Single plasma sampling to predict oral clearance of CYP3A probe midazolam

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KEY WORDS cytochrome P-450 CYP3A; midazolam; 1'-hydroxymidazolam; pharmacokinetics

ABSTRACT

AIM: To find out a single plasma sampling to estimate oral clearance of midazolam (MDZ) and CYP3A activity, and explore the pharmacokinetics of midazolam hydroxylation in Chinese subjects. METHODS: The pharmacokinetics of midazolam was assessed in ten healthy male individuals after an oral dose of 7.5 mg midazolam. RESULTS: A significant correlation (r = 0.7, P < 0.05, n = 10) was found between plasma MDZ clearance and the plasma ratio of 1-hydroxymidazolam to midazolam, which was assessed at 1 h after MDZ intake in the volunteers. Pharmacokinetics parameters of midazolam were as follows: Cmax (191 ± 17) nmol/L, tmax (1.01 ± 0.14) h, τ1/2 (3.2 ± 0.4) h, AUC0-∞ (681 ± 43) nmol·h·L⁻¹, Clc (0.54 ± 0.04) L·h⁻¹·kg⁻¹, K1 (0.2415 ± 0.0021) h⁻¹, K2 (0.82 ± 0.16) h⁻¹. CONCLUSION: Single plasma sampling of 1 h after 7.5 mg oral MDZ intake can be used to predict the oral clearance of midazolam.

INTRODUCTION

As the major constitutive enzymes in liver and intestine, cytochrome P450 isoforms are responsible for the metabolism of a majority of therapeutic compounds. CYP3A activity is highly variable, causing difficulty in the therapeutic use of CYP3A substrates. A measure of CYP3A activity relative to first pass metabolism is provided following an oral dose, whereas, after intravenous administration, CYP3A-mediated hepatic metabolism is primarily obtained. A practical in vivo probe method that characterizes both intestinal and hepatic CYP3A activity would be useful.

Several approaches including crythromycin breath test, midazolam clearance, 6β cortisol/free cortisol ratio in urine, nifedipine metabolite production in urine, dapson metabolite urine ratio, and lignocaine metabolite blood assay were used to measure CYP3A activity, but all have its limitations.

The primary midazolam metabolite is 1'-hydroxy-midazolam. Minor metabolites formed by CYP3A metabolism are 4-hydroxymidazolam and 1,4-hydroxymidazolam. Both CYP3A4 and CYP3A5 are capable of catalyzing midazolam hydroxylation. The biotransformation of midazolam to 1'-hydroxymidazolam, has been proposed as a probe for CYP3A activity in vivo. Especially as measured by oral clearance of midazolam, is very sensitive to modulation of the enzyme’s level of activity. Ideally, only one or a few blood samples would be required to describe MDZ metabolism, but the test always involves measuring a full plasma level-time profile over 6–8 h to get the clearance of midazolam. Therefore, finding a single blood sample at an appropriate time that can be used as the prediction of CYP3A activity is particularly needed.

In this study, we elucidated the pharmacokinetic behavior of midazolam in Chinese subjects, and verified the single plasma sampling to predict the oral clearance of the CYP3A probe midazolam.

MATERIALS AND METHODS

Chemicals Midazolam (MDZ) and 1-hydroxymidazolam (1'-OH-MDZ) were purchased from Ultrace Chemical Company (Manchester, UK). Nortriptyline was purchased from Sigma Chemical Co (St Louis, USA). Acetonitrile and methanol of HPLC grade and doubly distilled water were required for HPLC with UV detector. All other chemicals were of AR grade available from commercial sources.
Subjects Ten normal male volunteers (age, 19 to 21 y; weight, 55 to 80 kg) were used for the study. The experimental protocol was approved by the Ethical Committee of Hunan Medical University and all subjects gave written, informed consent before commencing the study. All subjects were in good health as indicated by medical history, routine physical examination, and biochemical testing. All subjects were asked to abstain from alcohol, caffeine and grapefruit juice for a week before the study. All subjects were non-smokers and ate normal diet.

Experimental protocol After an overnight fast, each subject received 7.5 mg midazolam (Dormicum, Hoffman-La Roche Ltd, Basel, Switzerland) orally along with 100 mL water. Food was prohibited for 2 h except water intake. Blood samples (8 mL) were withdrawn through an indwelling heparin cannula previously inserted into an antecubital vein, at the following times: pre-dose (control), 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, and 8 h after oral administration. Urine was collected at the following times: pre-dose (control), 0–4, 4–8, 8–12, 12–24 h after drug administration. Total volumes were measured and aliquots (5 mL) subsequently stored at −20 °C until required for analysis.

