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LINEAR-QUADRATIC DOSE KINETICS OR DOSE-DEPENDENT REPAIR/MISREPAIR

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Linear-Quadratic Dose Kinetics or Dose-Dependent Repair/Misrepair

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ABSTRACT: Models for the response of cells exposed to low LET radiation can be grouped into three general types on the basis of assumptions about the nature of the interaction which results in the shoulder of the survival curve. The three forms of interaction are 1) sublethal damage becoming lethal, 2) potentially lethal damage becoming irreparable, and 3) potentially lethal damage "saturating" a repair system. The effects that these three forms of interaction would have on the results of specific types of experiments are investigated. Comparisons with experimental results indicate that only the second type is significant in determining the response of typical cultured mammalian cells.

1. INTRODUCTION

A nearly universal characteristic of the dose-response relationship of eukariotic cells exposed to ionizing radiation is an increase in effectiveness per unit dose with increasing dose, known as the shoulder of the survival curve. This shoulder is a clear indication of some form of interaction involving the products of successive energy deposition events. Most of the models which have been proposed assume one of three basic types of mechanism which would result in this interaction. These mechanisms are 1) interaction of sublethal damage to produce lethal damage, 2) radiation induced misrepair of potentially lethal damage, and 3) saturation of the processes repairing potentially lethal damage. Each of these interaction mechanisms is compatible with a wide variety of "repair" mechanisms which reduce the amount of damage and result in reduced effect with decreasing dose rate, dose fractionation, or delayed cell growth following irradiation.

The purpose of this note is to explore the characteristics of the interaction mechanisms, independent of the type of repair, and show the type of experimental data which can be used to distinguish between these mechanisms.

2 MECHANISMS OF INTERACTION

To the extent possible the three types of models will be described in terms of the nature of the interaction which results in the sholder of the survival curve, without limiting them by applying specific types of repair kinetics.

2.1 Interaction of Sublethal Damage

A wide variety of models, (Braby and Roesch 1978) have been based on repair of sublethal damage. The term "sublethal damage" is generally applied to damage that must interact with additional damage from an independent energy deposition event in order to inactivate a cell. This results in a dose-squared dependence and an initial slope of a survival curve equal to zero. A non-zero initial slope occurs if either a) products formed by a single charged-particle track interact or b) there is a second type of damage which does not require interaction. Considering just sublethal damage, the probability of interaction must depend on the concentration of damage at the time the next energy deposition event occurs. In a split-dose

experiment, repair during the interval between doses has the effect of reducing the concentration of damage which remains to interact with damage produced by events making up the next dose. Thus at low doses where the concentration of damage following the first dose fraction is very low, further reduction will have very little effect on the probability of interaction, and on the lethality.

We should also consider the effects of repair between energy deposition events and compare it with the effect of repair after irradiation has ended. Repair in these two situations can be evaluated using split-dose experiments and "delayed-plating" experiments. Although there will be repair following irradiation in both types of experiments, repair between events can be virtually eliminated in delayed-plating experiments by giving the irradiation at a high dose rate. Repair of sublethal damage between events can clearly reduce lethality by reducing the probability of interaction, while removal of this type of damage after irradiation is complete will have no effect since there will be no additional damage produced with which it can interact. If cells also have the capacity to repair potentially lethal damage produced by interaction of sub-lethal damage, they will also show an independent delayed-plating effect. In either case, fractionating a given dose results in more repair than can be achieved by a plating delay. Since all models assume that equal doses produce equal amounts of initial damage, the sublethal-damage interaction model predicts a higher final survival following a split-dose exposure than following the same exposure with a plating delay. Furthermore, it predicts plating delay will become ineffective at low doses because there will be very little potentially-lethal damage formed by the interaction of sublethal damage.

2.2 Misrepair of Potentially Lethal Damage

Models based on dose-dependent misrepair, characterized by the LPL model (Curtis 1986), assume that all damage is potentially lethal but that new potentially lethal lesions can interact with those remaining from previous energy deposition events to produce irreparable damage. Unrepaired potentially lethal damage remaining in the cell is lethal. In this type of model, repair of damage between events reduces the probability of interaction and thus reduces the amount of irreparable damage produced by a given total dose. Again, the concentration of damage is lower in the split-dose experiment so less damage is made irreparable, and there is higher survival than in a delayed-plating experiment which provides the same amount of time for repair of potentially lethal damage.

