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Phase I clinical and pharmacokinetic study of cyclophosphamide administered by five-day continuous intravenous infusion

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Summary. A total of 14 patients, 7 male and 7 female, received in all 21 evaluable courses of cyclophosphamide administered by 5-day continuous infusion. Cyclophosphamide doses were escalated from 300 to 400 mg/m^2 per day for 5 days and repeated every 21–28 days. The patient population had a median age of 55 years (range 38–76) and a median Karnofsky performance status of 80 (range 60–100). Only 1 patient had not received prior therapy; 5 patients had received only prior chemotherapy, 1 had received only prior radiotherapy, and 7 had received both. Tumor types were gastric (1), lung (2), colon (4), urethral adenocarcinoma (1), cervical (2), chondrosarcoma (1), melanoma (1), uterine leiomyosarcoma (1), and pancreatic (1). The dose-limiting toxicity was granulocytopenia, with median WBC nadir of 1700/μl (range 100–4800) in 8 heavily pretreated patients treated at 350 mg/m^2 per day for 5 days. One patient without heavy prior treatment received two courses at 400 mg/m^2 and had WBC nadirs of 800/μl and 600/μl. WBC nadirs occurred between days 9 and 21 (median 14). Drug-induced thrombocytopenia occurred in only one patient (350 mg/m^2 per day, nadir 85000/μl). Neither hyponatremia nor symptomatic hypoosmolality was observed. Radiation-induced hemorrhagic cystitis may have been worsened in one patient. Nausea and vomiting were mild. Objective remissions were not observed. The maximum tolerated dose for previously treated patients is 350 mg/m^2 per day for 5 days. This dose approximates the doses of cyclophosphamide commonly used with bolus administration. Plasma steady-state concentrations (Css) of cyclophosphamide, measured by gas liquid chromatography, were 2.09–6.79 μg/ml. Steady state was achieved in 14.5 ± 5.9 h (mean ± SD). After the infusion, cyclophosphamide disappeared from plasma monoexponentially, with a t ½ of 5.3 ± 3.6 h. The area under the curve of plasma cyclophosphamide concentrations versus time (AUC) was 543 ± 150 μg/ml h and reflected a cyclophosphamide total-body clearance (CL_{TB}) of 103 ± 31.6 ml/min. Plasma alkylating activity, assessed by *p*-nitrobenzyl-pyridine, remained steady at 1.6–4.3 μg/ml nor-nitrogen mustard equivalents. Urinary excretion of cyclophosphamide and alkylating activity accounted for 9.3% ± 7.6% and 15.1% ± 2.0% of the administered daily dose, respectively. The t ½ and AUC of cyclophosphamide associated with the 5-day continuous infusion schedule are similar to those reported after administration of cyclophosphamide 1500 mg/m^2 as an i.v. bolus. The AUC of alkylating activity associated with the 5-day continuous infusion of cyclophosphamide is about three times greater than the AUC of alkylating activity calculated after a 1500-mg/m^2 bolus dose of cyclophosphamide. Daily urinary excretions of cyclophosphamide and alkylating activity associated with the 5-day continuous infusion schedule are similar to those reported after bolus doses of cyclophosphamide.

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Introduction

Cyclophosphamide has been used in varying doses and in a variety of schedules. The two major modes, however, have been high-dose, i.v. bolus therapy and low-dose oral daily therapy. Recently, several other drugs with known activity when given in intermittent bolus doses have been shown to have equal or augmented activity when given as continuous infusions, sometimes with attenuation of toxicity [18, 19]. In these cases, the antitumor effect appears to be critically dependent not only on the concentration of drug achieved, but also on the duration of exposure. Cytosine arabinoside, etoposide, bleomycin, vincristine, and 5-fluorouracil are notable examples [1, 4, 5, 7, 22]. Alkylating agents, and more specifically cyclophosphamide, by continuous infusion schedules have had minimal testing. Although cyclophosphamide is active against cells in all phases of the cell cycle, it is more effective against dividing cells [6]. Thus, exposing tumors to cyclophosphamide for relatively long periods may increase cell kill in tumors with a relatively low growth fraction.

