Mind the time : 24-hour rhythms in drug exposure and effect

Kervezee, L.

CHAPTER

Population pharmacokinetic model characterizing 24-hour variation in the pharmacokinetics of oral and intravenous midazolam in healthy volunteers

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ABSTRACT

Daily rhythms in physiology may affect the pharmacokinetics of a drug. The aim of this study was to evaluate 24-hour variation in the pharmacokinetics of the CYP3A substrate midazolam. Oral (2mg) and intravenous (1mg) midazolam was administered at six time points throughout the 24-hour period in twelve healthy volunteers. Oral bioavailability (population mean value (RSE%) of 0.28 (7.1%)) showed 24-hour variation that was best parameterized as a cosine function with an amplitude of 0.04 (17.3%) and a peak at 12:14 in the afternoon. Absorption rate constant was 1.41 (4.7%) times increased after drug administration at 14:00. Clearance (0.38L/min (4.8%)) showed a minor 24-hour variation with an amplitude of 0.03 (14.8%) L/min and a peak at 18:50. Simulations show that dosing time minimally affects the concentration time profiles after intravenous administration, while concentrations are higher during the day compared to the night after oral dosing, reflecting considerable variation in intestinal processes.

STUDY HIGHLIGHTS

What is the current knowledge on this topic?

- The pharmacokinetics of the CYP3A4 substrate midazolam may be subject to 24-hour variation, but previous studies did not assess all pharmacokinetic parameters simultaneously and yielded conflicting results.

What question did this study address?

- How do the pharmacokinetics of oral and intravenous midazolam depend on time of administration?

What this study adds to our knowledge?

- Oral bioavailability and absorption rate constant of midazolam show considerable 24-hour variation, while clearance shows minor fluctuations.
- Concentration-time profiles of midazolam are affected by dosing time after oral administration, but not after intravenous administration.

How might this change clinical pharmacology and therapeutics?

- Our design, with appropriate control for unperturbed circadian rhythmicity and semi-simultaneous oral and intravenous administration, combined with population pharmacokinetic modelling can be applied to study 24-hour variation in the pharmacokinetics of other model compounds, yielding detailed information on the effect of time of administration on the concentration profile.

INTRODUCTION

Many physiological processes including gene expression, metabolism and organ function exhibit 24-hour variation (Meijer et al., 2012). As a result of these rhythms, the pharmacokinetics of drugs may vary over the day (Dallmann et al., 2014). Although different chronopharmacological studies have shown that the pharmacokinetics of several drugs depend on the time of administration (Baraldo, 2008; Bruguerolle et al., 2008; Kaur et al., 2013), this source of variability has not been evaluated systematically. A possible approach to methodically assess 24-hour variation in pharmacokinetic parameters is to study a model drug representing a group of drugs that are absorbed, distributed, metabolized and/or eliminated in a similar way. Such an approach requires a strict standardized study protocol with external validators to ensure that the research is performed with minimal or no disturbance of the physiological rhythms.

Midazolam is extensively metabolized by both hepatic and intestinal cytochrome P450 3A (CYP3A) and is considered a probe of CYP3A enzyme activity (Fuhr et al., 2007; Gorski et al., 1998; Lee et al., 2002; Thummel et al., 1996; Tsunoda et al., 1999). CYP3A is an important drug metabolizing enzyme, metabolizing 30% of clinically used drugs (Zanger and Schwab, 2013). In vitro research shows that hepatic CYP3A activity fluctuates during the 24-hour period (Froy, 2009; Takiguchi et al., 2007). Moreover, in vivo CYP3A activity in humans measured by urinary 6βhydroxy-cortisol to cortisol ratio showed diurnal variation by an average of 2.8 fold (Ohno et al., 2000).

Several chronopharmacokinetic studies on midazolam have been published (Bienert et al., 2013; Klotz and Reimann, 1984; Klotz and Ziegler, 1982; Koopmans et al., 1991; Tomalik-Scharte et al., 2014). In most of these studies, however, midazolam was administered either orally (Koopmans et al., 1991) or as an intravenous infusion (Bienert et al., 2013; Klotz and Reimann, 1984; Tomalik-Scharte et al., 2014), and therefore not all pharmacokinetic parameters (absorption rate constant, bioavailability and clearance) could be assessed separately. To distinguish between bioavailability, systemic clearance and volume of distribution, oral and intravenous administration should be combined in one single study. In the current study, we aimed to evaluate 24-hour variation in the pharmacokinetic parameters of midazolam after semi-simultaneous oral and intravenous administration in healthy volunteers.

METHODS

Study design and data

Healthy, non-smoking Caucasian male subjects, aged between 18 and 50 and a body mass index (BMI) between 18 and 30 kg/m^2 were recruited for this study, which took place at the Centre for Human Drug Research in Leiden, the Netherlands. Subjects were excluded from participation if any clinically significant abnormality was found in medical history, routine laboratory tests or 12-lead ECG recordings or if they used any medication, could be characterized as an extreme morning- or evening-type as determined by the Horne-

Ostberg Chronotype Questionnaire (Horne and Ostberg, 1976), made transmeridian flights or did shift work from a month prior to the start of the study. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center and was carried out according to the ICH guidelines for good clinical practice(ICH).

