Sequence-dependent Synergism between Dichloromethotrexate and 5-Fluorouracil in a Human Colon Carcinoma Cell Line

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ABSTRACT

Prior to their use in patients with hepatic metastasis of colon cancer, we studied the cell-killing effects of 5-fluorouracil, 5-fluoro-2'-deoxyuridine, 3',5'-dichloromethotrexate (DCM), and combinations of these fluoropyrimidines with DCM on a human colon carcinoma cell line (HCT-8). Sequential exposure of these cells to DCM followed by 5-fluorouracil resulted in significant synergistic cell killing at every dose and time studied, and the synergy was more evident with increasing doses of both drugs. In contrast, simple additive effects were observed when DCM was given together with or following 5-fluorouracil. When the antifolate was given before 5-fluoro-2'-deoxyuridine, simple additive effects, rather than synergy, were observed. Mild antagonism and even strong antagonism were found when 5-fluoro-2'-deoxyuridine preceded DCM administration or when the cells were exposed to the drugs simultaneously, respectively.

INTRODUCTION

The fluoropyrimidines FUra and FdUrd, as well as DCM, are potentially useful drugs for the treatment of patients with hepatic metastasis due to gastrointestinal cancer (12). These drugs are less toxic to nondividing hepatic parenchymal cells than to dividing tumor cells; in addition, they are extensively metabolized by normal liver (3,6) and thus cause less systemic toxicity when administered via the hepatic artery as compared to i.v. administration.

Since the sequential use of MTX and FUra has been shown to give synergistic tumor cell kill in vitro (1,5) as well as in vivo (2,11,15) experimental systems and is in clinical use (8,16,18), we studied the cell-killing effects of the more rapidly metabolized MTX analogue DCM alone and in combination with FUra and FdUrd on the human colon tumor cell line HCT-8, prior to use in patients with hepatic metastasis of colon carcinoma.

MATERIALS AND METHODS

FUra and FdUrd were obtained from commercial sources and were the materials available for clinical use. DCM was a gift of Lederle Laboratories and was greater than 95% pure, as measured by high-pressure liquid chromatography.

Tissue culture cells utilized in this investigation were from the continuously growing human colon adenocarcinoma line HCT-8 (19). Cells were maintained in 25-sq cm sterile plastic flasks (Costar, Cambridge, Mass.) as monolayer cultures in Roswell Park Memorial Institute Tissue Culture Medium 1640 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% horse serum and subcultured weekly. Under these conditions, the doubling time was 18 hr.

Clonogenic Assay. A monolayer clonal growth technique was used (17). Cell suspensions were obtained by trypsinization of stock cultures for 5 min. A high degree of monocellular dispersion was obtained by pipette trypsinized cells followed by passages through needles of decreasing size, from 19 to 25 gauge. Portions of 500 cells in 5 ml of medium containing 10% horse serum were pipetted into sterile 60-mm Petri dishes (Costar) and incubated at 37° and 100% humidity, with 7.5% CO2. Eighteen hr later, when the cells were firmly attached to the bottom of the Petri dish but had not yet divided, 0.1 ml of an appropriate dilution of drug in PBS was added to each dish. Control dishes received the same volume of PBS. After the designed incubation period, the medium was decanted, the dishes were washed twice with 5 ml of PBS, and 5 ml of fresh medium were replaced. In sequential treatment experiments, this procedure was repeated after the exposure to the second drug. Ten days after the initial plating, colonies were stained with orcein, and those colonies with more than 30 cells were counted at x10 using a dissecting microscope. Each experimental point was determined in triplicate with 5 replicate controls, and experiments were repeated at least 3 times.

Experimental Design. Times and sequences of exposure to DCM, FUra, and FdUrd are outlined in Tables 1 and 2. The tables also show some of the doses selected for drug combination experiments; in general, they were chosen to give a cell kill between 30 and 70%, so that maximum synergy could be observed. The quantitation of drug interaction was done by calculating the ratio between the product of the survival fraction of each individual drug and the survival fraction of the drug combination (14). Values greater than one were considered to show synergism, those less than one, antagonism, and those of about one, an additive effect.

RESULTS

Cell Kill as a Function of Concentration and Time of Exposure to DCM, FUra, and FdUrd. HCT-8 cell kill produced by DCM, FUra, and FdUrd as a function of concentration and time of exposure is summarized in Charts 1 to 3. The ED50 for DCM and FUra decreased by one log when HCT-8 cells were exposed to either of these drugs for 24 hr as compared to 4 hr. Cell kill by FdUrd was even more time dependent; the ED50 was 300 times lower when cells were exposed to this drug for 24 hr, as compared to a 4-hr exposure. The ED50 values for a 24-hr exposure to FdUrd, DCM, and FUra were 0.006, 0.4, and 3.5 μM, respectively. While exposure of these cells for longer than 24 hr produced a substantial increase in cell kill in the case of DCM, relatively modest increments of cell kill were obtained with FUra and FdUrd.

