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ORIGINAL ARTICLE

Phase I and pharmacokinetic study of cisplatin and troxacitabine administered intravenously every 28 days in patients with advanced solid malignancies

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Abstract
Purpose To assess the feasibility of administering troxacitabine, an L-nucleoside analog that is not a substrate for deoxycytidine deaminase, in combination with cisplatin, to identify pharmacokinetic interactions, and to seek preliminary evidence of antitumor activity.
Methods Patients with advanced solid malignancies were treated with cisplatin intravenously over an hour followed by troxacitabine intravenously over 30 min on day 1 every 28 days at the following cisplatin/troxacitabine (mg/m^2) dose levels 50/4.8, 75/4.8, 50/6.4, 75/6.4, and 75/8.0. Plasma and urine sampling were performed to characterize the pharmacokinetic parameters of troxacitabine in combination with cisplatin.
Results Thirty-one patients received 77 courses of cisplatin/troxacitabine at five dose levels. Grade 4 neutropenia

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lasting more than 5 days and/or grade 4 thrombocytopenia were consistently experienced by minimally pretreated (MP) and heavily pretreated (HP) patients at doses exceeding 75/6.4 and 50/4.8 mg/m^2, respectively. Mean values for the volume of distribution at steady state and clearance of troxacitabine were 196–396 L and 7.2–9.8 L/h, respectively. A patient with metastatic non-small cell lung cancer experienced a 42% reduction in extent of disease for 6 months.

Conclusions The combination of cisplatin and troxacitabine produces dose-limiting myelosuppression at lower doses of troxacitabine than single agent doses. The recommended phase II doses of cisplatin/troxacitabine are 75/6.4 and 50/4.8 mg/m^2, every 4 weeks, for MP and HP patients, respectively. The addition of cisplatin did not substantially alter the pharmacokinetic behavior of troxacitabine.

Keywords Cisplatin · Troxacitabine · Phase I · Pharmacokinetics

Introduction

Troxacitabine (*β*-L-OddC, BCH-4556, Troxatyl) is an unnatural L-nucleoside analog with a broad range of antitumor activity in preclinical in vitro and in vivo models [1]. The distinct L-configuration confers unique mechanistic properties relative to natural D-nucleoside analogs, such as cytarabine and gemcitabine. Its cellular membrane permeation is non-carrier mediated [2]. It is resistant to deamination [1], phosphorylated from diphosphate to triphosphate by 3-phosphoglycerate kinase [3], and excised from DNA by human apurinic/apyrimidinic DNA endonuclease [4]. Troxacitabine causes chain termination after it is incorporated into DNA because it lacks a hydroxyl group and is a potent inhibitor of DNA polymerases [5].

In four phase I trials, treatment with troxacitabine on various schedules resulted in objective antitumor responses in patients with refractory myeloid leukemia, renal cell carcinoma, and ocular melanoma, and its principal toxicities were neutropenia, skin rash, and hand-foot syndrome [6–9]. Given as a single agent every 3 weeks, the recommended phase II dose for troxacitabine is 10 mg/m^2 [8]. In vitro studies demonstrate synergy between nucleoside analogs and cisplatin. This synergism may be due to several mechanisms, including increased formation of DNA-cisplatin adducts and reduced DNA repair [10–13]. The clearance of troxacitabine is predominantly renal [6–9] and thus the combination with cisplatin may lead to altered pharmacokinetics of troxacitabine. This study was designed to evaluate whether the clearance of troxacitabine was notably affected by cisplatin, particularly since evaluations of the drug combination seemed reasonable based upon preclinical synergy, the broad range of antitumor activity of each agent, and non-overlapping toxicities.

Materials and methods

Patients with pathologically or cytologically documented advanced solid malignancies that were refractory to conventional therapy or for whom no effective therapy existed were candidates for this study. Eligibility criteria also included: age ≥18 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; a life-expectancy of at least 12 weeks; no chemotherapy, radiotherapy, or other experimental therapy within 4 weeks of treatment (6 weeks for nitrosourea and mitomycin-C); adequate hematopoietic [absolute neutrophil count (ANC) ≥1,500/μL, platelet count ≥100,000/μL, hemoglobin ≥9.0 g/dL], hepatic (total bilirubin ≤1.5 mg/dL, AST and ALT ≤3 times the upper normal limit or ≤5 times the upper normal limit if liver metastasis was present), and renal (creatinine ≤1.5 mg/dL or estimated creatinine clearance ≥45 mL/min) functions; no severe medical comorbidity; no brain metastasis; and no active serious infection. All patients gave written informed consent according to federal and institutional guidelines. The study was conducted in accordance with the ethical principles stated in the Declaration of Helsinki and the applicable guidelines on good clinical practice.

Dosage and dose escalation

Three patients were treated at the initial dose level. If no cycle-1 dose-limiting toxicities (DLTs) were observed, three additional patients were treated at the next dose level. If one of three initial patients experienced a DLT at any given dose level, then three additional patients were treated at the same dose level. If a DLT occurred in at least two patients at any given dose level, then the dose escalation of that drug was halted, and the next three patients enrolled

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were treated at the next lower dose level of that drug. The other drug was then escalated in the same way to maximize the single agent doses of each drug that could be given in combination.

