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Clinical report

Phase I and pharmacologic study of i.v. hydroxyurea infusion given with i.p. 5-fluoro-2′-deoxyuridine and leucovorin

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Preclinical data suggests that the action of fluoropyrimidines may be enhanced by the addition of hydroxyurea. We developed a phase I trial to determine the maximum tolerated dose and pharmacokinetics of i.v. hydroxyurea (HU) in combination with i.p. 5-fluoro-2′-deoxyuridine (FUdR) and leucovorin (LV). Eligible patients had metastatic carcinoma confined mostly to the peritoneal cavity, and adequate hepatic, renal and bone marrow function. Patients were treated with a fixed dose of FUdR (3 g) and LV (640 mg) administered on days 1–3. HU was administered as a 72-h infusion starting simultaneously with i.p. therapy on day 1. The following dose levels were studied: 2.0, 2.5, 3.0 and 3.6 g/m^2/day. Pharmacokinetics were studied in blood and peritoneal fluid. Twenty-eight patients were accrued. Steady-state plasma and peritoneal fluid HU levels increased with increasing dose, and steady state was achieved within 12 h of continuous dosing. The steady-state HU plasma:peritoneal fluid concentration ratio ranged from 1.06×10^3 to 1.25×10^3 and the plasma HU clearance ranged from 4.63 to 5.81 l/h/m^2. Peritoneal fluid AUC = 137 639 $\pm$ 43 914 μg/ml·min, $t_{½}$ = 100.9 $\pm$ 56.4 min and Cl = 25.29 $\pm$ 10.88 ml/min. Neutropenia represented the dose-limiting toxicity. We conclude that i.p. FUdR and LV in combination with i.v. HU is well tolerated. The addition of systemic HU increased the incidence of myelosuppression. [© 2001 Lippincott Williams & Wilkins.]

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Introduction

The pharmacokinetic and theoretical advantages of administering chemotherapy drugs by the peritoneal route have been well described.[1] Although it has been shown that many drugs can be safely administered via this route, until recently no clear clinical advantages had been demonstrated.[2] A large, randomized study comparing i.v. versus i.p. cisplatin (both accompanied by i.v. cyclophosphamide) as first-line therapy for ovarian cancer has shown a survival advantage for the i.p. arm;[3] a second randomized study[4] with a similar design of up-front i.p. (after two cycles of i.v. carboplatin) versus i.v. cisplatin, both with i.v. paclitaxel, by the Gynecologic Oncology Group, shows an advantage in progression-free survival for the i.p. arm.[5] Moreover, encouraging preliminary results have been seen in pilot studies of i.p. fluoropyrimidines in gastric[6,7] and colorectal cancer,[8] and of i.p. consolidation with platinum-based combinations,[9–13] fluoropyrimidines,[14] interferon[15] or interleukin-2[16] in ovarian cancer.

The deoxynucleoside 5-fluoro-2′-deoxyuridine (FUdR) is a direct precursor of fluorodeoxyuridine phosphate (FdUMP), which is considered the key anabolite mediating 5-fluorouracil (5-FU) action. Both clinically available fluoropyrimidines (5-FU and FUdR) exert part of their antitumor effects via FdUMP inhibition of thymidylate synthase (TS), which in turn results in reduced availability of thymidine (from dUMP), cessation of DNA synthesis, DNA strand breaks and eventually programmed cell death. TS inhibition by FdUMP takes place via its substitution for dUMP in a ternary complex with the enzyme and the folate cofactor 5,10-methylene-

tetrahydrofolate.[17] In fact, excess reduced folate has resulted in enhanced TS inhibition and cytotoxicity of fluoropyrimidines against *in vitro* cell lines.[2,18] On the other hand, such inhibition also results in high concentrations of dUMP which may overcome TS inhibition by FdUMP.[5] However, some have hypothesized that dUTP 'overshoot' resulting from release of normal nucleoside/nucleotide pool feedback inhibition, induced by fluoropyrimidine alterations, overwhelms uracil glycosylase excisional repair, resulting in fraudulent incorporation of dUTP into the DNA, subsequent strand breaks and increased cytotoxic effects.[19] Experiments in support of such a hypothesis are the schedule-dependent effects of dipyridamole—an inhibitor of nucleoside uptake and modulator of intracellular nucleoside pools—on the cytotoxicity of fluoropyrimidines.[20]