Analytical procedures Plasma samples were assayed for parent midazolam and for 1'-OH-MDZ. To 1 mL plasma, 100 μL of internal standard (nortriptyline, 100 nmol/L), 1 mL buffer glycine (0.75 mol/L, pH 9), and 4 mL diethyl ether were added. The samples were extracted and centrifuged for 10 min at 2500 × g. The organic phase was extracted and transferred to glass tubes and evaporated to dryness under a stream of nitrogen at 37 °C. The residue was dissolved in 100 μL of mobile phase, and 20 μL were injected into the HPLC column.

The urine aliquots were assayed for the main urinary metabolite, 1'-OH-MDZ, after enzymatic deconjugation with glucuronidase-sulphatase. Urine 200 μL was mixed with 0.8 mL of 0.2 mol/L acetate buffer pH 5.3 and incubated at 37 °C overnight after addition of 20 μL glucuronidase-sulphatase. After incubation, urine was adding 1 mL buffer glycine (0.75 mol/L, pH 9), then extracted with diethyl ether.

The HPLC equipment consisted of LA-10A pump, SPD-10A ultraviolet detector, automatic injector, and C-R7A Chromato-Integrator. All above apparatus were purchased from Shimadzu (Tokyo, Japan). MDZ and 1'-OH-MDZ were separated on a C8 column (4.6 mm × 150 mm, 5 μm particle size, Hewlett). The composition of the mobile phase was 32 % acetonitrile : 3 % methanol : 65 % 0.1 mol/L buffer acetate (v/v/v) (pH 4.34). The flow rate through the column at 35 °C was 1.1 mL/min, and MDZ and 1'-OH-MDZ were monitored by ultraviolet absorbance at 234 nm.

Plasma standard curves were linear in the concentration range of 11–344 nmol/L for MDZ and 8–246 nmol/L for 1'-OH-MDZ. Urine standard curves were linear in the concentration range of 11–86 μmol/L for MDZ and 8–61 μmol/L for 1'-OH-MDZ. The intra-day and inter-day coefficients of variation were <8%.

Data analysis Noncompartmental techniques were used in the pharmacokinetic analysis (Figr1 perfect software, 1990). The weight-normalized systemic oral clearance was calculated as $Cl_{oral} = \text{Dose} / \text{AUC}^{\text{oral}}$, where AUC denotes the area under the drug concentration-time curve determined by the logic trapezoidal rule extrapolated to infinity. The rate constant of elimination ($K_e$) was determined by least squares regression analysis of the post-distribution phase of the plasma concentration time profile and the corresponding half-life ($t_{1/2}$) determined as $0.693 / K_e$. $K_o$ was determined by residue analysis of distribution phase of the plasma concentration time profile.

Correlation between plasma MDZ clearance and 1'-OH-MDZ : MDZ plasma ratio of different time were examined by linear regression, with $P < 0.05$ accepted as statistically significant.

RESULTS

Plasma levels of midazolam after oral administration The plasma concentration versus time curve of MDZ and its main metabolite 1'-OH-MDZ after a single dose (7.5 mg) of MDZ after oral administration was shown in Fig 1.

Pharmacokinetic study Pharmacokinetics parameters of MDZ and 1'-OH-MDZ in ten healthy male volunteers were shown in Tab 1.

Correlation analysis A significant correlation between weight-normalized plasma MDZ clearance and plasma concentration ratio of 1'-OH-MDZ to MDZ$^{10}$ measured at 1 h after intake of a single 7.5 mg oral dose of MDZ in ten healthy male volunteers was shown in Fig 2 ($r = 0.70$, $P < 0.05$, $n = 10$). The correlation between weight-normalized plasma MDZ clearance and the plasma concentration ratio of 1'-OH-MDZ to MDZ measured at different time points was shown in Tab 2. The amount of 1'-OH-MDZ in 0–4 h, 0–8 h, 0–12 h.
Fig 1. Plasma levels of the MDZ and 1'-OH-MDZ concentration after a single oral dose (7.5 mg) in ten healthy male volunteers. n = 10. x ± s.

Tab 1. Pharmacokinetics of midazolam in healthy male subjects after oral administration of midazolam (7.5 mg). The variability in oral clearance was threefold. n = 10. x ± s.