The maximum-tolerated dose (MTD) and the recommended treatment dose were defined as the highest dose level at which less than two of six patients experienced DLT in cycle 1. More than six patients could be treated at the recommended dose level to obtain additional information about the tolerability of the dose. Patients were stratified into heavily (HP) and minimally pretreated (MP) categories so that a recommended dose could be defined for each group. HP patients were defined as those who had been previously treated with ≥6 courses of alkylating agent-containing chemotherapy (except low-dose cisplatin) or ≥4 courses of carboplatin, ≥2 courses of mitomycin-C or nitrosourea, or radiation therapy to >25% of hematopoietic reserves. DLT was defined as any of the following occurring during cycle 1: grade 4 neutropenia lasting more than 5 days or associated with fever (≥38.5°C); grade 4 thrombocytopenia; any grades 3 or 4 non-hematologic toxicity except for alopecia and suboptimally treated nausea or vomiting; and unresolved drug-related toxicity delaying re-treatment more than 2 weeks. Toxicity was graded before every cycle according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 2.0.

Drug administration

Troxacitabine was supplied by BioChem Pharma, Inc., Laval, Quebec, as a lyophilized powder in 25 mg vials. Cisplatin was commercially available and was supplied in 10 mg (20 mL) and 50 mg (50 mL) vials. Before infusion, cisplatin was diluted in 500 mL 0.9% saline and troxacitabine reconstituted in 0.9% saline. All patients were pretreated with 0.5 L of 0.9% saline infused over 1 h starting 1 h prior to the administration of cisplatin. Subsequently, cisplatin was infused over 1 h, followed by a 30-min infusion of troxacitabine. Afterwards, all patients received 1 L of 0.9% saline over 2 h. Courses of treatment were repeated every 4 weeks. Antiemetic therapy was administered 30 min before cisplatin chemotherapy and consisted of ondansetron 8 mg intravenously or 16 mg orally, granisetron 1 mg intravenously or orally, or dolasetron 100 mg intravenously or 200 mg orally. Dexamethasone 20 mg intravenously was also given followed by dexamethasone 8 mg orally every 6 h for four doses. Prochlorperazine was administered on an as needed basis. Filgrastim use was permitted for grade 4 neutropenia for more than 5 days or associated with fever (≥38.5°C). Filgrastim was discontinued at least 24 h before the start of the next course. Patients receiving recombinant erythropoietin prior to study entry were allowed to continue this therapy during the study.

Pretreatment assessment and follow-up studies

Medical histories were collected and physical examinations and routine laboratory studies were performed pretreatment and weekly. Routine laboratory studies included complete blood cell counts, differential leukocyte counts, liver and renal function tests, and serum electrolyte levels. Pretreatment studies also included relevant radiologic studies for evaluation of all measurable or evaluable lesions of malignancy, as well as an assessment of relevant tumor markers. Radiologic studies for disease status assessments were repeated after every other course or as needed to confirm response. Patients were able to continue treatment if they did not develop progressive disease unless they had intolerable toxicity in spite of two dose reductions or wished to voluntarily withdraw from the study. A complete response was defined as the disappearance of all disease on two measurements separated by a minimum of 4 weeks. A partial response required more than a 50% reduction in the overall sum of the bidimensional products of all measurable lesions determined by two observations not less than 4 weeks apart. Progressive disease was defined as an increase of at least 25% in the overall sum of the bidimensional product of measurable lesions compared with baseline or the appearance of new lesions.

Pharmacokinetic sampling and analytic methods

Plasma samples for troxacitabine pharamacokinetic analysis were obtained during cycle 1 before dosing and at 0, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72, and 168 h after the end of the 30-min intravenous infusion of troxacitabine. Urine was also collected over 72 h during the following time intervals: 0–4, 4–8, 8–12, 12–24, 24–48, and 48–72 h after the start of the troxacitabine infusion. Plasma and urine samples were analyzed for troxacitabine at Phoenix International Life Science, Inc. (Saint-Laurent, QC) using a liquid chromatography in tandem with mass spectrometry method validated over the concentration ranges of 0.6–100 ng/mL and 10–5,000 ng/mL, respectively [7].

Pharmacokinetic analysis

Pharmacokinetic parameters were estimated from the concentration–time data using non-compartmental analytical methods as implemented by WinNonLin standard version 4.1 (Pharsight, Inc., Mountain View, CA). The maximal plasma concentration (C_{max}) and time to maximal plasma concentration (t_{max}) were determined by visual inspection of the data sets. Actual recorded sampling times were used instead of nominal planned target sampling times. The area under the concentration versus time curve from time 0 to the last sampling time t at which the concentration could be

measured (AUC_{0-t}) was calculated using the linear trapezoidal rule. The terminal elimination rate constant (K_e) was calculated by linear regression of the terminal portion of the elimination curve plotted on a log-linear scale. The apparent terminal elimination half life ($t_{1/2}$) was calculated from the formula: $t_{1/2} = 0.693/K_e$. The area under the concentration versus time curve extrapolated to infinity (AUC_{0-inf}) was estimated by adding the AUC_{0-t} to the extrapolated area out to infinity as determined by C_{last}/K_e, where C_{last} is the last measured plasma concentration. Total body clearance (CL) was estimated from the dose divided by AUC_{0-inf} and the volume of distribution at steady state (V_{ss}) was calculated from the formula: V_{ss} = dose (AUMC)/$(AUC)^2$ – $[R_0T^2/2(AUC)]$ where AUMC is the area under the first moment curve, T is the duration of the infusion, and R_0 is the dose rate of infusion [14]. Plasma concentrations below the limit of quantitation were not used in the pharmacokinetic analysis. Urinary excretion was determined by multiplying the urine collection sample drug concentration by the urine collection volume when available. The total excretion of drug over the urine collection period was expressed as a percentage of the total drug administered during that dosing period.

