Evaluation of CYP3A activity in humans using three different parameters based on endogenous cortisol metabolism

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Aim: Currently, there is considerable debate as to which method is more accurate for measuring the activity of CYP3A in vivo: cortisol 6β-hydroxylation clearance (Clm(6β)) or the urinary ratio of 6β-OHF to F (6β-OHF/F). Furthermore, the value of measuring endogenous levels of cortisol over a 24 h period (AUCf) needs to be confirmed. The aim of the present study was to determine which method was most effective at measuring changes in the in vivo activity of CYP3A: AUCf, Clm(6β), or 6β-OHF/F.

Methods: A two phase, cross-over design was adopted in this study. A total of 24 subjects (12 males and 12 females) were randomly assigned to one of two groups: the test group subjects were given 250 mg clarithromycin tablets twice a day for a period of 4 d, whereas the control group received a placebo twice daily for a similar period. On d 5 of the study, the last dose of either clarithromycin or placebo was supplemented with an oral dose of 7.5 mg midazolam (MDZ); blood and urine samples were then collected at various times. All samples collected at the same sampling times on d 4 were used to evaluate the effects of MDZ administration on cortisol levels and metabolism. The ratio of 1-hydroxymidazolam (1-OHMDZ) concentration to MDZ concentration at 1 h (MR) was taken as a measure of the in vivo CYP3A activity. AUCf, Clm(6β), and 6β-OHF/F were also used as biomarkers for CYP3A activity.

Results: No correlations were found (either before or after inhibition) between CYP3A activity and any of the following measures: AUCf, Clm(6β), or 6β-OHF/F (r<0.4, P>0.05). After 4 d of clarithromycin administration, CYP3A activity (MR) decreased by 75% (P=0.000), whereas AUCf increased by 19% (P=0.040), and Clm(6β) and 6β-OHF/F decreased by 54.2% (P=0.000) and 50% (P=0.003), respectively. No significant changes in AUCf (P=0.178), or in the amount of urinary 6β-OHF (P=0.169), or in F (P=0.391) were found over a 24 h time period, either with or without MDZ administration.

Conclusion: Although Clm(6β) and 6β-OHF/F can reflect the decline in CYP3A activity, the impression they provide is neither accurate nor complete. AUCf is completely ineffective for evaluating variations in CYP3A activity. MDZ administration had no evident effects on either cortisol metabolism or excretion over a period of 24 h.

Keywords: CYP3A activity; cortisol; 6β-hydroxycortisol; midazolam

Introduction

Cytochrome P450 3A (CYP3A), which is highly expressed in the liver and in the intestine, is the most abundant cytochrome isoform in humans. CYP3A accounts for approximately 60% of the total cytochrome content in the liver. Nearly half of all current clinical drugs are substrates for CYP3A[1, 2]. Overall, the activity of the CYP3A subfamily in adults is comprised mostly of CYP3A4 and CYP3A5[2]. Because of the wide variability in CYP3A activity, it is necessary to study the CYP3A phenotype in order to predict the optimal dosage range of drugs (ie, that which can improve therapeutic effects but minimize adverse effects)[3, 4].

Several assessments of the CYP3A phenotype have been carried out in vivo; these include midazolam (MDZ) plasma clearance, the erythromycin breath test and the 6β-hydroxycortisol/free cortisol (6β-OHF/F) ratio in urine. Of these markers, the most widely used probe drugs are MDZ and erythromycin; however these biomarkers all have limitations.

MDZ is biotransformed primarily to 1-hydroxy-midazolam (1-OHMDZ) in vivo. This procedure is catalyzed by CYP3A enzymes. Both intestinal and hepatic CYP3A activity can be characterized by MDZ oral clearance, and this is typically used as a reliable index for CYP3A phenotyping[5]. However, in order to measure the oral clearance of MDZ, several blood...
samples are often required. Zhu et al.\(^6\) used single plasma sampling to predict CYP3A activity in ten healthy male volunteers in vivo. A significant correlation was demonstrated between weight-normalized MDZ oral clearance and the plasma concentration ratio of 1-OHMDZ to MDZ (MR) that was measured 1 h after intake of a single (7.5 mg) oral dose of MDZ (\(r=0.7, P<0.05\)). However, due to its pharmacodynamic activity as a central nervous system depressant, this technique is not convenient for the evaluation of CYP3A activity.

