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ORIGINAL ARTICLE

Pharmacokinetics of decitabine administered as a 3-h infusion to patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS)

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Abstract
Purpose In this study, pharmacokinetics (PK) of decitabine administered as a 3-h intravenous infusion of 15 mg/m^2 every 8 h for 3 days (cycles repeated every 6 weeks) was evaluated in patients with MDS or AML.
Methods The PK of this dosing regimen was evaluated in sixteen patients with MDS or AML. Plasma samples were obtained pre-dose and during the first 8-h dosing interval on each dosing day during Cycle 1, and at pre-dose and just prior to the end of infusion during Cycle 2. PK samples were assayed for decitabine by a sensitive and specific validated liquid chromatography-tandem mass spectrometry method.
Results The mean maximum observed plasma concentration (C_{max}), 64.8–77.0 ng/ml, and the mean area under the plasma concentration-time curve ($AUC_{0-\infty}$), 152–163 ng h/ml, were unchanged during dosing of decitabine for 3 days. The time to the maximum concentration (T_{max}) generally occurred at the end of infusion. The mean values for terminal phase elimination half-life (0.62–0.78 h), total body clearance (125–132 l/h per m^2), and volume of distribution at steady state (62.7–89.2 l/m^2), remained unchanged during the every 8 h dosing ($P > 0.05$). Cycles 1 and 2 C_{max} values for days 1, 2, and 3 were not significantly different as determined by paired two-tailed t test ($P > 0.05$). The primary toxicity of decitabine was myelosuppression, which was observed in all patients. Two deaths, from sepsis, were considered possibly related to decitabine.
Conclusions Decitabine dosed at 15 mg/m^2 iv every 8 h for 3 days resulted in a predictable and manageable toxicity profile in patients with MDS/AML. The repeated dosing did not result in systemic accumulation of the drug, and decitabine PK remained unchanged from cycle to cycle.

Keywords Decitabine · Myelodysplastic syndrome · Acute myeloid leukemia · Pharmacokinetics

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Introduction

Aberrant methylation of DNA has emerged as a significant epigenetic mechanism for the silencing of tumor suppressor genes in human cancers. The cytosine analogue, decitabine (Fig. 1, 5-aza-2′-deoxycytydine, Dacogen™, MGI PHARMA, Inc., Bloomington, MN, USA), is incorporated into DNA after phosphorylation and irreversibly inhibits DNA methyltransferase, leading to loss of DNA methylation. At high doses, decitabine exerts primarily a cytotoxic effect, but at lower doses its hypomethylating activity predominates, resulting in gene re-expression and cellular differentiation [1]. Decitabine has demonstrated activity in myeloid malignancies, including acute myeloid leukemia (AML) [2–5] and chronic myeloid leukemia (CML) [6, 7], and it has been extensively studied in myelodysplastic syndrome (MDS).

In Phase 2 studies in elderly patients with high-risk MDS, decitabine (45–50 mg/m^2 per day for three consecutive days) was well tolerated, and objective response rates of approximately 50% were observed [8–10]. Remarkably, major cytogenetic responses occurred in 31% of patients in these trials [11]. A Phase 3 trial confirmed the activity of decitabine in advanced MDS [12]. Patients randomized to

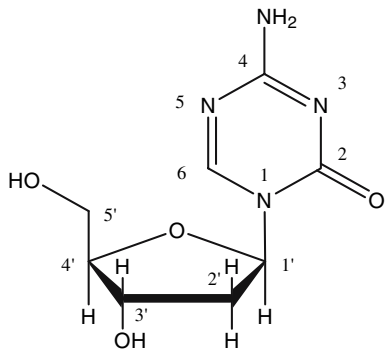


Fig. 1 Chemical structure of decitabine (4-amino-1-(2-deoxy-β-D-erythro-pentofuranosyl)-1,3,5-triazin-2(1*H*)-one); molecular weight: 228.21

receive decitabine at a dose of 15 mg/m^2 intravenous (iv) every 8 h for three consecutive days each cycle had a response rate of 17%, compared to the 0% response rate for patients randomized to receive supportive care alone. In addition, time to AML or death was significantly prolonged for patients with International Prognostic Scoring System (IPSS) intermediate-2/high-risk disease who were treated with decitabine.