Pharmacokinetic parameters were depicted using descriptive statistics. Linear least squares regression was used to assess the relationships between [1] troxacitabine dose and exposure (C_{max} and AUC_{0-inf}) [2] troxacitabine CL and creatinine clearance, and [3] troxacitabine CL and body surface area (BSA). Creatinine clearance was estimated according to the method of Cockcroft and Gault [15]. Parametric methods (t test) were used to examine the relationship between NCI–CTC-graded hematologic toxicity and troxacitabine AUC_{0-inf}. In addition, logistic regression analysis was used to explore the relationships between NCI–CTC-graded hematologic toxicity and troxacitabine AUC_{0-inf}, cisplatin dose levels, age, and gender. The a priori level of significance was set at 0.05. Statistical analyses were performed using Sigmastat, version 3.0 statistical software program (Systat, San Jose, CA).

Results

General

Thirty-one patients, whose pertinent characteristics are listed in Table 1, received 77 courses of cisplatin and troxacitabine through five dose levels between July 1999 and May 2002. The number of patients and courses at each cisplatin/troxacitabine dose level, as well as the rates of DLT as a function of dose level, are detailed in Table 2. The median number of courses administered per patient was 2 (range 1–7) and 8 (26%) patients received at least three courses. Dose reductions, due to toxicity that resulted in patients being treated with multiple intermediate dose levels, are outlined in Table 2.

Table 1 Patient characteristics

Characteristics	No. of patients
Total number of patients	31
Male/female	16/15
Age (years)	
Median	61
Range	20–73
ECOG performance status	
0	12
1	17
2	2
Prior therapy	
Chemotherapy	20
Chemotherapy and radiotherapy	8
Chemotherapy and immunotherapy	3
Tumor types	
Colorectal cancer	19
Gastric cancer	1
Hepatocellular carcinoma	1
Non-small cell lung cancer	1
Renal cell carcinoma	1
Cervical cancer	2
Ovarian cancer	1
Malignant melanoma	2
Others	3

ECOG Eastern Cooperative Oncology Group

Cisplatin and troxacitabine dose escalations proceeded in the following manner. For HP patients, three new patients received cisplatin/troxacitabine at the first dose level of 50/4.8 mg/m^2. One of them experienced grade 4 neutropenia more than 5 days and grade 3 hand-foot syndrome in the first course. Thus, three additional HP patients were treated at the same dose level of 50/4.8 without further evidence of DLT. Dose level escalation proceeded to 75/4.8 mg/m^2, which resulted in an unacceptably high rate of hematolgic DLT. One of five HP patients experienced both grade 4 neutropenia with fever in the first course and grade 4 thrombocytopenia, and another HP patient experienced grade 4 thrombocytopenia in the first course. Therefore, the recommended phase II dose level for HP patients was cisplatin 50 mg/m^2 in combination with troxacitabine 4.8 mg/m^2 on day 1, every 28 days. For MP patients, three new patients received cisplatin/troxacitabine at the dose level of 50/6.4 mg/m^2. One of them experienced grade 4 neutropenia more than 5 days in the first course. Thus, three additional MP patients were treated at the same dose level of 50/6.4 mg/m^2 and they tolerated cisplatin/troxacitabine

Table 2 Dose escalations

Dose (mg/m^2)		No. of patients			Patients with DLT		
Cisplatin	Troxacitabine	New	Reduced to this dose	Total	No. of courses	First course	All courses
Heavily pretreated patients							
50	3.2	0	1	1	1	–	–
50	4.8	6	0	6	18	1/6	1/6
75	4.8	5	0	5	9	2/5	2/5
Lightly pretreated patients							
50	4.8	1	1	2	10	0/1	0/1
50	6.4	6	0	6	16	1/6	1/6
75	6.4	6	1	7	11	0/6	0/6
75	8.0	7	0	7	12	2/7	2/7
Total		31			77		

without DLT. Dose level escalation proceeded to 75/6.4 mg/m^2, which was well tolerated in the first three patients. However, the next higher dose level, 75/8.0 mg/m^2, resulted in an unacceptably high rate of hematolgic DLT, which were believed to be troxacitabine-related. One of seven MP patients experienced both grade 4 neutropenia with fever and grade 4 thrombocytopenia in the first course, and another MP patient experienced both grade 4 neutropenia for more than 5 days and grade 3 maculopapular skin rash in the first course. Additional patients were treated at the preceding dose level of 75/6.4 mg/m^2 and tolerated cisplatin/troxacitabine well. Therefore, the recommended phase II dose level for MP patients was cisplatin 75 mg/m^2 in combination with troxacitabine 6.4 mg/m^2 on day 1, every 28 days.