DCM-FUra Combinations. A summary of the results obtained with combinations of DCM with FUra is shown in Chart 4. The ED50 values for DCM-FUra combinations were lower than the ED50 for either drug when given alone (Charts 1 and 2). However, the ED50 values for combinations of DCM with FdUrd were not substantially lower than the ED50 for FdUrd alone (Chart 3). The results are consistent with the interpretation that the DCM and FdUrd combinations are additive.

DCM-FdUrd Combinations. A summary of the results obtained with combinations of DCM with FdUrd is shown in Chart 5. The ED50 values are low for combinations of DCM and FdUrd, with synergistic cell killing observed at every dose and time studied, and the apparent ED50 values decrease progressively as the drug combinations become more synergistic. The ED50 values for combinations of DCM, FUra, and FdUrd are lower than those for any of the drugs given alone (Charts 2 and 3). The results are consistent with the interpretation that the DCM-FUra-FdUrd combinations are synergistic.
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Table 1
Percentage of survival of HCT-8 cells exposed to DCM, FUra, and their combinations
Survival was measured by a monolayer clonal growth technique and expressed as a percentage of untreated controls (see "Materials and Methods").

A. 4-hr exposure: sequence-dependent effects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM 0.3 µM</td>
<td>93.7 ± 5.7a</td>
</tr>
<tr>
<td>1.0 µM</td>
<td>69.8 ± 5.6</td>
</tr>
<tr>
<td>3.0 µM</td>
<td>50.9 ± 5.9</td>
</tr>
<tr>
<td>FUra (30 µM)</td>
<td>5.9</td>
</tr>
<tr>
<td>DCM (1.0 µM) + FUra (30 µM)</td>
<td>35.1 ± 3.4 (1.0)b</td>
</tr>
<tr>
<td>DCM (3.0 µM) + FUra (30 µM)</td>
<td>23.7 ± 2.5 (0.9)</td>
</tr>
<tr>
<td>FUra (30 µM) → DCM (0.3 µM)</td>
<td>53.6 ± 7.8 (1.1)</td>
</tr>
<tr>
<td>FUra (30 µM) → DCM (1.0 µM)</td>
<td>41.9 ± 3.5 (0.9)</td>
</tr>
<tr>
<td>FUra (30 µM) → DCM (3.0 µM)</td>
<td>26.3 ± 5.6 (1.0)</td>
</tr>
<tr>
<td>DCM (0.3 µM) → FUra (30 µM)</td>
<td>27.9 ± 9.0 (1.8)</td>
</tr>
<tr>
<td>DCM (1.0 µM) → FUra (30 µM)</td>
<td>20.2 ± 9.8 (2.3)</td>
</tr>
<tr>
<td>DCM (3.0 µM) → FUra (30 µM)</td>
<td>5.8 ± 5.0 (2.2)</td>
</tr>
</tbody>
</table>

B. Sequential DCM → FUra for various time periods

<table>
<thead>
<tr>
<th>% of survival</th>
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<tbody>
<tr>
<td>DCM (0.03 µM) → FUra (0.03 µM)</td>
</tr>
<tr>
<td>DCM (0.3 µM) → FUra (0.3 µM)</td>
</tr>
<tr>
<td>DCM (1.0 µM) → FUra (1.0 µM)</td>
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<tr>
<td>DCM (3.0 µM) → FUra (3.0 µM)</td>
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<tr>
<td>DCM (1.0 µM) → FUra (0.3 µM)</td>
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<tr>
<td>DCM (3.0 µM) → FUra (0.3 µM)</td>
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* Mean ± S.E. of at least 3 experiments.
Numbers in parentheses, coefficient of drug interaction. Only one value available.

These data clearly demonstrate synergistic cell kill on HCT-8 cells when DCM is given before FUra. These results are not surprising, in view of the data of Benz and Cadman (1), who also noted synergistic cell killing with sequential use of Mtx and FUra on the same cell line. The exact mechanism of this synergy observed in several experimental systems (2, 5, 11) is not entirely clear, but increased FUra nucleotide formation has been shown to correlate with increased levels of intracellular phosphoribosyl-1-pyrophosphate that result when certain cells are treated with DCM-FdUrd Combinations. Similar concentrations of DCM and 6 concentrations of FdUrd, ranging between 1 nM and 1 µM, were tested. Only data obtained with 0.003 and 0.3 µM FdUrd are shown (Table 2). A simple additive effect on cell kill was observed when DCM preceded FdUrd. Conversely, a mild antagonism occurred with the sequence FdUrd-DCM; the exposure to these drugs given simultaneously produced a very strong antagonism (Table 2A).

DISCUSSION

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DCM and Fluoropyrimidines

FUrA may be valuable for intraarterial therapy of hepatic metastasis of gastrointestinal carcinoma. When given by this route, these drugs are both metabolized moderately rapidly, and appropriate dose rates would not produce enhanced cell kill on normal renewal tissues, a problem encountered when MTX and FUra have been administered i.v. in some studies using these drugs in sequence (13, 20).

Since these data were obtained with a human colon carcinoma line propagated in culture, caution must be exercised in extrapolating these results to the clinical situation. Nevertheless, these data should be kept in mind when designing clinical protocols and should encourage further in vitro and in vivo testing of these regimens.

REFERENCES

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