Relatively limited pharmacokinetic (PK) trials of decitabine have been performed in patients with solid [13–15] and hematologic [16, 17] malignancies. Despite the extensive use of decitabine in clinical trials, no PK study had been undertaken in the past decade to determine the plasma concentrations of decitabine in patients with MDS and AML. Therefore, the objectives of this study were to determine the PK and safety of decitabine administered as a 3-h iv infusion every 8 h on three consecutive days, the approved dosing regimen, for two cycles in patients with MDS or AML.

Materials and methods

Study design

This single-arm, open-label, Phase I PK trial of decitabine enrolled 16 patients with MDS or AML. Patients were treated with decitabine, 15 mg/m^2 iv every 8 h for 3 days every 6 weeks for two cycles. PK determinations were performed for both cycles during the first 8-h dosing interval on days 1, 2, and 3. The sample size for this study was chosen to be sufficient to characterize the pharmacokinetics of decitabine and to determine intersubject variability in the patient population.

Patient eligibility

Patients were eligible for this study if they had AML (≥30% bone marrow blasts), except M3 or acute promyelocytic leukemia, or MDS (de novo or secondary) fitting any of the FAB classifications, or chronic myelomonocytic leukemia (with WBC $< 12{,}000\ \mu l^{-1}$). Patients with MDS were required to have an IPSS score of ≥1.5 as determined by complete blood counts, bone marrow assessment and bone marrow cytogenetics within 30 days of study entry. Additional inclusion criteria were age ≥ 18 years, ECOG performance status of 0–2, adequate renal and hepatic function, and life expectancy of >12 weeks. Patients were excluded if they had any of the following: CNS leukemia; radiotherapy, nitrosoureas, stem cell transplantation, anti-angiogenesis agents, decitabine, or 5-azacytidine within 6 weeks of study entry; chemotherapy within 4 weeks of study entry; immunosuppressive therapy, including corticosteroids, within 30 days of study entry; ongoing treatment with androgenic hormones; concurrent autoimmune hemolytic anemia or thrombocytopenia; other active malignancy; uncontrolled cardiac or pulmonary disease; HIV infection; or active viral, fungal, or bacterial infection. Women of childbearing potential were required to have a negative serum pregnancy test within 1 week of study entry and to practice a medically approved method of birth control. All patients signed an IRB-approved informed consent form.

Treatment plan

Decitabine was administered at a dose of 15 mg/m^2 as a 3-h iv infusion every 8 h for three consecutive days of a 6-week cycle. The total dose per treatment day was 45 mg/m^2; the total dose per cycle was 135 mg/m^2. Treatment was given on an inpatient basis. A maximum of a 3-week delay was allowed for recovery of myelosuppression and nonhematologic toxicities. No dose reduction was allowed. Treatment continued for a maximum of two cycles or until disease progression, intercurrent illness, or unacceptable adverse event. Patients were followed for adverse events for 56 days after the last dose of study drug. Patients who completed the PK sampling through Cycle 2 day 3 and who were experiencing treatment benefit were eligible to enroll in a second safety-only decitabine trial.

