Cancer Chemother Pharmacol (1989) 25: 124–130

Cancer Chemotherapy and Pharmacology

Effect of dipyridamole on fluorodeoxyuridine cytotoxicity in vitro and in cancer patients*

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Summary. Dipyridamole (DP) has previously been studied both in vitro and in vivo in combination with various antimetabolites, including methotrexate and 5-fluorouracil (5FU). We evaluated in vitro and clinically the effects of adding DP to fluorodeoxyuridine (FUDR) in colorectal cancer. Using a human colony-forming assay, we observed that 0.05 μM DP increased the cytotoxicity of FUDR by a median of 33.5-fold vs 1.5-fold for 5FU against human colon-cancer cell lines. The mechanism of the DP-enhanced antitumor activity of FUDR is not completely understood but appears to be related to a profound inhibition by DP of thymidine accumulation in and FUDR efflux from colon-cancer cell lines. On the basis of these in vitro results, 28 patients with metastatic colon cancer were entered in a clinical trial of monthly courses of 0.1 mg/kg FUDR daily for 14 days and 75 mg oral DP 5 times daily for 14 days starting on the 3rd day of continuous i.v. FUDR infusion. The pharmacokinetics of DP was studied in three patients; the results showed that 98% of total serum DP was protein-bound and that free DP levels were significantly lower than the concentrations necessary for the expected in vitro DP/FUDR modulation. Treatment was well tolerated, with only 12 patients developing mild to moderate toxicity. Of 27 evaluable patients, 4 achieved a partial response that lasted 2, 3, 5, and 6+ months. This relatively low response rate (15%), which is similar to that achieved with FUDR alone, may be explained by the low steady-state plasma concentrations of free DP achieved in our patients. Other means of DP administration, such as i.v., i.a., and i.p. injection, may be required to achieve free DP concentrations necessary for successful biochemical modulation of FUDR in patients.

Introduction

Colorectal carcinoma is among the most common malignancies in Western countries and is the second leading cause of cancer death in the United States [16]. Liver metastases are identified in approximately 20% of patients at diagnosis and represent the only site of metastatic disease in one-half of these patients [9]. There is no satisfactory therapy for metastatic colorectal carcinoma. 5-fluorouracil (5FU) and fluorodeoxyuridine (FUDR), a derivative of 5FU, are the most effective and most commonly used agents; they are associated with an average objective response rate of approximately 20% [9].

Strategies to improve response and survival in this disease involve the evaluation of new drugs and development of innovative methods aimed at increasing the efficacy of agents known to have activity in colon cancer (such as 5FU and FUDR). The latter approach has received considerable attention in recent years. A number of studies have used dipyridamole (DP), a nucleoside transport inhibitor, in combination with methotrexate [17], PALA [2], acivicin [19], *N*-10-propargyl-5,8-dideazafolic acid [11], and 5FU [5].

Grem and Fischer [5, 6] have studied the effect of DP on 5FU cytotoxicity against HCT 116, a human colon-cancer cell line. These authors showed that DP significantly altered the metabolism of 5FU, produced an increase in fluorodeoxyuridine phosphate (FdUMP) levels, and inhibited FUDR efflux [5]. On the basis of these data, we evaluated the effect of DP on 5FU and FUDR cytotoxicity against several human colon-cancer cell lines and conducted a phase I–II clinical and pharmacokinetic study of oral DP plus i.v. FUDR in patients with metastatic colorectal cancer.

Materials and methods

In vitro studies

Drugs. DP (Persantine) in powder form was a gift from Boehringer Ingelheim Ltd. (Ridgefield, Conn). FUDR was purchased from Hoffman-LaRoche (Nutley, NJ) and 5FU was obtained from Adria Laboratories (Columbus, Ohio). FUDR was reconstituted with saline at a concentration of 1 mg/ml, aliquoted, and stored at −80° C. DP was reconstituted in 100% DMSO at 100 μM, aliquoted, and stored at −80° C. 5FU was diluted with normal saline and stored at room temperature. [^{3}H]-FUDR (20 Ci/mM, 1 mCi/ml) was obtained from Moravek Biochemicals, Inc. (Brea, Calif), and [^{3}H]-thymidine (52 Ci/mM) was purchased from Amersham International (Arlington Heights, Ill).

