

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References

1. L.A. Dethlefsen and W.C. Dewey, Eds., Cancer Therapy by Hyperthermia, Drugs and Radiation, p. 550, Natl. Cancer Inst., Bethesda (1982).
2. V.I. Korogodin, Problems of Postradiation Recovery (in Russian), p. 391, Atomizdat, Moscow (1966).
3. R.R. Weichselbaum and J.B. Little, "Potential lethal damage recovery, prospects in radiotherapy," *Int. J. Radiat. Oncol. Biol. Phys*., **9**, 91 (1982).
4. A. Kumar, J. Kiefer, E. Schneider, and N.E.A. Crompton, "Inhibition of recovery from potentially lethal damage by chemicals in Chinese hamster V79 A cells," *Radiat. Environ. Biophys*., **24**, 89 (1985).
5. S.R.R. Musk and G.G. Steel, "The inhibition of cellular recovery in human tumour cells by inhibitors of topoisomerase," *Brit. J. Cancer*, **62**, 364 (1990).
6. H. Utsumi, M.L. Shibuya, and M.M. Elkind, "Novobiocin inhibits the repair of potentially lethal damage but not the repair of sublethal damage," *Radiat. Res*., **123**, 55 (1990).
7. H.O. Davidson, Biological Effects of Whole Body Gamma Radiation on Human Beings, p. 101, Johns Hopkins University Press, Baltimore (1957).

8. V.G. Petin and V.P. Komarov, "Mathematical description of synergistic interaction of hyperthermia and ionizing radiation," *Mathem. Biosci.*, **146**, 115 (1997).

9. V.G. Petin, J.K. Kim, G.P. Zhurakovskaya, and A.V. Rassokhina, "Mathematical description of synergistic interaction of UV light and hyperthermia," *J. Photochem. Photobiol. B: Biology*, **55**, 74 (2000).

Table 1. Radiobiological parameters of Chinese hamster cells recovery

Chemicals	Conditions of recovery	Irreversible component K	Recovery constant β, hr^{-1}
Without chemicals	Without chemicals	0.60	0.16
Pyruvate	10 mM immediately after irradiation	0.75	0.16
	20 mM immediately after irradiation	0.92	0.16
Lactate	10 mM immediately after irradiation	0.78	0.16
	20 mM immediately after irradiation	0.98	-
Nalidixic acid	5 μM immediately after irradiation	0.74	0.16
	10 μM immediately after irradiation	0.82	0.16
	20 μM immediately after irradiation	0.94	-
Novobiocin	5 μM immediately after irradiation	0.82	0.16
	10 μM immediately after irradiation	0.90	0.16
	20 μM immediately after irradiation	0.98	-

