BRIEF COMMUNICATION

X-RAY RESPONSE OF CHINESE HAMSTER OVARY CELLS DURING THE LATTER PART OF G_2

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ABSTRACT The ability of Chinese hamster ovary (CHO) cells to repair x-radiation damage during the transit from the late G_2 to early M cell cycle stages was investigated by conventional dose-fractionation techniques. Despite their relatively high radiation sensitivity, CHO cells positioned in late G_2 exhibit increased survival when a given dose of ionizing radiation is administered as two fractions (separated by 40–50 min) instead of as a single fraction. This increased survival apparently represents repair since neither cell cycle progression nor changes in the number of "effective targets" can account for the observed dose-sparing effect.

INTRODUCTION

It was previously reported (1) that HeLa S3 cells exhibit a sharp transition in radiation sensitivity at or near the x-ray arrest point (the last point in the cell cycle where x-irradiation is capable of delaying the progress of cells towards the ensuing mitosis). In fact, cells located just past the arrest point (postarrest G_2 cells) had an x-ray sensitivity that was indistinguishable from the maximally sensitive mitotic cells, while cells located just before the x-ray arrest point (prearrest G_2 cells) exhibited an x-ray response that was similar to maximally resistant late S-phase cells. A somewhat similar response has more recently been reported for yeast (2). Several investigators have postulated that the extreme radiation sensitivity of postarrest G_2 cells might reflect an inability of these cells to repair radiation damage (1–3). The present investigation was undertaken to determine if Chinese hamster ovary (CHO) cells also exhibit a transition in x-ray sensitivity at or near the arrest point, and to determine whether postarrest G_2 CHO cells can repair radiation damage. The results indicate that postarrest G_2 CHO cells are as sensitive as mitotic cells, while prearrest G_2 cells are much more resistant. In addition, on the basis of split-dose experiments, postarrest G_2 cells appear capable of repairing at least some radiation damage.

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MATERIAL AND METHODS

CHO cells were routinely grown in F10 medium (4) supplemented with 10% calf serum, 5% fetal calf serum, and kanamycin (100 μg/ml). Cultures were maintained at 37°C in an atmosphere of 5% CO_2 in air saturated with water vapor. Mitotic cells were collected from randomly dividing cultures (5) and plated for survival determinations. The plating efficiency of these cells ranged between 60 and 80% and the mitotic yield was always >80%. Cell survival was measured by determining the number of colonies (>50 cells) appearing in plastic dishes after 8–10 d of incubation.

X-irradiation was administered at room temperature at dose rates between 150 and 300 rad/min. The x-ray generator (General Electric Co., West Lynn, Mass.) was operated at 300 kVp, 20 mA, with 0.5 mm Cu + 1.0 mm Al added filtration, yielding a half-value layer of 1.25 mm Cu. Cultures were returned to 37°C incubators within 3 min after exposure or sham exposure. An electronic particle counter was used to determine cell number.

RESULTS

In the results shown in Fig. 1 mitotic cells were collected from randomly dividing cultures at various times after exposure or sham exposure. As expected (1, 6–8), when randomly dividing CHO cultures were irradiated with 500 rads, cells located past the arrest point continued to divide, in declining number, for ~1.0–1.5 h. As shown in the lower half of Fig. 1, all of these postarrest G_2 cells had approximately the same, relatively high, radiation sensitivity. Cells located before the arrest point at the time of the exposure did not begin entering mitosis until 4.5–6.0 h after exposure (Fig. 1; upper half). These cells were markedly more radiation-resistant. The slight increase in resistance that occurs in cells collected between 4.5 and 6.5 h after exposure might indicate, as others have reported (9), that for CHO cells radiation sensitivity rises slightly between late S and early G_2. Population mixing makes definitive conclusions concerning the behavior of the prearrest cells difficult, however.

As mentioned by several investigators (1–3), the high sensitivity of late G_2 cells may be due to an inability to repair radiation damage. Repair of radiation damage is one of the factors

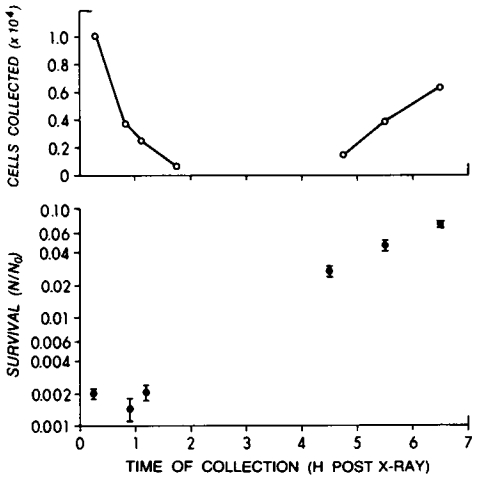

FIGURE 1 Response of randomly growing cultures of CHO cells to 500 rad. Number of cells collected (per milliliter) at various postexposure times (upper graph); survival of cells collected at various postexposure times (lower graph). Error bars represent standard errors of the mean.

responsible for cells exhibiting higher survival values after fractionated exposures. The results of several split-dose experiments are shown in Fig. 2. In these experiments, 40–50 min after administering a conditioning dose to randomly dividing cultures, a challenging dose was administered, and mitotic cells were immediately collected and plated for survival determinations. Thus, since challenging doses of the magnitude used in this study prevent prearrest G_2 cells from progressing towards mitosis for at least 2½ h, (7, 8), but have no effect on the progression of postarrest G_2 cells, the majority of cells would have received the conditioning exposure during late G_2, and the challenging exposure during early M. For a comparison with single (nonfractionated) exposures, cells were either collected immediately or 40–50 min after exposure (cultures exposed 40–50 min before collection received a sham exposure immediately before collection). The results, shown in Fig. 2, indicate that cells exposed to fractionated exposures had higher survival levels. The extent of increase was similar to the recovery observed when asynchronous cells were exposed to split exposures separated by a 40-min interval (results not shown).