* Supported in part by grants CA17094, CA23074, and CA39629 from the National Institutes of Health, Bethesda, Md 20205, and a grant from the Arizona Chronic Disease Commission. HSG is a recipient of an American Cancer Society Career Development Award

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Cell lines. Colon-cancer cell lines WiDr (derivative of HT29), Colo 205, HCT 15, LoVo, DLD-1, Colo 320 DM, SW 480, and SW 48 were obtained from ATCC (Rockville, Md). Cell lines were maintained in RPMI 1640 and supplemented with the same lot number of 10% nondialyzed fetal bovine serum with penicillin (100 IU/ml), streptomycin (100 μg/ml) and L-glutamine (2 m*M*) (Irvine, Santa Ana, Calif) at 37° C in 5% CO_2. Cell lines were harvested with trypsin-EDTA (0.025%) (Gibco, Grand Island, NY), with the exception of Colo 205 and Colo 320 DM, which were removed from flasks using a rubber policeman. Cell lines were used for no more than four passages after their removal from frozen stocks.

Tumor clonogenic assays. All cell lines were harvested during the exponential growth phase and processed into a single-cell suspension. The number of cells used in the clonogenic assay ranged from 5,000 to 20,000 cells/35-mm tissue-culture dish (NUNC, Kamstrup, Denmark), depending on the cell line.

Drug aliquots were removed from the −80° C freezer, thawed, and immediately diluted to appropriate concentrations with unsupplemented RPMI 1640. A DP control was run in each experiment at each concentration used for drug combinations. The final dimethylsulfoxide (DMSO) concentration for DP was 0.05% following dilution. Drugs were added at the time of plating to sufficient numbers of cells in supplemented RPMI that the clonogenic assay could be plated in triplicate at each drug concentration and control, as previously described [7, 8, 14]. Plates were incubated for 7–10 days at 37° C in 5% CO_2, after which 1 ml 1 mg/ml INT-[2-[*p*-iodophenyl-3-(*p*-nitrophenyl)]-5-phenyltetrazolium chloride] (Pfaltz and Bauer, Stamford, Conn) stain was added to the plates for 24 h [15]. Colonies were then counted by a Bausch and Lomb Image Analyzer [10].

Bone marrow samples. Bone marrow samples were obtained from normal healthy adult volunteers following written informed consent according to institutional guidelines. The marrow aspirate was diluted 1:5 with McCoy's 5A medium. The dilute bone marrow (2–5 ml) was then mixed with 2 ml 0.9% saline and 2 ml 6% gentian dextran (Travenol) and allowed to sediment at room temperature for 0.5–1 h.

The supernatant above the red blood cell layer was removed and washed twice with McCoy's 5A. Cells were then counted and the concentration was adjusted to 2.4×10^6 cells/ml in supplemented RPMI 1640. The clonogenic assay was carried out as previously described [7], using 400,000 cells/plate, with the exception of the addition of 100 μl GCT (giant-cell-tumor)-conditioned medium (Gibco, Grand Island, NY). Colonies were manually counted using an inverted microscope [12].

[^{3}H]-FUDR cellular efflux studies. Colo 205 cells (2×10^6 cells/ml) in supplemented RPMI 1640 were exposed to 2.0 μ*M* FUDR for 2 h at 37° C with gentle vortexing at 15-min intervals. The cells were then pelleted, washed three times in cold phosphate-buffered saline (PBS), and resuspended in supplemented RPMI 1640 media at 37° C. Incubation aliquots (1 ml) of the cells containing 2×10^6 cells were placed in 15-ml conical tubes. The samples were then divided into two groups: the first group contained only FUDR and served as a control, whereas DP was added to the second group to a final concentration of 5×10^{-8} *M*. At 0, 30, 60, and 120 min incubation, three tubes from each of the groups were processed to evaluate the amount of [^{3}H]-FUDR contained/10^6 tumor cells. The cells were washed in cold PBS to remove extraneous [^{3}H]-FUDR and then layered over 0.5 mg silicone oil in microfuge tubes. The cells were then centrifuged for 1 min at 12,000 *g*. The tip of the microfuge tube was snipped to obtain the cell pellet, which then was digested in 250 ml, 2 *M* sodium hydroxide for 4 h. The cells were neutralized with 0.5 *N* acetic acid and counted for [^{3}H] content/10^6 cells.