Hematologic toxicity

Myelosuppression, particularly neutropenia, was the principal toxicity of the cisplatin/troxacitabine combination. The distribution of grades of neutropenia as a function of dose is listed in Table 3. The median time to ANC nadir in course 1 was day 20 (range 9–28) and the recovery to ≥1,500/μL occurred by day 28 in 87% of patients. Neutropenia of grade 3 or grade 4 severity occurred in 34 (44%) of 77 courses and was dose limiting in 5 (6%) courses. Thrombocytopenia of grades 3 or 4 severity was observed in 7 (9%) of 77 courses. Two episodes (75/4.8 mg/m^2 of cisplatin/troxacitabine in one HP patient and 75/8.0 mg/m^2 of cisplatin/troxacitabine in one MP patient) were dose limiting. Drug-related anemia was generally grades 1 or 2 in severity, with three HP patients experiencing grade 3 anemia in five courses. Two patients required red blood cell transfusions. The dosage of troxacitabine required reduction for prolonged grade 4 neutropenia in three patients (50/4.8 mg/m^2 in 1 HP patient, 50/6.4 mg/m^2 in 1 MP patient, 75/8.0 mg/m^2 in 1 MP patient).

Non-hematologic toxicity

Non-hematologic toxicities were mild to moderate in severity in most patients and are summarized in Table 4. Troxacitabine treatment produced three types of cutaneous effects: skin rash, hand-foot syndrome, and hyperpigmentation. Overall, skin toxicity of grade 3 severity occurred in 4 (5%) courses, of which two were considered dose limiting. One HP patient at the dose level of 50/4.8 mg/m^2 developed grade 3 hand-foot syndrome (and grade 4 neutropenia >5 days) in the first cycle. One MP patient at the dose level of 75/8.0 mg/m^2 developed grade 3 maculopapular skin rash (also with grade 4 neutropenia lasting longer than 5 days) in the first cycle. Other commonly seen grade 3 non-hematologic toxicity included fatigue in four (5%) courses and nausea/vomiting in five (6%) courses, however, none of these were dose limiting.

Pharmacokinetics

Complete plasma sampling was obtained in all 31 patients. Pharmacokinetic parameters for each dose level are listed in Table 5. Mean C_{max} and AUC values increased linearly with troxacitabine dose (Table 5; Fig. 1). Troxacitabine pharmacokinetic parameters were dose-independent and characterized by mean (±standard deviation) values for V_{ss}, CL, and $t_{1/2}$ of 300 L (±163), 8.67 L/h (±2.23), and 56 h (±12), respectively. The mean troxacitabine plasma concentration–time plots for troxacitabine dose levels 4.8 and 6.4 mg/m^2 are shown in Fig. 2. Patients are grouped by the associated cisplatin dose level (either 50 or 75 mg/m^2). The troxacitabine plasma concentrations, such as CL, V_{ss}, and $t_{1/2}$ were not

Table 3 Hematologic toxicities

Dose level (cisplatin/ troxacitabine mg/m^2)	No. of new patients (courses)	No. of first courses (all courses) with toxicity										
		Neutopenia					Anemia			Thrombocytopenia		
		Grade 1–2	Grade 3	Grade 4	Grade 4 > 5 days	Grade 4 fever	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Heavily pretreated												
50/3.2	0 (1)	NA (0)	NA (0)	NA (0)	NA	NA	NA (1)	NA (0)	NA (0)	NA (0)	NA (0)	NA (0)
50/4.8	6 (18)	3 (11)	1 (3)	0 (4)	1	0	9 (13)	3 (5)	0 (0)	1 (2)	0 (1)	0 (0)
75/4.8	5 (9)	1 (5)	0 (0)	0 (1)	1	0	3 (9)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)
Lightly pretreated												
50/4.8	1 (10)	0 (3)	0 (0)	0 (0)	0	0	2 (7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
50/6.4	6 (16)	0 (6)	0 (3)	1 (2)	1	0	3 (15)	0 (0)	0 (0)	0 (2)	0 (0)	0 (0)
75/6.4	6 (11)	0 (2)	0 (0)	4 (7)	0	0	2 (8)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)
75/8.0	7 (12)	0 (4)	3 (4)	3 (5)	1	1	3 (10)	0 (0)	0 (0)	1 (2)	0 (0)	1 (2)

NA not applicable

Table 4 Non-hematologic toxicities

Toxicity	Number of courses (n = 77)			
Grade	1	2	3	4
Fatigue	23	21	4	0
Anorexia	12	7	1	0
Nausea	26	8	5	0
Vomiting	18	7	5	0
Diarrhea	12	2	0	0
Stomatitis	5	5	0	0
Skin rash	6	7	3	0
Hand-foot syndrome	6	2	1	–
Hyperpigmentation	15	0	–	–
Alopecia	10	11	–	–

different in patients treated with either dose of cisplatin (Tables 5, 6), suggesting that the pharmacokinetics of troxacitabine were similar for cisplatin dose level of 50 and 75 mg/m^2. However, because the study design did not include pharmacokinetic monitoring with or without cisplatin in each patient cohort, a formal analysis for a pharmacokinetic drug interaction between cisplatin and troxacitabine could not be performed.

