RECOVERY AFTER EXPOSURE TO NEAR-ULTRAVIOLET LIGHT OF CELLS CONTAINING 5-BROMODEOXYURIDINE

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ABSTRACT The survival of synchronized V79 Chinese hamster cells irradiated with near-ultraviolet light after a 1-h labeling with 5-bromodeoxyuridine (BrdUrd) is highly dependent upon the cells' position in the cell cycle at the time of irradiation (Hagan, M., and M. M. Elkind. *Biophys. J.* 1979. 27:75–86). In this report, we show that cells irradiated in the same S phase after BrdUrd incorporation demonstrate an ability to repair sublethal damage, in contrast to the lack of an increase in survival with dose fractionation in template-labeled cells (Ben-Hur, E., and M. M. Elkind. *Mutat. Res.* 1972. 14:237–245). In addition, we show that pulse-labeled cells in S phase can repair potentially lethal damage expressed by caffeine. The kinetics of these recovery processes and the absence of a caffeine effect on the repair of sublethal damage indicate that these two processes are to a large degree unrelated. We conclude that in template-labeled cells inadequate time to effect prereplicational repair precludes effective contributions to cell survival from other kinds of DNA repair processes.

INTRODUCTION

Mammalian cells exposed to near-ultraviolet (near-UV) or to visible fluorescent light after the incorporation of 5-bromodeoxyuridine (BrdUrd) are able to repair extensive damage in their DNA (Smets and Cornelis, 1971; Ben-Hur and Elkind, 1972 *b*; Makino and Munakata, 1979; for a review see Hutchinson [1973]). Both single-stranded breaks in human and in Chinese hamster cells and uracil photoproducts in human and in mouse cells have been shown to be rapidly removed during the first hour after light exposure (Ben-Hur and Elkind, 1972 *b*; Makino and Munakata, 1979; Smets and Cornelis, 1971). Although such observations of molecular repair were made with cells in which the DNA was presumably uniformly labeled with BrdUrd, the functional repair of proliferative capacity (i.e., increased cell survival) with dose fractionation was not observed (Ben-Hur and Elkind, 1972 *a*). Further, for template-labeled cells, little if any variation in survival was observed for irradiation at different positions, or ages, in the cell cycle (Ben-Hur and Elkind, 1972 *a*), in contrast to what is observed for other DNA strand-breaking treatments like x-irradiation (e.g., Sinclair and Morton, 1966). The lack of both a cyclic age-response variation and a survival increase with dose fractionation is consistent with the essentially exponential survival response of template-labeled, asynchronous cells. A shoulder-type survival curve can be observed, however, if

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Chinese hamster cells are briefly labeled with BrdUrd and then irradiated with near-UV light, either immediately after labeling or in the S phase of the next cell cycle after replication of the BrdUrd-substituted segment of DNA (Hagan and Elkind, 1979).

In the present work, we have further examined the properties of synchronized Chinese hamster cells pulse-labeled with BrdUrd at ages in their cell cycle when a shoulder-type survival curve appears. We show that such cells are able to repair sublethal as well as potentially lethal damage, and that the kinetics of these two processes differ. Hence, we conclude, as suggested earlier, that although some classes of lesions in DNA resulting from the exposure to light of asynchronous cells are repaired, such processes are ineffective in template-labeled cells, because inadequate time is available for the prereplicational repair of lesions lying just ahead of one or more replication forks (Hagan and Elkind, 1979).