The intravenous administration of stable isotopically labeled erythromycin in the erythromycin breath test also has its limitations. In particular, this technique is capable of measuring only hepatic CYP3A activity.

The measurement of endogenous cortisol (F) levels may represent a safe, simple and non-invasive assay of CYP3A activity. It has been suggested that the urinary ratio of 6β-OHF/F is also a useful marker of both the induction and the inhibition of hepatic CYP3A activity\(^7\). However, this measure does not appear to be an accurate marker of the pharmacokinetic properties of other substrates of CYP3A, such as the well-known probe drug MDZ and erythromycin\(^8-10\).

Recently, Furuta et al.\(^11\) found that cortisol 6β-hydroxylation clearance (\(Cl_{\text{in}6\beta}\)) could be a reliable index for in vivo CYP3A activity evaluation. In this study, a strong correlation between endogenous and exogenous 6β-hydroxylation clearance was shown in three healthy male volunteers. Furthermore, when the macrolide antibiotic clarithromycin was administered to another healthy male volunteer, the inhibitory effects of clarithromycin on in vivo CYP3A activity were clearly indicated by the 6β-hydroxylation clearance of endogenous cortisol, but not by the urinary 6β-OHF/F. It has also been suggested that urinary 6β-OHF/F does not always reflect the in vivo CYP3A activity\(^11\). However, significant shortcomings were associated with this study by Furuta et al.; these included the limited number of samples, and the lack of a comparison with probes such as MDZ and erythromycin.

In these studies, the relationship between the endogenous cortisol 6β-hydroxylation clearance and the in vivo CYP3A activity (as reflected by the oral clearance of MDZ) was examined. We have previously published the results obtained using a high-performance liquid chromatography with ultraviolet absorbance detection (HPLC-UV) method for the simultaneous determination of 6β-OHF and F in human urine or plasma\(^12\). Of the 12 subjects (6 males and 6 females) enrolled in our study, a good correlation between cortisol 6β-hydroxylation clearance and MDZ oral clearance was seen in only 6 male subjects. The unexpected outcome of this study was the discovery of a negative correlation between the ratio of the area under the plasma concentration-time curve of 1-OHMDZ to that of MDZ (AUC\(_{\text{MR}}\)) and the area under the plasma concentration-time curve of cortisol (AUC\(_{\text{F(0-24)}}\)). A strong correlation between plasma cortisol concentrations at 1, 4, 8, 10, and 24 h and AUC\(_{\text{F(0-24)}}\) was also determined by linear regression. Based on these earlier findings, we proposed that the measure of AUC\(_{\text{F}}\) that was calculated from plasma cortisol concentrations at 1, 4, 8, 10, and 24 h, represented a new biomarker for assessing in vivo CYP3A activity.

In conclusion, as the utility of \(Cl_{\text{in}6\beta}\) and 6β-OHF/F measures were still up for debate, our laboratory sought to determine the effectiveness of AUC\(_{\text{F}}\). Thus, the present study was undertaken to demonstrate whether AUC\(_{\text{F}}\), \(Cl_{\text{in}6\beta}\), and 6β-OHF/F provided accurate measures of variations in CYP3A activity in vivo. In addition, the effect of MDZ administration on cortisol metabolism and excretion over a 24 h time period was also investigated.

**Materials and methods**

**Chemicals and reagents**

MDZ, 1-hydroxy-midazolam, cortisol and 6β-hydroxycortisol (at least 98% purity) were purchased from Sigma-Aldrich. Acetonitrile and methanol were of HPLC grade. All other chemicals were of AR grade and were available from commercial sources.

**Subjects**

Twenty-four normal volunteers (12 males, 12 females; age: 22.1±1.2 years; weight: 58.6±5.4 kg; height: 165.9±7.0 cm) participated in this study. As indicated by their medical history, physical examinations, routine laboratory tests (hematology, blood chemistries and urinalysis), all subjects were in good health. The subjects were asked to abstain from the consumption of alcohol, caffeine and grapefruit juice for two weeks prior to the study. All of the subjects were non-smokers and consumed a normal diet. This experimental protocol was approved by the Ethical Committee of the School of Pharmaceutical Sciences, Central South University. Prior to commencing the study, written informed consent was obtained from each subject.