Renal excretion of unmetabolized drug was the principal mode of troxacitabine elimination. Matching urine volumes were available for the first nine patients. In these nine patients, 44.4% [±14.1 (standard deviation)] of the administered dose of troxacitabine was excreted unchanged in the urine over 24 h after drug administration. After 72 h, the percent of the total administered dose excreted in the urine had increased to 51.9% (±14.8). The troxacitabine CL is related to renal function as assessed by creatinine clearance ($R^2 = 0.3$, $P < 0.05$) (Fig. 3a). These data suggest that troxacitabine CL is related to renal function, that a substantial portion of the administered drug is excreted by the kidneys, and that dose adjustments may be necessary in patients with impaired renal function.

Table 5 Summary of troxacitabine pharmacokinetic parameters

Dose (mg/m^2)		No. of patients	C_{max} (ng/mL)	AUC_{0-inf} (ng h/mL)	V_{ss} (L)	CL (L/h)	$t_{1/2}$ (h)
Cisplatin	Troxacitabine						
50	4.8	7	446 (85)	1,400 (376)	196 (98)	7.2 (1.9)	43 (18)
75	4.8	5	251 (63)	1,023 (286)	396 (207)	9.8 (3.0)	62 (20)
50	6.4	6	516 (95)	1,611 (496)	392 (246)	8.6 (2.6)	68 (30)
75	6.4	6	456 (103)	1,378 (214)	297 (84)	9.1 (2.2)	55 (15)
75	8.0	7	717 (186)	1,634 (272)	262 (89)	9.1 (1.4)	55 (12)
Any	Any	31	–	–	300 (163)	8.67 (2.23)	56 (12)
50	Any	13	–	–	286 (201)	7.85 (2.27)	54 (26)
75	Any	18	–	–	311 (135)	9.27 (2.07)	57 (15)

C_{max} maximal plasma concentration, AUC_{0-inf} area under the plasma concentration versus time curve from time 0 to infinity, V_{ss} volume of distribution at steady state, *CL* total body clearance, $t_{1/2}$ elimination half life

The numbers in parentheses are standard deviation

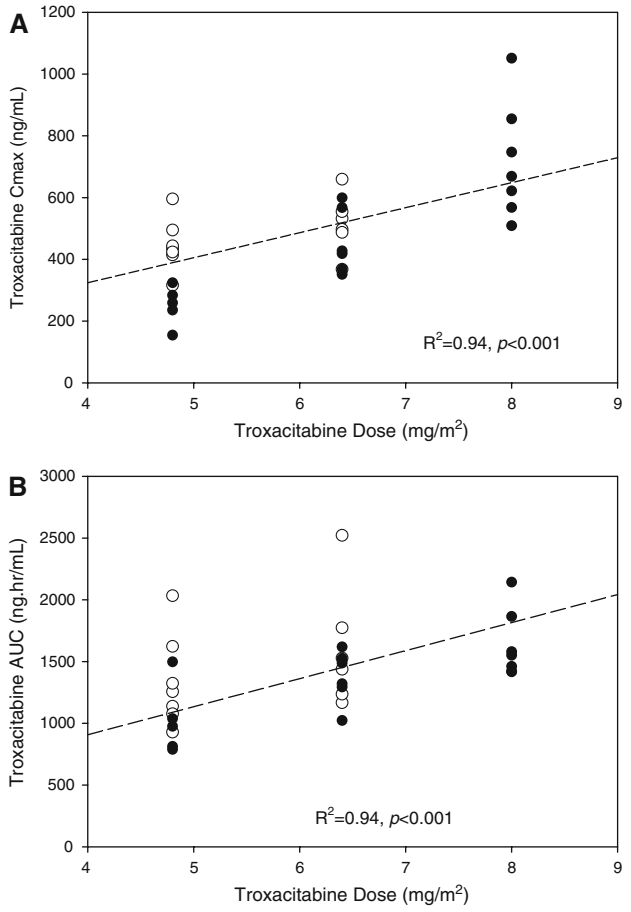

Fig. 1 Individual troxacitabine C_{max} (**a**) and AUC (**b**) values as a function of dose level. *Broken lines* represent the fit of linear regression model to the data. The *solid* and *open circles* represent the troxacitabine pharmacokinetics parameters with cisplatin dose level 50 and 75 mg/m^2, respectively