The assumption that residual unrepaired damage is lethal results in an initial slope for the dose-effect relationship. This means that repair continues to be effective even at very low doses since removing potentially lethal damage reduces lethality. Repair may actually become more effective because binary misrepair which makes damage irreparable becomes insignificant at low doses.

2.3 Repair Saturation

Another type of model is based on the assumption that damage is potentially lethal, and it is repaired by a system which requires use of a limited pool of repair capacity (Goodhead 1985). In this case a different response to split dose and delayed plating would be predicted. The magnitude of the depletion of repair capability depends on the amount of damage repaired; that is it depends on the dose but not on the concentration of the damage. A given dose will consume the same amount of repair capacity whether it is delivered at one time or in two or more fractions. Thus the total amount of damage that can be repaired in a given time is independent of the irradiation schedule, and the final survival (after sufficient time for all possible repair) in a delayed plating experiment must be equal to the final survival with a split dose.

Because the damage is assumed to be potentially lethal in this model, the effect at low doses is expected to be the similar to that of the LPL model, although for a different reason. That is, repair becomes more effective at low doses because the repair system is not saturated.

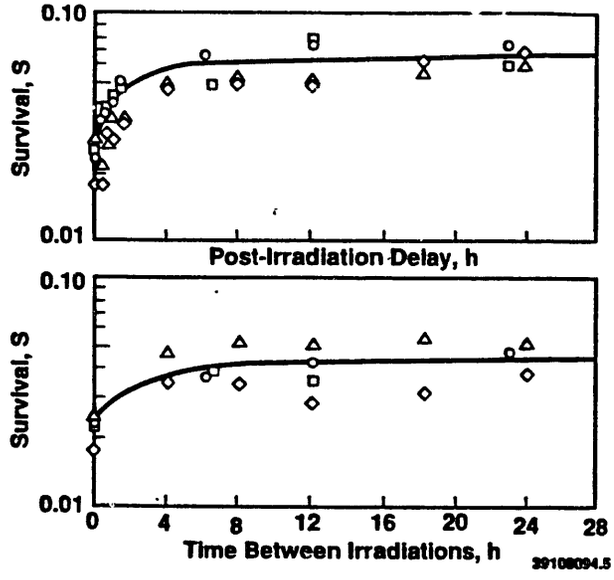

Figure 1 Split dose (two fractions, 4 Gy each) and delayed plating (8 Gy) repair in plateau phase CHO cells. Symbols refer to different experiments.

In order to obtain a split-dose effect with a repair-saturation model there must be some mechanism for replenishing the depleted supply of repair material. This replenishment can occur in many different ways, and only a few of the possibilities have been explored. These include release of repair enzymes after repair is completed, continuous turnover of the repair system through synthesis of new enzymes, and additional synthesis of repair enzymes in response to the damage. Release of enzymes after repair is complete and constant synthesis of new enzymes do not effect the relative amount of repair in split-dose and delayed-plating situations since they do not depend on the concentration of the damage. However, the induced synthesis of additional repair capacity might depend on the damage concentration. If it does, one would expect the synthesis of new repair capacity to increase with the damage concentration. This would result in more repair (higher final survival) in the delayed plating experiment where the damage concentration is higher.

3. EXPERIMENTAL OBSERVATIONS

The three types of models differ from each other with respect to the expected results of two types of experiments. The repair saturation model can be distinguished from the other two on the basis of the final survival in split-dose and delayed-plating experiments; the sublethal-damage model can be distinguished from models involving the interaction of potentially lethal damage on the basis of the amount of repair at very low doses. These comparisons, however, place unusual requirements on the experimental techniques.

