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Cisplatin preceded by concurrent cytarabine and hydroxyurea: a pilot study based on an in vitro model*

Kathy S. Albain[1], Lode J. Swinnen[1, 2], Leonard C. Erickson[1], Patrick J. Stiff[1], and Richard I. Fisher[1, 2]

[1] Section of Hematology and Oncology, Loyola University Stritch School of Medicine, Maywood, IL 60153, USA
[2] Hines Veterans Administration Hospital, Hines, IL 60141, USA

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Summary. As previously reported, cytotoxic synergy is produced when clinically achievable concentrations of cytarabine (Ara-C) and hydroxyurea (HU) are used as potential inhibitors of in vitro DNA repair in cisplatin (*cis*-Pt)-treated human colon carcinoma cells. This pilot study was subsequently designed to duplicate the in vitro dose and schedule and to determine the toxicity of this three-drug combination in two cohorts of patients. 21 patients had received prior chemotherapy and 19 were not previously treated. All patients had refractory solid tumors. They received monthly cycles of an oral loading dose of 800 mg/m^2 HU followed every 2 h by 6 oral doses of 400 mg/m^2, a 12-h continuous infusion of 200 or 250 mg/m^2/h Ara-C concurrent with the HU, and then 100 mg/m^2 *cis*-Pt over 1 h. A total of 95 cycles were given with the expected toxicities of nausea and vomiting and fatigue but no major acute toxicity observed. Thrombocytopenia was significant but transient and was dose-limiting only for patients who had received prior therapy. The median platelet nadir after one cycle was 43,000/μl for all patients and 67,000/μl for those who had not undergone prior treatment. Azotemia was treatment-limiting in responding and stable patients, suggesting the possibility of synergistic nephrotoxicity. Interestingly, there were early transient rises in both uric acid and lactate dehydrogenase (LDH). Partial responses were seen in 9 of 32 patients with measurable disease and there was significant improvement in 5 of 8 patients with only evaluable disease. The responses or improvement occurred in patients with non-small-cell lung cancer, breast carcinoma, glioblastoma, ovarian carcinoma, small-cell lung cancer, and mesothelioma. Of these 14 patients, 9 had failed prior chemotherapy regimens. Significantly, responses were observed in 3 of 8 patients who had previously received *cis*-Pt, suggesting that the HU/Ara-C combination modulated *cis*-Pt resistance. Because of these encouraging results, a second pilot study has been initiated with modifications dictated by the toxicity issues raised in this trial.

Introduction

Acquired or de novo drug resistance is a major factor limiting the success of chemotherapy regimens. Modulation of such resistance at the cellular level may significantly improve the therapeutic index of a variety of chemotherapeutic agents such as cisplatin (*cis*-Pt). We designed in vitro experiments so as to achieve cytotoxic synergy with clinically achievable concentrations of drugs that may inhibit a major mechanism of resistance to *cis*-Pt [29]. Cisplatin forms DNA adducts that lead to bifunctional lesions, including DNA-interstrand, DNA-intrastrand, and DNA-protein cross-links [21]. The DNA excision-repair system, which repairs lesions such as the thymine-thymine dimer induced by UV radiation, may also repair bulky DNA adducts [35] and is involved in the repair of DNA damage from *cis*-Pt [9, 10, 13, 16, 30]. The ability of a cell to excise UV-induced dimers is inhibited by cytarabine (Ara-C) and hydroxyurea (HU) [7, 8, 11, 12, 22, 26, 28, 34]. Thus, we hypothesized that the combination of Ara-C and HU could inhibit the tumor cell's capacity to repair *cis*-Pt DNA lesions and increase cytotoxicity over that of *cis*-Pt alone. When a 12-h exposure of human colon carcinoma cells (HT-29) to a combination of 10^{-6} M Ara-C and 10^{-3} M HU preceded a 1-h exposure to *cis*-Pt, the *cis*-Pt IC_{90} (the concentration of drug required to reduce colony-forming ability to 10% of control values) was reduced approximately 3-fold as compared with that of *cis*-Pt alone [29]. Increased levels and later persistence of DNA interstrand cross-links were observed with the three-drug combination as compared with *cis*-Pt alone. These data were consistent

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Offprint requests to: Kathy S. Albain, Section of Hematology/Oncology, Loyola University Medical Center, 2160 South First Avenue, Building 54 – Room 046, Maywood, IL 60153, USA

Table 1. Treatment schema

Time of dose (h)	–2	0	2	4	6	8	10	12	14
Time of day	6 p.m.	8 p.m.	10 p.m.	12 a.m.	2 a.m.	4 a.m.	6 a.m.	8 a.m.	10 a.m.
Hydroxyurea loading dose	X								
Hydroxyurea maintenance doses		X	X	X	X	X	X		
Ara-C continuous infusion		X →						Stop	
Normal saline hydration	X →								
						(to continue for an additional 12–48 h)			
Cisplatin 1-h infusion								X→	

with decreased repair of *cis*-Pt DNA adducts and/or an increased level of adduct formation.