Pharmacokinetic analysis

Approximately 3 ml of blood was collected in a 3-ml K_3 EDTA tube pre-loaded with 8 µl of tetrahydrouridine (THU 500 µg/ml), an inhibitor of cytidine deaminase. The blood sample was obtained from the arm opposite of drug administration. Serial venous blood samples were obtained on day 1 (1st dose), day 2 (4th overall dose), and day 3 (7th overall dose) of both treatment cycles. During the first cycle, samples were obtained immediately predose and at 1 h, 2 h, 2 h 55 min, 3 h 5 min, 3 h 15 min, 3 h 30 min, 4 h, 4 h 30 min, 5 h, 7 h, and 8 h after the start of the decitabine

infusion. During the second cycle, samples were obtained predose and 2 h 55 min after the start of the decitabine infusion. Blood samples were centrifuged (within 30 min after collection) at 1,800*g* for 10 min at 4°C. The resulting plasma was transferred into labeled screw-top 5-ml cryovials and stored at −70°C or below until analyzed. Samples were assayed within 30 days of collection.

Assay

Plasma concentrations of decitabine were determined using a validated liquid chromatography–tandem mass spectrometry (LC/MS/MS) method. In brief, in this method, decitabine and the internal standard, 5-azacytidine, were quantitatively extracted from 0.2 ml of human plasma sample using a solid phase extraction method. The extract was loaded onto a Kromasil column for separation. The HPLC effluent was introduced into a PE Sciex API-3000 Tandem Mass Spectrometer equipped with a Turbo IonSpray (electrospray) source. Positive ions were detected in the multiple reaction monitoring (MRM) mode with precursor→product ion pairs of 229.1→113.1 for decitabine and 245.3→112.9 for the internal standard. Calibration was accomplished by weighted ($1/x^2$) linear regression of the peak area ratio of the analyte to the internal standard. The assay had a dynamic range of 1–100 ng/ml.

For quality control samples, concentrations of decitabine were determined at low (QCL), mid (QCM), and high (QCH) levels and were used for the assay performance to determine precision and accuracy. Precision was determined as the percent coefficient of variance (%CV). Results of precision for decitabine spiked concentrations showed inter-day %CV values of 8.69% for QCL, 7.70% for QCM and 9.18% for QCH. Accuracy of the method was determined by the percent relative error (%RE). Results for accuracy in decitabine spiked concentrations showed inter-day %RE values of 4.67% for QCL, 3.25% for QCM and −1.00% for QCH. These results indicated an acceptable assay performance for the study.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated from plasma decitabine concentration-time data using noncompartmental methods by WinNonlin version 5.0. The maximum observed plasma concentration (C_{max}) and the time at which C_{max} was first observed (T_{max}) were determined by visual inspection of the individual data. The terminal phase elimination rate constant, K_{el}, was estimated from the slope of the concentration-time data during the log-linear terminal phase using least square regression analysis. The terminal phase elimination half-life ($T_{1/2}$) was calculated as $0.693/K_{el}$. The area under the plasma concentration–time curve from time 0 to the last measurable concentration at time T (AUC$(0-t)$) was calculated using the linear trapezoidal method with $AUC_{0\text{-}\infty}$ computed as AUC$(0-t)$ plus the extrapolation from the last time point to infinity using C_t/K_{el}, where C_t is the last quantifiable concentration. Total body clearance (CL_p) was calculated as Dose/$AUC_{0\text{-}\infty}$. The volume of distribution at steady state (V_{dss}) was calculated as $CL_p \times MRT$, where mean residence time (MRT) was calculated as $[AUMC/AUC_{0\text{-}\infty} - \tau/2]$, where τ is the duration of infusion. The area under the first moment curve (AUMC) was determined using the linear trapezoidal rule and extrapolated to infinity as: $AUMC_{0-t} + t \times C_t/K_{el} + C_t/(K_{el})^2$.

Plasma concentrations below the assay lower limit of quantification were treated as 0.0 ng/ml for purposes of calculating pharmacokinetic parameters. Actual times after the start of drug administration were used in the calculation of pharmacokinetic parameters. Plasma concentration-time data were also fitted using compartmental analysis. A two compartment body model with drug administration into the central compartment was applied using WinNonlin.