DP assay. The high-performance liquid chromatographic (HPLC) assay for DP in plasma was carried out at the Department of Human Oncology, University of Wisconsin, as described by Wolfram and Bjornsson [18], with minor modifications. The assay used a Spectra-Physics SP8770 pump, Gilson Spectroglo Fluorometric detector (285 nm excitation, 470 nm emission), LDC/Milton Roy model CI-10B integrator, and a Rheodyne injector with a 50-μl loop. A Waters Bondapak C-18 reverse-phase column was used with a mobile phase of 65% methanol: 35% water containing 5 m*M* heptane sulfonic acid and 0.1% acetic acid. At a flow rate of 1.8 ml/min, the retention time for DP was 4.1 min and that of the internal standard, RA 433, was 3.1 min. DP was extracted from 0.5 ml plasma with 10 ml 5% isobutanol in dichloromethane after the addition of 100 ng internal standard and 1.0 ml 2 *N* NaOH. The extract was dried and reconstituted in 100 μl mobile phase for chromatographic analysis.

Recoveries of DP and RA 433 averaged 67% and 81% and were linear between 0.5 and 20 μ*M*. The addition of known amounts of DP to patient samples yielded values averaging 98% of the expected amounts for both high (>10 μ*M*) and low (<5 μ*M*) levels of DP. To measure plasma levels of unbound DP, plasma ultrafiltrates were prepared using Amicon Centrifree filter units. The standard curve, obtained by adding DP to ultrafiltrates of normal plasma, was linear from 10 to 200 n*M*. These low concentrations of DP could be detected using the HPLC system previously described, with the addition of a more sensitive fluorometric detector (Shimadzu model RF-530) with excitation optimized at 286 nm and emission at 497 nm. The assay had a precision of <7%. Specificity of the method was demonstrated by the standard addition of known amounts of DP to previously assayed patient ultrafiltrates (yielding results that averaged 105% of the expected values).

Clinical study

Patient population. A total of 28 patients who had received no prior chemotherapy were entered in the study. All patients had bidimensionally measurable disease, histologically proven metastatic colorectal carcinoma, total serum bilirubin levels of <2.0 mg/dl, and serum creatinine levels of <1.5 mg/dl.

Drug supplies. FUDR was purchased from Hoffmann-LaRoche (Nutley, NJ) and DP was purchased from Geneva Generics (Broomfield, Colo).

Table 1. Biologic characteristics of human colon-cancer cell lines used in DP-fluoropyrimidine studies

Cell line	Cellular doubling time in suspension culture (h)	Tumor plating efficiency in soft agar (%)
LoVo	47.5	8
SW 48	55.4	5
SW 480	44.6	26
Colo 320 DM	37.0	13
HCT-15	41.4	8
Colo 205	24.6	14
DLD-1	40.2	30
WiDr	28.6	20

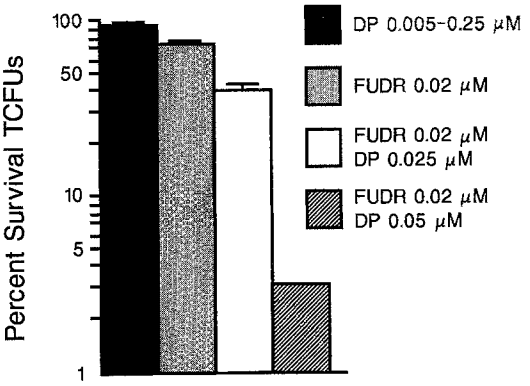

Fig. 1. Enhancement of FUDR cytotoxicity against the LoVo human color-cancer cell line with stepwise increases in DP concentrations. *Error bars* represent SE