Based on these in vitro results, this pilot study was initiated using a 12-h treatment with the two antimetabolites preceding a 1-h *cis*-Pt infusion. From published pharmacokinetic data, we chose doses and schedules for the three drugs that would achieve concentrations in vivo similar to those used in the in vitro model. The objectives of this pilot study were: (1) to determine the feasibility of the in vitro model in two clinical settings: patients who had failed standard chemotherapy programs (with or without *cis*-Pt) and those who had received no prior chemotherapy; (2) to assess the toxicity pattern, particularly to determine whether synergistic myelosuppression or another toxicity not expected from the individual drugs would occur; and (3) to determine whether at the predetermined drug doses this regimen could be recommended for phase II trials in tumors responsive and unresponsive to *cis*-Pt alone.

Patients and methods

Patients eligible for this trial included any adult with a diagnosis of refractory malignancy; a performance status (SWOG) of 0, 1, or 2; and a life expectancy of at least 8 weeks. This included patients who had either failed chemotherapy combinations with or without *cis*-Pt, radiation therapy, or other experimental therapy. Previously untreated patients with a solid tumor for which there was no effective standard treatment were also eligible. All patients had recovered from the side effects of prior therapy and had measurable or evaluable disease. Prestudy requirements included a complete blood count (CBC) with differential, platelet count, chemistry panel, serum magnesium determination, urinalysis, chest X-ray, an audiogram, and either a calculated or a 24-h urinary creatinine clearance (C_{Cr}) of $\geq$50 ml/min. Computerized tomographic scans and radionuclide scans were performed as indicated, depending on the type of tumor. Adequate marrow reserve was required (granulocytes, $\geq$1,000/μl; platelets, $\geq$100,000/μl. All patients gave written informed consent.

The treatment schema is shown in Table 1. Intravenous (i. v.) normal saline infused at $\geq$150 cm^3/h was begun prior to the initiation of therapy and was continued for a minimum of 12 h after completion of the *cis*-Pt treatment so as to achieve a urine output of at least 100 cm^3/h. Intake and urinary output measurements were obtained every 2 h for the first 24 h. A mannitol infusion of 25 g was given just prior to the *cis*-Pt infusion. Later in the study, the i.v. prehydration was increased to 24 h and lengthened to 24–48 h after treatment or until the patient maintained adequate oral (p. o.) intake. A standard polyantiemetic regimen was used. Dexamethasone eye drops (0.1%) were given in each eye every 6 h for six doses. A CBC and determinations of serum electrolytes, blood urea nitrogen (BUN), creatinine, calcium, magnesium, and uric acid (UrA) were obtained daily. Later in the trial, daily serum lactate dehydrogenase levels (LDH) and the fractional excretion of urinary UrA were measured before treatment and then every 6 h × 4.

The chemotherapy doses and schedules were derived from published pharmacokinetic data for each drug so as to duplicate the in vitro concentrations and exposure times required to achieve synergy, as summarized in Table 2 [1–6, 14, 17, 19, 23, 27, 32, 33]. The loading dose of HU (800 mg/m^2 p. o.) was rounded to the nearest 500 mg (capsule size); the total HU maintenance dose (400 mg/m^2 × 6 or 2.4 g/m^2) was rounded up to the nearest 500 mg and then divided among the 6 maintenance doses. Initially patients received 80% of the Ara-C dose (200 mg/m^2/h); subsequent escalation to the intended dose (250 mg/m^2/h) was planned if no grade III–IV toxicity was encountered. No additional escalation was intended, and dose escalation in a given patient was not permitted.

Granulocyte and platelet counts were monitored a minimum of 3–4 times during the 2nd–3rd week posttreatment. The dose modification for neutropenic infection or thrombocytopenia requiring transfusion (platelet count, <20,000/μl) was a 25% reduction of the previous Ara-C and HU

Table 2. Equivalence of the in vitro model and the pilot study design

Drug	Concentration required for in vitro synergy [29]	Optimal clinical dose and schedule to duplicate in vitro model	Achievable serum concentrations [reference]
Cytarabine	1 μM	250 mg/m^2/h for 12 h by continuous infusion	20 μM; rapid steady state [17, 27]
Hydroxyurea	1,000 μM	800 mg/m^2 p. o. loading dose, then 6 doses of 400 mg/m^2 p. o. every 2 h[a]	700–2,000 μM [1, 4–6, 23]
Cisplatin, total	10–50 μM	100 mg/m^2 infusion for 1 h	Peak (total): 10–30+ μM
free	0.2–1 μM[b]		Peak (free): 1–20 μM
			After 1–2 h (free):0.1–0.9 μM [2, 3, 14, 29, 32, 33]