Statistical analysis

Descriptive statistical analysis was performed on the plasma decitabine concentration data and pharmacokinetic parameters. Natural log-transformed AUC and C_{max} and untransformed (or raw) CL_p, V_{dss}, and $T_{1/2}$ were analyzed using an analysis of variance (ANOVA) model containing day as a fixed effect and subject as a random effect. A 5% level of significance was used to test for these effects. Adjusted means and their variances and covariances were used to estimate the adjusted mean difference between the day effects, their standard errors, and the 90% confidence intervals of the difference. For AUC and $C_{max,}$ the anti-log (exponent) of the differences and confidence limits were taken to estimate the ratio between treatment effects and the 90% confidence interval of the ratio. The 90% confidence intervals for the AUC and C_{max} ratios within the equivalence range of 80–125% were utilized to determine the clinically significant changes in the pharmacokinetics of decitabine on days 1, 2, and 3. For cycle to cycle changes in PK, a paired two-tailed t test for C_{max} was used.

Results

Patient characteristics

The characteristics of the 16 patients enrolled on this study are presented in Table 1. Eight patients (50%) had MDS with an IPSS score ≥ 1.5, of whom six had received no prior chemotherapy. Eight patients had relapsed ($n = 5$) or

Table 1 Patient characteristics

	Patients ($N = 16$) n (%)
Sex	
Female	4 (25)
Male	12 (75)
Age (years)	
Median (range)	70 (39–87)
Height (cm)	
Mean (range)	173 (157–183)
Weight (kg)	
Mean (range)	79 (58–108)
BSA (m^2)	
Mean (range)	1.9 (1.6–2.2)
Race	
White	13 (81)
Black	2 (13)
Other	1 (6)
Diagnosis	
MDS	8 (50)
AML	8 (50)
Prior chemotherapy regimens	
None	6 (38)
1	3 (19)
2	5 (31)
≥3	2 (12)

primary refractory ($n = 3$) AML. The patients with AML had received 1–5 prior chemotherapy regimens, and two had undergone prior stem cell transplantation. Twelve patients (75%) received the planned two cycles of therapy; four patients received only one cycle due to death ($n = 3$) or decline in performance status ($n = 1$).

Pharmacokinetic analysis

Of the 16 patients who received decitabine in Cycle 1, 14 were evaluable for PK analysis, and 2 were excluded based on plasma concentration time profiles indicated to have dosing errors during the administration of decitabine. Of the 12 patients who received decitabine in Cycle 2, 11 were evaluable for PK analysis and 1 was excluded for dosing errors. Mean plasma concentration-time plots after iv infusions for cycle 1 on days 1, 2, and 3 are shown in Fig. 2. Visual inspection of mean and individual log concentration versus time plots showed that after discontinuation of infusion, plasma concentrations of decitabine declined biexponentially and were measurable up to 2 h post infusion in the majority of subjects (plots are not shown). Steady state plasma concentration appeared to have been reached by the end of infusion.

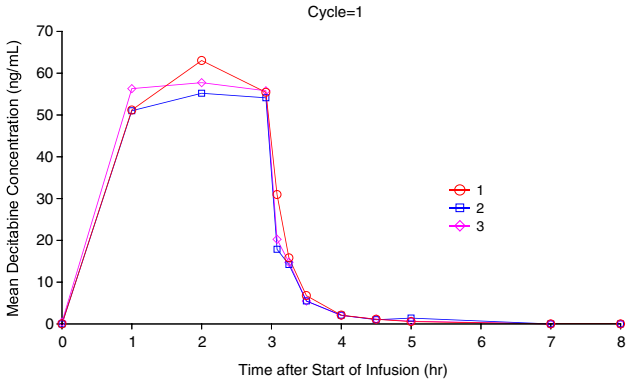


Fig. 2 Cycle 1 mean plasma decitabine concentrations following 15 mg/m² doses administered as a 3-h intravenous infusion every 8 h on days 1, 2, and 3 in AML/MDS patients