MATERIALS AND METHODS

Cell Culture

Our methods for synchronizing V79 Chinese hamster cells and for assessing cell survival by colony formation have been described (Hagan and Elkind, 1979). Briefly, two clones of V79 Chinese hamster cells (Elkind and Sutton, 1960), whose exposure to light after BrdUrd incorporation elicited similar responses, were grown in a humidified atmosphere containing 2% CO_2 at 37°C in Eagle's Minimum Essential Medium (Grand Island Biological Co., Grand Island, N. Y.) buffered with 20 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulfonic acid (HEPES) and one-third of the $NaHCO_3$ concentration normally contained in Earle's saline. The medium was supplemented with 15% fetal calf serum and with the additions described by Stanners et al. (1971), except that nucleosides were omitted. After synchronization, dishes were inoculated with single cells, which, after treatment, were allowed to grow for 7–8 d for colony formation. The medium was not changed during incubation for colony formation. The cell inoculum was adjusted to produce 100–300 colonies per 100-mm plastic tissue-culture dish (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). Synchrony, the selective retention of cells in the late G_1 to early S phase, was produced by the incubation of asynchronously growing cells for 3.5 h in growth medium containing 2 mM hydroxyurea (HU) (Sinclair, 1965). One hour after synchronization, cells were labeled with BrdUrd for 45 min at 37°C by the addition of 1×10^{-4} M BrdUrd and 1×10^{-6} M 5-fluorodeoxyuridine (FdUrd), after which the cells were rinsed with phosphate-buffered saline and then further incubated in medium containing 1×10^{-5} M thymidine. The surviving fraction was decreased to 0.36–0.40 by the HU treatment due to the killing of S phase cells by the HU (Sinclair, 1965). All of the data have been normalized to account for this cell killing. A further small reduction in the survival of BrdUrd-substituted cells (by 10–15%) was effected by the addition of 2 mM caffeine to the colony-formation medium; data involving caffeine treatment were also normalized to account for caffeine toxicity. In the figures, data points represent the mean of normalized colony counts from the cells in three dishes cultured as indicated above. Uncertainties, ±SE, are shown when larger than the points.

Drugs

5-BrdUrd and 5-FdUrd were purchased from the Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif. Caffeine (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 2 mM in growth medium. After the administration of caffeine, the medium was not changed during the incubation period for colony formation, unless indicated otherwise.

Near-ultraviolet Light Irradiation

Cells attached in plastic dishes were exposed at 44 cm from a bank of four FS-20 (Westinghouse Electric Corp., Pittsburgh, Pa.) tubular sun lamps. During irradiation, the dishes were rotated on a "lazy

Susan" apparatus to expose the cells uniformly. The near-UV light was filtered by the cover of a 100-mm Falcon dish (0.975 mm of polystyrene), as a consequence of which little if any cell killing resulted in the absence of BrdUrd labeling (Hagan and Elkind, 1979). All exposures were performed at a dose rate of 2.07 J m^{-2} s^{-1} as determined with a thermopile (Hilger-Watts, model FT 17.1 Engis Equipment Co., Chicago, Ill.) and a microammeter (Keithly Instruments Co., Clifton, N. J., model 150B). The spectrum to which cells were exposed has been published (Utsumi and Elkind, 1979 *a*).

RESULTS

Single-cell populations of Chinese hamster cells, exhibiting shoulder-type survival curves, were produced by labeling synchronized cells with BrdUrd at 1 h after the removal of the medium containing HU and irradiation of cells with near-UV light at later times in the same S phase. The time of the first near-UV light exposures corresponded to either 3 h ± 15 min (i.e., "mid"-S phase) or to 4.25 h ± 15 min (i.e., "late"-S phase) after synchronization, as noted in the figures.

Fig. 1 shows survival results for cells in mid-S phase that had incorporated BrdUrd from 1.0 to 1.75 h after HU removal and that were exposed to near-UV starting 1.25 h later (i.e., 3 h after HU removal). In addition to the obviously damage-accumulation-type, or threshold-type, survival curve for single exposures (filled squares), Fig. 1 also contains data for two-dose fractionation. The fractionation curve shows the result when, 60 min after a first dose of 1.0 kJ/m^2, graded second doses were given. The two sets of fractionation data show that the

