Bioavailability of Azacitidine Subcutaneous Versus Intravenous in Patients With the Myelodysplastic Syndromes

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The primary objectives of this study were to characterize the absolute bioavailability of azacitidine after subcutaneous (SC) administration and to compare the single-dose pharmacokinetics of azacitidine following SC and intravenous (IV) administration. Six patients with myelodysplastic syndromes were randomly assigned according to a crossover design to treatment A, consisting of azacitidine administered as a single 75-mg/m^2 SC dose, or treatment B, consisting of azacitidine administered as a single 75-mg/m^2 IV infusion dose over 10 minutes. A minimum of 7 days and a maximum of 28 days were permitted between treatments. The study demonstrated good bioavailability of a SC azacitidine dose compared to an IV infusion treatment. The exposure profiles following SC drug administration illustrate measurable azacitidine levels with bioavailability (AUC) values within 89% of those measured following IV administration (range, 70%-112%). The median IV half-life was 0.36 ± 0.02 hours compared to 0.69 ± 0.14 hours for SC administration. Regardless of the route of administration, a single dose of azacitidine, 75 mg/m^2, was generally well tolerated.

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Azacitidine, a ring analog of the pyrimidine nucleoside cytidine, has effects on cell differentiation, gene expression, and deoxyribonucleic acid (DNA) synthesis and metabolism and, through inhibition of DNA methyltransferase, induces reexpression of silenced genes in cancer cells.[1] Since the early 1970s, azacitidine has been investigated in the United States for the treatment of acute leukemia in both adults and children, and initial clinical trials in adults demonstrated the activity of this drug primarily in chemotherapy refractory patients with acute myelogenous leukemia (AML).[2] Subsequently, azacitidine was also evaluated in other types of neoplastic disease and benign hematologic disorders, including solid tumors, myelodysplastic syndromes (MDS), and hemoglobinopathies (eg, thalassemia and sickle cell anemia).[2]

Treatment options for MDS have been largely ineffective because allogeneic stem cell transplantation is the only curative therapy and a viable option for only 5% of patients. Most patients receive supportive care alone for palliation, which does not alter the natural course of the disease. MDS ultimately progresses to death, either due to bone marrow failure or transformation to AML. The rationale for using azacitidine to treat MDS is based on the ability of azacitidine to reverse epigenetic gene silencing and its effects as a hypomethylating agent, which induces cell differentiation in vitro. Two phase II studies demonstrated that azacitidine could improve hematopoiesis, which manifests as increases in peripheral blood counts, decreases in transfusion requirements, and risk of hemorrhage and infection.[3] These effects on hematopoiesis were confirmed in a phase III randomized study (Cancer and Leukemia Group B [CALGB 9221]), in which azacitidine administered subcutaneously (SC), 75 mg/m^2, daily for 7 days every 28 days was shown to be sig-

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nificantly superior to best supportive care and appeared to alter the natural course of MDS.[4,5] The risk of transformation to acute leukemia was also markedly reduced in this study.

The maximum tolerated dose (MTD) of azacitidine following SC administration has not been determined, although SC doses as high as 100 mg/m^2 were administered in the CALGB MDS studies. In contrast, the MTD for intravenous (IV) administration of azacitidine was reported to be in the range of 150 to 200 mg/m^2/d when given daily for 5 days every 14 to 21 days in children aged 2 to 17 with AML.[6] The MTD IV dose was reported to be as high as 500 mg/m^2 when given once weekly or 150 mg/m^2 when given twice weekly to patients with solid tumors.[7,8] Pharmacokinetic data were not collected in these studies, likely due to a lack of reliable analytical methods.

The pharmacokinetics and drug disposition of azacitidine in humans were initially evaluated using a radiolabeled compound, but the azacitidine and drug metabolites could not be distinguished.[9,10] More recently, however, a sensitive and selective high-performance liquid chromatography with mass spectrometric detection (LC-MS/MS) method has been successfully developed and validated to quantify plasma concentration of azacitidine.[11]

The aim of the present study was to characterize the absolute bioavailability of azacitidine after subcutaneous administration and to compare the single-dose pharmacokinetics of azacitidine given SC with IV administration. Using LC-MS/MS, we analyzed plasma samples collected from MDS patients treated with a single dose of azacitidine 75 mg/m^2 given SC or 75 mg/m^2 given IV over a period of 10 minutes. Both the SC and IV doses used in this study were based on previously published literature. The SC dose of azacitidine (75 mg/m^2) was the same as that used in CALGB studies 8921[3] and 9221.[4] The IV dose was based on a study by Israili et al,[10] in which azacitidine was administered at doses of 150 to 250 mg/m^2 as an 8- to 10-minute intravenous bolus to 5 subjects.