**Experimental protocol**

A two-way crossover design was adopted in this test. A total of 24 volunteers (12 males and 12 females) were randomly assigned to one of two groups. After an overnight fast, either 250 mg clarithromycin tablets (experimental group) (Huiren, China) or placebo (control group) were given twice daily (at 08:00 and 20:00) to each subject, for a period of 4 d. On d 5, the last dose of either clarithromycin or placebo was given with an oral dose of 7.5 mg MDZ (Roche, China). Blood samples were then collected both prior to and at 1, 4, 8, 10, 24 h after drug administration. Urine samples were collected at 0−4, 4−8, 8−10, 10−24 h time periods. To evaluate the effects of MDZ administration on cortisol levels and metabolism, the samples that were collected on d 4 were compared with those obtained on the day of MDZ treatment. With the exception of water intake, food was prohibited for 2 h after MDZ administration. To separate the plasma, blood samples were centrifuged at 3500×g for 10 min as the volume of urine was recorded. The plasma and urine samples were stored at -20 °C until required for analysis. After a washout period of two weeks, two groups switched treatments respectively. Blood and urine samples were collected in the same way.
Analytical methods

6β-OHF and F in plasma or urine

F and 6β-OHF in urine or plasma were measured using the HPLC-UV method that was developed in our laboratory[13]. The lower limit of quantitation was 6.9 µg/L for 6β-OHF and 2 µg/L for F in urine. The lower limit of quantitation was 7.8 µg/L for F in plasma. The accuracy, determined for three different concentrations, ranged between 95% and 107%.

MDZ and 1-OHMDZ in plasma

MDZ and 1-OHMDZ in plasma were determined using a previously established HPLC method with minor modifications[13]. Briefly, to act as the internal standard, a solution of chloridiazepoxide was added to 1.0 mL of human plasma. After the addition of 100 µL of 1.0 mol/L sodium hydroxide, the plasma samples were extracted with 5.0 mL of cyclohexane/diethyl ether (3:7). HPLC analysis was performed using a Shimadzu LC-2010CHT high-performance liquid chromatography apparatus (Shimadzu, Kyoto, Japan). The separation of MDZ and 1-OHMDZ was achieved on a Hypersil GOLD column (4.6 mm×150 mm, 5 µm, Thermo Electron, USA) using a mobile phase of methanol/10 mmol L⁻¹ phosphate buffer (pH 7.4) (57:43, v/v). The flow rate was 1.0 mL/min and the ultraviolet absorbance was monitored at 254 nm. The lower limit of quantitation was 2 µg/L for 1-OHMDZ and 4 µg/L for MDZ. The coefficients of variation for both intra-day and inter-day precision for each compound were less than 3.9%.

Data analysis

The plasma concentration ratio of 1-OHMDZ to MDZ (MR) at 1 h was used as a measure of the in vivo CYP3A activity. AUC<sub>F</sub> at each sample time was calculated from the plasma cortisol concentrations using a trapezoid ruler. Cl<sub>m(6β)</sub> was calculated from the amount of urinary excreted 6β-OHF over a period of 24 h, divided by AUC<sub>F</sub>. 6β-OHF/F was calculated from the ratio of urinary 6β-OHF to F.

Parameter values obtained in the same subjects under different conditions were compared using Wilcoxon’s rank sum test, whereas AUC<sub>F</sub> both before and after inhibition, was logaritically transformed, and then evaluated using the variance analysis method. Correlations between MR and AUC<sub>F</sub>, between MR and Cl<sub>m(6β)</sub> and between MR and 6β-OHF/F were determined using Spearman’s rank correlation analysis. All statistical analyses were performed using SPSS 12.0 statistics software, and P values less than 0.05 were considered to be significant.

Results

CYP3A activity variation with clarithromycin pretreatment

The in vivo CYP3A activity, reflected by MR, was reduced by 75% (P=0.000) following clarithromycin pretreatment. The significant change in MR is shown in Figure 1. Plasma MDZ, 1-OHMDZ concentration and CYP3A activity (MR) both before and after inhibition by clarithromycin are included in Table 1.

AUC<sub>F</sub> variation and correlation with CYP3A activity

MDZ administration resulted in a 25% decrease (P=0.033) in plasma cortisol concentrations at 1 h after MDZ administration, but there were no changes in concentration at other sampling times. AUC<sub>F</sub> was increased by 19% (P=0.040) when CYP3A activity was inhibited by clarithromycin (Table 2 and Figure 2). No correlations were found between AUC<sub>F</sub> and CYP3A activity either before or after inhibition (r=0.342, P=0.102; r=0.391, P=0.059; Figure 3). There was also no correlation between the change in AUC<sub>F</sub> and CYP3A activity (r=0.260, P=0.220) (Figure 3).