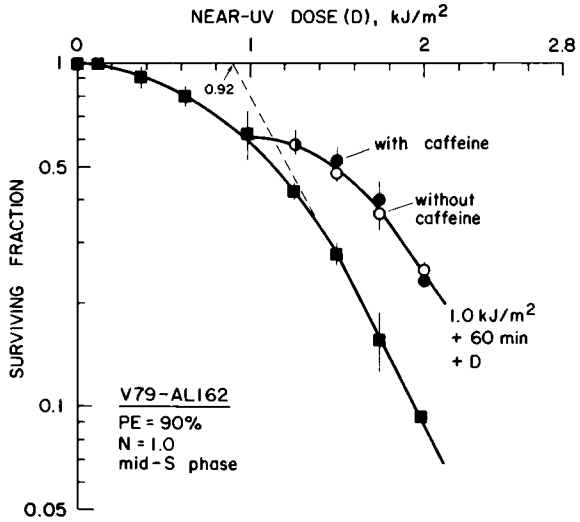

FIGURE 1 Single-cell survival curves for synchronized Chinese hamster cells, clone V79-AL162, exposed to near-UV light starting at mid-S phase, i.e., 3 h after the removal of HU. Cells were made light sensitive by incubation in medium containing BrdUrd (see text). The squares refer to single doses (without caffeine), and the circles to graded second doses after 1.0 kJ m^{-2}. 2 mM caffeine was present only between dose fractions. The dashed curve extrapolates to the shoulder width, 0.92 kJ m^{-2}, the single-dose curve has a D_0 = 0.44 kJ m^{-2}, the dose that reduces survival by a factor of $1/e$ along the terminal exponential portion of the curve. *PE*, the plating efficiency of unirradiated cells relative to the proportion of cells that survived the HU treatment. *N*, multiplicity. *D*, the second fraction of near-UV dose to give the total doses shown by the circles. Uncertainties are standard errors.

enhancement of survival is independent of whether the medium used in the interfraction interval contained 2 mM caffeine. Thus, as further described in reference to Fig. 5, caffeine does not affect the ability of cells to repair the sublethal damage due to a first dose of 1.0 kJ/m^2.

Fig. 1 demonstrates the presence, and the repair, of sublethal damage for cells in mid-S phase after sensitization to near-UV light induced by BrdUrd. To test for the presence of potentially lethal damage during the S-phase, posttreatment with caffeine was used. From other studies, we know that caffeine in the millimolar range will enhance cell killing in V79 Chinese hamster cells when added to the growth medium after nonionizing (far- or near-UV light) or after ionizing radiations (x-rays or fission-spectrum neutrons) (H. Utsumi and M. M. Elkind, unpublished observations). The survival curve data in Fig. 2 show that 2 mM caffeine results in the expression of a large sector of potentially lethal damage in mid-S-phase cells (compare the filled squares with the filled squares in Fig. 1) and also in late-S-phase cells (compare with data in Fig. 4). Further, in the case of late-S-phase cells, an 8-h delay in the addition of caffeine after light exposure still results in nearly the same lethal enhancement. The results illustrated in Fig. 3 show that for mid-S-phase cells the potentially lethal damage expressible by caffeine is slowly repaired, compared to sublethal damage (see Fig. 5), although somewhat more rapidly than for late-S-phase cells (Fig. 2).

In Figs. 4 and 5, the influence of dose fractionation with and without caffeine is examined to determine whether a relationship exists between the repair of sublethal and potentially lethal damage. In Fig. 4, late-S-phase cells were incubated in the presence of 2 mM caffeine for colony formation after their exposure to two equal light doses separated by 60 min. The data show that essentially no effect on net survival results from dose fractionation, since the