Study design. Written informed consent was obtained from all patients according to institutional guidelines. Prior to treatment initiation, a Port-A-Cath$_{TM}$ (Pharmacia Nu Tech, Inc., Piscataway, NJ) was installed in a subclavian vein in all patients to enable continuous i.v. outpatient treatment with a portable pump. Treatment consisted of FUDR given by continuous i.v. infusion at a dose of 0.10 mg/kg daily for up to 14 consecutive days every 4 weeks, and oral DP at a fixed dose of 75 mg five times daily for 14 days (i.e., the highest DP dose tolerated for 14 consecutive days without any dose reduction in preliminary studies), starting on the 3rd day of FUDR infusion and ending 3 days after completion of FUDR treatment.

DP administration was delayed for 3 days after the initiation of the FUDR infusion because the results of our in vitro cellular FUDR influx studies (unpublished data) and those of Grem and Fischer [5] revealed DP inhibition of cellular FUDR influx. DP administration was continued for an additional 3 days after the completion of FUDR infusion in an attempt to decrease FUDR cellular efflux.

Toxicity was graded according to previously reported Southwest Oncology Group criteria [13]. Patients experiencing grade III or IV toxicity underwent a treatment delay until toxicity cleared; their treatment resumed with a reduction in the duration (i.e., number of consecutive days) of FUDR infusion by 25% and 50%, respectively.

Tests carried out prior to the beginning of treatment included complete blood counts with platelet and differential counts, SMA-20 chemistry panel, carcinoembrionic antigen (CEA), chest X-ray, and computerized tomography or abdominal ultrasonography as warranted to assess tumor status. Patients were examined every 4 weeks, at which time the above-mentioned blood tests were repeated. Objectively measurable disease was evaluated every 2 months, or earlier if evidence of progression was suspected. Patients whose disease progressed during therapy were removed from the study. In responders, treatment was continued until evidence of disease progression.

Response criteria. A complete response (CR) was defined as the complete disappearance of all measurable and evaluable disease as well as the normalization of CEA values for at least 1 month. A partial response (PR) was defined as a reduction of $>50\%$ in the size of all measurable lesions for at least 1 month. Patients who did not fulfill these criteria were considered to be nonresponders. Progression was defined as the appearance of new lesions or an increase of $>25\%$ in existing lesions. Duration of response was measured from the time the patient fulfilled criteria for response until progression of disease.

Blood sampling for DP pharmacokinetics. Blood samples (10 ml) were obtained from 3 patients who were randomly selected from the 27 who were treated with FUDR plus DP. Samples were collected in heparinized tubes at 8 a.m. (i.e., 8 h after the last DP dose) and 12 noon (i.e., 4 h after the last DP dose) during 2 days of FUDR therapy (i.e., day 5 and day 9 or 12). The blood samples were immediately placed on ice and centrifuged at 3,000 *g*, and the separated plasma was frozen in polyethylene tubes at $-20°$ C until assayed for free and bound DP.

Results

In vitro studies

In Table 1 are listed the biologic characteristics of the eight colon-cancer cell lines used in the evaluation of the DP-fluoropyrimidine interaction studies. The tumor-cell doubling times in suspension culture ranged between 24.6 and 55.4 h, and their plating efficiencies in soft agar ranged between 5% and 30%.

Effect of DP on FUDR cytotoxicity against human colon-cancer cell lines. The interaction between DP and FUDR is shown in bar-graph form in Fig. 1. When the FUDR concentration was held constant at 0.02 μM (continuous exposure), progressively increasing concentrations of DP from 0.005 to 0.25 μM were associated with stepwise enhancement of FUDR cytotoxicity against the LoVo tumor cell line. A DP concentration of 0.05 μM was consistently associated with an enhancement of FUDR inhibition of tumor colony-forming units of >1 log, as shown in Fig. 2. The addition of DP (fixed dose, 0.05 μM) resulted in marked potentiation of FUDR cytotoxicity in six of eight human colon-cancer cell lines, with a median ID_{50} ratio of FUDR plus DP to FUDR alone of 33.5 (range, 0.7–1,200) (Table 2). At concentrations up to 0.5 μM, DP alone had no cytotoxic effect in any of the eight cell lines tested.