Examples in the literature of patients receiving continuous infusion therapy with cyclophosphamide include patients with carcinoma who received 300–750 mg/m^2 per day i.v. for 3 days and patients with acute leukemia who were treated with 400 mg/m^2 per day i.v. for 4 days [3, 21]. The dose-limiting toxicity in each study was reversible myelosuppression, and especially leukopenia.

Thus, our purpose was further evaluation of the use of cyclophosphamide given by 5-day continuous i.v. infusion, with particular attention to determining the maximum tolerated dose, identifying the toxicities associated with such treatment, and elucidating the pharmacokinetics

of cyclophosphamide associated with such drug administration.

Materials and methods

Patient selection and evaluation. All patients entered into this study had advanced, histologically documented cancer refractory to conventional therapy and had recovered from toxic effects of all other prior treatments. Patients were ineligible if they had received radiotherapy or chemotherapy in the preceding 4 weeks (8 weeks for those receiving nitrosoureas or mitomycin C), and if they had a Karnofsky performance less than 60%. All patients had adequate pretreatment bone marrow function (WBC ≥ 3500 µl and platelets $\geq 100\,000/\mu l$), liver function (bilirubin ≤ 2.0 mg/dl), and renal function (creatinine ≤ 2.0 mg/dl). Patients with a history of congestive heart failure were excluded. Patients receiving phenobarbital, phenytoin, allopurinol, or adrenal steroids were excluded. Pretreatment evaluation included a complete history and physical examination, tumor measurements when applicable, documentation of weight, body surface area and performance status, and the following laboratory studies: complete blood count with differential and platelet count; urinalysis; serum urea nitrogen, creatinine, alkaline phosphatase, bilirubin, aspartate aminotransferase, glucose, calcium, and phosphorus; chest roentgenograph and electrocardiogram. Additional roentgenographic, radionuclide and computer-assisted tomographic studies were performed as needed for assessment and measurement of disease status.

After treatment, patients had weekly evaluations, which included physical examination, determinations of weight, performance status, complete blood count, serum creatinine, serum urea nitrogen, serum bilirubin and glutamine-oxaloacetic transaminase; and assessment of toxicity and response to therapy. Prior to entry into this study all patients were informed of the investigational nature of the treatment and signed an informed consent form approved by the Institutional Review Board. Measurable disease was not required, but was measured serially when present.

Drug administration. The initial dose level was 300 mg/m^2 per day by continuous i.v. infusion for 5 days. The daily dose of cyclophosphamide was divided in half and diluted in 1000 ml of 5% dextrose in water and/or 0.077 *M* or 0.154 *M* NaCl solution. Each 1000 ml solution was administered by continuous infusion over 12 h with an IVAC 960 infusion pump (IVAC Corp., San Diego, Calif) or Travenol Flo-Gard 8000 volumetric infusion pump (Travenol Labs., Deerfield, Ill). Patients were hospitalized for the entire 5-day duration of the infusion. Treatments were repeated every 21–28 days, provided toxicity had reversed and there was no tumor progression. Doses were escalated by 50 mg/m^2 per day in subsequent patients, the objective being to reach a maximum tolerated dose with acceptable, reversible toxicity. At least three patients were treated at any dose level before dose escalation. No escalations were made in individual cases, except for one patient in whom the initial dose of 300 mg/m^2 per day for 5 days was escalated to 375 mg/m^2 per day for 5 days. Antiemetics were used when needed. During the 5 days of infusion, serum osmolality and serum sodium were measured daily.

