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INDUCTION OF MUTATIONS BY 5-FLUORODEOXYURIDINE: A MECHANISM OF SELF-POTENTIATED DRUG RESISTANCE?

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In medium containing concentrations of deoxycytidine that occur in vivo, 5-fluorodeoxyuridine induced mutation frequencies 6-90 fold greater than spontaneous mutant frequencies at two genetic loci in Chinese hamster cells. In medium lacking deoxycytidine, 5-fluorodeoxyuridine was more cytotoxic but induced no mutants. Hence, the effectiveness of cancer therapy with 5-fluorodeoxyuridine may be limited by self potentiated development of 5-fluorodeoxyuridine-resistant mutants and enhanced and prolonged by manipulating deoxycytidine metabolism.

The success of cancer chemotherapy is often limited by the development of drug resistance in neoplastic cells. Two categories of mechanisms by which the resistance develops can be envisaged: 1) selection of pre-existing resistant cells; 2) induction of heritable alterations in the phenotypes of progeny of the original tumor cells (1). The second category should be especially relevant when the drugs used are known mutagens, such as alkylating agents, which may self-potentiate the emergence of resistant mutant cells. Although, this category of mechanisms is not normally considered to be important in the development of resistance to antimetabolites such as 5-fluorodeoxyuridine (FdUrd), treatment of cells with FdUrd does result in a powerful inhibition of thymidylate synthetase, the only de novo source of deoxythymidine triphosphate (dTTP). Therefore, FdUrd can be expected to decrease the availability of TTP for DNA synthesis (2) and hence effectively to perturb intracellular deoxyribonucleotide pools. Since deoxyribonucleotide pool imbalances have been shown to be mutagenic in cultured mammalian cells (3), it is possible that FdUrd might, by this mutagenic mechanism,

AG, 8-azaguanine; dCyd, deoxycytidine; dCTP, deoxycytidine triphosphate; FdUrd, 5-fluoro-2'-deoxyuridine; HAT, medium containing 10^{-5}M hypoxanthine, 10^{-6}M aminopterin and 5 x 10^{-5}M thymidine; oua, ouabain; r, resistant; s, sensitive; TG, 6-thioguanine; TTP, deoxythymidine triphosphate.

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cause the development of FdUrd resistant cells. In fact, it has previously been reported that in Chinese hamster V79 cells treated in S phase with FdUrd, 8-azaguanine resistant (AG^r) mutant-frequencies about 2 fold greater than the spontaneous frequencies occurred (4). However, in standard assays for mutagens in bacteria (5) and V79 cells, (6), FdUrd was not mutagenic.

We now report experiments in which FdUrd is strikingly mutagenic, producing mutations detected by the well characterized markers 8-azaguanine (AG)-, 6-thioguanine (TG)- and ouabain (oua)-resistance (6) in asynchronous Chinese hamster V79 cells. The rationale for our experiments was as follows: In the presence of imbalanced precursor pools, mammalian DNA polymerases can continue to catalyze DNA synthesis, producing replication errors in the process (7). Therefore, treating Chinese hamster cells with FdUrd in medium containing dCyd, which increases intracellular deoxycytidine triphosphate (dCTP) concentrations (8), it should be possible to produce conditions such that the cells' DNA polymerases have sufficient pyrimidine deoxyribonucleotides to catalyze DNA synthesis, while the dCTP/dTTP ratio in the cells is increased because of the inhibition of thymidylate synthetase. Under these conditions, an increasing amount of DNA replication errors and mutations should occur.

Materials and Methods

Chinese hamster V79 cells were cultured in Dulbecco's medium supplemented with dialyzed fetal calf and calf serum and plated for cytotoxicity and mutagenesis assays as previously described (9). At 6 hr after plating the cells were treated for 2 hr with FdUrd (Sigma Chemical Co.) in medium containing or lacking 10 μM dCyd (Sigma) and, after being washed twice with medium, were incubated in medium with dCyd or without dCyd, respectively. Cytotoxicity was determined by the plating efficiency methods described previously (9). For measurements of AG-resistant and TG-resistant mutant frequencies, previously described methods, with an expression time of 6 days and replating of the cells (9), were used, and mutant selection was accomplished with 40 μg/ml AG or 10 μg/ml TG in medium without dCyd. For measurements of Oua^r mutant frequencies with expression times of 1-2 days the cells were not replated, but for longer expression times cells were replated (10^5 cells/100 mm dish), and 10^{-3} M ouabain in medium without dCyd was used for mutant selection (6).

Results and Discussion

In medium containing 10 μM dCyd, 0.15--0.5 μM FdUrd produced a dose dependent increase in AG^r mutant frequencies. In this medium, 0.5 μM FdUrd produced 10.3 $\pm$ 3.4 AG^r mutants/10^5 survivors, which is more than 10 fold greater than the spontaneous mutant frequency (0.7 $\pm$ 0.2 mutants/10^5 survivors). Moreover, in medium