METHODS

Study Design

This study was a multicenter, randomized, open-label, 2-treatment, 2-period, complete crossover design. Up to 10 male or female patients with MDS (any of the 5 French-American-British [FAB] classification subtypes) were to be enrolled to achieve the target of 6 completed patients. Patients ≥40 years, with a life expectancy >3 months, an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, and normal hepatic and renal function who signed a study-specific informed consent were eligible. Patients who were pregnant, had a history of severe cardiac or pulmonary disease, or received radiation therapy, chemotherapy, or other investigational drugs within the previous 30 days were excluded from the study. Concomitant medications considered strong microsomal enzyme inducers (eg, omeprazole, phenobarbital, rifampin, dexamethasone) or inhibitors (eg, selective serotonin reuptake inhibitors, macrolide antibiotics, fluoroquinolones, codeine) were to be avoided. Following screening procedures and baseline assessments (performed within 22 days prior to administration of study drug), the patients were randomly assigned to treatment A, consisting of azacitidine administered as a single 75-mg/m^2 SC dose, or treatment B, consisting of a single-75 mg/m^2 IV infusion dose administered over 10 minutes. The protocol allowed a minimum of 7 days and maximum of 28 days between treatments.

All patients were enrolled at 2 US investigational sites, Ohio State University and Mount Sinai Medical Center. The Western Investigational Review Board and Mount Sinai Medical Center Investigational Review Board reviewed and approved the study protocol for the 2 investigative sites.

Drug Administration

The study drug, prepared and packaged by Ash Steven (Riverview, Mich), was supplied as 100-mg azacitidine powder vials. To obtain azacitidine suspension for SC administration, the powder vials were reconstituted with 4 mL of sterile water for injection, to yield a final concentration of 25 mg/mL. Subcutaneous doses up to 100 mg (4 mL) were administered in a single injection. For doses greater than 100 mg, the dose was equally divided between 2 syringes for SC injection administered at the same time in 2 different body regions. The SC injection was to be administered within 45 minutes following preparation of the azacitidine suspension. To prepare the IV solution, 4 mL of this azacitidine suspension was diluted up to 50 mL with Lactated Ringer's solution. The azacitidine dose was infused at a rate of 5 mL/min, followed by a 10-mL saline infusion. The IV infusion was to be administered over 10 minutes.

All patients received single doses of azacitidine 75 mg/m^2 as a SC injection (treatment A) or IV infusion (treatment B) no later than 10 AM on day 1 of each study period.

Blood Sampling

Plasma concentrations of azacitidine were measured in blood samples collected through either an indwelling catheter or by peripheral venipuncture in tubes containing potassium EDTA. To minimize aqueous degradation of azacitidine during sampling, the collected blood samples were immediately processed or stored in an ice bath following the blood draw and processed within 30 minutes. Azacitidine is stable up to 4 days when extracted from plasma directly into acetonitrile that contains zinc sulfate. Stability is further enhanced by evaporating the acetonitrile and storing azacitidine at –70°C as a dried residue.

For SC dosing, samples were collected at 30 minutes and 1, 2, 4, 8, 12, 24, and 48 hours following the SC dose. For IV dosing, samples were collected at 5, 11, and 30 minutes and 1, 2, 4, 8, 12, 24, and 48 hours following the beginning of the IV infusion. Because samples collected after 12 hours of dosing were below the quantifiable limit (BQL) in the first 2 patients, the protocol was then amended to exclude samples drawn at 12, 24, or 48 hours postdosing in subsequent patients. Once collected, the samples were vortexed, centrifuged, and stored at –70°C until shipment (within 2 days).