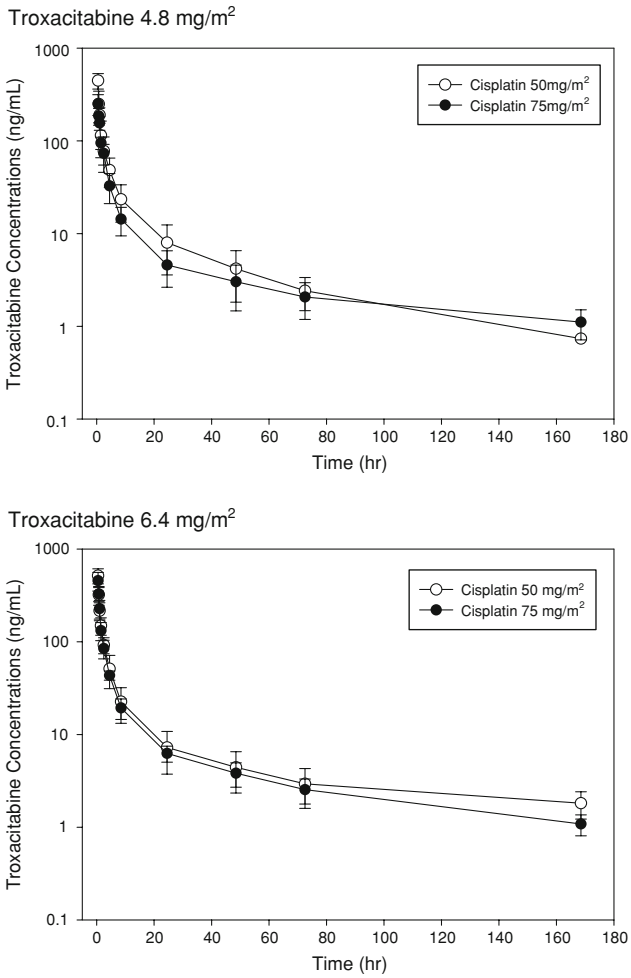

Fig. 2 Mean troxacitabine plasma concentration profiles after administration of troxacitabine 4.8 and 6.4 mg/m^2 with two different dose levels of cisplatin

Troxacitabine is dosed based on BSA in cancer patients [16]. Therefore, the relationship between the troxacitabine clearance and BSA was explored. Troxacitabine CL increases linearly with BSA (Fig. 3b) supporting the rationale of BSA-based dosing of troxacitabine in cancer patients.

Pharmacodynamics

Data from all 31 patients were available for clinical pharmacodynamic assessment. When compared with the mean values of patients with grades 0–3 neutropenia in any cycle (not limited in the first cycle), patients with grade 4 neutropenia in any cycle had a higher AUC value (1,541 vs. 1,262 ng/mL, $P < 0.05$) (Fig. 4). Further analysis using a logistic regression model suggested that troxacitabine AUC_{o-inf} was the best predictor for the grade 4 neutropenia. Age, gender, and cisplatin dose levels were not predictive for the occurrence of grade 4 neutropenia. The patients, who had grade 4 thrombocytopenia in any cycle had a trend toward a higher mean AUC value than those patients who had grades 0–3 thrombocytopenia in any cycle, but the difference was not statistically significant ($P > 0.05$).

Antitumor activity

A minor response that lasted for 6 months occurred in a 69-year-old man with non-small cell lung cancer (NSCLC) metastatic to the left adrenal gland. His disease had previously progressed on paclitaxel/carboplatin and paclitaxel/gemcitabine. The patient experienced a 42% reduction in the extent of his disease after two courses of cisplatin/troxacitabine at the dose of 50/4.8 mg/m^2. Progressive disease was confirmed radiologically after the eighth course.

Discussion

Troxacitabine is a novel cytosine analog with an unnatural stereochemical orientation. Cisplatin is an active agent

Table 6 Pharmacokinetic summary of troxacitabine

	Cisplatin/ troxacitabine	Troxacitabine alone (8)
Dosage (mg/m^2)	50/4.8	4.8
Mean AUC (ng h/mL)	1,400 (376)	794 (191)
Clearance	7.2 (1.9)	11.5 (1.7)
Dosage (mg/m^2)	75/4.8	4.8
Mean AUC (ng h/mL)	1,023 (286)	794 (191)
Clearance (L/h)	9.8 (3.0)	11.5 (1.7)
Dosage (mg/m^2)	50/6.4	6.4
Mean AUC (ng h/mL)	1,611 (496)	1,586 (428)
Clearance (L/h)	8.6 (2.6)	6.5 (1.6)
Dosage	75/6.4 (mg/m^2)	6.4 (mg/m^2)
Mean AUC (ng h/mL)	1,378 (214)	1,586 (428)
Clearance (L/h)	9.1 (2.2)	6.5 (1.6)
Dosage (mg/m^2)	75/8.0	8.0
Mean AUC (ng h/mL)	1,634 (272)	1,536 (446)
Clearance (L/h)	9.1 (1.4)	10.5 (2.5)

The numbers in parentheses are standard deviation

against several advanced solid malignancies. In this study, the combination of cisplatin and troxacitabine produced dose-limiting myelosuppression at lower doses of troxacitabine (6.4 and 4.8 mg/m^2 in MP and HP patients, respectively) than single agent doses (10 mg/m^2). The addition of cisplatin did not substantially alter the pharmacokinetic behavior of troxacitabine.

The observed toxicity of this regimen was expected based on the toxicity profiles of the individual agents [8]. In contrast to gemcitabine (another nucleoside analog) combined with cisplatin [17], myelosuppression was the dose-limiting toxicity of troxacitabine plus cisplatin. Clinically, significant grades 3 or 4 neutropenia complicated 29 and 43% of the treatment cycles overall in LP and HP patients, respectively. Clinically significant grades 3 or 4 thrombocytopenia complicated 14 and 4% of the treatment cycles overall in LP and HP patients, respectively. Myelosuppression actually precluded further dose escalation of troxacitabine to the recommended phase II dose of the single agent (10 mg/m^2 30-min infusion once in every 3 weeks). Similar situations also occurred in combination with troxacitabine and other cytotoxics in acute leukemia [18].

