The Prediction of the Area under the Curve and Clearance of Midazolam from Single-Point Plasma Concentration and Urinary Excretion in Healthy Volunteers

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There are large inter- and intra-individual variations in CYP3A4 activity. Midazolam, which is predominantly metabolized to 1'-hydroxymidazolam and 4-hydroxymidazolam by CYP3A4, is considered an effective probe for CYP3A4. To determine the area under the curve (AUC) of midazolam or midazolam clearance for CYP3A4 activity, multiple plasma samples of midazolam are required. This study aimed to evaluate whether measurement of a single plasma concentration or urinary excretion of midazolam could be used to predict the AUC of midazolam in healthy volunteers. We conducted a retrospective analysis of two pharmacokinetic studies. Nineteen volunteers received intravenous (5, 15, and 30 µg/kg) and oral (15, 50, and 100 µg/kg) administration of midazolam on sequential days. The midazolam concentration in plasma and urine was determined by LC-MS/MS. Plasma midazolam concentrations showed a good correlation with the AUC at all blood sampling points after the administrations. The coefficient of determination was highest at 1–2 and 2–4 h after intravenous (>0.96) and oral administration (>0.94), respectively, among all the sampling times. The errors for bias and accuracy of prediction were the lowest at 1.5 and 4 h after intravenous and oral administration, respectively. In case of urinary excretion, a significant positive correlation between midazolam and the AUC was observed only after oral administration. Thus, the AUC of midazolam can be evaluated by blood sampling at 1.5 h after intravenous administration and at 4 h after oral administration.

Key words midazolam; single-point sampling; urinary excretion; CYP3A4

INTRODUCTION

CYP3A4 is involved in the metabolism of various drugs. The activity of CYP3A4 varies with sex and age and intra-individual variations are also known to arise from diverse pathological conditions.1–3 Moreover, many drug interactions with CYP3A4 have been reported and are often clinically problematic.3

Several CYP3A substrates have been suggested as probes to determine CYP3A4 activity, including alprazolam, erythromycin, midazolam (MDZ), and verapamil.4,5 MDZ is considered a particularly useful probe.5 MDZ is predominantly metabolized to 1'-hydroxymidazolam and 4-hydroxymidazolam (1'-OHMDZ and 4-OHMDZ) by CYP3A4 and CYP3A5. Therefore, MDZ was considered to be a probe of CYP3A4 in the clinical trial. However, in an attempt to perform in vivo activity of CYP3A4 is characterized by the collection of multiple plasma samples of MDZ to determine the area under the curve (AUC) or clearance (CL). The collection of multiple plasma samples is costly and inconvenient. Therefore, the use of minimized sampling for CYP3A4 activity could provide useful information for the pharmacotherapy of patients while being less invasive. In addition, as urinary samples can be collected noninvasively, this sampling method can reduce the burden on the patient. Several reports have performed the evaluation of CYP activity with a reduced number of blood collections.6,7 However, it has not been established whether single-point blood sampling can evaluate CYP3A4 activity. Because CYP3A4 activity commonly uses the plasma concentration of MDZ, there are few studies that use urinary samples.

The aim of this study was to evaluate whether a single-point plasma concentration and the urinary excretion of MDZ and its metabolites accurately predicted the AUC and CL of MDZ in healthy volunteers.

MATERIALS AND METHODS

Study Designs We conducted a retrospective analysis of two pharmacokinetic studies involving a total 29 administrations in 19 healthy volunteers.8,9

The first study was conducted in three phases, distinguished by different doses of MDZ.9 On day 1, in the low-dose phase, subjects received a single intravenous (i.v.) bolus injection of 5 µg/kg MDZ. The blood samples were obtained at 0, 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 10, and 24 h after i.v. MDZ. On day 2, the subjects received a single oral dose of 15 µg/kg MDZ and the blood samples were collected 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h later. The urine samples were collected until 24 h after administration on days 1 and 2. The middle-dose phase was initiated, with subjects administered 15 µg/kg i.v. MDZ on day 1 and 50 µg/kg per os (p.o.) MDZ on day 2.

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The high-dose phase was initiated, with subjects administered 30 µg/kg i.v. MDZ and 100 µg/kg p.o. MDZ on days 1 and 2, respectively. The blood and urine samples were collected as described for the low-dose phase.

The second study was a randomized, open-label, crossover study design with two phases. The volunteers took either ursodeoxycholic acid (UDCA) or placebo three times a day for 9 d. The probe drugs, 5 µg/kg i.v. MDZ and 15 µg/kg p.o. MDZ, were administered after an overnight fast on days 8 and 9, respectively. From this study, we evaluated only placebo data.