From one week prior to each study visit, subjects were instructed to maintain a stable sleep-wake schedule (waking times between 07:00-08:00, bedtimes between 23:00-00:00). Subjects kept a sleep diary and wore an Actiwatch (CamNtech Actiwatch Light®, UK) to monitor their daily activity profiles. Subjects refrained from heavy exercise for 24 hours prior to a scheduled study visit and were not allowed to use products that interfere with CYP3A metabolism (such as grapefruit, banpeiyu, pomegranate, star fruit, black berry, and wild grape) for two weeks prior to the study, and no caffeinated drinks, alcoholic drinks, honey and cruciferous vegetables for 72 hours prior to the drug administration until 48 hours thereafter.

The study consisted of three study visits at which the subjects received a 2 mg oral midazolam solution and 1 mg intravenous midazolam (separated by 150 min) twice a day at a 12 hour interval. The clock times of midazolam administration differed for each study visit, so that data were collected at six different time points throughout the 24-hour period (oral administration at 10:00, 14:00, 18:00, 22:00, 02:00 and 06:00) in each of the 12 volunteers (Fig. 1a), with a washout period of at least two weeks between the study visits. Throughout the study visits, subjects remained in a semi-recumbent position. At night (23:30 until 07:30), lights were dimmed and subjects wore an eye mask. From two hours prior to drug administration, subjects fasted. A light meal was served at t=395min and a snack at t=540min after oral administration. Water was allowed as required.

Samples (2.7mL) to determine midazolam concentrations in serum were collected at t= 0, 15, 30, 45, 58, 65, 70, 75, 80, 90, 120, 148, 155, 165, 180, 210, 240, 270, 330 and 390 minutes after oral administration, as well as at t= 715 minutes in case it involved the first 12 hours of a study visit. Midazolam concentrations were measured using a validated liquid chromatographic tandem mass spectrometric (LC-MS/MS) assay (van Erp et al., 2011). Within-day and between-day inaccuracy and imprecision were less than 5% and the lower limit of quantitation (LLQ) was 0.3 µg/L (van Erp et al., 2011).

Samples to determine thyroid stimulating hormone (TSH) concentrations in serum (1.2 mL) were collected hourly during the study visits. TSH concentrations (µIU/mL) were measured by an electrochemiluminescence immunoassay (ECLIA, Cobas, Roche Diagnostics GmbH, Mannheim, Germany) on an Elecsys immunoassay analyser (Roche Diagnostics GmbH, Mannheim, Germany), calibrated against the World Health Organization Second Standard International Reference Preparation (80/558). The LLQ was 0.005µIU/mL. Blood pressure and heart rate were measured every two hours during the study visits.

Single component cosinor analysis was performed to evaluate the presence of a 24-hour rhythm in blood pressure, heart rate and endogenous TSH levels using R software (v2.15; R Foundation for Statistical Computing, Vienna, Austria). Cosinor analysis is a statistical method to fit a cosine function to longitudinal data. If the period assumed to be known

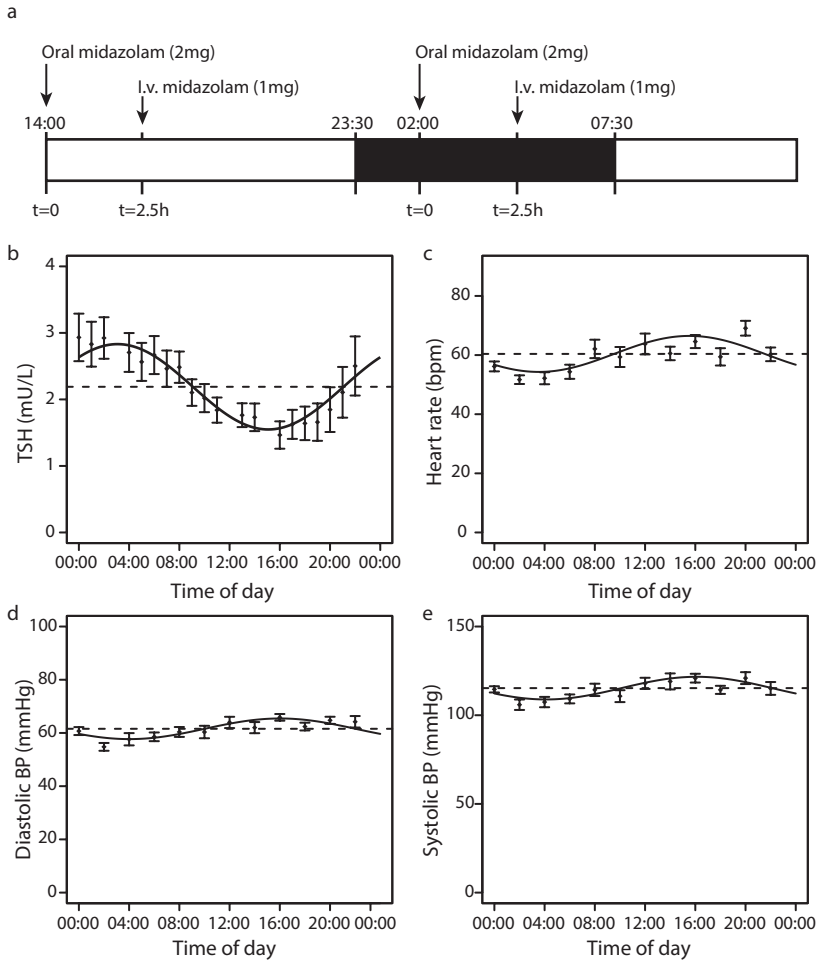

Figure 1 (a) Schematic representation of the drug administration protocol per study visit. Subjects completed two occasions, separated by 12 hours. At t=0, subjects received 2mg midazolam (MDZ) orally. At t=2.5h, subjects received 1mg midazolam intravenously. After 12 hours, the procedure was repeated. In each of the three study visit, drug administration took place at two different clock times (t=0 at 14:00 and 02:00 in this example), so drug administration occurred at six different clock times throughout the 24-hour period. The order of time of drug administration was randomized. The dark box indicates the clock times during which the subjects were instructed to sleep. **(b-e)** Mean values of TSH levels (b), heart rate (c), diastolic (d) and systolic blood pressure (e) obtained during the study visits across the 24-hour period (n=12 subjects). The solid lines show the cosine curve with a period of 24-hour that best fits the data, obtained through cosinor analysis.

