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The Repair of Radiation Injury in L-Cells Treated by Adriamycin

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Abstract—*The effect of adriamycin on the radiation survival curve before, during and after treatment was studied in L-cells. The radiation survival curve became exponential when the cells treated with sufficient concentration of adriamycin, were irradiated at a specific time. The 'time' factor therefore must also be considered an essential parameter for interaction. The time dependence (kinetics) of survival was determined by treating the cells with* 0.5 μg/ml (0.1 μg/ml) *adriamycin for* 4 hr (6 hr) *and irradiating them at various intervals with* 600 rad (700 rad). *The most intensive interaction was found to be* 3 hr *after addition of the drug. No reparatory survival increase was observed in the double dose experiment immediately following addition of the drug. This is not due to the actual absence of repair, but is rather caused by the growing intensity of the adriamycin–radiation interaction. When the interaction was maximal and stationary during the double dose experiment, no suppression of early repair was observed.*

INTRODUCTION

ADRIAMYCIN ('Adria' in further text), an antibiotic isolated from *Streptomyces peucetius* var. caesius [1, 2] has proven to be an antitumor agent [3–6] and has been used as a cytocidal drug alone or in combination with other drugs or radiation. Besides its antitumor activity, Adria has also shown to be a potentiator of radiation response [7–9].

Adria increases the killing potential of ionizing radiation in mammalian cells in culture [10–12], but does not affect the slope of the exponential part of the survival curve. The increased killing ability, however, is due to the diminution of the extrapolation number (capability to accumulate the sublethal damage). For a certain period of time and at suitable concentration levels [12], the radiation survival curve becomes purely exponential under the action of Adria. For this reason the 'concentration' and the 'time' factors must be considered important parameters of interaction, just as the changes in n and D_0. The present communication places special emphasis on certain aspects of time dependence—the kinetics of Adria–radiation interaction.

The survival increase in double dose experiments (Elkind repair) is usually explained [13, 14] in terms of shoulder reappearance on the radiation survival curve. The disappearance of the shoulder, therefore, may have implications of the cell's ability to repair the radiation injury. Belli and Piro [10] and Hellman and Hannon [11] investigated the effect of Adria on the repair of radiation injury but did not observe any changes. Similar results were reported by Byfield *et al.* [15], in spite of the inhibition effect of Adria on the repair replication [16].

Certain new data are reported in this paper to obtain a better understanding of the repair phenomena in relation to the kinetics of the Adria–radiation interaction.

MATERIALS AND METHODS

L-cells grown in MEM + 10% calf serum were used in our experiments. The cell culture was maintained at 37°C in a humified atmosphere supplemented with 5% CO_2. Irradiation by 70 kVp X-rays produce a survival curve with $n=2.0$ and $D_0=160$ rad. The cells were plated in Petri dishes 20 hr before the experiments started. The number of inoculated cells was determined by assuming that approximately 100 cells survive, according to the colony forming techniques of Puck and Marcus [17]. Adriamycin, made by

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Farmitalia in Milano, was applied in appropriate concentrations. The survival response under various conditions has been determined by a number of authors (e.g. [10, 12, 18, 19]) for various mammalian cell lines in culture. The response was a sigmoidally shaped toxicity curve with exponential behaviour inside the survival range 0.5–0.01. Cells surviving treatment underwent the maximal sublethal damage. In our experiments the cells were exposed to Adria, either 0.5 μg/ml, 4 hr or 0.1 μg/ml, 6 hr. For both expositions the toxicity was approximately 0.1 (10%) and this survival is within the range of the exponential portion of the toxicity curve. The exposition to Adria was stopped by washing the cells and adding fresh medium. The colonies were scored 12 days after cells were inoculated. In order to obtain comparable and consistent results, certain experiments were performed simultaneously, starting with the same cell suspension. Results of experiments are shown together in the same graph. In all experiments the specific action of Adria and irradiation upon cells was determined separately. In this way we could evaluate whether the combined treatment survival was a consequence of interaction.

RESULTS

To determine the effect of Adria on the radiation survival curve, cells were exposed to various doses of irradiation before, during and after Adria treatments. For simplicity purposes, we have defined the moment of Adria addition as 0 h, while the negative and positive values are corresponding time points before and after 0 h. According to this time scale, the cells were irradiated at −3 h, 3.5 h and at 8 h. The shoulder of the survival curve disappeared at 3.5 h (Fig. 1), while curves obtained at −3 h and 8 h did not show any change either in the shoulder size or in the slope of the exponential part, as compared to the original survival curve. The last two curves, however, shifted downwards corresponding to the toxicity factor of Adria. No significant effect on the slope of the experimental part at any time was observed.