[a] Maintenance dose interval chosen to maintain a steady state for 12 h and to allow for a variable range in half-life, peak levels, and interpatient variability following a single oral dose

[b] Based on 98% protein binding in vitro

Table 3. Patient characteristics

Number enrolled/evaluable	40/40
Sex (M/F)	25/15
Median age (range) in years	61 (36–80)
Performance Status (SWOG):	
0	13
1	23
2	4
Previous therapy:	
None	13
Interleukin-2 plus LAK cells	1
Radiation therapy	6
Chemotherapy	7
Chemotherapy and radiation therapy	13
Prior cisplatin	8
Type of solid tumor:	
Non-small-cell lung carcinoma	17
Small-cell lung carcinoma	5
Breast carcinoma	7
Colorectal carcinoma	5
Melanoma	2
Ovarian carcinoma	1
Brain (glioblastoma)	1
Mesothelioma	2
Disease status:	
Measurable	32
Evaluable	8

dose. A CBC, chemistry panel, serum magnesium determination, an audiogram, and a chest X-ray were obtained just prior to each cycle. Monthly cycles were planned, with treatment being repeated when the granulocyte count was >1,500/μl; the platelet count, >100,000/μl; and the C_{Cr} or serum creatinine value, ≥50 ml/min or ≥2 mg/dl, respectively. There was no reduction of *cis*-Pt doses due to azotemia.

Response to therapy was assessed in patients with measurable disease according to standard criteria (SWOG). In patients with only evaluable disease, a significant improvement was defined as the resolution of all symptoms, a decrease of at least 50% in serum markers (if present), and radiographic improvement of evaluable disease. Progression was defined as the appearance of new lesions, an increase of ≥25% in the area of measurable lesions, and/or an increase in evaluable disease or bone pain. Patients were considered to be evaluable for toxicity and response if they completed at least one cycle. Patients were continued on therapy until the occurrence of disease progression or prohibitive toxicity or until they refused further treatment.

Results

Patient characteristics and treatment summary

The characteristics of the 40 enrolled and evaluable patients are summarized in Table 3. In all, 13 patients had received no prior therapy; 6, prior radiotherapy; 20, prior chemotherapy (including *cis*-Pt in 8 cases) with or without radiotherapy; and 1, interleukin-2 plus LAK cells. A total of 95 cycles were given to the 40 patients, and the median number of cycles per patients was 2 (range, 1–7). Overall, 15 patients received one course; 10, two courses; 5, three courses; 7, four courses; 2, five courses; and 1, seven courses. Subsequent cycles were given every 3, 4, or 5 weeks to 80% of the patients (median for all patients, every 29 days). The hydration program and urinary output goals were achieved without morbidity in all patients.

Toxicity

The majority of patients tolerated the 15 h of chemotherapy well. In 40% of courses, 1–3 episodes of emesis occurred during the first night, requiring a repeat of the HU dose if the emetic event took place within 1 h of the dose or if capsules were seen in the emesis. After discharge, 85% of the cycles resulted either in no additional symptoms or in mild nausea that resolved by day 8. The remaining 15% of the cycles resulted in an emesis pattern not unlike that described for other *cis*-Pt regimens. Anorexia and malaise occurred to varying degrees in 42% of patients and delayed subsequent therapy by 1 week in 12 of 95 cycles. During the first 24–48 h of therapy, 9 patients (12 cycles) developed diarrhea that resolved by the time of discharge. A transient, low-grade fever occurred in 26 courses at 12–24 h after the completion of chemotherapy. A mild, diffuse erythema of the skin or a fine, erythematous macular rash was often observed on days 2–3. Two patients had a dramatic erythematous reaction in previously irradiated areas of recurrent subcutaneous metastases. Three other patients experienced an erythematous skin reaction in the area of a previous radiation port that was not involved with tumor. Following discharge, 50% of cycles resulted in a mild, desquamative rash of the hands and feet. Two patients with brain metastases who had received prior radiation had a transient period of confusion. There were no treatment-associated deaths.

Severe thrombocytopenia was one of the two major toxicities observed. Table 4 summarizes the myelotoxicity encountered in the 19 and 21 patients (with and without prior chemotherapy, respectively) treated at each of the two Ara-C dose levels. Treatment with the target Ara-C dose (250 mg/m^2) in two previously treated patients produced severe thrombocytopenia. Thus, all other previously treated patients were accrued at the lower Ara-C dose. Previously untreated patients tolerated the higher dose of Ara-C well: 13 subjects received 30 cycles, with a median platelet nadir of 67,000/μl (range, 21,000–250,000/μl) following cycle 1 and a median of 44,000/μl for all 30 cycles (range, 8,000–275,000/μl). In all patients, the median platelet nadir did not fall below 25,000/μl until the fourth cycle, occurring between day 9 and day 11 (range, days 9–16), with a median duration of 3 days. No patient experienced a hemorrhagic complication. Severe prolonged thrombocytopenia (50,000–80,000/μl) occurred at 6 weeks in 3 patients who had undergone extensive prior chemotherapy, requiring the cessation of treatment. The median granulocyte nadirs, presented in Table 4, occurred between day 14 and day 20 (range, days 8–22) and lasted 3–5 days. Only five cycles resulted in readmission for treatment of a neutropenic fever. A reduction of 25% in the HU and Ara-C dose prevented neutropenic complications after subsequent cycles.