Sample acquisition. Heparinized blood samples were drawn before therapy and at 4, 12, and 20 h during the 1st day of cyclophosphamide infusion. On days 2–5, samples were drawn daily at 12-h intervals. A sample was drawn at the time of completion of infusion and at 1, 2, 3, 4, 6, 8, 18, and 24 h after completion. After collection, samples were centrifuged for 10 min at 1000 *g* and the resulting plasma supernatants were transferred into plastic tubes, frozen, and stored at -20 °C until the time of assay.

Urine was collected on ice as 6-h aliquots for 6 days, starting at the time of initiation of treatment. At the end of each 6-h collection the volume of urine was measured and a 10-ml sample was frozen and stored at -20 °C in plastic tubes until the time of assay.

Analysis of cyclophosphmide and alkylating activity in plasma and urine. Unchanged cyclophosphamide was quantified by gas-liquid chromatography after extraction into $CHCl_3$ and derivatization with trifluoroacetic acid as described previously [14]. Alkylating activity in plasma and urine was assessed spectrophotometrically by the *p*-nitrobenzyl-pyridine technique of Friedman and Boger [10, 15]. Computer modeling of the decline in plasma concentrations of cyclophosphamide was performed with MLAB [17], a computer program which performs iterative, weighted, nonlinear, least-squares regression. Based on visual inspection, initial estimates of concentration and coefficient of excretion were made by graphic analysis and curves were fitted to a monoexponential decline with weighting by the reciprocal of the observation variance. Total-body clearance (CL_{TB}) was calculated as the dose divided by the area under the curve of plasma cyclophosphamide concentrations versus time (from 0 to infinity) (AUC).

Results

Patient population

A group of 14 patients received a total of 24 courses of cyclophosphamide, 21 of which were evaluable for toxicity. One patient refused further treatment on the 2nd day of the first course of treatment and left the hospital. One patient received only half of her second course because of suspected cyclophosphamide-exacerbated hemorrhagic cystitis. One patient received a second course of therapy with a pretreatment platelet count of 75 000/µl, thereby rendering that course inevaluable for hematologic toxicity. Patient characteristics and the tumor types represented are shown in Table 1.

Hematologic toxicity

Leukopenia was the dose-limiting toxicity (Table 2). Significant leukopenia was noted at the dose of 300 mg/m^2 per day. One of four patients treated at this level developed a WBC nadir of 200/µl and fever, but recovered with antibiotic therapy. It is of note that three of the four patients treated at 300 mg/m^2 per day received extensive radiation therapy and chemotherapy prior to enrollment in the current study.

Three of seven patients treated with 350 mg/m^2 per day developed fever while leukopenic, but all three recovered with antibiotic therapy. One patient had a perirectal infection, one had infection of widely ulcerated skin melanoma lesions of a lower extremity, and one had transient fever of unknown etiology.

Table 1. Patient characteristics

Number of patients	14
Females/males	7/7
Age-median (range)	55 (38–76)
Karnofsky performance status median (range)	80% (60–100)
Previous treatment	
Chemotherapy only	5
Radiotherapy only	1
Chemotherapy & radiotherapy	7
No prior therapy	1

Tumor type	No. of patients	No. evaluable for response*
Gastric adenocarcinoma	1	1
Lung- non small cell carcinoma	2	2
Colon adenocarcinoma	4	4
Urethral adenocarcinoma	1	1
Cervocal carcinoma	2	2
Chondrosarcoma	1	1
Melanoma	1	1
Uterine leiomyosarcoma	1	1
Pancreatic adenocarcinoma	1	0

* Criteria for evaluation for response included measurable disease and one course of treatment

The one patient who received 375 mg/m^2 per day had tolerated a previous first course at 300 mg/m^2 per day well (WBC nadir of 2800/μl), but developed a WBC nadir of 600/μl at the higher dose. In addition, this patient developed thrombocytopenia very early during his second course. In fact, his second course day-1 platelet count was 75000/μl, and it declined to 35000/μl by day 5. He did not fully recover platelet production, but did recover leukocyte production. Both clinically and at autopsy, the cause of his prolonged thrombocytopenia was felt to be related to widespread gastric carcinomatosis with disseminated intravascular coagulation.