The plasma concentrations until 10 h after MDZ administration from the two studies were used for the analysis of plasma concentrations; the analysis was based on a total of 285 data points for intravenous administration and 250 data points for oral administration. The urine concentration was analyzed using data from 29 samples.

### Pharmacokinetic Analysis

The pharmacokinetics parameters for MDZ and its metabolites were referred from the previous reports. The metabolic ratio in plasma defined as the concentration of the ratio of the metabolite, 1'-OHMDZ, to MDZ, was calculated using each time point. The metabolic ratio in urine defined as the concentration of metabolites (1'-OHMDZ and 4-OHMDZ) to MDZ was calculated using urine samples taken 24 h after dose. In plasma samples only low levels of 4-OHMDZ were detected.

### Cross-Validation and Predictability Analysis

We performed cross-validation to evaluate the validity of regression of AUC or CL prediction. Briefly, after we excluded data obtained from one administration (test set) of the analyzed these from 29 administrations from regression analysis, we performed regression analysis using the remaining data from 28 administrations (training set) and calculated the predicted curve. Based on the predicted curve equation, the predictive values of AUC and CL (AUCpred, CLpred) were calculated using one administration excluded. We calculated the AUCpred or CLpred using all administrations. The mean prediction error (MPE), mean absolute error (MAE), and root mean squared prediction error (RMSE) were determined from the following expressions by using a predicted value and an observed value (AUCobs, CLobs).

\[
\text{MPE} = \frac{1}{n} \sum_{i=1}^{n} (\text{AUC}_{\text{pred}} - \text{AUC}_{\text{obs}}) \\
\text{MAE} = \frac{1}{n} \sum_{i=1}^{n} |(\text{AUC}_{\text{pred}} - \text{AUC}_{\text{obs}})| \\
\text{RMSE} = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (\text{AUC}_{\text{pred}} - \text{AUC}_{\text{obs}})^2}
\]

### Statistical Analysis

Statistical analysis was conducted using GraphPad Prism software (version 5.0; GraphPad, San Diego, CA, U.S.A.). The criterion for significance was \(p \leq 0.05\). The correlations between the AUC and the plasma concentrations at each time point, the CL or CL/F and the urinary metabolic ratios were evaluated by unweighted linear regression. The \(p\) value was calculated by using Pearson correlation coefficients.

### RESULTS

#### Relationship of Plasma Concentration of MDZ and the AUC after Intravenous Administration, and the Metabolic Ratio in Plasma and CL

A significant positive correlation was observed between the plasma concentrations of MDZ at 0.08–10 h and the AUC after the intravenous administration of midazolam.

![Graph](image1.png)

Each point represents the value of each volunteer. The point fit the liner equation, \(y = 1.40x + 3.89\); 0.25 h, \(y = 1.95x + 2.89\); 0.5 h, \(y = 2.72x + 1.55\); 1 h, \(y = 3.94x + 2.32\); 1.5 h, \(y = 5.48x + 2.63\); 2 h, \(y = 7.09x + 2.67\); 4 h, \(y = 13.78x + 3.81\); 6 h, \(y = 28.15x + 0.57\); 8 h, \(y = 44.49x + 1.37\); 10 h, \(y = 51.98x + 6.36\), where \(y\) is AUC of midazolam and \(x\) is plasma concentration of midazolam. MDZ: midazolam and AUC: area under the curve.
was found between the plasma concentration of MDZ and the AUC at all blood sampling points (Fig. 1, Table 1). The coefficient of determination was highest at 1–2 h after intravenous administration (>0.96) among all the sampling times. In addition, the errors for bias and accuracy of prediction (MPE, MAE, RMSE) were the lowest at 1.5 h. (Table 1).

The relationship between the metabolic ratio in plasma and the AUC after Intravenous Administration from Plasma Concentration of Midazolam and its Metabolic Ratio in Plasma, Respectively, at Each Sampling Point