After preclinical studies showed increased cytotoxicity of 5-FU and FUdR when combined with leucovorin (LV),[2,18] some clinical trials,[21,22] but not all,[23] demonstrated improved survival in patients with colorectal cancer treated with 5-FU and LV as compared to 5-FU alone. A meta-analysis does support an advantage in response rates but not survival in advanced colorectal cancer.[24] Nevertheless, interest has developed in new forms of modulation instead of, or to be added to, LV. Such efforts at modulation may also be applicable to orally administered prodrugs of 5-FU by themselves[25] or with inhibitors of 5-FU catabolism, a subject of current investigations.[26–29]

We have previously established the suitable dose for phase II/III trials for i.p. FUdR, at 3 g for 3days.[30] A multicenter randomized phase II study confirmed the tolerance findings and suggested an advantage for the use of i.p. FUdR over mitoxantrone in patients with ovarian cancer.[14] We also showed that the addition of LV to this regimen did not substantially modify the pharmacokinetics or toxicity profile.[31,32] The current study was based on the premise that the addition of hydroxyurea (HU) may enhance the TS mechanism of action of fluoropyrimidines by decreasing dUMP levels.[5,33–35] Additional potentiation could be expected if LV were to be added.[2] Since the optimal sequencing for the fluoropyrimidine–HU combination is unknown, we chose the simultaneous administration for practical reasons. We sought to establish the maximally tolerated dose (MTD) of i.v. HU given by continuous infusion for 3 days, together with i.p. FUdR and LV. We also sought to characterize the toxicities of this combination, determine pharmacokinetic parameters and obtain preliminary information regarding clinical activity.

Material and methods

Patients

Eligible patients had histologically confirmed residual, recurrent or metastatic carcinoma, predominantly confined to the peritoneal cavity; no symptomatic disease was present elsewhere. Patients with ovarian cancer had received prior treatment with carboplatin/cisplatin and paclitaxel prior to entry. To qualify all patients had to have adequate hepatic (bilirubin <2.0 mg/dl, SGOT <2 times the upper limit of normal), renal (serum creatinine <2.0 mg/dl) and bone marrow (leukocyte >3500 cells/mm^3 and platelets $>100\,000$ cells/mm^3) function. A Karnofsky performance status (KPS) of $\geqslant 60$ was required. The protocol was approved by the Institutional Review Board of the University of Southern California and all patients gave written informed consent. The study was opened for accrual between July 1993 to October 1995.

Dosage and administration

Eligible patients had an i.p. catheter surgically placed, and were treated with fixed doses of FUdR and LV. Every 3 weeks FUdR was administered for 3 consecutive days at a daily dose of 3 g given i.p. in 2 l of normal saline. On days 2 and 3 the volume could be reduced to 1–1.5 l if residual peritoneal fluid could not be drained. The i.p. LV, at a dose of 640 mg, was mixed together with each FUdR dose. HU was administered as a 72-h continuous i.v. infusion, starting simultaneously with i.p. therapy on day 1. Dose levels of HU that were studied in this dose-escalation design are shown in Table 1, with a minimum of three patients entered at each dose level. In the event of any grade 3 or 4 toxicities (NCI Common Toxicity Criteria, 1992) considered dose limiting and occurring on cycle 1, the levels were expanded to six patients, unless a second severe event was recorded. With such an event, the next lower dose level was to be expanded in order to determine the recommended dose for phase II study. Dose-limiting toxicities consisted of

Table 1. Dose levels

Level	FUdR (i.p.) (g/day)	LV (i.v.) (mg/day)	HU (i.v.) (g/m^2/day)	*N*
1	3.0	640	2.0	10
2	3.0	640	2.5	6
3	3.0	640	3.0	7
4	3.0	640	3.6	5

grade 4 neutropenia lasting 5 days or more, grade 3 or 4 neutropenia with fever, grade 4 thrombocytopenia and any grade 3 or 4 non-hematologic toxicities. Cycles were repeated every 3 weeks or when recovery of all toxicities occurred.