(in this case 24 hours), a cosine function can be rewritten as a linear function and the data can be fitted via least squares regression (Cornelissen, 2014). The mesor, amplitude and acrophase can be calculated from the estimated intercept and coefficients.

Population pharmacokinetic modeling

The pharmacokinetic data were analyzed using non-linear mixed effects modeling

(NONMEM v7.2; ICON Development Solutions, Hanover, MD, USA)(Beal et al., 2009) and R (v2.15) (R Development Core Team, 2008), Pirana (v2.7.1), Xpose (4.5.0) and PsN (3.6.2) (Keizer et al., 2013) were used to visualize the data. The first-order conditional estimation method with interaction was used throughout model development.

Structural and statistical model

Pharmacokinetic models incorporating either two or three compartments with first order, zero order or combined first- and zero order oral absorption were investigated. Furthermore, the addition of one or more transit compartments or an oral absorption lag time was evaluated (Savic et al., 2007). Interindividual variability (IIV) in pharmacokinetic parameters was assumed to be log-normally distributed. Residual variability was investigated using proportional, additive or combined proportional and additive error models.

Twenty-four hour variation

Twenty-four hour variation in the different structural pharmacokinetic parameters was first explored by incorporating interoccasion variability (IOV), representing the variability between the six different times of administration, on each of these parameters of interest using the following equation (Karlsson and Sheiner, 1993):

$$\theta_{ij} = \theta_{mean} * e^{ni} + k_{ij} \qquad \text{(Equation 1)}$$

where is the individual parameter estimate at the j^{th} occasion, is the population mean, η_i is a random variable for the ith individual (IIV) and k_{ij} is a random variable for the i^{th} individual at the j^{th} occasion (IOV). Both η_i and k_{ij} were assumed to be independently normally distributed with mean of zero and variances ω^2 and π^2 , respectively. The k values used in IOV plots are empirical Bayes estimates (EBEs) of the interoccasional random effect (NONMEM ETA) of the parameter involved.

If a 24-hour rhythm was visually identified in IOV plots, a cosine function with a period of 24 hours (1440 minutes) was implemented in the model as follows:

$$P = \theta_i + \theta_{AMP} * \cos(2\pi * (t - \theta_{ACROPHASE}) / 1440) \qquad \text{(Equation 2)}$$

where P represents the studied pharmacokinetic parameter, θ_i the mesor (individual value of the pharmacokinetic parameter around which it oscillates), θ_{AMP} the amplitude and $\theta_{ACROPHASE}$ the acrophase (time of the peak of the cosine function). t represents the time in minutes starting at midnight of the first study visit and continuing until the end of the third study visit. It was assumed that the cosine function described the data accurately when no residual trend of diurnal variation was left in the IOV plots upon inclusion of the function and it resulted in a reduced IOV value. Twenty-four hour variation was also evaluated by estimation of different multiplication factors on the pharmacokinetic parameters for the six time-points of administration (10:00, 14:00, 18:00, 22:00, 02:00 and 06:00).

If no full 24-hour variation could be identified for a pharmacokinetic parameter, but only an increase at a certain time interval of the day, this was parameterized as half a cycle of a

sine function:

$$INC = \theta_{AMP} * \sin(2\pi * (TSIN - \theta_{ON}) / \theta_{FR}) \quad \text{(Equation 3)}$$

where INC represents the increase in a parameter, θ_{AMP} the amplitude, θ_{FR} the frequency of the oscillations (minutes), TSIN the clock time in minutes after 12:00 (noon) and θ_{ON} represents the onset of the increase in the parameter. The end of the increase in the pharmacokinetic parameter was calculated as follows:

$$END = 0.5 * \theta_{FR} + \theta_{ON} \quad \text{(Equation 4)}$$

Model selection and internal model evaluation

Model development and selection was guided by comparison of the objective function value (OFV, i.e. -2 log likelihood (-2LL)) between nested models, precision of parameter estimates and visual improvement in goodness-of-fit plots split by the six times of administration (observed versus individual-predicted concentrations, observed versus population-predicted concentrations, conditional weighted residuals versus time after dose and conditional weighted residuals versus population-predicted concentrations plots and individual plots). A p-value of <0.05 (ΔOFV=-3.84 for one degree of freedom) was considered statistically significant. For internal model evaluation, a bootstrap analysis was performed using 250 replicates and visual predictive checks (VPCs), stratified by the six times of administration, were created using 1000 simulated datasets.

Simulations

The final population pharmacokinetic model was used to simulate the concentration-time curves of a subject dosed at 6 different administration times of a 7.5 mg oral dose or a 2 mg intravenous bolus dose.

RESULTS

Study participants

Twelve healthy Caucasian male volunteers participated in the study. Their demographics are summarized in Table 1. One subject withdrew consent during the study due to personal reasons and was replaced by another study subject who was dosed according the same randomization order.

