Single Plasma Concentrations of 1'-Hydroxymidazolam or the Ratio of 1'-Hydroxymidazolam:Midazolam Do Not Predict Midazolam Clearance in Healthy Subjects

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*The 30-minute ratio of 1'-hydroxymidazolam:midazolam plasma concentrations has been used as a measure of midazolam clearance in liver transplant patients. This study determined if a single concentration of 1'-hydroxymidazolam or the ratio of 1'-hydroxymidazolam:midazolam could be used to predict midazolam clearance in healthy subjects. Plasma midazolam and 1'-hydroxymidazolam concentrations from three previous studies were used for analyses. Data obtained predose and at 5, 30, 60, 120, 240, 300, and 360 minutes following intravenous doses of midazolam in 61 adults were divided and used to derive and validate equations to predict midazolam clearance. Equations were derived using linear regression and then validated by comparing predicted to observed clearance. Only one equation was related to midazolam clearance as a function of 1'-hydroxymidazolam, but it did not predict midazolam clearance (*r = *0.29,* p = *0.31). Single sampling of 1'-hydroxymidazolam or 1'-hydroxymidazolam:midazolam plasma concentrations cannot be used to predict midazolam clearance in healthy adults.*

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Cytochrome P450 (CYP) phenotyping describes actual drug-metabolizing enzyme activity at any point in time.[1] Phenotyping studies are used to estimate enzyme activity by correlating biomarker clearance to the amount of enzyme present.[2] Intravenous (IV) midazolam is used as a hepatic cytochrome P450 3A (CYP3A) phenotyping biomarker.[2,3] Conventionally, CYP3A activity is characterized by collecting multiple midazolam plasma concentrations over a 6- to 8-hour period to determine midazolam total systemic clearance (CL).[2,4] Multiple plasma samples are costly and inconvenient, and therefore the use of minimized sampling has been investigated. Minimized sampling of plasma midazolam has been shown to be highly predictive ($r^2 = 0.95$) of midazolam AUC using three samples.[5] However, the use of a single plasma sample would be optimal. It would minimize assay costs and subject and staff time involvement. Data in liver transplant patients suggest that the plasma 1'-hydroxymidazolam:midazolam concentration ratio 30 minutes following administration of an intravenous bolus dose of midazolam is predictive ($r^2 = 0.76$) of midazolam CL.[6]

The purpose of this study was to determine if a single 1'-hydroxymidazolam sample or 1'-hydroxy-

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midazolam:midazolam plasma sample ratio predicts midazolam CL in healthy adults.

MATERIALS AND METHODS

Data from three previous studies, collected at two institutions (Bassett Healthcare and Vanderbilt University), were compiled and used for these analyses. Details of the original studies have previously been published.[1,4,7] A total of 61 healthy adults received an IV bolus of midazolam. The doses ranged from 1.0 to 3.1 mg. Subjects at Bassett Healthcare received midazolam 0.025 mg/kg, while subjects at Vanderbilt University received a 1 mg dose regardless of body weight.

Subjects

Bassett Healthcare. Data from 33 healthy adults were used for these analyses. In brief, subjects underwent a medical history and physical exam prior to study participation. Subjects were nonsmokers and consumed no more than one 12-ounce beverage of beer or alcoholic equivalent per day. Subjects received IV midazolam 0.025 mg/kg (Versed®: 2 mg/ml for injection, Hoffman-LaRoche, Nutley, NJ) infused over 60 seconds via an antecubital vein. Then, 7 ml blood samples were collected into EDTA-containing tubes predose and at 5, 30, 60, 120, 240, 300, and 360 minutes following midazolam administration via an IV catheter placed in the contralateral arm. Blood samples were centrifuged. The plasma was frozen at –80°C until analysis.

Vanderbilt University. Data from 28 healthy volunteers were used for the analyses. Subjects were nonsmokers and were medication free for the duration of the study. Subjects had medical histories, physical examinations, and blood work analyses prior to study participation. Subjects were given 1 mg of IV [$^{15}N_3$]-labeled midazolam infused over 15 to 30 seconds. Blood samples were obtained predose and at 5, 10, 15, 30, 60, 90, 120, 180, 240, 300, 360, and 480 minutes following IV midazolam administration. Collected samples were centrifuged. Plasma was frozen at –20°C until analysis.