Table 4. Treatment summary and myelotoxicity

Treatment category	Number of patients/ cycles	Median nadir platelet count/μl (range) after cycle 1	Median nadir platelet count/μl (range) over all cycles	Number of first cycles requiring platelet transfusion (platelets, <20,000/μl)	Median nadir granulocyte count/μl (range) after cycle 1
All patients	40/95	43,000 (7,000–250,000)	30,000 (4,000–275,000)	7 (18%)	340 (0–5,000)
Ara-C dose category, patients with prior chemotherapy:					
200 mg/m^2	19/45	31,000 (7,000–91,000)	26,000 (4,000–115,000)	6 (32%)	300 (0–1,920)
250 mg/m^2	2/6	26,000 (18,000–35,000)	19,000 (8,000–35,000)	1 (50%)	520 (400–640)
Ara-C dose category, patients without prior chemotherapy:					
200 mg/m^2	6/14	68,000 (31,000–90,000)	46,000 (7,000–99,000)	0	1,125 (40–3,400)
250 mg/m^2	13/30	67,000 (21,000–257,000)	44,000 (8,000–275,000)	0	320 (0–1,000)

Table 5. Characteristics of patients or cycles with respect to the degree of azotemia

Parameter[a]	Maximal serum creatinine (mg/dl)		
	Normal –2.0 (number ≤1.5)	2.1–2.9	≥3.0
Number of patients	20 (8)	12	8
Median age (years)	57	63	66
% aged ≥65 years	25%	33%	62%
Number of cycles	71 (49)	16	8
% with contrast dye within 20 days	46%	44%	75%
Baseline creatinine (mg/dl)	0.9	1.1	1.2
Baseline creatinine clearance (ml/min)	85	69	76
Baseline BUN/Cr ratio	17:1	19:1	15:1
Baseline uric acid (mg/dl)	5.0	5.8	6.2
Maximal uric acid increase[b]	1.0	1.9	2.0

[a] Except as indicated, all data represent mean values
[b] Occurred on days 3–5 following treatment
BUN, blood urea nitrogen; Cr, creatinine

Azotemia was the other major toxicity observed as shown in Table 5. There was only mild (maximal Cr value, ≤2.0 mg/dl) azotemia, if any, in 20/40 patients or after 71 cycles (76%). In the other 20 patients, significant azotemia occurred after 1 cycle in 8 cases, after 2 cycles in 5 subjects, after 3 cycles in 2 cases, after 4 cycles in 4 subjects, and after 7 cycles in one patient. Because of a response to treatment, 10 of these 20 patients were continued on therapy despite marginal renal function. A 75-year-old man required dialysis after one cycle, and he remained on dialysis with stable metastatic non-small-cell lung cancer 1.5 years later. Resolution of significant azotemia to a Cr value of <2.0 mg/dl occurred in all but 3 patients and delayed the next cycle by 2–3 weeks in 7/95 cycles. No patient with significant azotemia received concurrent aminoglycosides or other nephrotoxic drugs. Of the 40 patients, 7 had previously received *cis*-Pt and 1, carboplatin and *cis*-Pt; 5 of these 8 had a maximal Cr value of >2.0 mg/dl. However, 3 elderly patients who had previously received high-dose *cis*-Pt received 4, 5, and 7 cycles without experiencing nephrotoxicity. There was a 26% decrease in the number of cycles causing moderate and severe azotemia after the pre- and posttherapy hydration periods were lengthened, although this was not statistically significant. Other pre- and posttreatment parameters were compared among patients and among cycles according to three categories of maximal azotemia (Table 5). Differences in these parameters among the three nephrotoxicity categories were analyzed using either chi-square analysis or analysis of variance. This analysis does not account for the fact that a patient receiving multiple cycles may be represented in more than one azotemia category. Nevertheless, it does appear that greater toxicity occurred in cycles in which patients had a higher baseline Cr value ($P = 0.0008$) and in those associated with a greater increase in UrA levels ($P = 0.028$). The other potential associations with severe azotemia suggested in Table 5 (e.g., advanced age, higher baseline UrA values, and a higher percentage of contrast dye within 20 days) were not statistically significant, although numbers were small in the category representing Cr values of >3 mg/dl. We cannot rule out the possibility that certain combinations of these parameters may have had additional adverse impact.