Local abdominal toxicity (chemical peritonitis) was graded as follows: none (grade 0), mild discomfort, fullness or bloating requiring no treatment (grade 1), moderate discomfort requiring analgesics (grade 2) and severe discomfort requiring narcotic (grade 3). In the event of any grade 3 or 4 (except local peritoneal) toxicities FUdR was reduced by 33% and LV by 50%. If despite this modification grade 3 or 4 toxicities appeared, then HU was decreased by one dose level. Local toxicity did not require dose modifications but in the event of grade 3 abdominal toxicity or complaints, distribution of peritoneal fluid was assessed by CT scan of the abdomen. If no abnormal distribution was seen, patients were retreated but were taken off study if grade 3 toxicity recurred. Commercially available FUdR (Floxuridine; Roche Laboratory), in the form of 500 mg sterile powder in a 5 ml vial, was employed. LV, commercially available, in the form of 50 and 100 mg vials was used. HU as an i.v. formulation was supplied by the CTEP/NCI.

Pretreatment and follow-up studies

At baseline history and physical exam, performance status, complete blood cell count (CBC), serum chemistries, tumor markers (CEA, CA 125 or CA 19-9 as clinically indicated), electrocardiogram, urinalysis, chest X-ray and appropriate radiological exams for measurable disease were recorded. CBC was obtained weekly. Patients remained on therapy for four cycles in the absence of disease progression or unacceptable toxicity. After four cycles patients could continue on therapy without the i.v. HU if there was persisting evidence of peritoneal disease. Standard SWOG response criteria were used.[36]

Pharmacokinetics

Pharmacokinetic studies were conducted in a minimum of two patients at each dose level. Blood samples (6 ml, heparinized tubes) were obtained on day −1 at t = 0 (pre-dose), and at 1, 2, 3, 4, 6 and 24 h following completion of i.p. administration of FUdR. All blood samples were centrifuged immediately upon collection and the plasma transferred to polypropylene freezer vials and frozen at −20°C until assayed. Peritoneal fluid samples (10 ml) were drawn on day −1 at t = 0 (pre-dose), and at 1, 2, 3, 4, 6 and 24 h post-dose. FUdR, 5-FU and LV were determined in plasma and peritoneal fluid samples using validated HPLC methods.[31] HU was determined by a HPLC method using electrochemical detection.[37] Plasma concentration–time data for FUdR were fitted using the polyexponential curve-fitting program, RSTRIP, version 4.05 (MicroMath Scientific Software, Salt Lake City, UT), and pharmacokinetic parameters determined. Steady-state HU concentrations in plasma and peritoneal fluid were determined after 24 h of continuous infusion. The plasma concentration:peritoneal fluid concentration ration at 24 h was determined and the HU plasma clearance was calculated using the following equation:

$$\mathrm{Cl_{p1}} = k_0 / C_\mathrm{p}\mathrm{ss}$$

where k_0 is the i.v. infusion rate in mg/h/m^2 and $C_\mathrm{p}$ss is the HU plasma concentration at t = 24 h.

Results

At the first dose level, two dose-limiting toxicities were encountered and the level was expanded to six patients. Because at this level no dose-limiting toxicities were seen in previously untreated patients, further dose escalation proceeded in two separate cohorts: previously treated and previously untreated. On dose level 4 only untreated patients were entered.

Twenty-eight patients received a total of 111 courses and the median number of cycles per patient was 4. Patient characteristics are shown in Table 2. Sixteen patients completed 4 or more cycles of treatment. Reasons for withdrawal in the other 12 patients were progressive disease (n = 8), physician discretion (n = 3) and patient refusal (n = 1). One patient with breast cancer and ascites, who received

Table 2. Patient characteristics (n = 28)

Age (years)	
median (range)	49.5 (24–80)
Sex	
male	11
female	17
Prior chemotherapy	13
PS	
90	8
80	15
70	4
Primary site	
colorectal	13
unknown	5
appendix	4
others	6[a]

[a]Ovary = 2, stomach = 2, small bowel = 1, breast = 1.

only one course of treatment, was ineligible since she had to be started on concomitant radiation therapy to the spine for bone metastases that became symptomatic just after entry. Only five patients had measurable disease. The others had no clinical or radiological evidence of disease (i.e. presence of disease was established only with a surgical procedure) and therefore were not evaluable for response to therapy.