Analytical Procedure

Plasma samples were analyzed for midazolam and 1'-hydroxymidazolam concentrations using liquid chromatography/tandem mass spectrometry (LC/MS/MS). Analyses of the plasma samples collected at Bassett Healthcare were performed at Oneida Research Services, Inc. (Whitesboro, NY). Details of these procedures have previously been published.[4,8] Interassay accuracy of the LC/MS/MS assay ranged from 5.73% to 9.20% of nominal values. Interassay precision of the method was ≤ 9.89% at quality control sample concentrations of 0.75, 7.5, and 75.0 ng/ml. Analyses of these plasma samples did not include a β-glucuronidase incubation period. As a result, only unconjugated plasma 1'-hydroxymidazolam was quantified.

Samples collected at Vanderbilt University were assayed on site. The LC/MS/MS assay interday reproducibility was < 12%. Analyses of the plasma samples included a β-glucuronidase incubation period, allowing for quantification of total 1'-hydroxymidazolam.[7]

Since the 1'-hydroxymidazolam concentrations were not comparable, the data from each institution were analyzed separately. Subsequent data groups are designated as unconjugated 1'-hydroxymidazolam and total 1'-hydroxymidazolam.

Pharmacokinetic Analysis

Midazolam $AUC_{0-\infty}$ were determined using the trapezoidal rule with extrapolation to infinity of the last measured midazolam concentration. The unconjugated 1'-hydroxymidazolam data were analyzed using TOPFIT 2.0 (Gustav Fischer Verlag, Stuttgart, Germany). The total 1'-hydroxymidazolam data were analyzed using NCOMP.[7] Midazolam clearance was determined as (midazolam dose)/$AUC_{0-\infty}$.

Data Analysis

Models to predict midazolam CL as a function of a single postdose concentration of 1'-hydroxymidazolam or the ratio of 1'-hydroxymidazolam:midazolam concentrations were derived using stepwise multiple linear regression (SAS 6.12, SAS Institute, Cary, NC). Sixteen of the 33 subjects from the unconjugated 1'-hydroxymidazolam group and 14 of the 28 subjects from the total 1'-hydroxymidazolam group were randomly selected and their data used to generate model equations. p-values ≤ 0.05 were considered statistically significant. Regression coefficients (r^2) for the derived equations were determined. The derived equations were then validated with data from the remaining subjects by comparing predicted CL to observed CL using the Pearson correlation coefficient (r). The Wilcoxon signed rank test (SigmaStat 2.03, SPSS, Inc., Chicago) was used to determine statistically significant differ-

Table I Demographic Information and Midazolam Clearance by Data Analysis Group for 61 Healthy Adults

	Unconjugated 1'-Hydroxymidazolam Group		Total 1'-Hydroxymidazolam Group		
	Derivation	Validation	Derivation	Validation	Total
Total subjects	16	17	14	14	61
Male	7	7	14	14	
Female	9	10	0	0	
Age range (years)	25-51	22-56	25-44	24-43	
Age (mean ± *SD*)	39 ± 8.6	36.7 ± 9.3	33.6 ± 6.1	33.4 ± 5.8	
Weight range (kg)	57-102.6	57-124.5	68.2-115.9	75.7-102.7	
Weight (mean ± *SD*)	77.0 ± 16.8	77.8 ± 16.7	88.4 ± 2.2	85 ± 9.1	
Height range (cm)	158-184	157-188	165-185	165-193	
Height (mean ± *SD*)	169.4 ± 7.7	170.5 ± 8.9	178.0 ± 7.2	177.5 ± 7.5	
Race, *n* (%)					
Caucasian	16(100)	17(100)	7(50)	8(57)	48(79)
African American	0(0)	0(0)	7(50)	6(43)	13(21)
Midazolam clearance					
Range (ml/min)	404-718	437-836	194-381	198-391	
Mean ± *SD*	539 ± 87	608 ± 118	282 ± 65	297 ± 55	
Median	534	581	266	296	

No statistically significant differences in demographic information ($p > 0.05$) exist between derivation and validation subsets of either group.

ences between demographic data for each group (derivation and validation).