An unusual finding involved an early elevation of UrA and LDH serum values. Of 93 cycles, 80 resulted in a mean increase in UrA of 1.7 on days 3–5, which resolved by the time of discharge. This occurred despite i.v. hydration, the absence of diuretic use, and a drop in the BUN/Cr ratio by day 2. The fractional excretion of uric acid (FEUrA) was measured during 15 cycles. In 13 courses there was an increase in the FEUrA from a mean pretreatment value of 10% (range, 7%–14%) to a mean peak of 22% (range, 14%–43%). This occurred on day 2, often prior to the peak rise in UrA, and returned to the baseline level after 12 h.

Table 6. Characteristics of patients with a partial response or significantly improved evaluable disease

ID	Age (years)	Sex	Type of tumor	PR or Imp	Location of active disease	Prior therapy	Cycles received (*n*)	Reason for removal from study
1	63	F	Breast	Imp	Multiple chest wall nodules	CMFP, AC, tamoxifen, RT	3	Cr = 2.0
13	71	F	Breast	Imp	Inflammatory skin, chest wall	FAC, tamoxifen, RT	1	Cr = 2.0
25	72	F	Breast	PR	Lung nodules, supraclavicular nodes	CMF, tamoxifen	3	Patient stopped therapy
26	39	F	Breast	PR	Lung nodules	CMF, tamoxifen, RT	4	Progressed
6	66	M	Squamous lung	PR	Lung, adrenal	Carboplatin, *cis*-PT+VP-16	3	Cr = 2.1
17	56	F	Adenocarcinoma lung	PR	Lung, lymphangitic spread	None	4	Cr = 2.1
18	80	M	Squamous lung	PR	Lung, liver, nodes	None	4	Cr = 2.2
19	76	M	Squamous lung	PR	Lung, nodes	None	1	Cr = 2.7
23	57	M	Large-cell lung	Imp	Lung, bone	*cis*-Pt +VP-16, RT	7	Patient stopped therapy
34	57	F	Large-cell lung	PR	Bilateral lung masses, nodes	None	4	Cr = 2.0
28	65	M	Small-cell lung	PR	Nodes, brain	CAV + VP-16, RT	1	Orthostatic, plt = 76,000/μl
4	41	M	Brain	PR	Brain	High-dose AZQ with ABMT, RT	3	plt = 80,000/μl
30	68	F	Ovarian	Imp	Omentum, mesentery	*cis*-Pt + C	5	Cr = 1.8, C_{Cr} = 45 ml/min
39	66	M	Pleural mesothelioma	PR	Multiple masses and large effusion	None	2	Cr = 2.2

C, cyclophosphamide; M, methotrexate; F, 5-fluorouracil; P, prednisone; A, Adriamycin; V, vincristine; *cis*-Pt, cisplatin; VP-16, etoposide; plt, platelets; RT, radiotherapy; Cr, serum creatinine (mg/dl); C_{Cr}, creatinine clearance; ABMT, autologous bone marrow transplantation; AZQ, aziridinylbenzoquinine; ID, patient identification number; PR, partial response in patients with measurable disease; Imp, objective improvement of evaluable disease

Daily serum LDH levels were followed during 31 cycles in 15 patients whose LDH value had not previously been elevated due to disease. There was a consistent increase (mean, 73 units) within 4 days. Of these 15 patients, 8 subsequently showed a response.

Responses

The characteristics of the 14 patients who responded (n = 9) or significantly improved (n = 5) are shown in Table 6. Response of measurable disease or significant improvement in evaluable disease occurred in 4 of 7 (57%) patients with refractory breast carcinoma; in 6 of 17 (35%) subjects with non-small-cell lung cancer; and in 1 patient each with refractory small-cell lung cancer, glioblastoma, ovarian carcinoma, and extensive pleural mesothelioma. Of the 14 cases of response or objective improvement, 9 occurred in patients who had failed one or more standard chemotherapy regimens, including 3 of 8 subjects who had failed prior treatment with moderate- to high-dose *cis*-Pt.

All 9 responses in 32 (28%) patients with measurable disease were partial. Five other patients had a significant decrease in evaluable disease with improvement of their symptoms: patient 13 exhibited a significant improvement in extensive inflammatory skin and arm involvement after one cycle; patient 1 achieved resoluton of chest wall nodules; patient 4 showed a marked decrease in a nonmeasurable brain mass plus edema; patient 30 displayed a decrease in the ovarian carcinoma serum marker CA-125 from 523 to 26 after four cycles; and patient 23 experienced resolution of bone pain and significant decrease in a nonmeasurable primary lung mass with associated atalectasis. Patients who showed a tumor response or improvement experienced a degree of myelotoxicity and UrA elevation similar to that exhibited by the group as a whole, although 8 of the 14 responders (57%) as opposed to 7 of 26 (27%) nonresponders had a transient LDH elevation (chi-squaretest = 3.55; P = 0.06). The median duration of response was 4.5+ months (range, 1+–8+ months). In 9 of the 14 responding or improved patients, treatment was discontinued due to azotemia (Table 6).