Physiological parameters

Several physiological variables, used to verify that the approach of our study is suited to assess diurnal rhythmicity in physiological processes, fluctuated over the 24-hour period (Fig. 1b-e). TSH levels showed significant 24-hour variation with a relative amplitude of 29% and peak levels around 03:05 at night (r^2=0.13, $p<0.0001$). Heart rate and diastolic and systolic blood pressure also exhibited a significant 24-hour rhythm (r^2=0.14, $p<0.0001$ for all three parameters) with relative amplitudes of 10%, 6.3% and 5.6%, respectively, and peaks

Table 1: Subject demographics

	N	Mean	SD	CV (%)	Median	Range
Age (years)	12	21.8	3.19	14.6	22	18-27
Weight (kg)	12	76.0	8.65	11.4	75.4	63.4-92.9
Body mass index (kg/m^2)	12	22.3	2.37	10.6	21.9	18.8-25.8

N: number of subjects; SD: standard deviation; CV: coefficient of variation

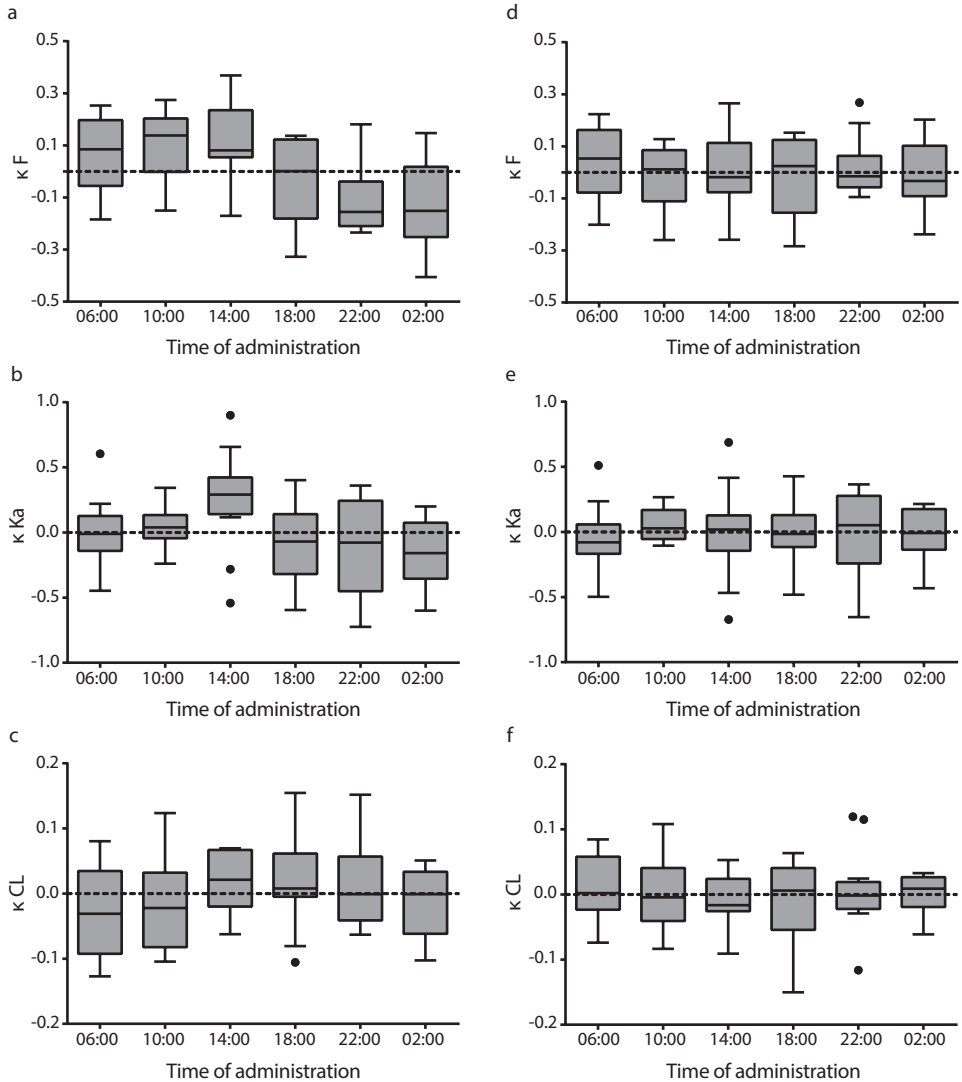

Figure 2 Interoccasion variability (κ, kappa) versus time of administration of midazolam for oral bioavailability (F) **(a, d)**, absorption rate constant (Ka) **(b, e)** and clearance (CL) **(c, f)**. Left column represents IOV (κ) versus time plots of the simple model in which no cosine function was incorporated (a,b,c) and right column represents IOV (κ) versus time plots of the models after implementation of a cosine function for oral bioavailability (d), a multiplication factor at the 14:00 hour administration time for absorption rate constant (e) and a cosine function for clearance (f). The k values used in these IOV plots are empirical Bayes estimates (EBEs) of the interoccasional random effect (NONMEM ETA) in the parameter involved (oral bioavailability, absorption rate constant or clearance).

around 16:00.