<table>
<thead>
<tr>
<th>MDZ</th>
<th>1'-OH-MDZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{max} (nmol·L^{-1})</td>
<td>191 ± 17</td>
</tr>
<tr>
<td>t_{max} (h)</td>
<td>1.01 ± 0.14</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td>AUC_{0-t} (nmol·h·L^{-1})</td>
<td>0.01 ± 0.04</td>
</tr>
<tr>
<td>V_{ss} (L·h^{-1}·kg^{-1})</td>
<td>0.54 ± 0.06</td>
</tr>
<tr>
<td>K_{e} (h^{-1})</td>
<td>0.24 ± 0.021</td>
</tr>
<tr>
<td>K_{e} (h^{-1})</td>
<td>0.82 ± 0.18</td>
</tr>
</tbody>
</table>

0–24 h urine has no correlation with the oral clearance of midazolam (P > 0.05).

DISCUSSION

A large number of orally administered drugs exhibit low systemic availability because of extensive first-pass metabolism catalyzed by the microsomal cytochrome P450 superfamily of enzymes. Many of these drugs are substrates of CYP3A. CYP3A4 and CYP3A5 are major CYP3A isoforms expressed in adults. Overlapping substrate specificities between CYP3A4 and CYP3A5 have previously made it difficult to separate metabolism by these isoforms[11]. Locating in liver and intestinal epithelium mostly, CYP3A activity exhibits considerable interindividual variability. Several studies have examined the validity of MDZ CI as a phenotyping measure of CYP3A[12]. In an in vitro/in vivo comparison. Thumel et al demonstrated a correlation between hepatic microsomal CYP3A content and midazolam clearance/kg body weight after iv administration among liver transplant recipients[13]. Correlations between CYP3A activity (1'-OH-MDZ formation rate) and protein content within an intestine were significant[13]. The interindividual variability in the pharmacokinetics of oral administered MDZ is in part determined by interindividual variability in the hepatic microsomal V_{max} for the 1'-hydroxylation of MDZ. However, the relationship between the disposi-

Tab 2. Correlation between MDZ clearance and the concentration ratio of 1'-OH-MDZ to MDZ plasma in different time points. n = 10.

<table>
<thead>
<tr>
<th></th>
<th>0.25 h</th>
<th>0.5 h</th>
<th>1 h</th>
<th>1.5 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio (x ± s)</td>
<td>0.49 ± 0.22</td>
<td>0.52 ± 0.24</td>
<td>0.48 ± 0.16</td>
<td>0.45 ± 0.13</td>
<td>0.42 ± 0.13</td>
<td>0.38 ± 0.06</td>
<td>0.40 ± 0.12</td>
<td>0.36 ± 0.14</td>
<td>0.48 ± 0.12</td>
</tr>
<tr>
<td>r</td>
<td>0.299 ± 0.295</td>
<td>0.296 ± 0.293</td>
<td>0.244 ± 0.170</td>
<td>0.212 ± 0.135</td>
<td>0.216 ± 0.126</td>
<td>0.204 ± 0.124</td>
<td>0.250 ± 0.149</td>
<td>0.306 ± 0.192</td>
<td>0.481 ± 0.300</td>
</tr>
<tr>
<td>p</td>
<td>0.001 ± 0.002</td>
<td>0.003 ± 0.001</td>
<td>0.012 ± 0.010</td>
<td>0.024 ± 0.020</td>
<td>0.049 ± 0.043</td>
<td>0.204 ± 0.197</td>
<td>0.406 ± 0.301</td>
<td>0.681 ± 0.490</td>
<td>0.901 ± 0.901</td>
</tr>
</tbody>
</table>
tion of midazolam administered po and hepatic CYP3A content is weaker than that reported after iv administration, indicating the importance of the contribution of intestinal CYP3A to the in vivo disposition of MDZ administered po.15

Intravenous MDZ was used to phenotype hepatic CYP3A. Thunnell et al showed a strong correlation between hepatic CYP3A content and the plasma 1'-OH-MDZ/MDZ concentration ratio of 30 min after intravenous administration in liver transplant recipients. But the good correlation observed between MDZ clearance and the metabolite/parent drug ratio may be dependent on drug-induced variability, therefore, the 1'-OH-MDZ/MDZ plasma concentration ratio may be of limited utility as a CYP3A probe in a "normal, healthy population,"16. In order to examine the usefulness of MDZ as a CYP3A probe to predict cyclosporine clearance, Villeneuve et al chose to assess MDZ metabolism from a single blood collection obtained 60 min after administered intravenously.10 Kim et al found that plasma samples 5, 30 and 360 min post-dose accurately predicts midazolam AUC after a single iv dose, and predict AUC using two samples (obtained at 30 and 360 min) correlated highly with actual AUC.24