Discussion

The first objective of this pilot study was to determine the feasibility of our in vitro model [29] in the clinical setting. The doses and schedules of the three drugs previously tested in vitro were deliberately chosen as being clinically achievable. The pilot study was then designed to use the optimal doses and schedules of Ara-C, HU, and *cis*-Pt described in the pharmacologic literature that would achieve the levels that demonstrated synergy in vitro. Our second goal was to fully characterize the toxicity pattern, and our final objective was to determine whether this regimen could be used in phase II studies in both previously untreated patients and those resistant to *cis*-Pt. To address these objectives, accrual of a sufficient number of patients with and without prior chemotherapy was mandatory. Thus, this pilot study was neither a phase I nor a phase II trial in the classic sense, similar to other recent attempts at modulation of clinical drug resistance. We found that administration of this three-drug program was feasible and did not result in major acute toxicity. The majority of patients were either older or had received extensive prior therapy such that some of the treatment-related effects were not unexpected. However, the two major toxicities of dose-limiting thrombocytopenia and treatment-limiting azotemia deserve further comment.

Treatment at the intended Ara-C dose was clearly feasible in the untreated population but was not possible due to thrombocytopenia in patients who had received prior chemotherapy. However, the duration of the platelet nadir was extremely short; in fact, it would have been missed had CBCs been obtained only on days 8 and 15 following therapy. None of the three drugs used alone at these doses should cause significant thrombocytopenia. The combination of Ara-C and HU is synergistic in vitro [28, 34], and two clinical trials testing different doses and schedules in refractory leukemia and lymphoma patients reported varying degrees of myelosuppression [15, 25]. Thus, additive or possibly synergistic myelotoxicity cannot be ruled out in the current study.

Azotemia occurred in 20/40 patients, was unrelated to cycle number in a given patient, and was treatment-limiting in 9 of 14 responding patients. Once azotemia was noted, patients were removed from study instead of decreasing the *cis*-Pt dose, since there is little evidence that further renal toxicity is avoided by *cis*-Pt dose modification. It remains to be determined whether the nephrotoxicity seen in this study was attributable to *cis*-Pt alone or to a synergistic effect of the three-drug combination. In 14 previously described trials involving patients given oral or i.v. HU, nephrotoxicity did not develop, although one study reported hyperuricemia, uricosuria, and a 5%–25% decrease in phenolsulfonphthalein excretion [24]. In the present trial, azotemia occurred more frequently in cycles in which patients showed a higher baseline creatinine value and higher posttreatment UrA elevations. An adverse *additive* interaction may have occurred at the renal tubular level among (1) the uricosuric effect of HU, (2) a possible mild tumor lysis (transient increase in LDH and UrA values) that may have raised the serum and urinary UrA values, and (3) the direct toxic effect of *cis*-Pt. Alternatively, *synergistic* renal toxicity from the three drugs may have occurred. A review of 25 studies of Ara-C plus *cis*-Pt and a few trials of *cis*-Pt and HU combinations did not find an increase in nephrotoxicity over that obtained using *cis*-Pt alone. There is recent evidence that the levels of *cis*-Pt-DNA adducts in normal human tissue are similar to those achieved in tumors and that increased nephrotoxicity may occur in patients with higher adduct levels in kidney tissue [18, 20]. Therefore, the azotemia in this trial might also partly be explained by inhibition by Ara-C and HU of the repair of *cis*-Pt-DNA adducts in kidney tissue.

We recognize that this pilot study does not prove that the anti-tumor effect of *cis*-Pt was enhanced and/or that *cis*-Pt resistance was overcome in tumor tissue. However, the trial was not designed to formulate such conclusions. Many responses observed in this trial could be attributed to *cis*-Pt alone. Nevertheless, several of the responses appear to provide preliminary support for the hypothesis on which the in vitro model was based. In particular, three of eight patients who had previously failed *cis*-Pt treatment either responded or showed significant improvement in evaluable disease. Two of the other five patients had small-cell carcinoma that had progressed after initial treatment with *cis*-Pt and VP-16; one subject achieved a 30% decrease in measurable disease after one cycle of this therapy but refused additional treatment, and the other patient showed a response in the liver but progressed in bone after four cycles. The majority of patients who responded or improved exhibited an elevation in LDH and UrA values. In addition, flare reactions were observed in areas of subcutaneous metastases when present. An acute lysis syndrome was previously described in patients with refractory lymphoma who were treated with *cis*-Pt, Ara-C, and dexamethasone [31] and may have occurred in some patients in the present trial.