| Time (h) | Concentration | Metabolic ratio |
|---------|---------------|-----------------|
|         | Correlation*  | Prediction      | Correlation** | Prediction     |
|         | $r^2$         | $p$             | MPE (%)       | MAE (%)        | RMSE (%)       | $r^2$ | $p$ | MPE (%) | MAE (%) | RMSE (%) |
| 0.08    | 0.867         | <0.001          | 10.36         | 35.74          | 44.00          | 0.075 | 0.158 | 7.14     | 19.48    | 27.34     |
| 0.25    | 0.930         | <0.001          | 9.09          | 22.71          | 28.08          | 0.084 | 0.126 | 6.55     | 18.52    | 26.35     |
| 0.5     | 0.948         | <0.001          | 6.51          | 19.31          | 25.38          | 0.069 | 0.460 | 7.22     | 20.98    | 30.88     |
| 1       | 0.964         | <0.001          | 5.27          | 16.20          | 20.67          | 0.060 | 0.201 | 7.07     | 18.94    | 28.05     |
| 1.5     | 0.964         | <0.001          | 4.41          | 12.20          | 14.50          | 0.044 | 0.277 | 7.25     | 19.59    | 28.57     |
| 2       | 0.966         | <0.001          | 4.81          | 13.81          | 16.48          | 0.146 | 0.041 | 6.41     | 19.17    | 26.65     |
| 4       | 0.786         | <0.001          | 18.93         | 25.62          | 18.93          | 0.318 | 0.001 | 4.63     | 19.05    | 23.58     |
| 6       | 0.943         | <0.001          | 18.23         | 24.19          | 18.23          | 0.473 | <0.001| 3.80     | 19.56    | 24.42     |
| 8       | 0.862         | <0.001          | 23.69         | 30.08          | 23.69          | 0.389 | 0.001 | 2.66     | 17.91    | 26.52     |
| 10      | 0.522         | <0.001          | 43.48         | 63.56          | 43.48          | 0.020 | 0.488 | 4.63     | 16.82    | 25.58     |

*$^* This symbol represents coefficient of determination ($r^2$) and $p$ value between plasma concentration of midazolam at each sampling point and its AUC. ** This symbol represents coefficient of determination ($r^2$) and $p$ value between metabolic ratio in plasma at each sampling point and its CL. AUC, area under the curve; CL, total clearance; MPE, mean prediction error; MAE, mean absolute error; RMSE, root mean squared prediction error.

![Fig. 2. The Relationship between the 1'-Hydroxymidazolam/Midazolam Ratio at 0.08–10 h and the Total Clearance of Midazolam after the Intravenous Administration of Midazolam](image-url)

Each point represents the value of each volunteer. The point fit the linear equation, 0.08 h, $y = 34.87x + 6.89$; 0.25 h, $y = 10.09x + 6.42$; 0.5 h, $y = 3.28x + 7.06$; 1 h, $y = 8.88x + 5.98$; 1.5 h, $y = 6.05x + 6.17$; 2 h, $y = 11.43x + 4.85$; 4 h, $y = 12.93x + 4.13$; 6 h, $y = 9.14x + 4.87$; 8 h, $y = 5.45x + 6.16$; 10 h, $y = 0.84x + 6.83$, where $y$ is total clearance of midazolam and $x$ is metabolic ratio in plasma (1'-hydroxymidazolam/midazolam). MDZ: midazolam and 1'-OHMDZ: 1'-hydroxymidazolam.

Relationship between Plasma Concentration of MDZ after Oral Administration and AUC and the Metabolic Ratio in Plasma and CL/F Similarly, for oral administration, the AUC of MDZ was significantly correlated with the plasma concentration at each sampling point (Fig. 3). The coefficient of determination at 2–4 h was >0.94. MPE, MAE and RMSE showed lower values at 4 h after oral administration (Table 2).

The relationship between the metabolic ratio in plasma and CL/F was analyzed and found to show a significantly positive correlation between 2 and 6 h after administration (Fig. 4).

Relationship between Urinary MDZ and Metabolite Excretion and the AUC and the Metabolic Ratio in Urine and CL The investigation of the results from the urine sample
showed that urinary MDZ excretion was not significantly correlated with AUC after intravenous administration. In contrast, a significant correlation was observed after oral administration (\(y=6.07x+2.22, r^2=0.727\)) (Table 3).

**DISCUSSION**

It is suggested that MDZ AUC can be evaluated by blood sampling at 1.5 h after intravenous administration and at 4 h post oral administration. In our results, the optimal sampling time after oral administration was similar to previous reports. The coefficient of determination in the metabolic ratio was lower than that in plasma and AUC, which is not necessarily a good indication. Our results suggest that in the analysis using MDZ, it is better to use the plasma concentration than the metabolic ratio.

In our analysis, the bias and accuracy of prediction, measured by the MPE, MAE, and RMSE, were estimated. The bias and accuracy 1.5 h after intravenous administration were <15%, which was within acceptable limits for the evaluation reported in previous studies. However, prediction accuracy (MAE, RMSE) at 4 h after oral administration was relatively high compared with reported acceptable limits, although prediction bias (MPE) was within the limit. It has been reported that the prediction models using multiple sampling points obtain smaller errors of bias and accuracy for prediction of MDZ AUC in comparison with that using the single sampling point. However, multiple sampling is difficult from patients in clinical settings. The collection of single plasma samples is more appropriate to perform in vivo activity of CYP3A4 for
pharmacotherapy. Interestingly, Misaka et al. reported that low doses of MDZ do not result in the development of clinical effects or side effects. Therefore, single-point blood sampling after low-dose MDZ administration can be safely used for patients.