Cl<sub>m(6β)</sub> variation and correlation with CYP3A activity

MDZ administration caused increases in 6β-OHF urinary excretion of 51% (P=0.022), 75% (P=0.002) and 103% (P=0.019) at 0–4, 4–8, and 8–10 h time-periods, respectively. However, there were no increases in excretion and no alterations in Cl<sub>m(6β)</sub> at 10–24 and 0–24 h time-periods. Following clarithromycin administration, there was a decrease in 6β-OHF urinary excretion at all time-periods, and a reduction in Cl<sub>m(6β)</sub> of 54.2% (P=0.022; Table 3 and Figure 2). However, there were no correlations found between Cl<sub>m(6β)</sub> and MR, either before or after

Table 1. Mean values of plasma 1-hydroxy-midazolam, midazolam concentrations, and CYP3A activity (MR) before and after inhibition by clarithromycin, n=24. Data are given as means±SD.

| Time (h) | 1-OHMDZ (µg/L) | MDZ (µg/L) | MR |
|----------|---------------|------------|----|
|          | Before inhibition | After inhibition | Before inhibition | After inhibition | Before inhibition | After inhibition |
| 1        | 12.38±4.47    | 10.36±4.49  | 26.96±8.82  | 95.25±52.73  | 0.49±0.19     | 0.13±0.06       |

MR: the plasma concentration ratio of 1-OHMDZ to MDZ at 1 h.
inhibition ($r=0.107$, $P=0.619$; $r=-0.317$, $P=0.132$, respectively; Figure 4). There was also a lack of a correlation between the change in $C_l(6\beta)$ and MR ($r=0.019$, $P=0.931$; Figure 4).

Table 2. Plasma cortisol concentration variations before and after clarithromycin administration. $n=24$. Data are given as mean±SD.

| Time (h) | Cortisol concentration (μg/L) | Before inhibition | With MDZ | After inhibition |
|---------|-------------------------------|------------------|----------|-----------------|
|         | Non MDZ                       | With MDZ         | AUC (μg·h·L$^{-1}$) |                   |
| 1       | 77.26±40.10                   | 57.90±18.63      | 61.35±22.96 |
| 4       | 44.46±33.84                   | 50.28±17.40      | 55.98±27.92 |
| 8       | 46.48±37.56                   | 50.35±18.55      | 61.65±28.02 |
| 10      | 41.36±18.13                   | 46.13±14.57      | 57.29±23.34 |
| 24      | 97.02±49.23                   | 113.90±32.45     | 136.72±48.62 |
| AUC (μg·L$^{-1}$) | 1459.56±696.24 | 1609.17±357.89 | 1918.97±620.25 |

$AUC_\gamma$: the area under the plasma concentration-time curve of cortisol at various sampling times.

Table 3. Variations in $6\beta$-OHF urinary excretion before and after clarithromycin inhibition. $n=24$. Data are given as mean±SD.

| Time period (h) | $6\beta$-OHF urinary excretion (μg) | Before inhibition | With MDZ |
|----------------|-----------------------------------|------------------|----------|
| Non MDZ | With MDZ | AUC (μg·h·L$^{-1}$) |                   |
| 0–4     | 12.72±8.58 | 19.15±10.77 | 10.30±5.23 |
| 4–8     | 5.78±3.48 | 13.26±6.89 | 5.56±3.26 |
| 8–10    | 5.70±2.89 | 11.56±18.00 | 3.61±1.63 |
| 10–24   | 24.27±12.78 | 20.98±9.74 | 12.92±7.65 |
| 0–24    | 24.27±12.78 | 20.98±9.74 | 12.92±7.65 |
| $C_l(6\beta)$ (mL/min) | 0.67±0.32 | 0.69±0.30 | 0.32±0.16 |

$C_l(6\beta)$: cortisol $6\beta$-hydroxylation clearance.