Analytical Method

A validated LC-MS/MS method was used for the determination of azacitidine in human plasma. The instability of azacitidine dictated that a portion of the sample processing occur at the sites. Prelabeled, capped polypropylene tubes were sent to each site in preparation for sample collection. Each tube contained 2 mL of acetonitrile and approximately 200 mg of zinc sulfate to stabilize azacitidine in plasma. Following vortexing and centrifugation of each blood collection tube (Vacutainer), a 100-mL aliquot of plasma was transferred from each blood collection tube to the appropriate prelabeled sample collection tube. All tubes were vortexed, centrifuged, and stored at –70°C until shipment (within 2 days). Upon arrival at the bioanalytical laboratory, internal standard (uracil beta-D-arabinofuranoside [Ara U]) was added to each tube, which was subsequently vortexed and centrifuged. The organic layer (acetonitrile), containing the extracted azacitidine, was transferred to a second appropriately labeled polypropylene test tube and evaporated to dryness. Sample tubes were stored at –70°C until analysis by LC-MS/MS with electrospray ionization. This assay was validated over a range of 10.0 to 600 ng/mL. The in-process accuracy acceptance criteria for quality control samples were set to 20% of the theoretical value. Accuracy, as expressed by mean percent recovery relative to theoretical concentrations of calibration standards, was 95% to 105%, and precision, expressed as percent coefficient of variation, was 4% to 7%.

Pharmacokinetic Analysis

Pharmacokinetic (PK) parameters were calculated using noncompartmental techniques with WinNonlin Professional Version 4.01 (Pharsight Corporation, Mountain View, Calif). Graphics were prepared with SAS Version 8.2 or SigmaPlot 7.101 (SPSS, Chicago, Ill).

Pharmacokinetic parameters calculated from plasma concentrations included C_{max}, t_{max}, $AUC_{0-\infty}$, AUC_{0-z}, AUC_{ext}, λ_z, $t_{1/2}$, and absolute bioavailability (F). Apparent plasma clearance (CL_{sc}) was calculated following the SC treatment only; systemic plasma clearance (CL) and volume of distribution (Vd) were calculated following the IV treatment only.

Statistical Analysis

Descriptive statistics were presented by treatment for the concentration data at each scheduled sample collection time point and for the derived pharmacokinetic parameters. To assess the absolute bioavailability of azacitidine, the 75-mg/m^2 SC dose (treatment A) was compared to the 75-mg/m^2 IV infusion dose (treatment B), with treatment B as reference. The comparisons were evaluated with an analysis of the natural log-transformed data. An analysis of variance (ANOVA) with terms for sequence, period, and treatment as fixed effects and subject nested within sequence as a random effect was performed for AUC_{0-z}, $AUC_{0-\infty}$, and C_{max}, from which a 90% confidence interval for the ratio of treatment means was obtained.

RESULTS

Patients

Of a total of 8 patients who were enrolled in this study, 6 met entry criteria and were randomized to receive either treatment A or B, followed by a crossover to the other treatment not previously received. The 6 patients (3 men and 3 postmenopausal or surgically sterile women) were Caucasian and nonsmokers, 57 to 83 years of age, and had a mean body weight of 76.8 ± 14.4

Table I Summary of Arithmetic Mean ± SD Pharmacokinetic Parameters Following Subcutaneous (SC) and Intravenous (IV) Dose Administration (n = 6)

Route	C_{max}, ng/mL	$AUC_{0-\infty}$, ng•h/mL	AUC_{0-z}, ng•h/mL	$t_{1/2}$, h	CL, L/h	Vd, mL
SC	750.0[a] ± 403.3	960.53 ± 458.06	923.88 ± 473.61	0.69[b] ± 0.14	167.48 ± 48.69	
IV	2750.0 ± 1069.0	1044.26 ± 285.67	1025.11 ± 298.06	0.36[c] ± 0.02	146.70 ± 46.91	76076.45 ± 25515.25

Apparent clearance (CL_{sc}) after SC dosing and systemic clearance (CL) following IV infusion; Vd following IV infusion only; AUC_{0-8}, area under the plasma concentration time 0 to infinity, calculated by log-linear trapezoidal summation and extrapolated to infinity by addition of the last quantifiable plasma concentration divided by the elimination rate constant λ_z; AUC_{0-z}, area under the plasma concentration time curve from time 0 to the last measurable time point, calculated by the log-linear trapezoidal summation.

a. Approximately 3 µM.
b. Approximately 41 minutes.
c. Approximately 22 minutes.