Effect of DP on 5FU cytotoxicity against human colon-cancer cell lines. As shown in Table 3, at 0.5 μM (i.e., 10-fold greater DP concentrations than were used with

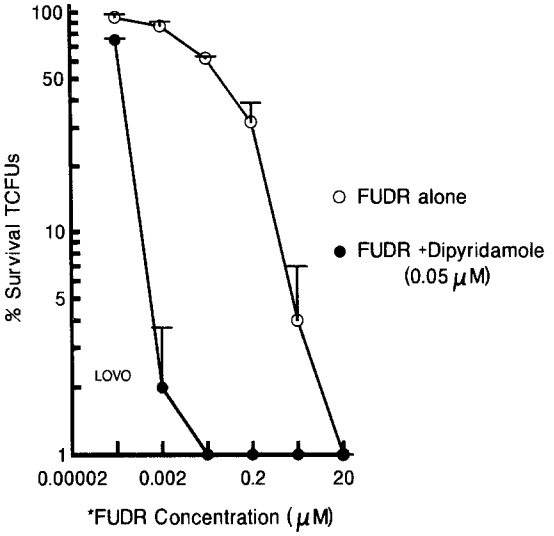

Fig. 2. FUDR dose-response curve against the LoVo human colon-cancer cell line in the absence or presence of DP at a concentration of 0.05 μ*M*. *Error bars* represent SE

Table 2. ID_{50} of FUDR with and without DP at 0.05 μ*M* against human colon-cancer cell lines[a]

Cell lines	ID_{50} FUDR (μ*M*)	ID_{50} FUDR + DP (μ*M*)	ID_{50} ratio of FUDR to FUDR + DP
LoVo	0.02[b]	0.0035[b]	57.0
SW 48	0.02[d]	0.002[d]	10.0
SW 480	0.18 ± 0.08[c]	0.059 ± 0.039[c]	3.1
Colo 320 DM	2.4[b]	0.002[b]	1,200.0
HCT 15	0.32 ± 0.31[c]	0.049 ± 0.03[c]	6.5
Colo 205	0.36 ± 0.093[c]	0.002 ± 0.001[c]	180.0
DLD-1	2.0 ± 1.2[c]	0.03 ± 0.012[c]	66.7
WiDr	0.02[d]	0.028[d]	0.7
Median ID_{50} ratio =			33.5

[a] FUDR and FUDR plus DP dose-survival curves for each tumor cell line were constructed on the basis of triplicate experiments (i.e., at each FUDR and DP concentration tested) conducted on the same day
[b] Average of two separate experiments
[c] Average of three separate experiments
[d] Single experiment

FUDR), DP produced minimal potentiation of 5FU cytotoxicity compared with FUDR. The median ID_{50} ratio of 5FU plus DP (0.5 μ*M*) to 5FU for the eight cell lines was only 1.5 (range, 1.1–6.7).

Effect of DP on FUDR cytotoxicity against human bone-marrow CFU-C. The addition of DP (0.5 μ*M*) significantly enhanced FUDR cytotoxicity against bone-marrow CFU-C grown from all six volunteers evaluated, with a median enhancement in FUDR activity of >20-fold (Table 4). Marked variation in the degree of enhancement of FUDR cytotoxicity was observed.

Tumor cell efflux of FUDR. The amount of [^{3}H]-FUDR remaining in Colo 205 tumor cells at 30 min after incubation at 37° C in supplemented RPMI 1640 (without [^{3}H]-FUDR) was only 15.6% of control values, as shown in Fig. 3. In contrast, the presence of DP at a concentration of 0.05 μ*M* significantly delayed the efflux of [^{3}H]-FUDR from Colo 205 tumor cells, with 88% of control values remaining at 30 min, 62% remaining at 60 min, and 27% remaining at 120 min.