RESULTS

Demographic data for the derivation and validation groups are shown in Table I.

Single sampling strategies were investigated at all time points (i.e., 5, 30, 60, 120, 240, 300, and 360 min) following the dose of midazolam. No model derived from the unconjugated 1'-hydroxymidazolam data, using 1'-hydroxymidazolam or 1'-hydroxymidazolam:midazolam, was predictive of midazolam CL.

No model derived from the total 1'-hydroxymidazolam data to predict midazolam CL as a function of a single time point 1'-hydroxymidazolam:midazolam was predictive of midazolam CL. One model predicted midazolam CL as a function of total 1'-hydroxymidazolam. The equation is as follows:

$$CL = 445.9 - 65.1 \text{ (total 1'-hydroxymidazolam}_{300\ min}\text{)}.$$

The corresponding $r^2 = 0.29$ had a $p < 0.0001$. This derived equation was then validated with data from the remaining 14 participants. Pearson correlation was used to compare predicted midazolam CL to observed midazolam CL (Figure 1). The derived equation could not be validated ($r = 0.29$, $p = 0.31$).

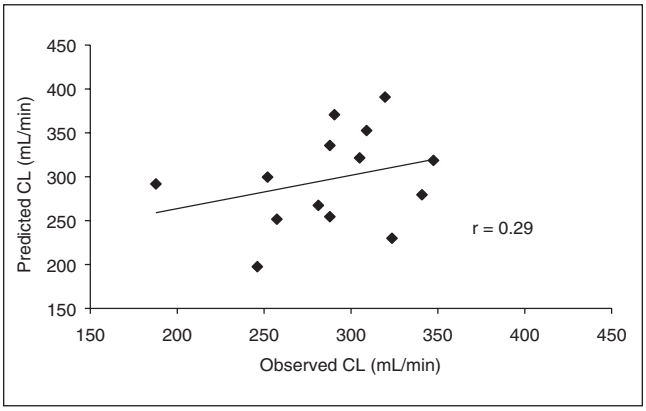

*Figure 1. Observed midazolam clearance versus predicted midazolam clearance based on the 300-minute total 1'-hydroxymidazolam concentration with corresponding Pearson correlation coefficient (*r*).*

DISCUSSION

Limited sampling has been demonstrated with the use of midazolam,[6,9] and single sampling of omeprazole and 5-hydroxyomeprazole concentrations 3 hours following drug administration is often used for CYP2C19 phenotyping.[10,11] While the limited sampling strategy for midazolam is more convenient than standard sampling, single sampling would be optimal.

A previous study showed that a single sampling approach was predictive of midazolam clearance in liver transplant patients. However, this correlation was dependent on steroid-induced variability.[6] As a result, the use of this 30-minute ratio would most likely have minimal utility in predicting midazolam clearance in healthy subjects. The results of our data analyses showed failure of any single sampling time point of 1'-hydroxymidazolam or the ratio of 1'-hydroxymidazolam:midazolam to predict midazolam clearance. A possible reason for this failure is intersubject variability associated with genetic and environmental factors. Also, midazolam is lipophilic, and its volume of distribution is increased in obese subjects.[12] Some study participants were obese and therefore have larger volumes of distribution. In addition, midazolam is moderately extracted by the liver, where the hepatic extraction ratio (E_H) is $0.3 \leq E_H \leq 0.7$. As such, the clearance of midazolam is dependent on CYP3A activity, hepatic blood flow, and the fraction of unbound midazolam in the plasma. The influence of all of these factors will add intersubject variability to resulting midazolam clearance.

Single sampling strategies using 1'-hydroxymidazolam:midazolam or 1'- hydroxymidazolam concentrations cannot be used to predict midazolam CL in healthy adults. Minimized sampling (i.e., 5, 30, 360 min) of midazolam concentrations remains a more reliable method for estimating midazolam AUC. Midazolam CL can then be calculated and correlated to hepatic CYP3A activity.

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