The overall pharmacokinetic summary is presented in Table 2. The inter-subject variability in C_{max} and $AUC_{0-\infty}$ as measured by CV% ranged from 60 to 81% indicating moderate to high variability in these parameters. The ratio of geometric least squares mean values for day 2 to day 1 treatments for C_{max} was 87.8% and for $AUC_{(0-\infty)}$ the ratio was 94.7%. Similarly, the ratio of geometric least squares mean values for day 3 to day 1 treatments for C_{max} was 97.0% and for $AUC_{(0-\infty)}$ the ratio was 96.3%. These results indicate that the C_{max} and $AUC_{0-\infty}$ values were unchanged during 8-h TID dosing of decitabine for 3 days. This was also consistent with comparison of day 2 and day 3 to day 1 AUC_{0-t} accumulation ratio of (mean ± SD) 0.96 ± 0.21 and 0.99 ± 0.29, respectively. The T_{max} generally occurred at the end of infusion.

For days 1, 2, and 3, the mean $T_{1/2}$ values were 0.62, 0.67, and 0.78 h, respectively. The difference between day 1 and day 2 $T_{1/2}$ was −0.05 h (P value = 0.555) and the difference between day 1 and day 3 $T_{1/2}$ was −0.16 h (P value = 0.0689). As the $T_{1/2}$ is approximately 35 min, steady state in plasma was reached by the end of infusion. The mean total body clearance values were 125, 130, and 132 l/h per m^2, respectively, with moderate inter-subject variability (CV% 52–57%). The difference between day 1 and day 2 CL_p was −5.1 l/h per m^2 (P value = 0.441) and the difference between day 1 and day 3 CL_p was −6.5 l/h/m^2 (P value = 0.328) indicating CL_p remained unchanged during the 3 days of dosing. The mean values of volume of distribution at steady state ranged from 62.7 to 89.2 l/m^2. The difference between day 1 and day 2 V_{dss} was 9.08 l/m^2 (P value = 0.635) and the difference between day 1 and day 3 V_{dss} was −17.5 l/m^2 (P value = 0.364). These results indicate that the decitabine $T_{1/2}$, CL_p, and V_{dss} remained unchanged during the TID dosing.

For the 11 patients who received decitabine for two cycles, C_{max} values (mean ± SD) for cycle 1 on days 1, 2, and 3 were 71.6 ± 51.1, 63.6.3 ± 41.3, and 78.5 ± 69.4 ng/ml,

Table 2 Cycle 1 pharmacokinetic summary of decitabine following 15 mg/m² doses administered as a 3-h intravenous infusion every 8 h on days 1, 2, and 3 in AML/MDS patients

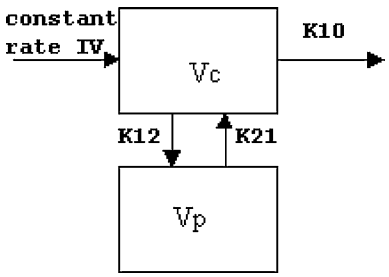
Day	C_{max} (ng/ml)	T_{max} (h)	$AUC_{(0-t)}$ (ng h per ml)	$AUC_{0-\infty}$ (ng h per ml)	$T_{1/2}$ (h)	CL_p (l/h per m^2)	V_{dss} (l/m^2)	AUC ratio[a]
1 (n = 14)								
Mean	73.8	2.49	162	163	0.62	125	71.8	
SD	48.6	0.70	101	101	0.31	66.7	62.7	
CV%	66	28	62	62	49	53	87	
	62.1[b]	–	137[b]	139[b]	–	–	–	
2 (n = 14)								
Mean	64.8	2.53	150	152	0.67	130	62.7	0.96
SD	40.2	0.69	90.4	90.5	0.30	67.4	36.4	0.21
CV%	62	27	60	60	44	52	58	22
	54.5[b]	–	129[b]	131[b]	–	–	–	
3 (n = 14)								
Mean	77.0	2.29	157	158	0.78	132	89.2	0.99
SD	62.5	0.63	101	101	0.39	74.7	100	0.29
CV%	81	27	64	64	50	57	112	30
	60.3[b]	–	132[b]	133[b]	–	–	–	–