Table 3. ID_{50} of 5FU with and without DP at 0.5 μ*M* against human colon-cancer cell lines[a]

Cell lines	ID_{50} 5FU (μ*M*)	ID_{50} 5FU + DP (μ*M*)	ID_{50} ratio of 5FU to 5FU + DP
LoVo	2.0 ± 1.2[c]	0.3[b]	6.7
Colo 320 DM	4.5 ± 2.3[c]	3.1[b]	1.5
SW 480	1.4 ± 1.0[c]	1.2 ± 0.9[c]	1.2
WiDr	1.6 ± 0.46[c]	0.85[b]	2.1
HCT 15	1.5 ± 1.3[c]	1.4[b]	1.1
DLD-1	0.19 ± 0.22[c]	0.096[b]	2.0
Colo 205	0.41[b]	0.27[b]	1.5
Median ID_{50} ratio =			1.5

[a] 5FU and 5FU plus DP dose-survival curves for each tumor cell line were constructed on the basis of triplicate experiments (i.e., at each 5FU and DP concentration tested) conducted on the same day
[b] Average of two separate experiments
[c] Average of three separate experiments

Table 4. ID_{50} of FUDR with and without DP at 0.5 μ*M* against human bone-marrow CFU-C from normal volunteers[a]

Volunteer number	Plating efficacy (%)	ID_{50} FUDR (μ*M*)	ID_{50} FUDR + DP (μ*M*)	ID_{50} ratio of FUDR to FUDR + DP
1	0.07	2.1	0.07	30.0
2	0.04	6.5	0.033	197.0
3	0.37	0.61	0.020	30.5
4	0.14	0.23	0.057	4.0
5	0.03	2.3	0.19	12.1
6	0.09	0.25	0.057	4.4
Median ratio =				21.1

[a] FUDR and FUDR plus DP dose-survival curves for each bone-marrow CFU-C were constructed on the basis of triplicate experiments (i.e., at each FUDR and DP concentrations tested) conducted on the same day

Clinical studies

Based on our in vitro studies revealing DP's consistent enhancement of FUDR cytotoxicity against bone-marrow CFU-C, we shortened the duration of FUDR treatment and attained a somewhat lower overall dose in the first ten patients treated in the study. In all, 1 patient received FUDR for 8 days; 1, for 10 days; 8, for 12 days; and the remaining 17, for 14 days.

Of the 28 patients registered, 1 was nonevaluable because of the lack of measurable disease. Clinical characteristics of evaluable patients are shown in Table 5. All had a performance status (Karnofsky rating) of >70%, except two patients whose performance statuses were 55% and

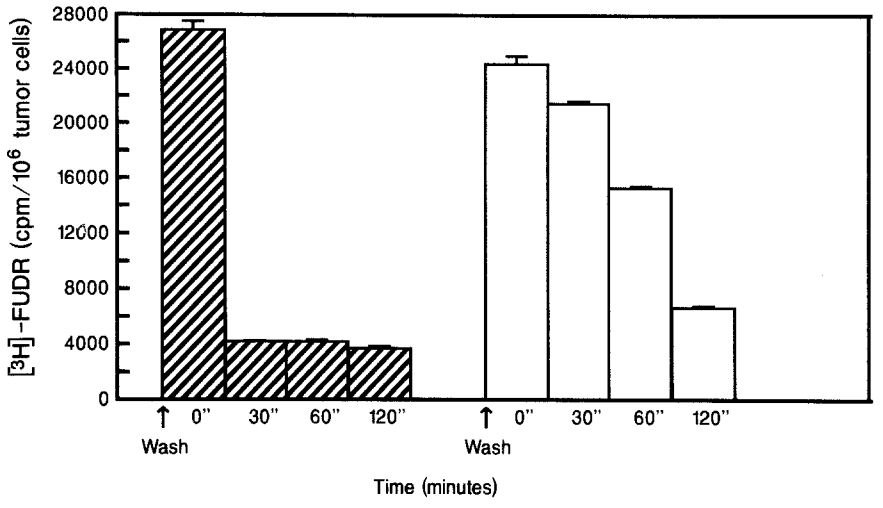

Fig. 3. Efflux of [^{3}H]-FUDR from human Colo 205 colon-cancer cells over a 120-min period in the absence (*hatched*) or presence (*blank*) of DP at 0.05 μ*M*. *Error bars* represent SE