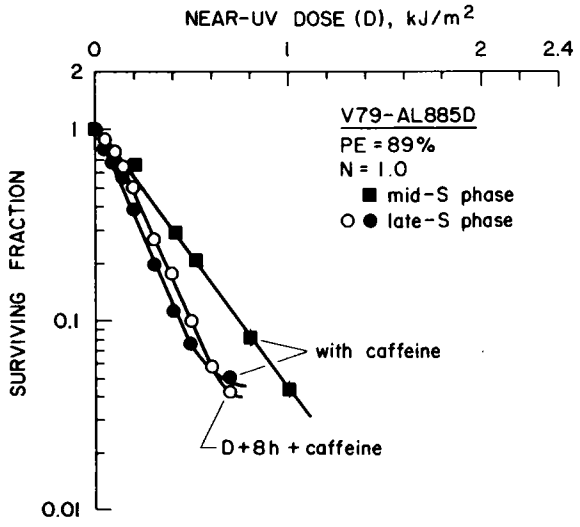

FIGURE 2 The effect of 2 mM caffeine on the survival of synchronized Chinese hamster cells, clone V79-AL885D. After BrdUrd incorporation to make cells sensitive to near-UV light, cells were exposed to light in mid-S phase or in late-S phase, after which medium containing caffeine was added as indicated. The D_0 doses are mid-S-phase cells, 0.32 kJ m^{-2}; and late-S-phase cells, 0.19 kJ m^{-2}. In this figure, D stands for a single dose of near-UV light. Other details as for Fig. 1.

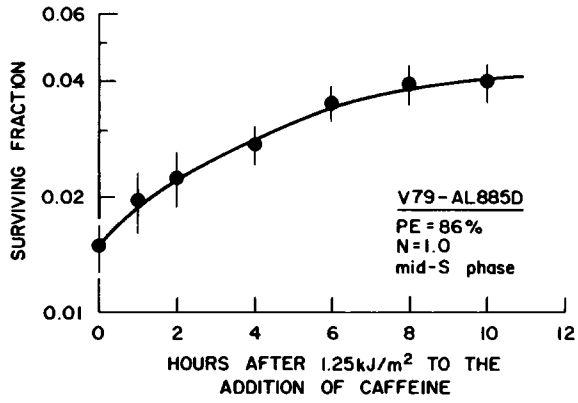

FIGURE 3 The kinetics of the repair of potentially lethal BrdUrd/near-UV light damage, in mid-S-phase V79-AL885D cells, expressible by 2 mM caffeine and made evident by the progressive delay in the addition of caffeine to the colony formation medium. Other details as for Fig. 1.

survival points fit the same curve as that for late-S-phase cells in Fig. 2. The results in Fig. 4 also show, however, that in the absence of caffeine late-S-phase cells have a threshold-type single-dose survival curve and that dose fractionation results in an increase in net survival. Consistent with the smaller shoulder width of late-S-phase cells compared to mid-S-phase cells (Fig. 1), the increase in survival after an interfraction interval of 60 min is less for the former compared to the latter cell age. This difference would probably be even more apparent

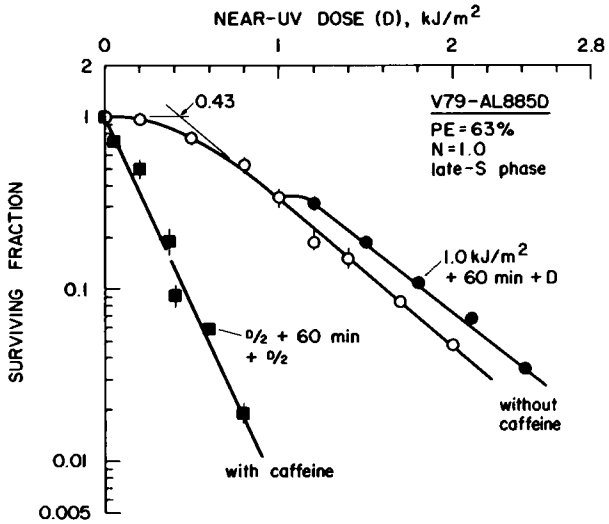

FIGURE 4 The effect of 2 mM caffeine on the fractionation response of synchronized Chinese hamster cells, clone V79-AL885D. After synchronization and incubation with BrdUrd to induce light sensitivity, cells were first irradiated at late-S phase and then incubated in the absence of 2 mM caffeine (upper curves) or in its presence after second doses (lower curve). The shoulder width of the single-dose curve is indicated, 0.43 kJ m^{-2}; its D_0 = 0.51 kJ m^{-2}. The data points are plotted according to the total doses received; for the closed circles, the total dose is 1.0 kJ m^{-2} + D (D_0 = 0.54 kJ m^{-2}), and for the squares the first and second doses were one-half of the total doses (D_0 = 0.20 kJ m^{-2}). Other details as for Fig. 1.