The dependence of interaction intensity on time can be suitably followed up by irradiating cells with a fixed dose (700 rad) at various intervals before, during and after the presence of Adria. Thus, the samples undergo double treatment: irradiation at various times and exposure to Adria during a common time period 0–4 h. The resulting function of time (Fig. 2) represents the Adria–radiation interaction kinetics. In order to evaluate the degree of interaction at any moment, two control samples were introduced for single treatment. One (A) was only irradiated by 700 rad, whereas the other (B) was exposed only to Adria (the exposures to radiation and Adria were equal to the amounts used in combination treatment). By multiplying the survival fractions in the control samples A and B, we obtained the survival level corresponding to the independent mode of interaction I = A × B (level indicated by the horizontal dotted line in Fig. 2).

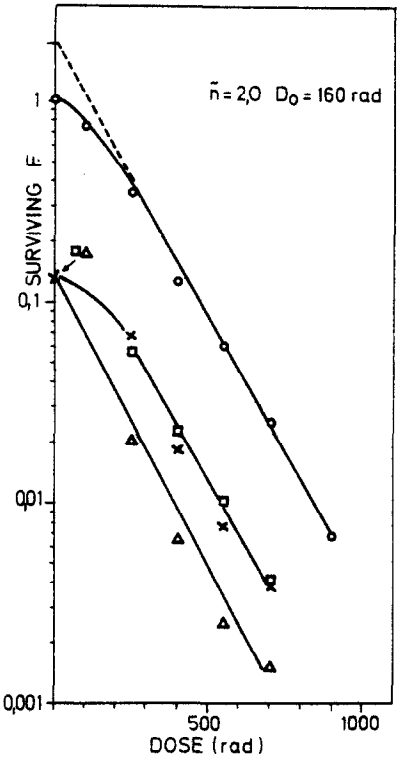

Fig. 1. Effect of Adria (0.5 μg/ml, 4 hr) on the radiation survival curve. (○—○) regular radiation survival curve. Adria was added at 0 h and the following radiation survival curves were obtained: (□—□) at −3 h; (△—△) at 3.5 h; (×—×) at 8 h.

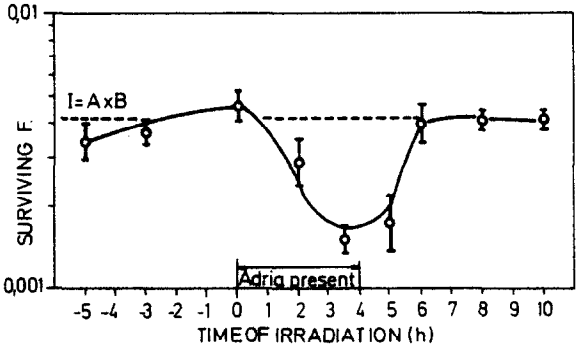

Fig. 2. Curve of interaction kinetics. Adria (0.5 μg/ml, 4 hr) was added at 0 h and the cells were irradiated at indicated times (700 rad). The dotted line represents the independent level survival.

The curve of interaction kinetics (Fig. 2) shows that the combined action of irradiation given before administration of Adria is equal to the independent level action. When irradiation is given after the administration of Adria, the survival progressively declines, achieving its minimum value at 3.5 h. Finally, when irradiation is administered after removal of the drug, the survival curve gradually reaches the independent level action again at 6–7 h. Since no change was observed in the slope of the exponential part of the survival curve (Fig. 1), the minimum survival at 3.5 h should be explained by the disappearance of the shoulder of the survival curve.