By oral administration, Carrile et al found a significant correlation (r = 0.89, P < 0.0068, n = 8) between plasma MDZ clearance and the 1'-OH-MDZ/MDZ plasma ratio, assessed at 0.5 h after MDZ intake in the volunteers.10 But in the study of Kinirons et al., uncertain results were found that there is significant correlation between the oral clearance of the drug and the ratios at both 30 min (r = 0.48, P = 0.03) and 60 min (r = 0.7, P = 0.001). Since CYP3A is abundant in the intestines, orally administration MDZ is subject to both intestinal and hepatic CYP3A metabolism. Both the in vitro and in vivo MDZ data suggest that the small intestine can be major source of interindividual variability in oral bioavailability.22 Therefore to determine an optimal single plasma sampling to predict oral clearance of the CYP3A probe MDZ will be useful.

In this study, we confirmed that there was a significant correlation (r = 0.7, P < 0.05, n = 10) between plasma MDZ clearance and the 1'-OH-MDZ/MDZ plasma ratio, assessed at 1 h after MDZ intake in the volunteers (Table 2). This finding was interesting because it would provide a simpler estimate for measuring liver and intestinal CYP3A activity, with a single blood measurement. Further investigations in larger populations should be carried out. In addition, there was good correlation between the oral clearance of MDZ and the ratios at 1.5 h (r = 0.63, P = 0.049). So it seemed possible to estimate MDZ clearance from any single plasma 1 - 1.5 h postdose, which still need to be verified.

It showed that there were differences among the correlation of MDZ clearance and the ratios in different time points (Table 2). In absorption phase before peak concentration, the ratios were uncorrelated with the clearance of MDZ because clearance was a parameter that was used to evaluate drug elimination. In elimination phase, the ratios determined from a single blood collection were also affected by multiple factors, but the clearance of MDZ was relatively fixed. For example, the rate of elimination expedited when drug concentration increased, but the clearance kept constant.

In this study, we also estimate the pharmacokinetics of midazolam after oral administration in Chinese subjects and the variability in oral clearance was threefold. Compared to the results from literature, the AUC and clearance of midazolam by oral administration of 7.5 mg were (514 ± 217) nmol·h·L⁻¹ and (0.51 ± 0.18) L/(h·Kg) respectively for white subjects, (681 ± 43) nmol·h·L⁻¹ and (0.54 ± 0.04) L/(h·Kg) respectively for Chinese subjects. AUC of Chinese subjects are higher than that of white subjects, but there is no significant difference of weight-normalized oral clearance between these two racial groups.

In conclusion, we have established a simple method to investigate CYP3A activity in humans, which will benefit for population study of CYP3A activity.

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单点采血反映口服 CYP3A 探针咪达唑仑的代谢清除率

关键词 细胞色素 P-450 CYP3A；咪达唑仑；1'-羟化
咪达唑仑；药代动力学

目的：研究中国男性健康受试者口服咪达唑仑后其
1'-羟化代谢的药代动力学规律，并寻找合适的单个
采血点血浆中 1'-羟化咪达唑仑/咪达唑仑的浓度比
值来反映咪达唑仑的血浆清除率。方法：10 名受试
者禁食 8 小时后清晨空腹口服 7.5 mg 咪达唑仑，利
用非房室模型计算药代动力学参数。结果：咪达唑
仑药代动力学参数 Cmax 为 (191 ± 17) nmol/L, tmax 为
(1.01 ± 0.14) h, t1/2 为 (3.2 ± 0.4) h, AUC0∞ 为 (612 ±
43) nmol·h·L-1, Cl red 为 (0.54 ± 0.04) L/(h·kg), Kp 为
(0.2415 ± 0.0021) h-1, Ke 为 (0.082 ± 0.018) h-1。1 小时血浆中咪达唑仑与其代谢产物 1'-羟化
咪达唑仑的比值与其清除率的相关性统计学上具有
显著意义 (r = 0.7, P < 0.01, n = 10)。结论：可用
口服咪达唑仑后 1 小时采一个采血点的代谢产物 1'-羟
化咪达唑仑与咪达唑仑浓度的比值来反映其血浆清
除率，应用于 CYP3A 活性测定的人群试验。

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