Population pharmacokinetic model and internal model evaluation

The mean concentration time-profiles of midazolam after oral and intravenous administration at the six time points is shown in Supplementary Fig. 1. A three compartment PK model with equalized peripheral volumes of distribution best described the data. The peripheral volumes were equalized, as these values were almost equal and the model resulted in a similar objective function ($p>0.05$). Oral absorption of midazolam was best described by a one transit compartment absorption model, where oral absorption rate constant and transit compartment rate constant were equalized. Residual variability was best described by using a proportional error model for both oral and intravenous data.

To explore 24-hour variation in the different pharmacokinetic parameters, IOV was sequentially incorporated on oral bioavailability, absorption rate constant and systemic clearance (Supplementary Table 1). The presence of a 24-hour rhythm was most evident for oral bioavailability (Fig. 2a, $p<0.001$, ΔOFV -349). After implementation of IOV on absorption rate constant an increase in this parameter was identified after administration at 14:00 (Fig. 2b, $p<0.001$ ΔOFV -258). The magnitude of a possible 24-hour rhythm in clearance of midazolam seemed lower compared to oral bioavailability and absorption rate constant (Fig. 2c, $p<0.001$, ΔOFV -93). The η-shrinkage for the EBEs of the interoccasional random effect was higher than 30% for oral bioavailability and absorption rate constant (33% and 55%, respectively, Supplementary Table 1), resulting in potentially unreliable EBEs (Karlsson and Savic, 2007). Therefore, these observations necessitated further analysis by implementation of a cosine function on each of these parameters evaluated by objective function.

The 24-hour variation in bioavailability was accurately described by a cosine function (Equation 2), resulting in a significant improvement in OFV compared to the IOV on bioavailability model ($p<0.001$, ΔOFV -28) and in a reduced IOV value (from 20 to 15.4%, Supplementary Table 1). Alternatively, 24-hour variation in bioavailability was estimated by implementing different multiplication factors on this parameter for each of the six time points of administration. This multiplication factor model showed a similar fluctuation over the 24-hour period compared to the cosine model (Supplementary Fig. 2a) and had a similar OFV (2431 for the cosine model with 2 additional parameters versus 2430 for the multiplication factor model with 5 additional parameters, $p>0.05$ for 3 degrees of freedom). The cosine model was preferred over the multiplication factor model, because both the IOV model (Fig. 2a) and multiplication factor model (Supplementary Fig. 2a) revealed a cosine function in bioavailability and the cosine model required less parameters to be estimated, while having larger predictive value. After implementation of the cosine function for bioavailability, there was no remaining trend in IOV confirming the appropriateness of the cosine model for this parameter (Fig. 2d).

After implementation of the cosine function for bioavailability, the variation in absorption rate constant was modeled, which was best described by the estimation of a multiplication factor at 14:00 ($p<0.01$, ΔOFV -9, Supplementary Table 1). After

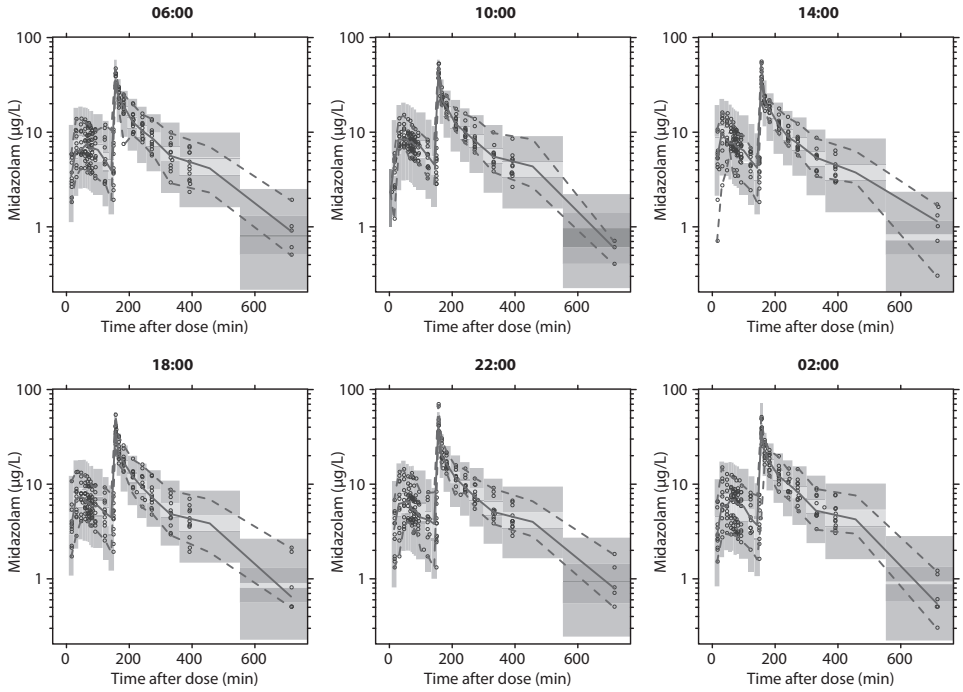

Figure 3 Visual predictive checks of the final model stratified by time of midazolam administration (06:00, 10:00, 14:00, 18:00, 22:00 and 02:00). Observed concentrations are shown as half open circles with solid and lower and upper dashed lines showing the median, 2.5th and 97.5th percentiles of the observed data, respectively. The shaded areas represent 95% confidence intervals for the model predicted median, 2.5^{th,} 97.5th percentiles constructed from 1000 simulated datasets of individuals from the original dataset.