In the evaluation using urinary excretion, a significant positive correlation of MDZ was observed with the AUC only after oral administration.

Streetman et al. reported that the metabolic ratio in urine was useful as an indicator of CYP3A4 inhibition. However, the metabolic ratio in urine did not correlate with the CL of MDZ. Streetman et al. attributed this to the large variation in CL that was observed from drug–drug interactions. In this study, we altered the concentration in the blood by changing the dosage, but we observed a small variation in CL. Therefore, it was concluded that there was no correlation between the metabolic ratio and CL. In the future, it is necessary to study the variation of CL owing to drug interactions. As this study is retrospective, we believe that prospective research with a focus on drug interactions is necessary.

**CONCLUSION**

Our results suggest that the best way to predict the AUC of MDZ from single-point plasma concentration of MDZ at 1.5 and 4 h after intravenous and oral administration, respectively. These methods can be used for the AUC prediction and can be applied to in vivo activity of CYP3A4.

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**Conflict of Interest** The authors declare no conflict of interest.
REFERENCES

1) Schwartz JB. The influence of sex on pharmacokinetics. Clin. Pharmacokinet., 42, 107–121 (2003).

2) Rodighiero V. Effects of liver disease on pharmacokinetics. An update. Clin. Pharmacokinet., 37, 399–431 (1999).

3) Dresser GK, Spence JD, Bailey DG. Pharmacokinetic–pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin. Pharmacokinet., 38, 41–57 (2000).

4) Thummel KE, Shen DD, Podoll TD, et al. Use of midazolam as a human cytochrome P450 3A probe: I. In vitro–in vivo correlations in liver transplant patients. J. Pharmacol. Exp. Ther., 271, 549–556 (1994).

5) Lee JI, Chaves-Gnécco D, Amico JA, Kroboth PD, Wilson JW, Frye RF. Application of semisimultaneous midazolam administration for hepatic and intestinal cytochrome P450 3A phenotyping. Clin. Pharmacol. Ther., 72, 718–728 (2002).

6) Kim JS, Nafziger AN, Tsunoda SM, Choo EE, Streetman DS, Kashuba AD, Kulawy RW, Beck DJ, Rocci ML Jr, Wilkinson GR, Greenblatt DJ, Bertino JS Jr. Limited sampling strategy to predict AUC of the CYP3A phenotyping probe midazolam in adults: application to various assay techniques. J. Clin. Pharmacol., 42, 376–387 (2002).

7) Chaobal HN, Kharasch ED. Single-point sampling for assessment of constitutive, induced, and inhibited cytochrome P450 3A activity with alfentanil or midazolam. Clin. Pharmacol. Ther., 78, 529–539 (2005).

8) Misaka S, Uchida S, Imai H, Inui N, Nishio S, Ohashi K, Watanabe H, Yamada S. Pharmacokinetics and pharmacodynamics of low doses of midazolam administered intravenously and orally to healthy volunteers. Clin. Exp. Pharmacol. Physiol., 37, 290–295 (2010).

9) Yan B, Yang Y, Uchida S, Misaka S, Luo J, Takeuchi K, Inui N, Yamada S, Ohashi K, Watanabe H. Effects of ursodeoxycholic acid on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam in healthy volunteers. Naunyn Schmiedebergs Arch. Pharmacol., 377, 629–636 (2008).

10) Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. J. Pharmacokinet. Biopharm., 9, 503–512 (1981).

11) Masters JC, Harano DM, Greenberg HE, Tsunoda SM, Jang JJ, Ma JD. Limited sampling strategy of partial area under the concentration-time curves to estimate midazolam systemic clearance for cytochrome P450 3A phenotyping. Ther. Drug Monit., 37, 84–89 (2015).

12) Mueller SC, Drewelow B. Evaluation of limited sampling models for prediction of oral midazolam AUC for CYP3A phenotyping and drug interaction studies. Eur. J. Clin. Pharmacol., 69, 1127–1134 (2013).

13) Streetman DS, Kashuba AD, Bertino JS Jr, Kulawy R, Rocci ML Jr, Nafziger AN. Use of midazolam urinary metabolic ratios for cytochrome P450 3A (CYP3A) phenotyping. Pharmacogenetics, 11, 349–355 (2001).