Split-dose experiments have been performed with two equal dose fractions delivered at a specified interval, T_i, with the cells irradiated in plateau phase and replated in growth conditions immediately after the last dose. Cells generally have a time, T, available to repair damage before this damage becomes fixed after replating. This means that in the split-dose experiment with immediate replating, the cells have T_i + T to repair damage occurring during the first dose and T to repair damage produced during the second dose. On the other hand, cells in a delayed plating experiment are held in plateau phase for a time, T_p following a single exposure and then trypsinized and replated. The time T_p + T is then available for repair of all of the damage. Figure 1 shows the results of these two types of experiments for plateau-phase CHO cells irradiated and held at room temperature. Since we do not know the exact value of T we can only compare the results for $T_i = T_p$. In the split-dose experiment the first half of the dose will have the same time for repair as the damage produced in the delayed-plating experiment, but the damage produced in the second half of the exposure will have only time T for repair. In spite of there being less total opportunity for repair, the final survival is significantly higher for the split-dose experiments. Split-dose experiments have also been performed with replating delayed several hours after the second dose. These result in a slightly higher survival, but cannot be interpreted unambiguously because the first half of the dose was available for repair for a time exceeding the maximum time for repair in the delayed-plating experiments.

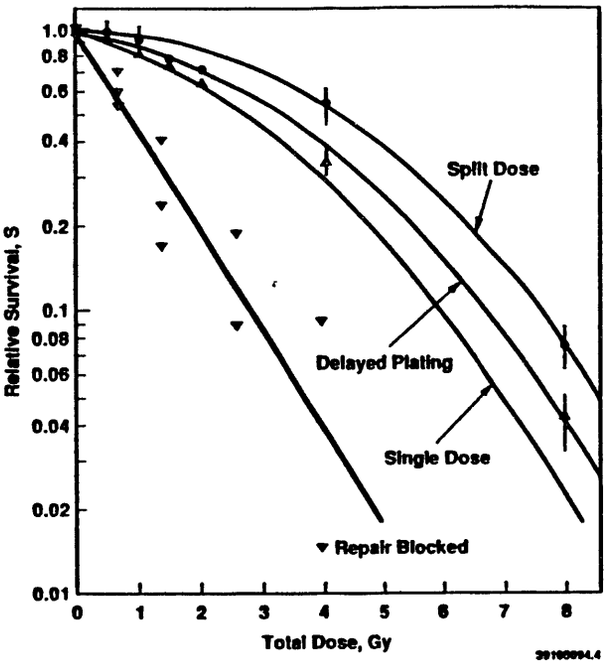

Figure 2 Effect of 24 hour split dose and delayed plating repair as a function of dose for plateau phase CHO cells.

The comparison between the sublethal damage and potentially lethal damage is more difficult because it requires evaluation of the total amount of damage repaired at low doses in delayed-plating experiments. This is difficult because the majority of the damage is repaired during T under normal experimental conditions. The only way to determine the total amount of repair is to make a measurement with repair blocked. The drug β-ara-A prevents repair, but there is concern that if present before or during irradiation it may also alter the production of damage. To avoid this problem experimental procedures were developed to use β-ara-A added immediately after exposure to low doses of x rays (Nelson et al 1991). The results of delayed plating experiments with doses as low as 0.5 Gy are compared with split-dose survival, and with the survival when all repair has been blocked are shown in figure 2. They clearly show that there is a significant amount of damage that can be removed during a plating delay, even at low doses where the split-dose effect has become insignifigant.

4. CONCLUSIONS

Saturation of repair has been demonstrated at relatively high doses (Wheeler 1991) and is clearly a significant factor when large amounts of damage occur in cells. It is also possible that some specific chemical changes in the DNA or nuclear matrix which are not lethal in themselves may interact with additional products to produce lethal combinations when the dose is high enough. However, the real concern in evaluating models for the risks of exposure to ionizing radiation is the effect at the very low doses which may be encountered in the environment or as a result of energy production.

The three types of models most commonly used to describe the response of cells to ionizing radiations have been used to predict the response of cells in low dose experiments. It is shown that they predict significantly different responses. A limited set of experiments, with a single cell type, indicates that only the assumptions that the relevant damage is lethal if not repaired, and that it can interact with additional damage to produce an irreparable product are consistent with the actual response of cells.

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