implementation of this multiplication factor, IOV on absorption rate constant was removed from the model, because of the high η-shrinkage of the EBE of the interoccasional random effect (55%, Supplementary Table 1). Addition of multiplication factors on absorption rate constant at other time-points of administration did not further improve the model ($p>0.05$, Supplementary Fig. 2b). Alternatively, a cosine function was tested, but this model did not result in adequate prediction of the increased absorption rate constant at 14:00. Furthermore, inclusion of half a cycle of a sine function to describe the peak in absorption rate constant (Equation 3 and 4) resulted in a peak at 14:59 and an amplitude of 0.056min-1 (increase of 106%) and an onset and offset of the peak at 14:12 and 15:45, respectively. However, this model was very sensitive to initial parameter estimates and did not result in a significant improvement in OFV compared to the model with a multiplication factor at 14:00 ($p>0.05$, ΔOFV -3.7, 2 degrees of freedom). Therefore, the model with a multiplication factor at 14:00 was selected. No rhythm remained in the IOV plot after implementation of this factor (Fig. 2e). However, this plot should be viewed with caution because of the high ETA shrinkage and IOV on the absorption rate constant was therefore removed from the model, as described above. The multiplication factor estimated by this model was 1.46 (resulting in an absorption rate constant of 0.08 min-1), indicating a strong increase in absorption rate

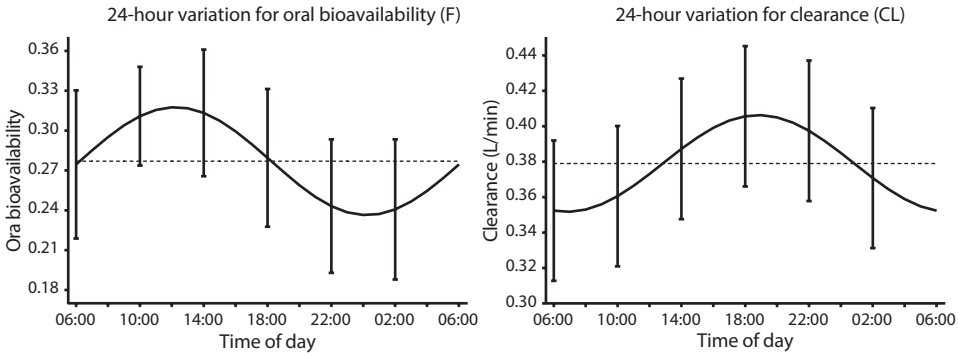

Figure 4 Twenty-four hour fluctuation for oral bioavailability (F) and clearance (CL) according to the final model with the 95% confidence interval of the empirical Bayes estimates (EBEs) for F (IIV+IOV) and CL (IIV) at each administration time. For oral bioavailability, the time of the peak was estimated at 12:14 with an estimated amplitude of 0.041 (14.7% increase) (**left panel**). For clearance, the time of the peak was estimated at 18:50 with an estimated amplitude of 0.027 L/min (7.2% increase) (**right panel**).

constant after administration at 14:00.

After implementation of a cosine function for bioavailability and a multiplication factor for absorption rate constant, 24-hour related changes in clearance were modelled. For this parameter, 24-hour variation was best described by a cosine function (Equation 2), resulting in a significant decrease in OFV compared to the IOV model for clearance ($p<0.001$, ΔOFV -26, Supplementary Table 1). Since the IOV value was substantially smaller than the IIV on clearance, IOV on clearance was removed from the model. Clearance could also be described by estimation of different multiplication factors for each of the six times of drug administration (Supplementary Fig. 2c), resulting in similar variation over the 24-hour period as the cosine model. After implementation of the cosine function for clearance, there was no remaining trend in IOV on this parameter (Fig. 2f) (η shrinkage of 20%), confirming the appropriateness of the cosine model for clearance.

Hence, the final model selected to describe 24-hour variation in midazolam concentration profiles included a cosine function for bioavailability and clearance and a multiplication factor to describe the increase in absorption rate constant at 14:00. The model parameter values are summarized in Table 2. Observed versus individual predicted concentrations and observed versus population predicted midazolam concentrations of the final pharmacokinetic model for all six time-points of administration are shown in Supplementary Fig. 3. The final model was evaluated using bootstrap analysis, confirming that the model parameters could be estimated with good precision (Table 2). Furthermore, VPCs stratified by time of administration indicated good predictive performance for both oral and intravenous data with good agreement between observed data and model simulated confidence intervals for the median, 2.5th and 97.5th percentiles (Fig. 3). Fig. 4 shows the 24-hour variation in bioavailability and in clearance of the final model.The cosine function on bioavailability has a relative amplitude of 14.7% with a peak at 12:14, while the cosine function on clearance has a relative amplitude of 7.2% and a peak at 18:50.

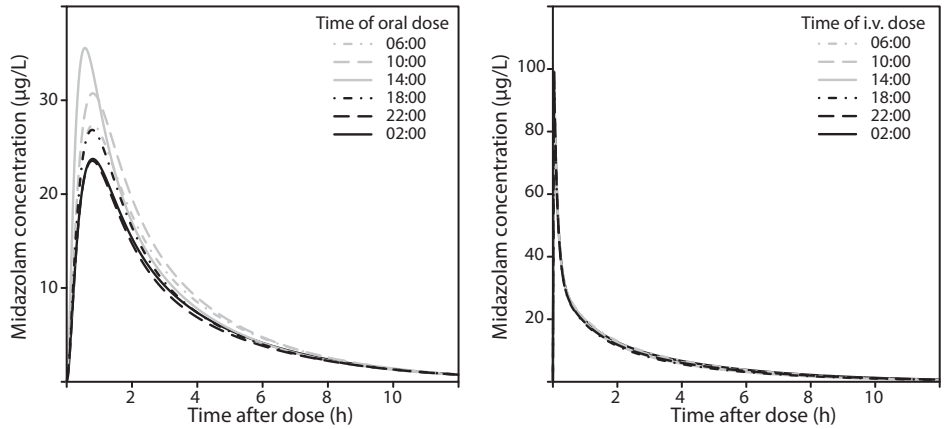

Figure 5 Population predicted midazolam concentrations over time after 7.5 mg oral administration (**left panel**) and a 2 mg intravenous bolus (**right panel**) at 06:00, 10:00, 14:00, 18:00, 22:00 and 02:00.