On the basis of these results, the maximum tolerated dose (MTD) for heavily pretreated patients was established at 350 mg/m^2 per day for 5 days. Further dosage escalation was restricted to patients who had not received extensive prior radiation therapy or more than one course of prior cytotoxic chemotherapy. A single patient was treated with cyclophosphamide at a dose of 400 mg/m^2 per day for 5 days. At this dosage, the patient developed WBC nadirs of 800/μl and 600/μl after his first and second courses of therapy, respectively (Table 2). This patient recovered uneventfully from each instance of leukopenia and experienced no infectious complications. During neither course of cyclophosphamide infusion did the patient's platelet nadir decrease below 265000/μl (Table 2).

Seven patients received more than one course of therapy at the same dose level. Six of these did not show differences in the degree of myelotoxicity after each course. The remaining patient had a WBC nadir of 1900 after the first course and a WBC nadir of 200 after the second course.

Nonhematologic toxicities

Mild to moderate nausea and vomiting were observed in seven patients and made antiemetics necessary in two (Table 3).

One patient with cervical cancer, who had received prior pelvic irradiation and had a long history of intermittent hematuria, was felt to have an increase in the severity of hematuria on day 2 of her second course of therapy at 350 mg/m^2 per day. She underwent cystoscopy, which showed hemorrhagic cystitis, and the drug was discontinued. Her hematuria later resolved. This patient did not require transfusions and did not receive treatment specifically directed at her hemorrhagic cystitis.

In 11 patients, serum osmolalities were measured daily during the 5 days of therapy. In 8 of these patients, serum osmolality remained within normal limits (275–295 mosmol). Three patients had decreases in serum osmolality from 280 to 271 mosmol, 291–270 mosmol, and 282–273 mosmol, respectively. There were no symptoms associated with these laboratory findings. Serum sodium was measured daily during therapy in all patients, and no changes were detected. Fluid retention developed in 2 patients and was attributed to the fluid and sodium load associated with drug infusion. This was easily reversed with diuretic therapy. There were no observed renal or hepatic toxicities associated with 5-day continuous infusion of cyclophosphamide at the doses employed in this study.

Responses

No objective responses were observed among the 13 patients evaluable for response. One patient with colon

Table 2. Hematologic toxicity

Dose (mg/m^2/d × 5)	No. of patients	No. of evaluable courses	WBC median nadir (range)	Platelet median nadir (range)	Median days to WBC nadir (range)	Median days to WBC 3.5 × 10^3 (range)
300[a]	4	5	1300[b] (200–2800)	190000[b] (105000–229000)	14 (12–19)	21 (17–26)
350	8	14	1700 (100–4800)	322000 (85000–600000)	14 (9–21)	20 (17–28)
400	1	2	800 600	265000 281000	14 13	23 28

[a] One patient received a second dose at 375 mg/m^2 and later developed disseminated intravascular coagulopathy due to disseminated gastric carcinomatosis shown at autopsy. This second course was not evaluable because his initial platelet count was 75000/μl and constituted a protocol violation

[b] Cells per microliter

Table 3. Non-hematologic toxicity

Dose ($mg/m^2/d \times 5$)	No. of patients	Nausea and vomiting: No. of patients/grade	Hemorrhagic cystitis	Edema fluid retention
300[a]	4	1/I[b] 2/II	0	1
350	8	3/I	1[c]	1
400	1	1/I	0	0

[a] One patient had a second course of 375 $mg/m^2/d \times 5$
[b] ECOG toxicity scale
[c] This patient had a history of intermittent hematuria after pelvic irradiation. Her hematuria increased during the second course of therapy, which was interrupted on day 3

Table 4. Pharmacokinetics of cyclophosphamide and metabolites observed with 5-day continuous i.v. infusion