The three original objectives of this pilot study were achieved, but not entirely in a positive sense. Administration of this novel three-drug combination was feasible without major acute toxicity. The hematologic toxicity was of short duration and caused no morbidity in the majority of patients. The degree of thrombocytopenia was acceptable only in previously untreated patients at the target doses, suggesting that this regimen could only be brought to phase II trials in untreated patients. In addition, the degree of azotemia was treatment-limiting in the majority of responders, in other patients it significantly delayed subsequent cycles. Nevertheless, the anti-tumor responses were impressive.

These results suggest to us that additional pilot data is warranted before this regimen undergoes more widespread clinical testing. We deliberately did not modify the doses or the schedule during the trial because our goal was to attempt to duplicate and test the safety of the in vitro model. A major concern is that whereas we should have achieved the target concentrations in vivo based on the literature, the levels may have been either too low, in which case no synergy would be expected, or too high, resulting in unnecessary toxicity. We have therefore initiated a second pilot study in treated and untreated cohorts. A continuous infusion of HU has been substituted for oral HU to enable more effective achievement of a steady state

of HU. Serum levels of the three agents are being measured, with dose modification being undertaken if necessary. Pharmacologic manipulation of UrA with allopurinol is also being instituted to eliminate elevations in posttreatment UrA values as a possible cause of azotemia. The results of this second study will determine (1) the optimal phase II doses in the previously untreated subset and (2) whether the target drug levels can be achieved without prohibitive toxicity in patients who have undergone prior therapy. To determine whether synergy occurs among these drugs, trials comparing this regimen vs treatment with *cis*-Pt alone in *cis*-Pt-responsive tumors would be necessary. Although experimental evidence suggests that DNA repair is an important component of *cis*-Pt resistance, proof of the advantage of regimens such as this will ultimately require demonstration that repair inhibition plays the major role in de novo and acquired clinical *cis*-Pt resistance.