Simulations

Population predicted midazolam concentrations after a 7.5 mg oral dose and 2 mg intravenous bolus dose in a typical subject dosed at six different times during the day (10:00, 14:00, 18:00, 22:00, 02:00 and 06:00) were simulated using the final model (Fig. 5). The oral midazolam dose simulations show that the concentrations after administration in the late morning and early afternoon (10:00 and 14:00) are higher compared to the concentrations after administration in the late evening and early night (22:00 and 02:00). In addition, the time to maximum concentration (Tmax) is shorter when midazolam is administered at 14:00. In contrast to the oral dose simulations, the intravenous dose simulations show almost no variation during the 24-hour period.

DISCUSSION

This study aimed to evaluate the 24-hour variation in the pharmacokinetics of the CYP3A substrate midazolam after semi-simultaneous oral and intravenous administration at six different time points during the day (06:00, 10:00, 14:00, 18:00, 22:00 and 02:00). It was found that oral bioavailability and clearance are subject to 24-hour variation that could both be described by a cosine function. The peak of oral bioavailability was found at 12:14, with a relative difference between peak and trough values of 29.4%. The effect for clearance was found to be small with a peak at 18:50 and a relative difference between peak and trough levels of 14.4%. Furthermore, we found that absorption rate constant was increased 1.41 times after administration at 14:00.

Previous studies that investigated the diurnal variation of midazolam clearance in healthy volunteers did not yield consistent results (Bienert et al., 2013; Klotz and Reimann, 1984; Klotz and Ziegler, 1982; Koopmans et al., 1991; Tomalik-Scharte et al., 2014). In agreement with our results, Klotz and Ziegler found a higher clearance value in the evening compared

Table 2 Population pharmacokinetic parameters of the final model for midazolam and results of the bootstrap analysis (250/250 resamples successful).

Parameter	Model Estimates (RSE%)	Bootstrap Estimates (95% confidence interval)
$CL = CL_{mesor} + Amp * \cos((2\pi/1440)*(Time-Acrophase))$		
CL_{mesor} (L/min)	0.379 (4.8)	0.380 (0.344-0.417)
Amp (L/min)	0.027 (14.8)	0.028 (0.017-0.039)
Acrophase (min)	1130 (2.9)	1130.2 (1005.3-1204.7)
$V_{central}$ (L)	18.2 (5.4)	18.4 (15.3-20.9)
$V_{peripheral1} = V_{peripheral2}$ (L)	22.5 (2.5)	22.4 (20.2-26.2)
Q (L/min)	0.27 (6.8)	0.269 (0.209-0.334)
Q_2 (L/min)	1.31 (8.5)	1.29 (1.08-1.56)
Ka = Ktr (min^{-1})	0.053 (5.8)	0.053 (0.048-0.061)
Fraction Ka at 14:00	1.41 (4.7)	1.41 (1.07-1.78)
$F = F_{mesor} + Amp * \cos((2\pi /1440)*(Time-Acrophase))$		
F	0.277 (7.1)	0.275 (0.244-0.313)
Amp	0.041 (17.3)	0.041 (0.026-0.055)
Acrophase (min)	734 (5.3)	739.7 (667.0-821.0)
Interindividual variability		
CL (%)	16.2 (21)	15.2 (9.7-19.6)
Ka (%)	19.1 (21.9)	18.7 (10.7-24.2)
F (%)	23.3 (22.2)	22.7 (15.8-28.8)
Interoccasion variability		
F (%)	14.8 (10.5)	14.5 (11.5-17.9)
Residual proportional error		
σ_{oral} (%)	18.0 (5.6)	17.8 (15.8-19.8)
$\sigma_{intravenous}$ (%)	15.4 (6.1)	15.1 (13.2-17.3)
OFV (-2LL)	2299	2242 (1723-2730)

RSE = relative standard error (%); CL = systemic clearance of midazolam; Amp = amplitude; Acrophase = peak time of the cosine function in minutes after midnight; V = volume of distribution; Q = inter-compartmental clearance of midazolam between central and first peripheral compartment; Q2 = inter-compartmental clearance of midazolam between central and second peripheral compartment; Ka = oral absorption rate constant; Ktr= transit compartment rate constant; F = oral bioavailability; OFV = Objective Function Value

to the morning after intravenous administration (Klotz and Ziegler, 1982). More recently, Tomalik-Scharte et al. reported a cosine function in midazolam clearance over the day with a 10% increase at 15:00 (Tomalik-Scharte et al., 2014). This is consistent with our results, as we found a 7.2% maximum increase in clearance at 18:50. The small difference in peak time may be explained by the nature of the study; where Tomalik-Scharte et al. evaluated midazolam concentrations during the day upon a continuous intravenous infusion, we

studied an oral and intravenous bolus dose at 6 different times of administration. The fact that others found no influence of the time of administration on clearance may be explained by the low number of subjects in the study (Klotz and Reimann, 1984) and the fact that intensive care patients were studied, showing a disrupted circadian rhythm (Bienert et al., 2013). Hence, most chronopharmacokinetic studies about intravenous midazolam are in line with our findings of a relatively small 24-hour variation in midazolam clearance.