Cyclophosphamide	
Time to Css	14.5 ± 5.9 hr
Css	2.09–6.79 μg/ml
AUC	543 ± 150 μg/ml.h
t 1/2	5.3 ± 3.6 h
CL_{TB}	103 ± 31.6 ml/min
24-h urinary excretion	$9.3\% \pm 7.6\%$ of daily dose
Alkylating activity	
Css	1.6–4.3 μg/ml
AUC	1.08–2.9 μmol/ml.h
24-h urinary excretion	$15.1\% \pm 2.0\%$ of daily dose

carcinoma had minor decreases in lymphadenopathy and hepatomegaly; one patient with colon cancer had stable disease for 3 months; and one patient with a uterine leiomyosarcoma had stable disease for 4 months.

Pharmacokinetics

Plasma concentrations of cyclophosphamide and alkylating activity were measured in 13 courses administered to ten patients. Plasma steady-state concentrations (Css) of cyclophosphamide were achieved in all patients by 14.5 ± 5.9 h (mean $\pm$ SD) after initiation of the cyclophosphamide infusion and varied between 2.09 and 6.79 μg/ml among the various patients and courses studied (Table 4). Once established, cyclophosphamide Css remained relatively constant, with daily variations from the mean Css being $\leq 25\%$ in any given course. There was no consistent progressive increase or decrease in plasma concentrations of cyclophosphamide which might have reflected induction or impairment of any cyclophosphamide clearance process. After cessation of the cyclophosphamide infusion, plasma concentrations of cyclophosphamide decreased monoexponentially with t ½ of 5.3 ± 3.6 h. The cyclophosphamide exposure, as expressed by AUC, was 543 ± 150 μg/ml·h and reflected cyclophosphamide CL_{TB} of 103 ± 31.6 ml/min.

During the cyclophosphamide infusion, plasma concentrations of alkylating activity varied between 1.6 and 4.3 μg/ml nor-nitrogen mustard equivalents. After completion of the 5-day infusion, concentrations of alkylating activity rapidly declined to values below the limits of detection of the *p*-nitrobenzyl-pyridine assay. Although plasma concentrations of alkylating activity remained low throughout the course of the infusion, there was substantial exposure to alkylating activity, as expressed in the AUC of 1.08–2.9 μmol/ml·h.

Urinary excretions of cyclophosphamide and alkylating activity were measured on each day of nine courses administered to seven patients. Urinary excretion of cyclophosphamide accounted for $9.3\% \pm 7.6\%$ of the daily administered dose and was consistent from day to day for each patient. Urinary excretion of alkylating activity accounted for $15.1\% \pm 2.0\%$ of the daily administered dose.

Discussion

Manipulations of drug doses and schedules of administration are commonly used in animal and clinical studies to modify toxicity and efficacy. In some instances, marked therapeutic advantage [1] or reduction of toxicity [2] have been observed. Cyclophosphamide is usually administered in a low-dose, oral daily form or according to an intermittent, relatively high-dose schedule by the i. v. route. Very limited experience exists with cyclophosphamide used by a continuous infusion i.v. schedule [3, 16, 21]. Even less is known about the pharmacokinetics of continuous i.v. cyclophosphamide. Cyclophosphamide by continuous 5-day i.v. infusion has been tested in the treatment of acute lymphocytic leukemia [21], and continuous infusion of iphosphamide, a cyclophosphamide analogue, is being tested in soft tissue sarcoma [23]. Because cyclophosphamide is one of the most common and effective chemotherapeutic agents in use singly and in combination, it is critical that any modifications in its schedule of administration be based on adequate pharmacokinetic and toxicity information.