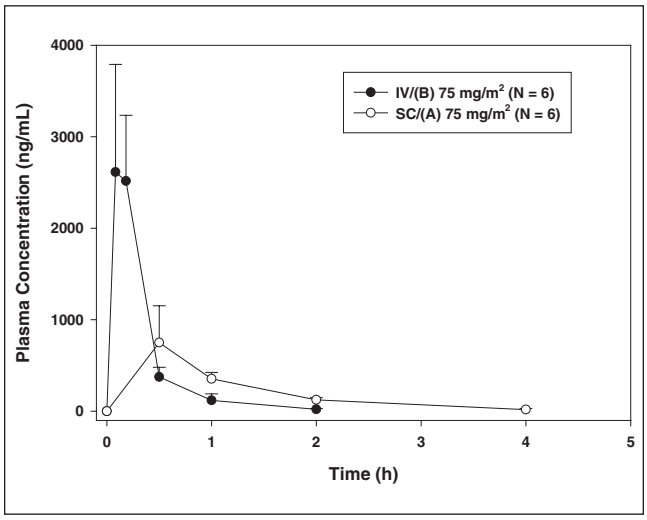

Figure 1. Mean azacitidine concentration-time profile for subcutaneous (SC) and intravenous (IV) treatments.

kg with a mean body surface area of 1.9 ± 0.2 m^2 (range, 1.68-2.20 m^2). According to the FAB classification at screening, 2 patients had refractory anemia (RA), and 4 patients had refractory anemia with excess of blasts (RAEB).

Pharmacokinetics

Following SC administration of azacitidine, maximum plasma concentrations were observed at 0.5 hours in all 6 patients. Following IV infusion, C_{max} was observed at the end of saline flush (11 minutes) in 4 of the 6 patients and 5 minutes into the infusion in 2 of the 6 patients. Mean maximum azacitidine plasma concentrations following IV infusion were approximately 4-fold higher than those observed following SC drug administration. $AUC_{0-\infty}$ values were well characterized by AUC_{0-z}, with an average of 2.3% and 4.8% of the $AUC_{0-\infty}$ extrapolated following IV and SC drug administration, respectively. For each route of administration, the mean plasma azacitidine concentrations with time are shown in Figure 1, and the PK results are summarized in Table I. Geometric mean peak plasma azacitidine concentration following SC administration was 687.30 ng/mL (approximately 3 µM). Mean IV half-life was 0.36 ± 0.02 hours (approximately 22 ± 1 minutes), whereas SC half-life was 0.69 ± 0.14 hours (approximately 41 ± 8 minutes). Apparent mean SC clearance (167.48 ± 48.69 L/h) was slightly higher than systemic (IV) mean clearance (146.70 ± 46.91 L/h). Volume of distribution following IV dosing was 76 ± 26 L. Systemic clearance exceeded the glomerular filtration rate and total renal blood flow, indicating that nonrenal elimination (eg, metabolism/hydrolysis/degradation) played a role in the elimination of parent drug.

Subcutaneous administration of azacitidine showed good bioavailability compared to IV administration. The average of the individual bioavailability estimates, based on ratios of $AUC_{0-\infty}$ and AUC_{0-z} arithmetic means, was 92% and 90%, respectively, with individual values ranging from 60% to 128% for $AUC_{0-\infty}$ and from 52% to 128% for AUC_{0-z}. Based on the SC/IV ratio of the geometric least squares (LS) means, the SC bioavailability was 89% and 86% using the pharmacokinetic parameters $AUC_{0-\infty}$ and AUC_{0-z}, respectively. The geometric LS mean ratio for C_{max} was 27%. The differences in C_{max} are consistent with higher maximum exposure expected following IV versus extra vascular drug administration. Absolute bioavailability of 89% was calculated based on the ratio of the $AUC_{0-\infty}$ SC geometric LS mean to the $AUC_{0-\infty}$ IV geometric LS mean with a 90% confidence interval of 70% to 112% (Table II).