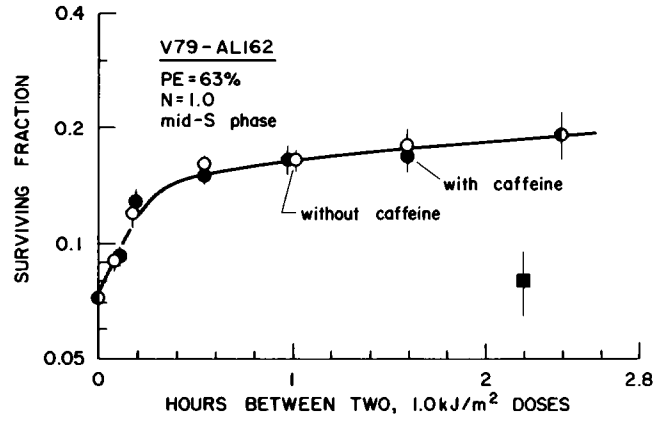

FIGURE 5 The kinetics of the repair of sublethal damage in V79-AL162 cells in mid-S phase made near-UV light sensitive by BrdUrd incorporation. 2 mM caffeine was present only between dose fractions. The filled square shows the survival after a single 2.0 kJ m^{-2} dose given at the time shown after the start of the fractionation sequence. Other details as for Fig. 1.

if survival equivalent doses were used at both ages. The results presented in Fig. 5 demonstrate for mid-S-phase cells that when caffeine is present during the interfraction interval only, no influence on two-dose survival is observed.

Thus, when the BrdUrd/near-UV light survival curve lacks a shoulder, a survival sparing does not result from dose fractionation (Fig. 4). When the survival curve has a shoulder, dose fractionation results in a survival increase the magnitude of which, for the same interfraction interval, increases with shoulder width (Figs. 1 and 4). In addition, caffeine can lead to the expression of potentially lethal damage—presumably by interfering with its repair—without affecting the repair of sublethal damage.

DISCUSSION

Although Chinese hamster cells uniformly labeled with BrdUrd, i.e., cells grown for several generations in the presence of a nontoxic concentration of BrdUrd, have a survival curve after exposures to near-UV light that is essentially exponential (Ben-Hur and Elkind, 1972 *a*), pulse-labeled cells have a damage-accumulation-type survival curve (Elkind and Whitmore, 1967) when they are irradiated in the same S phase after labeling or in the next S phase after the replication of the labeled DNA segments (Hagan and Elkind, 1979). The width of the shoulder, a measure of capacity for sublethal damage (Elkind and Whitmore, 1967), depends upon the cell's age in the S phase when it is exposed to light (Figs. 1 and 4). The foregoing is analogous to the age-dependent variations in sensitivity of Chinese hamster cells exposed to x-rays (Sinclair and Morton, 1966).

Dose fractionation of template-labeled cells does not result in an increase in survival (Ben-Hur and Elkind, 1972 *a*). This is consistent with the exponential character of the single-dose survival curve, since an exponential curve implies a lack of capacity for sublethal damage and, hence, a lack of damage to be repaired in an interfraction interval. However, pulse-labeled cells are able to repair sublethal damage (Figs. 1 and 4), consistent with the

threshold-type character of the survival curve of cells exposed during the S phase. In addition, the rate of repair appears to be quite rapid, i.e., from Fig. 5 the half repair time is estimated to be ~15 min, indeed, even more rapid than it is for x-ray sublethal damage, ~90 min (Elkind et al., 1967). Because of the dependence of shoulder width on cell age after pulse-labeling (e.g., note the quasithreshold doses in Figs. 1 and 4) and because of delays in aging induced by a first exposure, it is difficult to estimate if and when full repair of sublethal damage is achieved. This, too, is analogous to the situation in respect to the repair of x-ray-induced sublethal damage (Elkind and Redpath, 1977).