Table 5. Patient clinical characteristics

Evaluable patients (*n*)	27
Men	21
Women	6
Age:	
Range	31–78 years
Median	62 years
Performance status at presentation[a]:	
Range	55–95
Median	80
Site of disease:	
Liver only	21
Liver and lung only	2
Liver and other sites	3
Other	1
LDH at presentation (normal, 95–170 IU/l):	
≥300 IU/l	9
<300 IU/l	18
Tumor bulk (cm)[b]	
>7.5	4
5.0–7.5	7
2.5–4.9	11
<2.5	5

[a] Karnofsky rating
[b] Largest diameter of the largest measurable lesion
LDH, lactic dehydrogenase

Table 6. Plasma DP concentrations after 75 mg p.o. 5 times daily

Patient number	Day of DP dosing	Total DP (n*M*)	Free DP (n*M*)	% Free DP
1	5 (trough)[a]	920	<5	–
	5 (peak)[b]	1180	6.1	0.52
	9 (trough)	920	7.7	0.83
	9 (peak)	1740	17.3	0.99
2	5 (trough)	920	<5	–
	5 (peak)	1470	6.7	0.45
	12 (trough)	280	11.3	4.00
	12 (peak)	1280	7.1	0.55
3	5 (trough)	1290	–	–
	5 (peak)	1610	7.5	0.46
	12 (peak)	1240	20.8	1.68

[a] 8 h after the last DP dose
[a] 4 h after the first of q4h daily DP doses

60%. Of the 27 evaluable patients, 4 (15%) achieved a PR that lasted 2, 3, 5, and 6+ months; 2 of these patients showed the complete disappearance of liver metastases but were considered to be partial responders because their CEA values remained elevated.

Treatment was well tolerated, with 15 patients experiencing no significant toxicity. A total of 12 patients developed predominantly mild to moderate toxicity, which included diarrhea (1 patient, grade I; 1, grade II; 2, grade III), stomatitis (3 patients, grade I), nausea and vomiting (1 patient, grade I; 2, grade II), ataxia (1 patient, grade I), abdominal cramping (1 patient, grade III), and headache (1 patient, grade II). The FUDR dose in patients who experienced grade III toxicity was decreased by 25%, which resulted in diminished treatment toxicity in subsequent courses.

DP serum levels were measured in three patients. As shown in Table 6, >95% of DP was protein-bound. Interestingly, concentrations of free DP, the active form of the drug, were 2- to 10-fold lower than the critical DP concentration (i.e., 0.05 μ*M*) necessary for significant enhancement of FUDR cytotoxicity against the human colon-cancer cell lines.

Discussion

Our in vitro studies demonstrate that at 0.05 μ*M*, DP markedly potentiates FUDR cytotoxicity in six of eight human colon-cancer cell lines; however, at a concentration (i.e., 0.5 μ*M*) 1 log higher it did not significantly enhance 5FU cytotoxicity against the same cell lines. This indicates the heterogeneity of response of these cell lines to different fluoropyrimidines and the need to evaluate multiple cell lines before conclusions can be drawn concerning an in vitro drug interaction.

The mechanism of action of DP in enhancing FUDR cytotoxicity is not completely established. Laboratory studies by Grem and Fischer [6] suggest that the mechanisms associated with this interaction are probably multiple and involve to some degree a block of cellular thymidine uptake and FUDR efflux. The marked discrep-

ancy observed in our experiments in the capacity of DP to potentiate FUDR cytotoxicity in the eight cell lines tested is of particular interest. Such variations may be related to differences in DP's capacity to affect cellular thymidine uptake and/or FUDR influx or efflux. These possibilities are presently being evaluated in more depth in our laboratories and will be the subject of a separate publication.