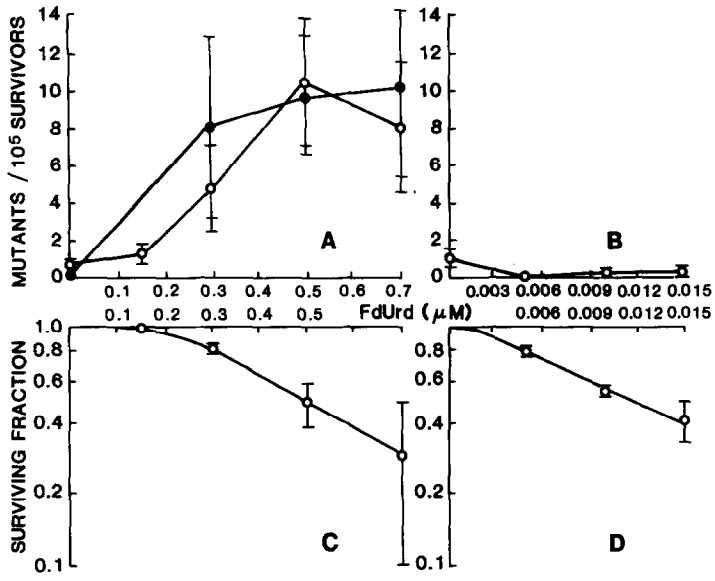

Figure 1: AG^r (o) and TG^r (●) mutant frequencies (A and B) and cytotoxicities (C and D) induced by 2 hr treatments with FdUrd in V79 cells in medium containing (A and C) or lacking (B and D) 10 μM dCyd. Each data point is the mean ± standard error of 2--5 independent experiments.

containing 10 μM dCyd, 0.3--0.7 μM FdUrd produced 50--90 fold increases in TG^r mutant frequencies over the spontaneous TG^r mutant frequencies (0.1 ± 0.1 mutants/10^5survivors) in this medium (Fig. 1A). In parallel experiments, in medium that did not contain dCyd, 6.8 μM MNNG induced 63.8 ± 18 AG^r mutants/10^5 survivors (5 experiments) and 65 ± 32 TG^r mutants/10^5 survivors (2 experiments). Thus, 0.5 μM FdUrd, in medium containing 10 μM dCyd, produced as many AG^r and TG^r mutants as would be expected to be induced by 1.0 μM MNNG. However, 10 μM dCyd alone did not increase the spontaneous AG^r mutant frequency, which was 1.0 ± 0.4 mutants/10^5 survivors (2 experiments) in medium without dCyd.

Figs. 1C and 1D show that 0.005--0.015 μM FdUrd in medium without dCyd were as cytotoxic as 0.3--0.7 μM FdUrd in medium with 10 μM dCyd. However, neither in our experiments (Fig. 1B) nor in previous studies (4,6) was FdUrd found to induce AG^r mutants in asynchronous V79 cells in medium without dCyd.

From dishes containing cells treated with 0.5 μM FdUrd in medium containing 10 μM dCyd, 5 colonies of cells that grew in 40 μg/ml AG were isolated and subcultured in medium without AG. The cells from all 5 colonies were unable to grow in medium

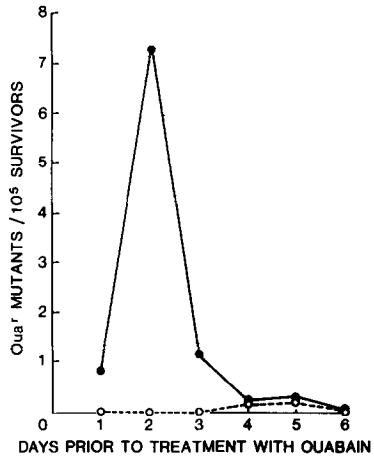

Figure 2: Oua^r mutant frequencies, in medium containing 10 μM dCyd, of cells treated (●) or not treated (o) with 0.5 μM FdUrd for 2 hr. Data from one representative experiment are shown.

containing 10^{-5}M hypoxanthine, 10^{-6}M aminopterin and 5 x 10^{-5}M thymidine (HAT) but grew to confluence and formed colonies (plating efficiency 43.8 ± 8.6%) in medium containing 40 μg/ml AG. This HAT^s, AG^r phenotype, which is characteristic of hypoxanthine (guanine) phosphoribosyl transferase deficient mutants (6), was retained by cells from all 5 colonies for at least 30 population doublings. Furthermore, with an expression time of 2 days (Fig 2), 4.0 ± 1.4 oua^r mutants/10^5 survivors (4 experiments) were produced by treating cells with 0.5 μM FdUrd in medium containing 10 μM dCyd, whereas the spontaneous oua^r mutant frequency in this medium was 0.6 ± 0.5 mutants/10^5 survivors (4 experiments). Thus Figs. 1 and 2 show that in medium containing 10 μM dCyd, FdUrd induced mutations at two genetic loci in V79 cells, but that in medium that did not contain dCyd, FdUrd was 30 fold more toxic but did not induce mutations.

The induction by FdUrd of mutants in medium containing dCyd is consistent with the rationale for our experiments, although further studies are required to fully elucidate the mechanism. Nevertheless, our findings, of mutant frequencies induced by FdUrd plus dCyd that are an order of magnitude greater than the spontaneous frequencies, suggests that self potentiated induction of FdUrd resistant mutants could be an important mechanism by which FdUrd resistance develops in mammalian cells exposed to FdUrd and concentrations of dCyd ~ 10 μM. Such concentrations of dCyd

have been found in rat plasma and in the ascites fluid of mice bearing L1210 ascites tumors, and up to 4μM dCyd has been found in human plasma (10), indicating that self potentiated induction of FdUrd resistant mutants could occur in vivo. Thus, treatments in which FdUrd is administered in combination with inhibitors of dCyd phosphorylation may effectively inhibit the emergence of FdUrd-resistant cells.

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