Caffeine added after light exposure during the S-phase of pulse-labeled cells shows that ordinarily such cells repair a large sector of potentially lethal damage (Fig. 2). The degree of potentiation of cell killing by caffeine depends upon cell age, as does the rate at which cells repair the potentially lethal damage expressed by caffeine. This is illustrated in Fig. 3, where the delayed addition of caffeine after light exposure shows that mid-S-phase cells repair the potentially lethal damage expressible by caffeine with an apparent half-time of 3–4 h. Because cells pulse labeled with BrdUrd become increasingly sensitive to light as they progress in their growth cycle (e.g., Figs. 1 and 4, and Hagan and Elkind, 1979), the results in Fig. 3 may be consistent with complete repair by 10 h of the potentially lethal damage expressible by caffeine, even though the survival reached by this time, 0.04, is appreciably less than that corresponding to a single dose of 1.25 kJ/m^2 as shown in Fig. 1. The possibility cannot be ruled out, however, that some damage with which caffeine can interact still remains by 10 h. In addition, little if any repair has occurred in late-S-phase cells by 8 h (Fig. 2).

Although various models of cell inactivation can be set up in which sublethal and potentially lethal damage are assumed to be functionally equivalent (e.g., see Alper [1977]), as has been shown for x-ray cell killing (Utsumi and Elkind, 1979 *b*; Ben-Hur et al., 1980), the results described do not support an unqualified association of this type for the inactivation of cells pulse-labeled with BrdUrd and exposed to near-UV light during the same S phase. Two major observations in this study illustrate the lack of a functional equivalence of these two types of cellular damage: (*a*) Caffeine present only during the interfraction interval does not alter the repair of sublethal damage (Figs. 1 and 5). (*b*) The repair of sublethal damage, e.g., in mid-S-phase cells, proceeds at a rate appreciably faster than the repair of potentially lethal damage expressible by caffeine (Figs. 3 and 5).

Added to this is the associated, internally consistent point that when caffeine posttreatment renders exponential an otherwise threshold-type survival curve, e.g., late-S-phase cells (Fig. 2), then dose fractionation is without effect (Fig. 4).

Though from the foregoing one can conclude that, in general, potentially lethal damage may not be related to sublethal damage, it is possible that in the absence of caffeine a relationship may exist between another kind of damage and damage that is functionally sublethal. Our data show that caffeine inhibits a repair process that is slow compared with the repair of sublethal damage, and that in so doing it appreciably increases the steepness of the survival curves of mid- and late-S-phase cells in addition to removing their shoulders. As a consequence, the status of sublethal damage expressible in the absence of caffeine becomes moot. What can be stated is that the interference with repair by caffeine renders BrdUrd pulse-labeled cells sufficiently sensitive to near-UV light that repair processes less critical for survival are functionally ineffective. Both the time-course of this repair and its inhibition by

caffeine (Lehmann, 1973) suggest that the process may be postreplicational repair (Rupp and Howard-Flanders, 1968). The absence of sublethal damage and its repair in template-labeled cells (Ben-Hur and Elkind, 1972 *a*) probably also result from an ineffectiveness of a postreplicational-type process in cells killed by the incomplete prereplicational repair of DNA lesions (Hagan and Elkind, 1979).

Future work is planned to examine the kinetics of repair of BrdUrd/near-UV light molecular lesions in order to clarify the relationships among various forms of potentially lethal and sublethal damage.

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