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References

1. Beckloff GL, Lerner HG, Frost D, Russo-Alesi FM, Gitomer S (1965) Hydroxyurea in biologic fluids: dose-concentration relationship. Cancer Chemother Rep 48: 57
2. Belliveau JF, Forastiere AA, Vogel W, Goren M, Posner M, O'Leary G (1987) Cisplatin pharmacokinetics: continuous infusion vs intermittent bolus in the treatment of head and neck cancer. Proc Am Assoc Cancer Res 28: 189
3. Belt RJ, Himmelstein KJ, Patton TF, Bannister SF, Sternson LA, Repta AJ (1979) Pharmacokinetics of non-protein-bound platinum species following administration of *cis*-dichlorodiammineplatinum(II). Cancer Treat Rep 63: 1515
4. Belt RJ, Haas CD, Kennedy J, Taylor S (1980) Studies of hydroxyurea administered by continuous infusion. Cancer 46: 455
5. Creasey WA, Capizzi RL, DeConti RC (1970) Clinical and biochemical studies of high-dose intermittent therapy of solid tumors with hydroxyurea. Cancer Chemother Rep 54: 191
6. Davidson JD, Winter TS (1963) A method of analyzing for hydroxyurea in biologic fluids. Cancer Chemother Rep 27: 97
7. Dunn WC, Regan JD (1979) Inhibition of DNA excision repair in human cells by arabinofuranosylcytosine: effect on normal and xeroderma pigmentosum cells. Mol Pharmacol 15: 367
8. Fram RJ, Kufe DW (1985) Effect of Ara-C and hydroxyurea on the repair of X-ray induced DNA single-strand breaks in human leukemic blasts. Biochem Pharmacol 34: 2557
9. Fram RJ, Cusick PS, Marinua MG (1986) Studies on mutagenesis and repair induced by platinum analogs. Mutat Res 173: 13
10. Fraval HNA, Rawlings CJ, Roberts JJ (1978) Increased sensitivity of UV-repair deficient human cells to DNA bound platinum products. Mutat Res 51: 121
11. Hiss EA, Preston RJ (1977) The effect of cytosine arabinoside on the frequency of single strand breaks in DNA of mammalian cells following irradiation or chemical treatment. Biochim Biophys Acta 478: 1
12. Kufe DW, Weishselbaum R, Egan EM (1984) Lethal effects of 1-β-D-arabinofuranosylcytosine incorporation into DNA during UV repair. Mol Pharmacol 25: 4334
13. Meyn RE, Jenkins SF, Thompson LG (1982) Defective removal of DNA crosslinks in a repair-deficient mutant of Chinese hamster ovary cells. Cancer Res 42: 3106
14. Patton TF, Himmelstein KJ, Belt R, Bannister SJ, Sternson LA, Repta AJ (1978) Plasma levels and urinary excretion of filterable platinum species following bolus injections and IV infusion of cisplatinum in man. Cancer Treat Rep 62: 1359
15. Pfeifle CE, Howell SB (1983) Phase I trial of cytarabine and hydroxyurea. Cancer Treat Rep 67: 1127
16. Plooy ACM, Dijk M van, Berends F, Lohman PHM (1985) Formation and repair of DNA interstrand crosslinks in relation to cytotoxicity and unscheduled DNA synthesis induced in conrol and mutant human cells treated with *cis*-diamminedichloroplatinum(II). Cancer Res 45: 4178
17. Plunkett W, Liliemark JO, Estey E, Keating MJ (1987) Saturation of ara-CTP accumulation during high-dose Ara-C therapy: pharmacologic rationale for intermediate-dose Ara-C. Semin Oncol 14 [Suppl 1] June: 159
18. Pourier MC (1989) DNA adduct formation in tissues of human cancer patients. Proc Am Assoc Cancer Res 30: 661
19. Reece PA, Strafford I, Russell J, Khan M, Gill PG (1987) Creatinine clearance as a predictor of ultrafilterable platinum deposition in cancer patients treated with cisplatin: relationship between peak ultrafilterable platinum plasma levels and nephrotoxicity. J Clin Oncol 5: 304
20. Reed E, Burt-Gupta S, Katz D, Poirier MC (1989) Platinum-DNA adducts measured at autopsy in multiple human tissues. Proc Am Assoc Cancer Res 30: 276
21. Roberts JJ, Thomson AJ (1979) The mechanism of action of antitumor platinum compounds. Prog Nucleic Acid Res Mol Biol 22: 71
22. Robichaud NJ, Fram RJ (1987) Prevention of Ara-C induced cytotoxicity by hydroxyurea in LoVo colon carcinoma cells. Biochem Pharamcol 36: 1673
23. Rosner F, Rubin H, Parise F (1971) Studies on the absorption, distribution, and excretion of hydroxyurea. Cancer Chemother Rep 55: 167
24. Samuels ML, Howe CD (1964) Renal abnormalities induced by hydroxyurea (NSC 32 065). Cancer Chemother Rep 40: 9
25. Schilsky RL, Williams SF, Ultmann JE, Watson S (1987) Sequential hydroxyurea-cytarabine chemotherapy for refractory non-Hodgkin's lymphoma. J Clin Oncol 5: 419
26. Snyder RD (1984) The role of deoxynucleoside triphosphate pools in the inhibition of DNA-excision repair and replication in human cells by hydroxyurea. Mutat Res 131: 163
27. Spriggs DR, Robbins G, Takvorian T, Kufe DW (1985) Continuous infusion of high-dose 1-β-D-arabinofuranosylcytosine. A phase I and pharmacological study. Cancer Res 45: 3932
28. Streifel JA, Howell SB (1981) Synergistic interaction between 1-β-D-arabinofuranosylcytosine, thymidine, and hydroxyurea against human B cells and leukemic blasts in vitro. Proc Natl Acad Sci USA 78: 5132
29. Swinnen LJ, Barnes DM, Fisher SG, Albain KS, Fisher RI, Erickson LE (1989) 1-β-D-Arabinofuranosylcytosine and hydroxyurea production of cytotoxic synergy with diamminedichloroplatinum(II) and modification of platinum-induced DNA interstrand crosslinking. Cancer Res 49: 1383
30. Swinnen LJ, Fisher SG, Erickson LC (1989) Ultraviolet irradiation produces cytotoxic synergy and increased DNA interstrand crosslinking with *cis*- and *trans*-diamminedichloroplatinum(II) Carcinogenesis 10: 1465
31. Velasquez NS, Cabanillas F, Salvador P, McLaughlin P, Fredrik M, Tucker S, Jagannath S, Hagemeister FB, Redman JR, Swan F, Barlogie B (1988) Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone. Blood 71: 117

32. Vermorken JB, Vijgh WJF van de, Klein I, Gall HE, Groeningen CJ, Hart GAM, Penedo H (1986) Pharmacokinetics of free and total platinum species after rapid and prolonged infusions of cisplatin. Clin Pharmacol Ther 39: 136
33. Vugrin M, Vugrin D, Goodwin B (1985) A study of the pharmacokinetics of cisplatin 100 mg/m^2 /day given by 20 minute infusion, 24-hour infusion, or 20 mg/m^2/day $\times 5$ in patients with non-small-cell lung cancer. Proc Am Soc Clin Oncol 4: 189
34. Walsh CT, Craig RW, Agarwal RP (1980) Increased activation of 1-β-D-arabinofuranosylcytosine by hydroxyurea in L1210 cells. Cancer Res 40: 3286
35. Warner HR, Demple BF, Deutsch WA, Kane CM, Linn S (1980) Apurinic/apyrimidinic endonucleases in repair of pyrimidine dimers and other lesions in DNA. Proc Natl Acad Sci USA 77: 4602