Although cisplatin pharmacokinetic evaluation has not been carried out in this study, the troxacitabine disposition appeared to be unaffected by cisplatin, since our data were consistent with those obtained in previous studies investigating the pharmacokinetic parameters of troxacitabine given as single agent [6–9]. The mean AUC value of troxacitabine alone (30-min infusion once every 3 weeks) from the previous study was 1,536 ng h/mL [8]. This is in accordance with the observation in this study with the mean

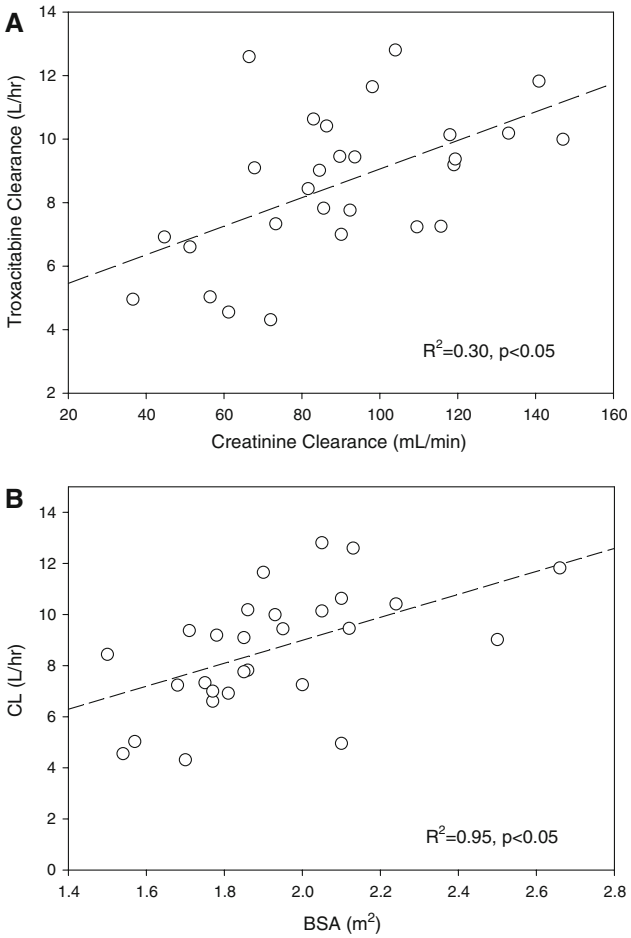

Fig. 3 Troxacitabine systemic clearance as a function of creatinine clearance (**a**) and body surface area (BSA) (**b**) (fit from linear least squares regression)

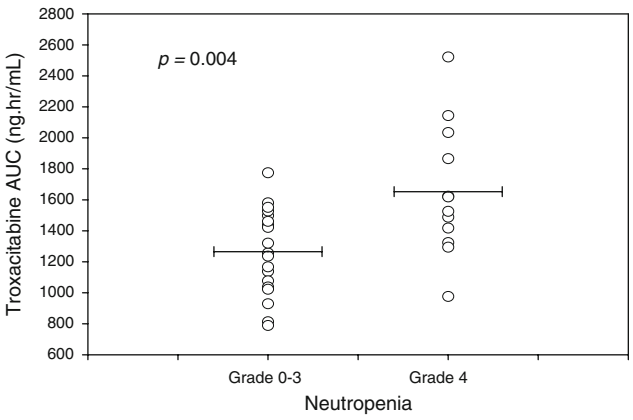

Fig. 4 The relationship between the worst grade neutropenia and troxacitabine AUC_{0-inf}. *Horizontal bars* represent mean values

AUC values ranging from 1,023 to 1,634 ng h/mL. The percentage of the total administered troxacitabine dose was excreted unchanged in the urine during the first 24 h in this study (44.4%) was also similar to that in the single agent

study (60%) [8]. Given the lack of pharmacokinetic interactions, the reason why the recommended phase II dose of troxacitabine in this combination is lower than that of single agent troxacitabine might lie in drug–drug interaction at the cellular level. However, the pharmacodynamic interaction between troxacitabine and cisplatin was not built in the study. Using mononuclear cells as a surrogate for the target, we might observe a cisplatin dose-dependent increase in cellular dFdCTP compared with single agent troxacitabine. From previous studies with troxacitabine alone, it is known that accumulation and retention of troxacitabine nucleotides by target cells are critical for the cytotoxicity of the drug [6–9].

The results of this study demonstrate one minor response in a patient with gemcitabine-refractory non-small lung cancer. Besides, single agent troxacitabine had only little to modest the activity in treatment-naive patients with NSCLC and pancreatic cancer, in which gemcitabine is a mainstay of treatment [19, 20]. Therefore, more data are required before pursuing further disease-directed evaluations of the combination of troxacitabine and cisplatin. Because xenograft experiments demonstrated that prolonged exposures to low micromolar concentrations of troxacitabine lead to significant inhibition of tumor growth without the need to achieve peak drug concentrations, the future development of troxacitabine will focus on different schedules, including delivery as a continuous infusion [21, 22].

