CAFFEINE ENHANCEMENT OF THE CYTOTOXIC AND MUTAGENIC EFFECT OF ULTRAVIOLET IRRADIATION IN A XERODERMA PIGMENTOSUM VARIANT STRAIN OF HUMAN CELLS

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SUMMARY

The term, xeroderma pigmentosum variants designates patients who suffer from the clinical manifestations of the disease, but whose cells have normal rates of excision repair of UV-induced lesions in DNA. In contrast to normal human fibroblasts, if cells from such variants are maintained in medium containing caffeine from immediately following exposure to UV until the survivors have undergone three doublings, the cytotoxic and mutagenic effect of UV light is dramatically increased. In the presence of 0.7mM caffeine, the slope of the UV survival curve increases ca. 3-fold. Similarly, the slope of the curve describing the frequency of mutations to azaguanine resistance induced by UV as a function of dose is ca. 3-fold steeper.

INTRODUCTION

Caffeine has the ability to interfere with DNA repair processes in bacteria (1,2) and certain non-human mammalian cells in culture (3-16). It has been reported to have no effect on the DNA repair of normal human cells (10,17). Lehmann et al., however, recently reported that caffeine interferes with the process by which cells derived from xeroderma pigmentosum (XP) variants (15) replicate DNA following ultraviolet irradiation. The term, "XP variant", has been used to designate patients who have the clinical manifestation of the disease but whose cells exhibit normal rates of incorporation of thymidine during excision repair. Experiments from our laboratory demonstrated that caffeine, at non-toxic or only slightly toxic concentrations, causes a dose-dependent increase in the cytotoxicity of ultraviolet light in cells derived from such XP variants, but not in normal human cells or in certain classical (excision repair defective) XP cells (18).

We have recently determined that cells derived from these XP variants show a higher than normal frequency of ultraviolet light-induced mutations to azaguanine resistance (19). Since these XP variant strains appear unique among diploid human cells in their response to the presence of caffeine following exposure to DNA-damaging agents, we wanted to determine what effect caffeine would have on the frequency of mutations induced in these cells by exposure to ultraviolet light. In V79 Chinese hamster cells, for example, caffeine has been shown to increase (5, 11) or decrease (5, 8, 14) the frequency of induced mutations depending on the dose of caffeine as well as the length of time the cells were exposed to caffeine. Fox has recently explained these different effects of caffeine by demonstrating that the compound lengthens the time required for the expression of mutations and pointing out that this fact has not been taken into consideration in determining the length of time between irradiation and the beginning of selection (14).

MATERIALS AND METHODS

Fibroblasts from a skin biopsy of a male XP variant, XP4BE, and from a patient with Lesch-Nyhan syndrome were obtained from the American Type Culture Collection, Rockville, Md. and were cultured and stored as described (20).

The cytotoxic effect of UV radiation was determined as described (2, 22) from the percent survival of the colony-forming ability of the irradiated cells compared to the unirradiated controls. From 10-14 replicate dishes were used to determine each point. The techniques used for irradation have been described (21). Cells were allowed no more than 10 hrs attachment time before irradiation. They were fed medium supplemented or not supplemented with freshly-dissolved caffeine (0.7mM) every other day from immediately after irradiation until the surviving cell population had undergone 3 doublings. They were then fed caffeine-free medium 3 times weekly until macroscopic colonies developed.

The techniques for determining the frequency of mutations to 8-azaguanine resistance induced by UV-irradation have been described (19, 20, 22). For each experimental point, a population of 1--3 x 10^6 cells was plated at a concentration of 8--22 x 10^3 cells per 60 mm dish. After 10-hrs for attachment, cells were rinsed, irradiated as described (20), and given medium supplemented or not supplemented with freshly-dissolved caffeine (0.7mM). The surviving cell population was allowed sufficient time to undergo 3 doublings before selection was begun. Each individual experiment included an unirradiated control population and two or more experimental irradiated populations cultured in the absence of caffeine as well as the corresponding series of an unirradiated control and two or more experimental irradiated populations cultured in the presence of caffeine during the expression period. Thus, a typical mutation experiment consisted of 800-1000 individual dishes. Fresh caffeine-free selection medium (Hams F-10 with 15% calf serum containing azaguanine 2 x 10^{-5}M)