Adverse Events

Overall, 5 of the 6 patients participating in the study experienced at least 1 adverse event following SC or IV administration. Adverse events were experienced in 5 patients following SC administration and 4 patients

Table II Comparison of Subcutaneous (SC) and Intravenous (IV) Azacitidine Dosing by Analysis of Variance

Parameter (Unit)	Treatment	Geometric Least Squares Mean	Ratio of Least Squares Means, %	90% Confidence Intervals, %
$AUC_{0-\infty}$, ng•h/mL	SC	896.37		
	IV	1011.22	88.6	70.2-111.9
AUC_{0-z}, ng•h/mL	SC	852.76		
	IV	987.28	86.4	64.1-116.4
C_{max}, ng/mL	SC	687.30		
	IV	2580.32	26.6	19.2-36.9

following IV administration. The most frequently reported adverse events were nausea (3/6) and vomiting (2/6), both observed with IV and SC administration; injection site bruising (2/6), SC administration only; and arthralgia (2/6), IV administration only. Most adverse events reported were grade 1 or 2 in intensity; grade 3 adverse events of hypertension (assessed as possibly related by the investigator) and worsening of low hemoglobin, which required transfusion (assessed as not related to study drug by the investigator), were reported in 1 patient each. There were no deaths, serious adverse events, or withdrawals from the study due to adverse events.

In general, there were no trends or clinically meaningful changes in laboratory parameters or vital sign values from baseline to endpoint. There were no clinically relevant changes in electrocardiogram findings or in physical examination findings.

DISCUSSION

The primary objectives of this study were to characterize the absolute bioavailability of azacitidine after subcutaneous administration and to compare the pharmacokinetics of single-dose azacitidine given subcutaneously with those of single-dose azacitidine given intravenously. Azacitidine appears to be a high-clearance, short half-life drug with good absolute bioavailability after SC administration (86%-89%, based on geometric LS mean ratios of AUC). Individual arithmetic bioavailability (F) values varied from patient to patient, ranging from 60% to 128% for $AUC_{0-\infty}$ and from 52% to 128% for AUC_{0-z}. These findings may be due to the inherent variability in the disposition of azacitidine, the bioanalytical measures, or the study design.

The instability of azacitidine has been well documented.[12] Therefore, substantial care was given with regard to sample handling and analysis. The instability of azacitidine necessitated that sample processing was performed in part at the sites. Personnel at each site were trained and required to pass a sample processing quality control test prior to processing samples. Nevertheless, it cannot be excluded that some variability in the data might have been introduced by personnel-dependent variations in sample processing. At least 3 quantifiable samples were obtained for each patient following SC dosing, and at least 4 quantifiable samples were obtained for each patient following IV dosing. For both treatments, distribution and elimination phases were defined by at least 2 data points. Despite variability of the pharmacokinetic parameter and bioavailability data, our study demonstrated measurable azacitidine levels following SC dosing, with bioavailability (AUC) values within approximately 90% of those measured following IV administration (range, 52%-128%).

Geometric mean SC and IV C_{max} concentrations were 687 ng/mL and 2580 ng/mL, respectively, which corresponds to 3 μM (SC) and 11 μM (IV). Half-life differences were noted between the 2 routes of administration. Subcutaneous half-lives (0.69 hours) were approximately 2-fold greater than IV half-lives (0.36 hours). Increased SC half-life of azacitidine might be due to the lack of adequately defining the distribution and elimination phases for both formulations. However, it is possible that following SC dosing, additional transient time was required for azacitidine to move from the SC compartment into the circulatory system. Another explanation is that the azacitidine remained both stable and bioavailable at the SC depot site until entering the plasma compartment.

The apparent (SC) clearance (167.48 L/h or 2791 mL/min) and systemic (IV) clearance (146.70 L/h) of azacitidine far exceeded the glomerular filtration rate (approximately 125 mL/min) and total renal blood flow (1200 mL/min) in healthy subjects.[13] This suggests that nonrenal elimination (eg, metabolism, hydrolysis, and/or degradation) plays a role in the elimination of parent azacitidine. The role of metabolism in the disposition of azacitidine is potentially quite significant, as can be derived by comparing our results with those obtained in prior bioavailability studies measuring ^{14}C.

The half-life calculated based on ^{14}C radioactivity appeared longer (3.4-6.2 hours) than that calculated for

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