Toxicity

There were no treatment-related deaths. As expected, hematological toxicity was dose limiting (Table 3). One episode of neutropenic fever was documented. In addition, one patient developed bacteremia due to *Klebsiella*, associated with a nadir granulocyte count of 768. Common non-hematological toxicities were nausea (85%), vomiting (39%), abdominal pain (61%), fatigue (53%), stomatitis (53%), fever (36%), diarrhea (21%) and skin (21%). However, with rare exception, these were only grade 1–2 (Table 4), easily controlled and in no instances were dose modifications required based exclusively on these toxicities. There was no suggestion of non-hematological toxicities related to dose level or cumulative dose. Among patients who experienced any degree of nausea or vomiting, only 25% had grade 3 symptoms. The majority experienced this toxicity on cycle 1 and symptoms were controlled on subsequent cycles with more aggressive antiemetic therapy. The presence or severity of nausea or vomiting did not appear to be related to dose level.

Only one patient experienced grade 3 local toxicity. This occurred on cycle 1 and following verification of adequate i.p. distribution, symptoms where controlled with narcotic analgesics on subsequent cycles. He received a total of four courses of treatment.

Responses

Five patients had measurable disease: three in the liver and two in the omentum. Of these, one patient with rectal cancer remained stable for 5 months manifesting a decline in CEA and clearing of positive cytology. Our largest group of patients had colorectal cancer: among the 13 patients in this group, seven of them achieved stable disease with a minimum duration of 4 months

Table 3. Hematologic toxicity by dose level

Toxicity	Dose level											
	1 (n = 10)			2 (n = 6)			3 (n = 7)			4 (n = 5)		
	0–2	3	4	0–2	3	4	0–2	3	4	0–2	3	4
WBC	7	3	0	3	3	0	6	0	1	0	4	1
AGC	8	2	0	3	2	1	3	3	1	1	1	3
Platelets	9	1	0	6	0	0	6	0	1	0	3	0
Hemoglobin	10	0	0	0	6	0	7	0	0	1	4	0

Table 4. Non-hematological toxicity by dose level

Toxicity	Dose level											
	1 (n = 10)			2 (n = 6)			3 (n = 7)			4 (n = 5)		
	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Nausea	7	3	0	3	2	0	5	0	0	3	1	0
Vomiting	2	2	0	3	0	0	2	1	0	1	0	0
Diarrhea	3	0	0	2	0	0	0	0	0	1	0	0
Fever	4	0	0	3	0	0	3	0	0	0	0	0
Headache	4	0	0	1	0	0	0	0	0	1	0	0
Stomatitis	5	0	0	3	1	0	3	0	0	3	0	0
Liver	3	0	0	2	0	0	0	0	0	1	0	0
Abdominal pain	8	0	0	3	1	0	4	0	0	1	0	0
Fatigue	3	1	0	4	1	0	4	0	0	1	1	0
Dizziness	2	0	0	0	0	0	0	0	0	0	0	0
Infection	1	1	0	0	0	0	0	0	0	0	0	0
Skin	2	1	0	1	0	0	0	0	0	2	0	0

(Table 5) and two of them are alive with disease with follow-up of more than 16 months. Five patients, all previously treated with 5-FU, remained alive for over 12 months. Additional patients with durable stable disease were one patient with unknown primary (6 months), one appendiceal (4 months) and one gastric cancer (4 months).

Pharmacokinetics

Figure 1 shows that steady-state plasma and peritoneal fluid levels were achieved following increasing doses of i.v. HU. The steady-state plasma and peritoneal fluid HU levels increased with increasing dose and steady-state was achieved within 12 h of continuous dosing. The steady-state HU plasma:peritoneal fluid concentration ratio was consistent ranging from 1.06×10^3 to 1.25×10^3 and the plasma HU clearance ranged from 4.63 to 5.81 l/h/m^2. Both the HU plasma:peritoneal fluid ratio and the HU plasma clearance were independent of the administered dose over the range of doses studied (Table 6).

The peritoneal fluid FUdR pharmacokinetics following a 3000 mg i.p. dose is shown in Table 7. The FUdR peritoneal fluid pharmacokinetic parameters that were determined in this study were consistent with a previous reported result for a 3000 mg dose.[24]

Table 5. Therapeutic effects in patients with colorectal cancer

Patient	Age	Duration of response (months)	Status	Prior 5-FU
EO	42	12	dead 34 months	yes
BK	59	7	alive 26 months	no
DW	65	5	dead 9 months	yes
PT[a]	37	5	alive +6 months	no
JB	46	4	dead 17 months	yes
ER[a]	54	+4	alive +17 months	yes
VB	58	4	dead 13 months	yes
HR	64	4	dead 13 months	yes

[a]Lost to follow-up.