was renewed 3 times weekly for 24-28 days until macroscopic azaguanine resistant colonies appeared. The mutation frequencies have been calculated as described in ref. 20. Since overcrowding of cells in the dishes can result in an apparent reduction in the mutation frequency caused by "metabolic cooperation" (23), in each experiment a known number of azaguanine resistant Lesch-Nyhan cells were seeded into 10 of the control and 10 of each set of experimental dishes and into 10 dishes containing no non-resistant cells to provide an estimate of the efficiency of recovery of mutants under the particular conditions used (24). The mutation frequency data have been corrected for the cloning efficiency of the cell strain determined for each individual experiment. (Cf. refs. 19, 20, 24. re. necessity of correction factors.) The cloning efficiency of XP4BE in these experiments ranged from 7-15 percent.

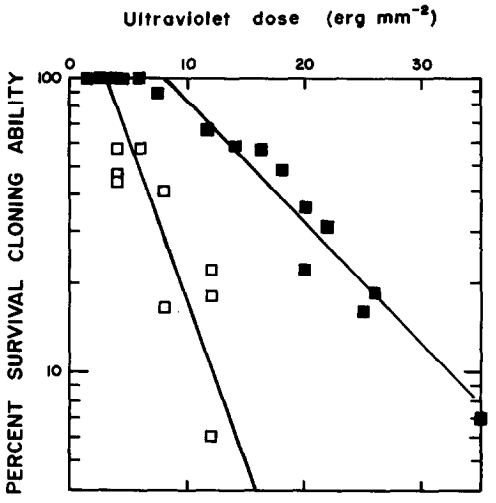

Figure 1. Effect of 0.7 mM caffeine on the cytotoxicity of UV in cells from varaint strain, XP4BE, as determined from the percent survival of the colony-forming ability. These survival data are taken directly from the cytotoxicity experiments which accompanied each of the mutagenicity experiments shown in Fig. 2. Cells were fed medium with caffeine at 0.7 mM (□) or without caffeine (■) from immediately following irradiation until surviving cells had undergone 3 cell doublings.

RESULTS AND DISCUSSION

The data presented in Fig. 1 show the significant increase in the cytotoxicity of UV light in the variant strain, XP4BE, if 0.7mM caffeine was added immediately following irradiation and cells were maintaine in medium containing caffeine until they had undergone three cell divisions. Caffeine at 0.7mM is only slightly toxic to these cells (survival of colony-forming ability, 60 percent (18)). The survival curve in Fig. 1 has been corrected for this toxicity.

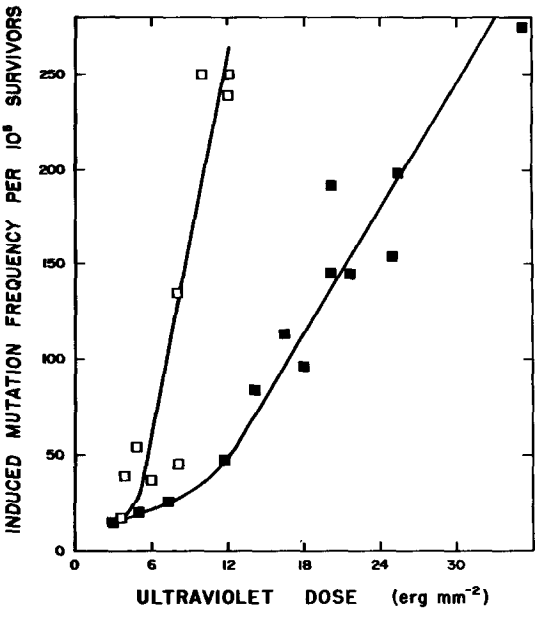

Figure 2. Effect of 0.7 mM caffeine on the frequency of mutations induced in XP4BE cells by UV as a function of dose. Surviving cells were allowed sufficient time to undergo 3 cell divisions in the absence (■) or presence (□) of caffeine before selection was begun.