The MTD in previously heavily pretreated patients in our study was 350 mg/m^2 per day for 5 days by continuous i.v. infusion, given every 3–4 weeks. The dose-limiting toxicity was leukopenia. Previously untreated patients were not entered in this trial in numbers sufficient to define an MTD in such a patient population. However, the one patient in this study who had not been heavily pretreated and who received two courses at 400 mg/m^2 per day for 5 days developed WBC nadirs of 800/μl and 600/μl. This is consistent with the earlier report by Solidoro et al., who used a 5-day continuous infusion schedule of cyclophosphamide to treat patients with acute lymphocytic leukemia [21]. These authors reported a mean WBC nadir of 700/μl and a mean duration of leukopenia of 3 weeks after cyclophosphamide doses of 400 mg/m^2 per day for 5

days. The MTD defined in our trial also agrees well with that reported by Bedikian et al. for a 3-day continuous infusion schedule [3]. These authors defined an MTD of 600 mg/m^2 per day for 3 days, administered every 3 weeks in patients with good bone marrow reserve. Although the dose-limiting toxicity with the 3-day continuous infusion schedule was also granulocytopenia, the 3-day schedule produced more severe nausea and vomiting than that observed in our trial.

Other toxicities encountered in our study were minor and reversible. Nausea and vomiting were mild to moderate and responded well to conventional antiemetic therapy. Pre-existing radiation-induced hemorrhagic cystitis may have been exacerbated by cyclophosphamide in one patient who had radiation therapy to the pelvis and had a long history of intermittent hematuria. There were no changes in serum sodium concentrations. Three patients had minor, transient, asymptomatic decreases in serum osmolality. Fluid retention developed in two patients and seemed to be due to the fluid load associated with the 5-day drug infusion.

Although there were no objective responses, our patient population consisted of heavily pretreated patients with tumors usually unresponsive to conventional therapy. Therefore, no conclusions about efficacy can be drawn from this study.

The pharmacokinetics of cyclophosphamide administered by 5-day continuous i.v. infusion were similar to those reported after i.v. bolus administration [2, 8, 9, 11–13, 15, 20]. The t ½ of 5.3 ± 3.6 h observed in this study for the decline in plasma concentrations of cyclophosphamide is comparable to the t ½ reported by ourselves [11] and others after i.v. bolus doses of cyclophosphamide [2, 8, 9, 13, 15, 20]. Although the continuous infusion schedule resulted in predictably lower plasma concentrations of cyclophosphamide, the cyclophosphamide exposures of patients in this trial were comparable to those in patients receiving the drug by bolus administration. The areas under the curve measured in patients in the current study equalled those we calculated for a patient population receiving 1500 mg/m^2 of cyclophosphamide as a bolus for the treatment of non-small cell lung cancer [21]. As expected, the 103 ± 31.6 ml/min total-body clearance of cyclophosphamide calculated for our current patient population is consistent with the total-body clearance reported by others after bolus dosing [15].

The plasma concentrations of alkylating activity measured in patients in this trial were very low and did not increase during the 5 days of treatment. Although plasma concentrations of alkylating activity remained very low, patients in this study experienced significant exposure to alkylating activity, as reflected in an AUC which was approximately three times greater than that measured after i.v. bolus doses of cyclophosphamide 1500 mg/m^2 [11]. The pharmacodynamic correlate of this pharmacokinetic observation was the marked myelosuppression associated with the 5-day continuous infusion of cyclophosphamide. Some caution must be expressed in comparing the relative areas under the curve associated with 5-day continuous infusion and bolus schedules, because the plasma concentrations of alkylating activity measured in our current study were much closer to the limit of detection of the *p*-nitrobenzyl-pyridine assay than were those measured after bolus cyclophosphamide. As a result, there may be a greater percentage error in individual measurements, which could be greatly amplified in subsequent calculations of pharmacokinetic parameters such as AUC.

Finally, the urinary excretions of cyclophosphamide and alkylating activity observed in patients treated with cyclophosphamide as 5-day continuous infusions agree well with those previously reported in patients treated with cyclophosphamide as an i.v. bolus [2, 8, 11–13, 15, 20].

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