Discussion

This study shows that full doses of i.p. FUdR and LV daily in combination with i.v. HU for 72 h at a dose of 3 g/m^2/day are well tolerated. A suggestion of beneficial antitumor effects was seen, primarily in patients with colorectal cancer metastatic to the peritoneum. However, these responses might have been achievable with the i.p. fluoropyrimidine alone. Although well tolerated, the addition of systemic HU modified, as expected, the toxicity profile, particularly as it related to hematological findings. In the current study, grade 3–4 hematological toxicity was seen as leukopenia 43%, neutropenia 46%, thrombocytopenia 18% and anemia 36% of patients. These findings contrast with the phase II study of i.p. FUdR in ovarian cancer where grade 3–4 leukopenia occurred in 11%, neutropenia 18%, thrombocytopenia 11% and anemia 7%.[14] In our previous study of FUdR and LV,

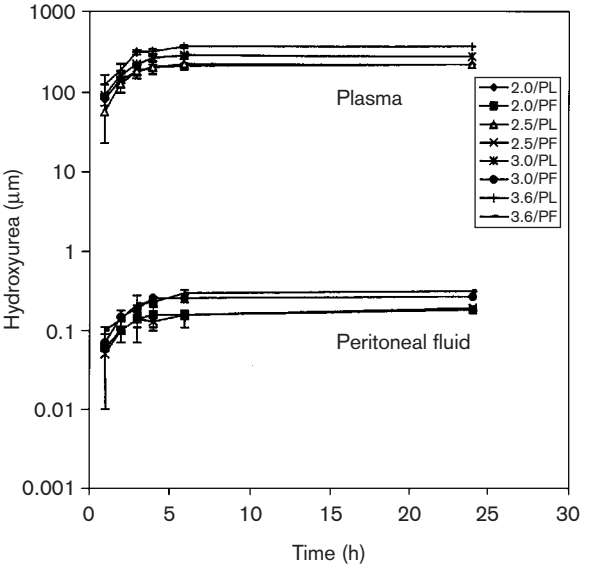

Figure 1. HU concentration against time in both plasma (PL) and peritoneal fluid (PF) for all dose levels.

Table 6. Steady-state HU plasma and peritoneal fluid concentrations following i.v. infusion at four dose levels

HU dose (g/m^2/day)	Steady-state HU concentration		Plasma:PF ratio	Clearance (l/h/m^2)
	Plasma concentration (μM/ml)	Peritoneal fluid (μM/ml)		
2.0	236.6 $\pm$ 33.8	0.19 $\pm$ 0.02	1.25×10^3	4.63
2.5	235.7 $\pm$ 17.0	0.20 $\pm$ 0.02	1.18×10^3	5.81
3.0	296.3 $\pm$ 16.3	0.28 $\pm$ 0.03	1.06×10^3	5.55
3.6	395.5 $\pm$ 18.4	0.33 $\pm$ 0.03	1.20×10^3	4.99

Table 7. Comparative mean FUdR peritoneal fluid pharmacokinetic parameters following 3000 mg i.p. dose

Parameter[a]	Current study	Previously published[21]
AUC (μg/ml·min)	137 639 ± 43 914	137 196 ± 111 916
$t_{½}$ (min)	100.9 ± 56.4	96.5
Cl (ml/min)	25.29 ± 10.88	30.58 ± 18.41

[a]Mean ± SD.

neutropenia grade 3–4 was seen in 16% of patients with no instances of severe thrombocytopenia.[31,32]

Some events related to non-hematological toxicity were also seen. Fever (36%), stomatitis (53%) and skin toxicities were fairly common in this population while they were rarely (< 20%) reported when HU was not used. Local toxicity, manifested as abdominal pain, was also more common in this trial compared to FUdR alone. However, mild abdominal pain was also common in our FUdR/LV studies.[31,32]

Conclusion

Recent studies suggest that i.p. chemotherapy may play an important role in the management of carcinomas that have a high tendency to spread in the peritoneal cavity (i.e. ovarian, stomach, colon). The tolerance and results achieved with i.p. FUdR in several studies warrant continued exploration of ways to enhance its antitumor effects through modulation or combinations of i.p. or systemic drugs and also efforts at documenting possible therapeutic roles. Whether HU is worthwhile as a concomitant modulator is not readily apparent from the data in this phase I study. Additional studies must determine the importance of sequencing[38] and the biochemical consequences of such modulation.

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