The effect of a certain drug on the cell's capability to repair radiation injury is usually studied in double radiation dose experiments which are performed under various drug treatment conditions. The drug may be present before the double dose experiment, or between radiation treatment pauses (e.g. [10, 20, 21, 22]) etc. The findings of the previous two experiments show that the potentiation effect is a time-dependent phenomenon. Consequently, the double dose (Elkind) repair could be only correctly understood if we know the degree of the potentiation effect during the entire double dose experiment. Three double dose experiments (300 rad + 300 rad), each lasting 4 hr, were performed successively, the first one before, the second one during and the third after administration of Adria (0.5 μg/ml, 4 hr). Throughout the entire period (12 hr), the drug–radiation interaction kinetics was followed using 600 rad of irradiation at various time intervals. In this experiment the drug was present in the samples in the same concentration and during the same time interval as in the repair experiment. In this way, certain samples received the same treatment as the control samples in the repair experiments. These results are shown in Fig. 3. The first repair curve started at −4 h. The survival reached a maximum value at −2 h and after that decreased. During this period, no significant deviations from the independent level of interaction were observed. In the second repair experiment the first dose was given immediately after Adria was injected into the samples. In order to understand what occurs in the next few hours (after administration of Adria), we must keep in mind two ongoing opposite processes. One is the repair process which increases the total surviving fraction of cells. The other one is the simultaneous, gradual disappearance of the survival curve's shoulder. This could be derived from the amount of interaction, since at that time the kinetics' curve descends under the independent level. These two processes compensate each other and the final result is an apparent suppression of the reparative survival increase. The third double dose experiment was started at 4 h. Immediately upon removal of Adria, the first dose of irradiation was given. At that time the maximum interaction is still in effect and the survival after the first dose occurs according to the exponential survival curve. In the next 2 hr, the shoulder reappears and at the same time a reparatory increase of the survival can also be observed.

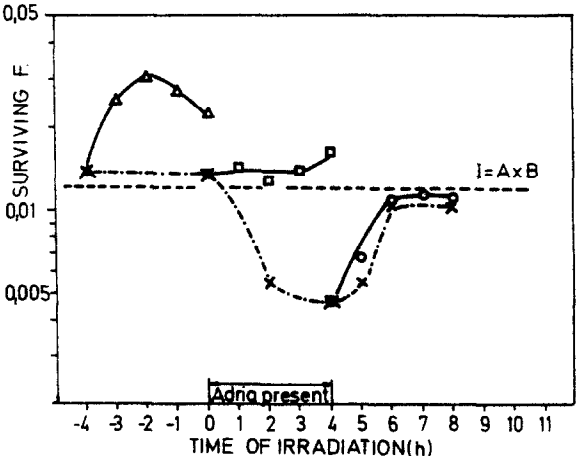

Fig. 3. Effect of Adria (0.5 μg/ml, 4 hr) *on early repair* (300 rad + 300 rad). *Adria was added at* 0 h, (△—△), (□—□), (○—○): *repair curves starting at* −4, 0 *and* 4 h, *respectively.* (×—×): *curve of interaction kinetics*, 0.5 μg/ml (4 hr) + 600 rad. *The dotted line represents the independent level survival.*

In the last double dose experiment, the survivals grew almost simultaneously, at the same rate as the interaction kinetics curve. This may mean that the survival increase in the double dose experiment is due to the gradual disappearance of interaction and not to the Elkind repair. The double dose survival increase, therefore, should be studied against the background of the Adria–radiation interaction level which does not change with time. This condition was achieved by increasing the duration of Adria-exposure up to 6 hr with a reduction in concentration to 0.1 μg/ml. Using this treatment and irradiation with 700 rad at various times, we obtained an interaction kinetics curve (crosses, Fig. 4) that was practically stationary at 3 h and continued to remain so. Three double-dose experiments were made again (Fig. 4). The first one starting at 0 h did not show any survival increase, similar to the case in

the previous experiment. The next two experiments, starting at 3 and 6 h showed a clearly expressed survival increase while the interaction kinetics function almost stayed at the same level the entire time. This result shows that in the last two cases, the survival increase is a pure reparatory phenomenon. Returning now to the previous experiment, it could be assumed that the reparatory increase (circles, Fig. 3) in the last experiment is not related to the termination of interaction but instead to the actual repair.