As previously mentioned, DP can also inhibit FUDR uptake into tumor cells, and this could potentially diminish its modulatory effects on FUDR-induced cytotoxicity. However, the data presented on DP inhibition of FUDR efflux from Colo 205 tumor cells (Fig. 3), taken together with the potentiation of FUDR cytotoxicity by DP in seven of eight human colon-cancer cell lines suggests that DP's inhibition of FUDR influx has minimal biological impact.

The favorable results of our in vitro studies prompted us to conduct a clinical evaluation of the relevance of the DP/FUDR interaction. The objective response rate of 15% and the overall toxicity observed in this trial are comparable to those obtained in other clinical studies using FUDR alone [9]. Thus, with the dose, schedule, and route used in our trial, the addition of DP did not appear to increase the response rate or the quality of FUDR response anticipated by the results of our in vitro studies. We believe that the major factor responsible for this negative clinical-trial result relates to the inability of the 75 mg/day DP dose to achieve the 0.05-μM level of unbound DP required for optimal interaction with FUDR in vitro. As shown in Table 6, although the *total* DP concentrations were within the range necessary to potentiate FUDR cytotoxicty, the concentrations of *free* DP, the active drug form, were significantly lower than the concentrations needed for this to occur in vitro. This point is of particular importance since DP given by the oral route is currently being used by other investigators evaluating the clinical value of DP/5FU interactions [1].

Although oral DP does not provide adequate plasma levels of free DP to potentiate FUDR cytotoxicity, Fischer et al. [4] have recently shown that i.v. DP given by continuous infusion at 23 mg/kg per 72 h (the maximally tolerated dose) resulted in steady-state concentrations of total and free DP of 11.9 and 0.028 μM, respectively. Of particular importance, the free DP levels (0.028 μM) obtained with i.v. dosing were in the range of levels necessary for optimal interaction with FUDR in vitro (i.e., approximately 0.05 μM).

The peritoneal cavity affords another potential route of administration for the achievement of high concentrations of anticancer agents. Chan et al. [3] have shown that DP injected i.p. results in high peritoneal concentrations of free DP, comparable with the concentrations used in our in vitro studies. These authors studied the pharmacokinetics of 50 mg/m^2 DP infused in 2 l normal saline in six ovarian cancer patients. DP concentrations in the intraperitoneal space peaked at 0.5–2.0 h after i.p. instillation. Peak, total intraperitoneal DP concentrations were 5- to 10-fold higher than the corresponding total DP plasma concentrations, whereas free DP concentrations in the peritoneal fluid were 10- to 20-fold higher than free plasma drug concentrations. Interestingly, DP in the peritoneal fluid was 58% free drug, in contrast to plasma DP, which was $<5\%$ free drug. This resulted in intraperitoneal concentrations of free DP in the range of 1 to 100 μM. Thus, the intraperitoneal space provides a marked pharmacologic advantage for DP over the i.v. route. The intrahepatic arterial infusion may afford another means of achieving high local concentrations of free DP. This strategy is particularly appealing in colon cancer patients in whom liver metastases are often the only site of active disease.

Despite the negative result of the present clinical trial, we feel that further studies exploiting the interaction between DP and FUDR are warranted. In this regard, laboratory studies evaluating the effect of DP on FUDR cytotoxicity against various human ovarian cancer cell lines as well as clinical trials using DP plus FUDR via the i.v., i.p., or intrahepatic arterial route would be of particular interest.

Acknowledgements. We would like to thank Dr. Paul Fischer (Department of Human Oncology, University of Wisconsin) for his technical assistance in the dipyridamole plasma assays. We would also like to thank Drs. Bernard Greenberg, Jonathan Schwartz, and Robert Pasterz for contributing patients to the clinical trial.

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Received 15 September 1989/Accepted 19 April 1989