[a] Ratio of day 3 to day 1 AUC(0-∞). n number of subjects

[b] Values are geometric means

respectively, and for cycle 2 on these days C_{max} values were 48.0 ± 34.4, 53.9 ± 59.9, and 42.5 ± 17.8 ng/ml, respectively. Paired two-tailed t test analysis for cycle 1 and 2 C_{max} values for days 1, 2, and 3 were not significantly different ($P > 0.05$), indicating decitabine PK was unchanged during both cycles. Additionally, plasma decitabine (mean ± SD) concentrations at the end of infusion for cycle 1 on day 1, day 2, and day 3 were 59.8 ± 51.3, 56.5 ± 33.7, and 54.1 ± 43.4 ng/ml, respectively and for cycle 2 these were 48.0 ± 34.4, 56.9 ± 60.0, and 42.5 ± 17.8 ng/ml, respectively. The corresponding values for each day were similar for both cycles supporting that there was no cycle-to-cycle variation in PK of decitabine in patients with AML/MDS.

Plasma concentration (C_p) time data from each patient was fitted to a 2-compartment body model with elimination from the central compartment (Vc)[14] to the $C_p = A^*(EXP(-\alpha^*t) - EXP(-\alpha^*t')) + B(EXP(-\beta^*t) - EXP(-\beta^*t'))$ equation, where A and B are coefficients, α and β are distribution and elimination rate constants, respectively [18], t is time during infusion, and t' is time after completion of infusion.



The estimated parameters for a representative patient 1, the apparent volume of distribution of the central compartment (Vc [8.12 l/m^2]), and the first-order intercompartmental rate constant from the central compartment (K21 [0.882 h^{-1}], α [11.6 h^{-1}], and β [0.80 h^{-1}]) were used to simulate the plasma concentration time profile of decitabine. As shown in Fig. 3, the model-predicted and the observed mean plasma concentration data are in good agreement.

Toxicity

As expected, the primary toxicity of decitabine was myelosuppression. Grade III–IV neutropenia and thrombocytopenia was observed in 94 and 100% of patients, respectively. Of note, most patients had pre-existing cytopenias; 69% of patients had grade ≥ 2 neutropenia and 81% had grade ≥ 2 thrombocytopenia upon entry into the study. Seven patients (44%) had at least one episode of febrile neutropenia, and five of these (31%) had a documented infection, including bacteremia, pneumonia, and skin infection (n = 1), bacteremia (n = 1), pneumonia (n = 2), and cellulitis (n = 1). The only other adverse event with a probable or definite relationship to decitabine was hypokalemia in one patient. There were three deaths in the study. Two deaths, from sepsis, were considered possibly related to decitabine, and one, from progressive disease, was considered unrelated. There was no evidence of cumulative toxicity and no correlation between PK and drug toxicity in this study.

Response to therapy

Two patients (13%) with MDS had a complete response to decitabine by standard criteria [19]. Neither had received prior chemotherapy for MDS. Two patients, with relapsed