Before it can be concluded that this increased survival represents repair, two possibilities must be considered. First, Lange (10) has shown that increased survival after fractionated exposures can occur in the absence of repair, if cells progress to a more resistant cell cycle stage by the time of the second exposure. Since there is no change in the radiation response of cells as they progress from late G_2 to M, such progression effects could not account for the increased survival values reported here. Second, Nagle and Humphrey (11) have shown that mitotic cells respond to radiation as if they were already in a functional two-cell or two-target

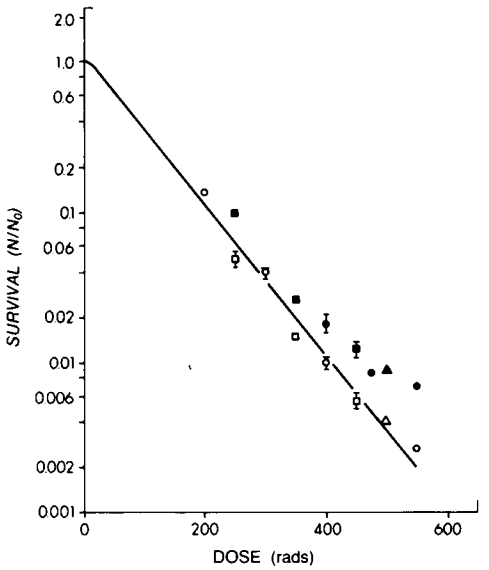

FIGURE 2 Survival of late G_2 CHO cells after x-irradiation. Randomly dividing cultures were given either single exposures (open symbols) or two exposures, separated by 40 to 50 min (closed symbols). (○) mitotic cells collected immediately after receiving single exposures; (□, △) mitotic cells collected 40–50 min after receiving single exposures. (●) conditioning dose of 300 rads; (■, ▲) conditioning dose equal to one-half total dose. Cells receiving two exposures were collected immediately after the second exposure. Survival is plotted on the basis of the total dose administered. Error Bars, where shown, represent standard errors of the mean. Where no error bars are shown, the values fit within the symbols.

state. If late G_2 cells responded as if they were in a single-target state, progression to mitosis would result in the creation of an additional functional target, and an increase in survival after fractionated exposures given in G_2 and M. To test this possibility, mitotic cells collected either immediately or 40 min after exposure were held in suspension for 1 h before plating. This results in the separation of most cell doublets and allows for the determination of whether, in terms of its response to radiation, a single cell exhibits a "functional" two-cell or single-cell response (11). The results (data not shown) indicate that there is no change in survival of cells collected 40 min or immediately after exposure to 250 rads (G_2 cells or M cells, respectively) when they are held in suspension for 1 h before plating.

DISCUSSION

The finding that postarrest G_2 cells exhibit increased survival after fractionated exposures is somewhat surprising since, based on the near shoulder less dose-response curve (Fig. 2), cells in this cell cycle stage appear unable to accumulate significant sublethal damage. It is clear, however, that factors other than repair of sublethal damage can be involved in split-dose recovery (10, 12). Two of these, progression to a more resistant stage of the cell cycle and changes in the number of "effective" targets, have already been shown not to be involved in the recovery reported here. It is important to note that repair of radiation damage occurs at exposures in the exponential region of dose-response curves. In particular, repair of potentially lethal damage (12, 13) occurs at such exposures, and often contributes to split-dose recovery. In the experiments reported here, attempts were made to expose cells receiving single exposures to the same experimental manipulations as cells receiving fractionated exposures. Thus, it is difficult to relate the split-dose recovery observed here to repair of potentially lethal damage. Two other possibilities appear more likely. First, a small subpopulation of resistant cells may exist within the larger population of sensitive late G_2 to M cells. The presence of a small subpopulation of cells exhibiting a shouldered response can be masked by the response of the other cells (14). More frequent collection of mitotic cells might determine whether such a subpopulation exists. Second, there is growing evidence that in eukaryotes as well as in prokaryotes certain repair processes can be induced by prior irradiation (15–18). If DNA repair processes can be induced in late G_2 cells, as appears to be the case after exposure to UV (18), this might account for the split-dose recovery observed. Certainly, careful biochemical and molecular studies of repair processes during late G_2 must be undertaken in mammalian cells to determine if such repair processes exist. Simply adding a protein synthesis inhibitor between the two exposures would not test this possibility, since such inhibitors alter the location of the x-ray arrest point (8) and alter the response of cells to unfractionated exposure (13).

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