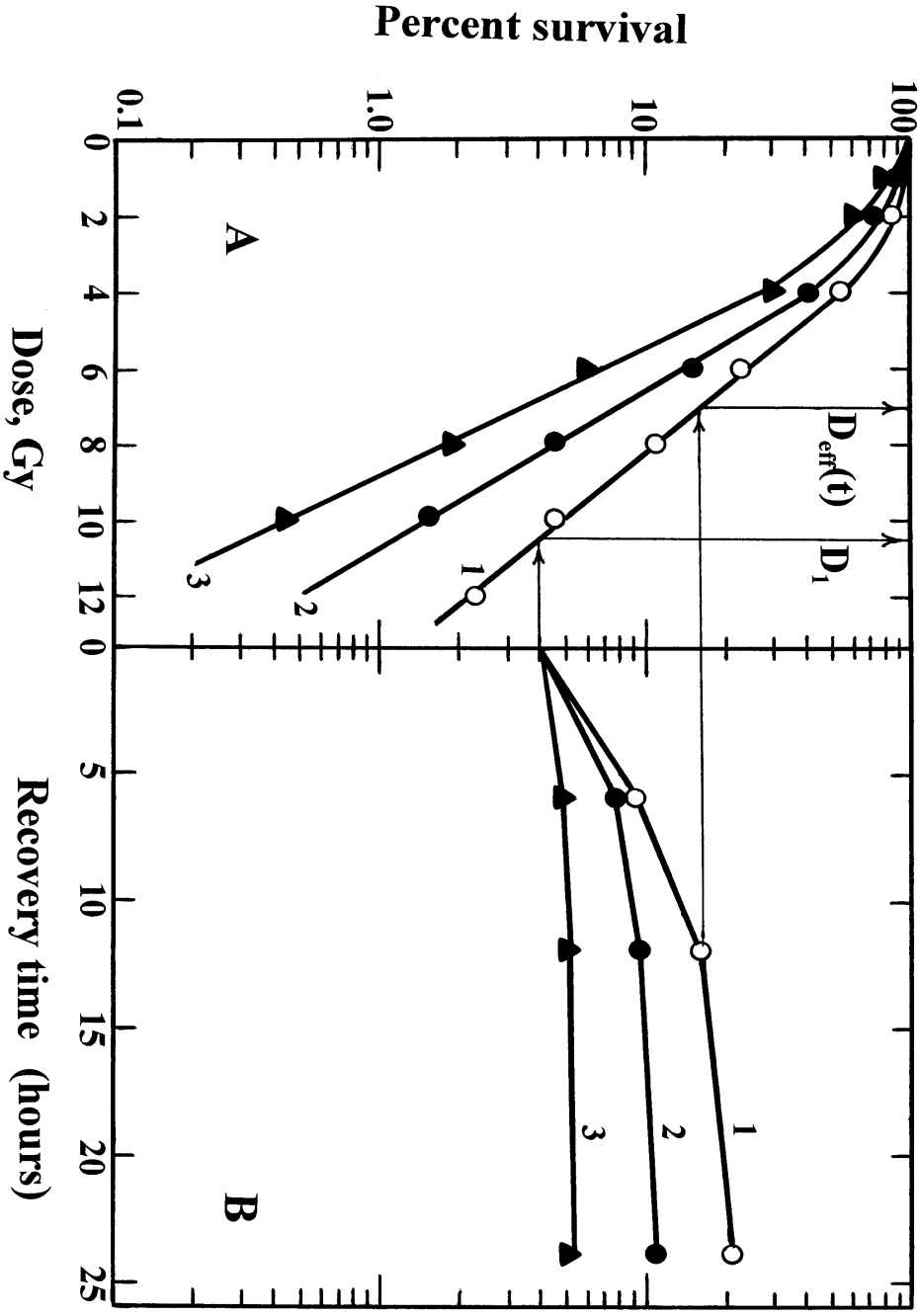

Fig. 1. Survival curves (A) and LHR recovery kinetics (B) of Chinese hamster V79 cells. A – cells were irradiated with ionizing radiation alone (curve 1) and combined with treatment for 24 h after irradiation with 10 mM (curve 2) and 20 mM (curve 3) of sodium pyruvate. B - the recovery process was occurred without chemical (curve 1) and in the presence of 10 and 20 mM pyruvate (curve 2 and 3, respectively). Arrows indicate example of the initial dose D_1 and effective dose $D_{eff}(t)$ determination.