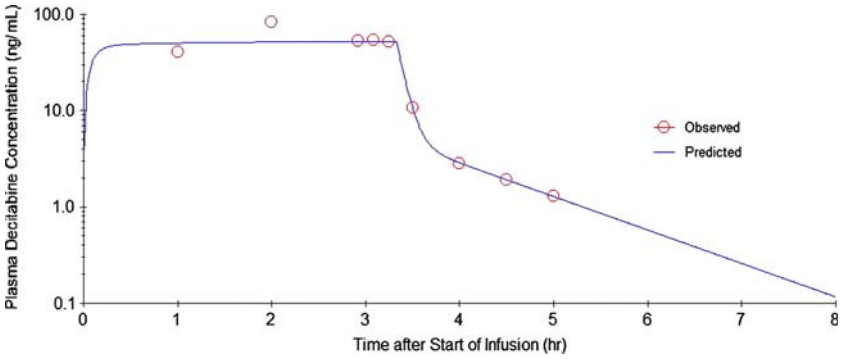


Fig. 3 Simulation of plasma decitabine concentrations after 15 mg/m^2 dosing using a 2-compartment pharmacokinetic model (*solid line*) and observed concentrations (*open circle*) for patient

AML and primary refractory AML, had progressive disease. Twelve patients (75%) had stable disease. Ten patients, including the two who achieved CR, have received a median 6 additional cycles of decitabine on the rollover, safety-only decitabine trial.

Discussion

Decitabine (5-aza-2′-deoxycytidine), an analogue of the natural nucleoside 2′-deoxycytidine, inhibits DNA methyltransferase enzymes, resulting in demethylation that restores gene expression. This is the first Phase 1 study of decitabine to characterize the PK of decitabine at the approved dosing regimen of 15 mg/m^2 three times a day for 3 days in patients with MDS/AML. In this study, the day 1 mean C_{max} values ranged from 64.8 to 77.0 ng/ml and mean $AUC_{0\text{-}\infty}$ values ranged from 152 to 163 ng h/ml. With repeated every 8-h dosing three times per day for 3 days, these parameters remained unchanged as determined by confidence intervals (within 80–125%) for geometric means for C_{max} and $AUC_{0\text{-}\infty}$. The inter-subject variability in C_{max} and $AUC_{0\text{-}\infty}$ was moderate to high.

As the plasma terminal phase elimination half-life was approximately 35 min, steady state in plasma was reached by the end of 3 h infusion and thereafter the decline in plasma concentration was bioexponential. The mean $T_{1/2}$ values did not change during 3 days of dosing ($P > 0.05$). Furthermore, due to the short decitabine $T_{1/2}$, there was no systemic accumulation of drug as determined by the day 3 to day 1 $AUC_{0\text{-}\infty}$ ratio of 0.99 ± 0.29. The mean total body clearance of decitabine ranged from 125 to 135 l/h per m^2 and remained unchanged during the three days of dosing. Similarly the C_{max} values did not vary significantly from cycle 1 to cycle 2 indicating that the CL_p remained unchanged from cycle to cycle, providing consistent exposure to the drug. The CL_p value was high and exceeded the hepatic blood flow (approximately 1,400 ml/min or 45 l/h per m^2), indicating an extrahepatic metabolism involved in elimination of decitabine, presumably by deamination of decitabine by cytidine deaminases. The steady state volume of distribution was large, and mean values ranged from 62.7 to 89.2 l/m^2.

In a previously published Phase 1 study, van Groeningen et al. [13] estimated a mean CL_p value of 126 ml/min per kg (265 l/h per m^2) and a mean V_{dss} value of 4.59 l/kg (161 l/m^2) following a 100 mg/m^2 dose of decitabine infused iv over 1 h. The higher value of CL_p observed by van Groeningen et al. is likely due to the nonspecific bioassay method, based on growth inhibition of L1210 cells, which they used to determine decitabine concentrations. In their study, the PK of decitabine at doses below 75 mg/m^2 could not be characterized, as decitabine concentrations were found to be below the level of detection in plasma samples taken during or at the end of infusion. The terminal phase elimination half-life (mean ± SD) of 35 ± 5 min was similar to the $T_{1/2}$ observed in the present study. The renal clearance was determined to be negligible, and therefore metabolism was postulated as the major route of total body clearance of decitabine.