The combination of troxacitabine and cisplatin produces dose-limiting myelosuppression at lower doses of troxacitabine than single agent doses. The recommended phase II doses of cisplatin/troxacitabine are 75/6.4 and 50/4.8 mg/m^2, every 4 weeks, for MP and HP patients, respectively. The addition of cisplatin did not substantially alter the pharmacokinetic behavior of troxacitabine. The development of troxacitabine continues in both solid and hematological malignancies.

References

1. Grove KL, Guo X, Liu SH et al (1995) Anticancer activity of beta-L-dioxolane-cytidine, a novel nucleoside analogue with the unnatural L configuration. Cancer Res 55:3008–3011
2. Gourdeau H, Clarke ML, Ouellet F et al (2001) Mechanisms of uptake and resistance to troxacitabine, a novel deoxycytidine nucleoside analogue, in human leukemic and solid tumor cell lines. Cancer Res 61:7217–7224
3. Krishnan P, Fu Q, Lam W et al (2002) Phosphorylation of pyrimidine deoxynucleoside analog diphosphates: selective phosphorylation of L-nucleoside analog diphosphates by 3-phosphoglycerate kinase. J Biol Chem 277:5453–5459
4. Chou KM, Kukhanova M, Cheng YC (2000) A novel action of human apurinic/apyrimidinic endonuclease: excision of L-configuration deoxyribonucleoside analogs from the 3' termini of DNA. J Biol Chem 275:31009–31015
5. Kukhanova M, Liu SH, Mozzherin D et al (1995) L- and D-enantiomers of 2′, 3′-dideoxycytidine 5′-triphosphate analogs as substrates for human DNA polymerases. Implications of the mechanism of toxicity. J Biol Chem 270:23055–23059
6. Giles FJ, Cortes JE, Baker SD et al (2001) Troxacitabine, a novel dioxolane nucleoside analog, has activity in patients with advanced leukemia. J Clin Oncol 19:762–771
7. de Bono JS, Stephenson J Jr, Baker SD et al (2002) Troxacitabine, an L-stereoisomeric nucleoside analog, on a five-times-daily schedule: a phase I and pharmacokinetic study in patients with advanced solid malignancies. J Clin Oncol 20:96–109
8. Belanger K, Moore M, Baker SD et al (2002) Phase I and pharmacokinetic study of novel L-nucleoside analog troxacitabine given as a 30-minute infusion every 21 days. J Clin Oncol 20:2567–2574
9. Lee CK, Rowinsky EK, Li J et al (2006) Population pharmacokinetics of troxacitabine, a novel dioxolane nucleoside analogue. Clin Cancer Res 12:2158–2165
10. Moufarij MA, Phillips DR, Cullinane C (2003) Gemcitabine potentiates cisplatin cytotoxicity and inhibits repair of cisplatin-DNA damage in ovarian cancer cell lines. Mol Pharmacol 63:862–869
11. van Moorsel CJ, Pinedo HM, Veerman G et al (1999) Mechanisms of synergism between cisplatin and gemcitabine in ovarian and non-small-cell lung cancer cell lines. Br J Cancer 80:981–990
12. Crul M, van Waardenburg RC, Bocxe S et al (2003) DNA repair mechanisms involved in gemcitabine cytotoxicity and in the interaction between gemcitabine and cisplatin. Biochem Pharmacol 65:275–282
13. Yang LY, Li L, Jiang H, Shen Y, Plunkett W (2000) Expression of ERCC1 antisense RNA abrogates gemicitabine-mediated cytotoxic synergism with cisplatin in human colon tumor cells defective in mismatch repair but proficient in nucleotide excision repair. Clin Cancer Res 6:773–781
14. Perrier D, Mayersohn M (1982) Noncompartmental determination of the steady-state volume of distribution for any mode of administration. J Pharm Sci 71:372–373
15. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine
16. Baker SD, Verweij J, Rowinsky EK et al (2002) Role of body surface area in dosing of investigational anticancer agents in adults, 1991–2001. J Natl Cancer Inst 94:1883–1888
17. Shepherd FA, Burkes R, Cormier Y et al (1996) Phase I dose-escalation trial of gemcitabine and cisplatin for advanced non-small-cell lung cancer: usefulness of mathematic modeling to determine maximum-tolerable dose. J Clin Oncol 14:1656–1662
18. Giles FJ, Kantarjian HM, Cortes JE et al (2003) Adaptive randomized study of idarubicin and cytarabine versus troxacitabine and cytarabine versus troxacitabine and idarubicin in untreated patients 50 years or older with adverse karyotype acute myeloid leukemia. J Clin Oncol 21:1722–1727
19. Dent SF, Arnold A, Stewart DJ et al (2005) Phase II study of troxacitabine (BCH-4556) in patients with advanced non-small-cell lung cancer. Lung 183:265–272
20. Lapointe R, Letourneau R, Steward W et al (2005) Phase II study of troxacitabine in chemotherapy-naive patients with advanced cancer of the pancreas: gastrointestinal tumors. Ann Oncol 16:289–293
21. Jimeno A, Messersmith WA, Lee CK et al (2008) Phase I study of troxacitabine administered by continuous infusion in subjects with advanced solid malignancies. Ann Oncol 19:374–379
22. Roboz GJ, Giles FJ, Ritchie EK et al (2007) Phase I/II study of continuous-infusion troxacitabine in refractory acute myeloid leukemia. J Clin Oncol 25:10–15