6$\beta$-OHF/F variations and correlation with CYP3A activity
MDZ administration produced increases in urinary $6\beta$-OHF/F of 102% ($P=0.017$), 185% ($P=0.006$) and 123% ($P=0.019$) at 0–4, 4–8, and 8–10 h time-periods, respectively; however, there was no increase in the urinary excretion of cortisol. The values of urinary $6\beta$-OHF/F at all time-periods decreased significantly when CYP3A was inhibited by clarithromycin, but no changes in the urinary excretion of cortisol were observed (Table 4 and Figure 2). No correlations were found between $6\beta$-OHF/F and MR, either before or after inhibition ($r=0.063$, $P=0.769$; $r=0.070$, $P=0.746$) (Figure 5). Furthermore, there was a lack of correlation between the change in $AUC_\gamma$ and CYP3A activity ($r=0.001$, $P=0.995$; Figure 5).

Effect of MDZ administration on endogenous cortisol levels
No significant changes were found in $AUC_\gamma$, either before or after MDZ administration ($P=0.178$; Table 2 and Figure 2). Meanwhile, no significant changes in the amount of urinary $6\beta$-OHF ($P=0.169$) and F ($P=0.391$) were measured over the 24 h time-period (Tables 3 and 4).
Discussion
The absence of any correlations between CYP3A activity and AUCₜₐₚₚ, Clₘₐₚₚ, and 6β-OHF/F
The relationships between CYP3A activity and several measures (AUCₜₐₚₚ, Clₘₐₚₚ, and 6β-OHF/F) both before and after clarithromycin inhibition were examined. However, there were no significant correlations between any of these measures and CYP3A activity (indicated by the secretion and metabolism of cortisol). It is now known that cortisol is produced by the adrenal cortex, and its secretion exhibits a circadian rhythm; the highest levels are secreted between 08:00 and 12:00 and the lowest levels are released at midnight[7]. The pathway underlying cortisol metabolism in humans is complex. It has been proposed that the conversions of cortisol

Table 4. Cortisol urinary excretion and 6β-OHF/F variations before and after clarithromycin inhibition. n=24. Data are given as mean±SD.

| Time period (h) | Cortisol urinary excretion (μg) Before inhibition | After inhibition | 6β-OHF/F Before inhibition | After inhibition |
|----------------|---------------------------------------------------|-----------------|---------------------------|-----------------|
|                | Non MDZ                                           | With MDZ        | Non MDZ                  | With MDZ        |
| 0–4            | 9.26±4.37                                         | 9.65±6.71       | 8.95±4.58                | 1.47±0.82       |
| 4–8            | 7.91±4.57                                         | 7.69±5.79       | 8.02±6.06                | 1.20±0.71       |
| 8–10           | 4.65±2.37                                         | 4.93±3.45       | 4.34±2.83                | 1.55±1.12       |
| 10–24          | 11.79±5.05                                        | 12.46±8.72      | 10.92±6.87               | 2.66±2.36       |
| 0–24           | 33.61±12.68                                       | 34.72±15.01     | 32.24±13.73              | 1.71±0.90       |

6β-OHF/F: urinary ratio of 6β-OHF/F.
to cortisol and 6β-hydroxycortisol to 6β-hydroxycortisone by 11β-HSD play important roles in cortisol metabolism. The metabolism of cortisone to 6β-hydroxycortisone is also catalyzed by CYP3A [16]. In addition, several other physiological factors might be involved in the disposition of MDZ and F. Firstly, when it is administered orally, MDZ is metabolized not only in the liver but also in the intestine. This affects the accuracy of measurements of AUCF, as this value mainly reflects hepatic CYP3A activity. Second, according to a previous study, the efflux of P-glycoprotein (P-gp) in vivo probably affects AUCF [19]. However, as MDZ exhibits characteristics of a class 1 drug in the biopharmaceutical classification system, the disposition of MDZ is influenced to a lesser degree by P-gp [16, 17].

Additionally, differences in renal function in different subjects may cause heterogeneity in 6β-OHF excretion that could affect the values of Cl(6β). It is also worth noting that CYP3A consists of at least three isoforms: CYP3A4, CYP3A5, and CYP3A7. Although CYP3A5 may also have played a role here, the relative contribution of each of these isoforms to cortisol metabolism has not been completely elucidated [7].