Fig. 2 gives the mutagenicity data. Caffeine at 0.7mM does not induce mutations in unirradiated cells but, when present during the time between irradiation and the beginning of azaguanine selection, causes a significant increase in the frequency of mutations induced in these cells by UV light. To compare the frequency of UV light-induced mutations in the presence and absence of caffeine, it is necessary to begin azaguanine selection under equivalent circumstances, e.g., after the surviving population has undergone the same number of cell divisions. (cf. ref. 19, 25). Cell division is necessary in order to "fix" the mutation and dilute the level of preexisting enzyme so that genotypic resistance to azaguanine can be manifested phenotypically. It is also necessary to use reconstruction experiments to correct for loss of mutants caused by overcrowding of cells in the dishes (26). The length of time required for three cell divisions is dependent on the dose of ultraviolet light and the

presence of caffeine accentuates this delay (14). Therefore, the length of this period was determined for each experimental point using the following procedure:

1. Pre-determine the number of surviving cells expected for each irradiation dose by the use of previously-generated survival curves. This can be done with an accuracy greater than 90 percent. In mutagenicity experiments, radiation doses which cause survivals less than 15 percent are not used because the large number of dead cells interferes with accurate cell count.
2. Plate cells at an initial density low enough that the surviving population can undergo three cell divisions (increase 8-fold) without causing the recovery of mutants to fall below 30 percent because of overcrowding. (cf. ref. 24).
3. Determine, by use of a Coulter counter, the number of cells attached to the dish at the time of irradiation.
4. Irradiate the population within ten hrs after plating to obtain accurate survival data.
5. By the use of a Coulter counter, determine every 12 to 24 hrs the number of attached cells per dish at each radiation dose and calculate the number of cell divisions undergone by surviving cells receiving that dose.

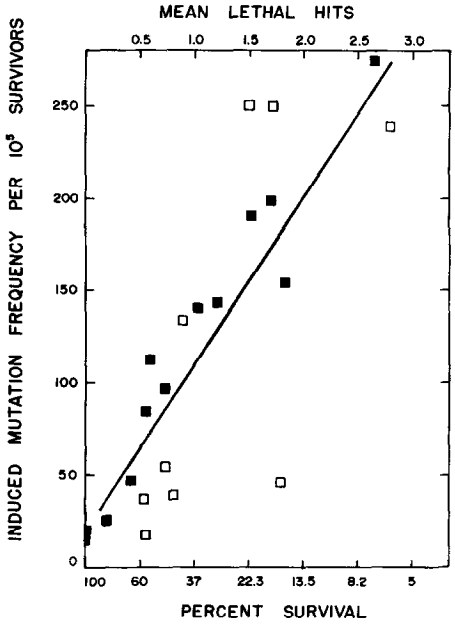

Figure 3. Relationship between frequency of UV light-induced mutations and its cytotoxic effect in cells which had 0.7 mM caffeine present (□) or absent (■) during the period following irradiation before selection was begun. The data are taken from those presented in Figs. 1 and 2. Assuming a random (Poisson) distribution of lethal events in the population, a survival of 37, 13.5, 8 percent corresponds to an average number of lethal events per cell of 1, 2 and 3, respectively. The line is that calculated by the least squares method from all the data, except the two points on the ordinate (100 % survival).

Our results with this XP variant strain, showing that caffeine increases the mutagenic effect of ultraviolet light, are in accord with those of Fox (14) with V79 Chinese hamster cells and support her conclusions drawn from that mammalian cell system. We compared the induced mutation frequencies in caffeine-free and caffeine-treated cultures at equal survival levels as shown in Figure 3. The line drawn in Figure 3 represents the least squares line for all the experimental points except the two points at doses which resulted in a survival level of 100 percent. It will be seen that there is no major difference between the caffeine-treated and caffeine-free populations. These results suggest that whatever caffeine is doing to increase the cytotoxic effect of the radiation is also affecting the production of radiation-induced mutations. An explanation consistent with our results has been suggested by Fox (14) following the hypothesis of Roberts et al. (11). This hypothesis suggests that most of the mutations induced by ultraviolet light in this strain are the result of small deletions arising during replication of DNA across from unexcised lesions, that caffeine by interfering with DNA replication in some way increases this damage, and that such interference results in both increased cell death and increased frequency of induced mutations in the caffeine treated population. Experiments to determine the types of mutations produced are underway to test this hypothesis.

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