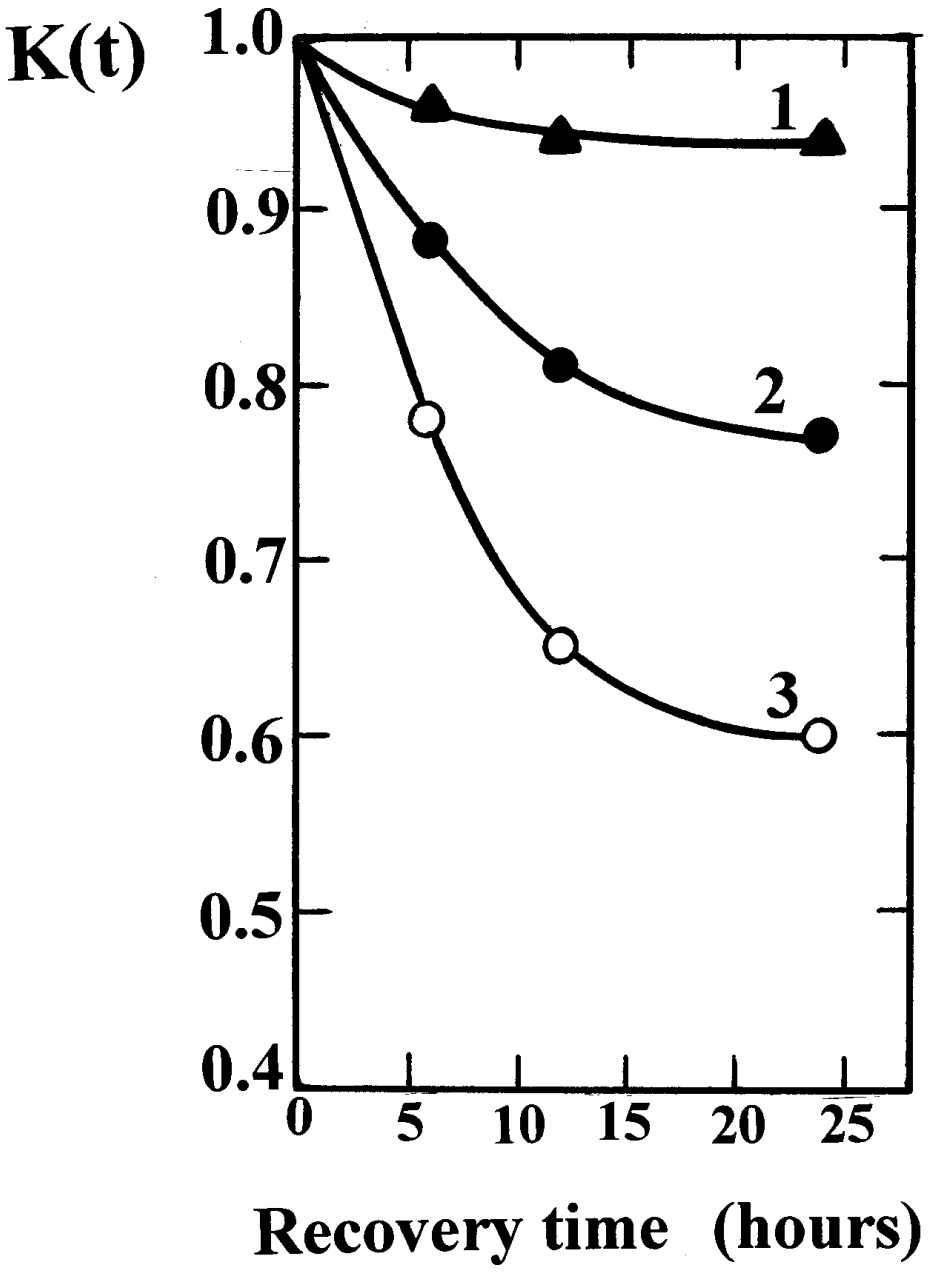

Fig. 2. The dependence of the relative fraction of irreversible damage $K(t) = D_{eff}(t) / D_1$ on recovery time of Chinese hamster V79 recovering after irradiation without chemical (curve 1) and in the presence of 10 mM (curve 2) and 20 mM (curve 3) of sodium pyruvate.

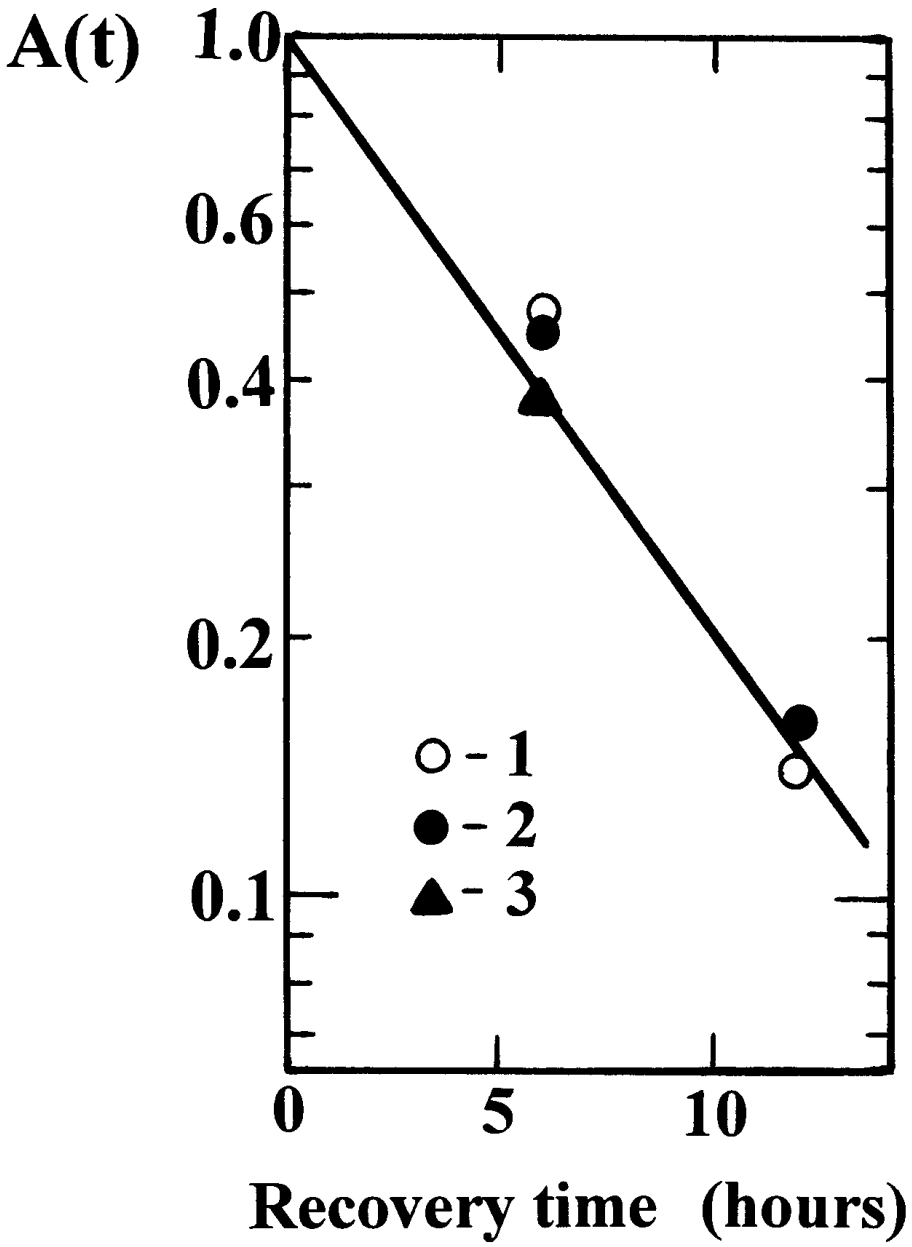

Fig. 3. The dependence of $A(t) = [D_{eff}(t) - D_{eff}(plateau)] / [D_1 - D_{eff}(plateau)]$ on recovery time of Chinese hamster V79 cells recovering after irradiation without chemical (1) and in the presence of 10 mM (2) and 20 mM (3) of sodium pyruvate.