In another study in pediatric patients with advanced malignancies [16], using the similar in vitro bioassay of inhibition of L1210 leukemic cells, plasma concentrations at the end of a 0.5–1 h infusion of 10 mg/kg ranged from 3.3 to 10 μg/ml, and $T_{1/2}$ values ranged from 11 to 13 min. With 24–40 h infusions, the mean steady state decitabine plasma concentration at the end of infusion was estimated to be approximately 0.5 μg/ml (range 0.3–0.9 μg/ml for the normalized infusion rate of 1 mg/kg per hour). Using the infusion rate and steady state concentrations, we estimated a CL_p of 70 L/h per m^2 in this study. In a longer infusion (72-h duration) study of decitabine at 20–30 mg/m^2 per day in adult patients with solid tumors (data on file), the steady state plasma concentrations ranged from 7.6 to 10.3 ng/ml as determined by the LC/MS/MS method. The CL_p values ranged from 105 to 116 l/h per m^2, similar to those observed in the present study. Therefore, the apparent differences in CL_p using the similar assay methodology of bioassay by Rivard et al. [16] and van Groeningen et al. [13] appear to be due to differences in patient population of pediatric and adult patients, respectively.

In another study by Momparler et al. [14], following 660 mg/m^2 iv infusion over 8 h of decitabine in patients with lung carcinoma, mean steady state concentration using a HPLC assay was 0.898 μg/ml (range 0.59–1.16 μg/ml). We estimate the CL_p based on the infusion rate and steady state concentration to be approximately 92 l/h per m^2 in this study. Plasma samples were also assayed using a bioassay of the growth inhibition of L1210 leukemic cells. These authors reported up to approximately 55% lower plasma decitabine concentrations with the use of the bioassay compared with the HPLC assay. This may explain the overestimation of CL_p using the bioassay in previous studies.

Recently a LC/MS/MS method for assay of decitabine has been published by Liu et al. [20]. This method was applied to assay plasma samples obtained from a Phase 1 study of decitabine combined with valproic acid in AML patients following a 1-h iv infusion dosing at 15 mg/m^2. No pharmacokinetic data were presented, but a plasma decitabine concentration of 103 ng/ml was reached at the end of a 1-h infusion, which appears to be consistent with the results of the present study in which the same dose administered over a longer period of 3 h resulted in a lower C_{max} of approximately 70 ng/ml. In addition to the differences in patient populations and assay methods, the instability of decitabine in aqueous solution, plasma sample collection method, handling, storage, and analysis within 1 month are critical factors for obtaining reliable pharmacokinetic data [20, 21].

In agreement with previous reports, decitabine was generally well tolerated in the patients treated on this trial. As was expected in this patient population, cytopenias and infections were the most frequent and significant toxicities. The majority of patients had cytopenias at baseline, and all patients experienced myelosuppression after treatment with decitabine. Forty-four percent of patients had febrile neutropenia with or without documented infection. Complete responses were observed in two of eight patients with MDS. Notably, ten patients (63%) derived clinical benefit from this treatment and continued therapy with decitabine beyond two cycles.

In conclusion, in this study pharmacokinetics and safety of decitabine have been characterized at the labeled dosing regimen of 15 mg/m^2 administered as a 3-h iv infusion three times a day for 3 days over two cycles of treatment of patients with MDS or AML. Decitabine treatment resulted in a predictable myelosuppression with Grade III–IV neutropenia and thrombocytopenia. The pharmacokinetic profile of decitabine was characterized by a terminal phase elimination half-life of approximately 35 min, and steady state plasma concentrations were reached at the end of infusion. There was no systemic accumulation of decitabine during repeated dosing due to the short $T_{1/2}$. The total body clearance was high (mean value up to 132 l/h per m^2) indicating extrahepatic metabolism involved in CLp of decitabine. The volume of distribution at steady state was large. The PK of decitabine also remained unchanged from cycle to cycle, thus providing a constant systemic exposure to patients for the treatment of MDS or AML. The toxicities and response rates in this study are consistent with those observed in previous trials of MDS patients.

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