**AUCF is not a suitable index for evaluating variations in CYP3A activity in vivo**

Our study reported that there were significant changes in both CYP3A activity (marked by MR) and AUCF after clarithromycin treatment in twenty-four healthy subjects. However, in response to a reduction in the values of MR of around 75%, the value of AUCF actually increased by 19%; no correlation was found between these measures. This suggested that AUCF was an entirely unsuitable index for evaluating variations in CYP3A activity. As discussed previously, this might be accounted for the regulation of cortisol feedback and physiological factors.

**Inhibitory effect on CYP3A activity in vivo can be partly reflected by Cl(6β) and 6β-OHF/F**

Although no correlations were found between Cl(6β) and CYP3A activity, or between 6β-OHF/F and CYP3A activity, significant changes were seen in Cl(6β) and 6β-OHF/F with clarithromycin treatment. The reduction in Cl(6β) of 54.2% accounted for approximately three-quarters of the decline in MR. Though it did not completely reflect the change in CYP3A activity, it was a more useful marker than AUCF. This inaccuracy might also be explained by the many factors that influence plasma cortisol levels. Similar to F, 6β-OHF is also a secretory product of the human adrenal system, and its secretion is thought to be controlled by adrenocorticotropic hormone [38]. Furthermore, individual differences in renal function may affect the degree of 6β-OHF excretion in different subjects, with a consequent effect on Cl(6β) values.

A measure of urinary 6β-OHF/F during the 0–24 h collection period might also be used to partly evaluate variations in CYP3A activity. After clarithromycin administration, 6β-OHF/F declined by about 50%. In this study, 6β-OHF urinary excretion declined by 50%, whereas cortisol excretion did not vary significantly during the 24 h collection period. This may be due to either decreased cortisol secretion or increased cortisol metabolism, one of which was the augmented conversion of cortisol to cortisone. Thus, measurements of the concentration of cortisone could provide an explanation for why the urinary cortisol level remained stable with clarithromycin pretreatment.

As discussed above, cortisol secretion exhibits a diurnal rhythm. In the present study, the intradividual variability in measures of Cl(6β) and 6β-OHF/F were 7.1- and 11.8-fold, respectively. This was clearly a major impediment for the establishment of a cortisol-based CYP3A parameter.

**Effect of MDZ administration on endogenous cortisol level**

In a previous study [19], the change in plasma cortisol level with MDZ anesthesia was examined in 11 patients that were undergoing abdominal hysterectomy. The plasma cortisol concentration decreased slightly at the induction of anesthesia and at the beginning of surgery. By the end of the surgery, the cortisol levels had increased significantly (the highest value was attained a few hours after the operation), although levels of this hormone approached the normal range on the morning following the operation. Misiolek et al. [20] observed the suppression of cortisol release when propofol and MDZ were used for anesthesia induction for non-toxic struma surgery in contrast to thiopentone administration. The results presented here showed the effect of MDZ administration on cortisol levels. With the exception of the plasma cortisol concentration at 1 h (25%, P=0.033), no significant changes were observed at other time points or in the measures of AUCF (Table 2). Meanwhile, variations in the amount of urinary 6β-OHF were detected during the collection periods of 0–4 h (51%, P=0.022), 4–8 h (75%, P=0.002) and 8–10 h (103%, P=0.019), but not in the 10–24 h (P=0.160), and 0–24 h (P=0.169) periods. There were also no differences in the Cl(6β) values during these periods (P=0.388; Table 3). There were no significant changes in the amount of urinary F at any time-period (Table 4). This might be explained by the existence of different mechanisms; it is possible that the reduced plasma cortisol levels at 1 h were mainly due to the enhanced conversion of cortisol to urinary 6β-OHF or to the substantially elevated F to E conversion which was followed by the transformation of E to 6β-OHE by CYP3A. This may have resulted in the enhanced conversion of 6β-OHE to 6β-OHF by 11-HSD. Because of this complex and slow procedure, the urinary excretion of 6β-OHF during the collection periods of 4–8 h and 8–10 h still remained at a high level. Further studies are required to either confirm or reject these hypotheses.

**Conclusion**

Although the inhibition of CYP3A activity can be detected by Cl(6β) and 6β-OHF/F values, these measures are lacking in accuracy. On the contrary, we have established that AUCF is an entirely inappropriate indicator of changes in CYP3A activity. We have also demonstrated that MDZ administration is without effect on either cortisol metabolism or excretion over
a 24 h time period.

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Author contribution
Ze-neng CHENG designed research; Xiao-min LI and Zhe-yi HU performed research; Xi LUO wrote the paper.

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