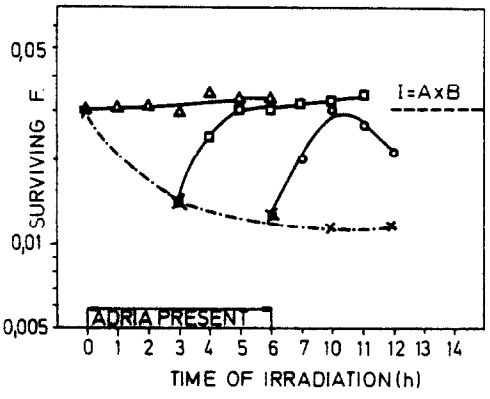

Fig. 4. Effect of Adria (0.1 μg/ml, 6 hr) *on early repair* (350 rad + 350 rad). *Adria was added at* 0 h. (△—△), (□—□), (○—○): *repair curves starting at* 0, 3 *and* 6 h, *respectively.* (×—×): *curve of interaction kinetics*, 0.1 μg/ml (6 hr) + 700 rad. *The dotted line represents the independent level survival.*

DISCUSSION

The experiments presented in this paper show that Adria modified the radiation survival curve of L-cells. Similar to the results obtained in other reports [10, 11], this was expressed as a disappearance of the curve shoulder at a suitable period of time with no change in the sensitivity of L-cells (change in slope of the exponential section). This effect, however, is explicitly dependent on the irradiation time table, with regard to the drug treatment period. There were certain periods (−3 h, 8 h) when no interaction was observed. These findings, as well as the data about interaction kinetics clearly reveal that the disappearance of the shoulder is a gradual process. The effect does not occur instantaneously but rather is a step by step process until the maximum interaction has been achieved at a specific time. Our results indicated that maximum potentiation occurs at 3.5 h after the addition of Adria which has been present for 4 hr.

Our data differ to some extent from the results which were obtained by Belli and Piro [10] in V-79 cells. These authors exposed the cells to an acute treatment with Adria (0.4 μg/ml, 1 hr) and obtained an interaction lasting over 24 hr. In our experiments a relatively lasting interaction was obtained at long (6 hr) exposures to Adria. These differences, however, seem to be of only quantitative nature due in part to cell line differences as well as to the different modes of treating with Adria.

The positive result of the reparative survival increase before and after the drug treatment period is an expected effect, since no interaction was observed during this period of time. Any possible repair inhibition should be related to the increased interaction intensity. As a matter of fact, in double-dose experiments performed immediately after addition of the drug, no significant increase of survival was observed with the increasing time interval between two doses. The absence of the survival increase in this double dose experiment must be, however, interpreted in terms of interaction kinetics. The first dose of irradiation was given at 0 h when the interaction did not yet appear. The series of second doses was given later during the growth of the interaction effect. Consequently the reparatory increase of survival is annihilated with a more or less equal increased survival deficit caused by the gradual appearance of interaction. In this way the apparent lack of survival increase during the rise of the interaction effect should not be interpreted as the absence of repair.

The experiment presented in Fig. 4 also shows that the cells completely repair sublethal damage during a whole period of the exponentially shaped survival curve. In conclusion we can assume that the reparative survival increase is not related to the presence of the shoulder on the survival curve. These results are in good agreement with the findings of other authors [10, 11, 15].

As demonstrated in this communication, the result of the combined action may not only be strongly dependent on the 'time' parameter, but also on the modes of exposure to the drug. Namely, we were not able to suppress the shoulder on the survival curve at Adria toxicities >0.3 (such experiments are not presented in current communication). On the basis of these data one could speculate on the molecular origin of the radiation–Adria interaction. The antitumor activity of Adria is probably related to its affinity for DNA, characterized by the intercalation between two successive base pairs [23]. In order to obtain an Adria–radiation interaction we need a high

Adria toxicity or relatively longer exposure time. This suggests that following such a treatment a more persistent binding of Adria to cell DNA could occur in surviving cells. This residual amount of bound Adria may be responsible for changes in DNA which could be lethal when combined with an additional sublethal radiation damage.

The experiments performed in this study are in fact an appropriate model for the phenomena playing an important role in combination drug–radiation therapy, especially considering the treatment time parameter. The *in vitro* conditions of our experiments do not allow the implication of anything concrete concerning actual therapy planning; however, we can draw some generalized conclusion which could be important from the point of view of a clinician and a radiobiologist as well.

First, the interaction of a drug and radiation could not be considered independently of the time parameter. The effect of the maximum interaction (potentiation) takes place at a certain moment in the treatment time table. The arbitrary sequence of radiation and drug exposure may not be necessarily a convenient way of treatment.

Secondly, the double dose repair phenomena cannot be understood correctly unless the interaction kinetics is followed up simultaneously. The absence of survival increase in a double dose experiment which is performed during the increasing interaction process, may not be an actual repair suppression. The capability to repair radiation injury must be, therefore, determined under conditions of stationary behaving interaction.

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