Our results about absorption processes of midazolam (24-hour variation in oral bioavailability and increase in absorption rate constant at 14:00) are not consistent with earlier chronopharmacokinetic studies on oral midazolam, finding no influence on Cmax, Tmax or oral bioavailability (Klotz and Ziegler, 1982; Koopmans et al., 1991). These discrepancies may be due to methodological differences. Klotz and Ziegler administered midazolam only at two different time points during the day (Klotz and Ziegler, 1982), and therefore the peak and trough may easily be missed. In the study of Koopmans et al., subjects were not allowed to lie down or sleep from 1 hour before to 8 hours after dosage (Koopmans et al., 1991), which could have disrupted the circadian rhythms in physiological processes of the subjects (Mullington et al., 2009). However, our finding of 24-hour variation in oral bioavailability of midazolam is supported by chronopharmacokinetic studies of other CYP3A substrates, such as nifedipine, tacrolimus and ciclosporin (Baraldo and Furlanut, 2006; Lemmer et al., 1991). Lemmer et al. showed an increased C_{max} and 35% increase in oral bioavailability after a morning dose of immediate release nifedipine compared to an evening dose (Lemmer et al., 1991). Furthermore, studies with oral tacrolimus and ciclosporin showed in general an increased C_{max} and AUC after morning dose compared to evening dosing (Baraldo and Furlanut, 2006; Iwahori et al., 2005; Min et al., 1996, 1997; Tada et al., 2003). Therefore, it seems that our findings on 24-hour variation in absorption processes are strengthened by the advanced study design that we used in comparison to previous oral midazolam studies that did not report these changes, and are supported by chronopharmacological studies of other CYP3A substrates.

Twenty-four hour variation in clearance and oral bioavailability as well as the increase in absorption rate constant can be explained by several physiological factors. Since midazolam is a typical probe for CYP3A activity (Gorski et al., 1998; Lee et al., 2002; Tsunoda et al., 1999), the rhythm in systemic clearance of midazolam may be explained by minor 24-hour variation in CYP3A activity. Multiple lines of evidence show that hepatic CYP3A activity fluctuates during the 24-hour period (Froy, 2009; Lu et al., 2013; Ohno et al., 2000; Takiguchi et al., 2007; Tomalik-Scharte et al., 2014). Like systemic clearance, 24-hour variation in oral bioavailability of midazolam may also be explained by variation in intestinal CYP3A activity, since CYP3A is present both in the gut wall and liver (Thummel et al., 1996). Another explanation for the variation in oral bioavailability may be the variation in splanchnic blood flow during the 24-hour period, which is supported by the findings of Lemmer et al., who demonstrated a 24-hour rhythm in hepatic blood flow (as a proxy for splanchnic blood flow) with a peak at 08:00 (Lemmer and Nold, 1991). This supports our finding that oral bioavailability is increased from the early morning until the end of the afternoon (Fig. 4).

An increased splanchnic blood flow will decrease the intestinal first pass effect, as it will carry the drug away from the enterocyte and the CYP3A enzyme (Patel et al., 2013; Yang et al., 2007). In contrast to oral bioavailability, the clearance of midazolam is not expected to be influenced by hepatic blood flow to such an extent, because midazolam is a low to intermediate extraction drug (extraction rate of 35%), making it relatively independent of hepatic blood flow (Tsunoda et al., 1999). The increase in absorption rate constant after oral administration at 14:00 may be explained by 24-hour variation in gastric emptying, gastrointestinal mobility and splanchnic blood flow (Dallmann et al., 2014; Hoogerwerf, 2010; Kumar et al., 1986; Lemmer and Nold, 1991), even though we could not identify a cosine function for absorption rate constant.

In this study, we utilized a semi-simultaneous design in which midazolam was administered as an oral and intravenous dose separated by 150 minutes (Lee et al., 2002). An advantage of this crossover approach is that intra-individual variability is limited, since the oral and intravenous dose are administered to the same individual at a relatively short time frame (Karlsson and Bredberg, 1989). By using six different time points of oral and intravenous midazolam administration, 24-hour variation in absorption parameters as well as clearance could be accurately identified. Moreover, we ensured that subjects had stable rest/activity patterns between the study days and controlled for the influence of eating and physical activity, both of which are known to have an impact on physiological rhythms (Froy, 2010). Another strength of our study design is that several endogenous markers, with known diurnal variation (heart rate, systolic/diastolic blood pressure and serum TSH levels) were used as external validators to verify that our approach, including the low dose of midazolam, did not interfere with normal circadian physiology of the subjects. We found that these endogenous markers show clear diurnal variation with peak and trough times that are comparable to values reported in the literature (Andersen et al., 2003; Guo and Stein, 2003). These findings indicate that the study population and design were well-suited to study diurnal variation of midazolam exposure.

As the pharmacokinetics of midazolam have been shown to be linear over a wide dose range (Halama et al., 2013; Misaka et al., 2010), we performed simulations on the basis of the final pharmacokinetic model using therapeutic doses. These simulations illustrate the findings of the current study by showing a substantial effect of time of administration on midazolam concentration-time profiles after oral administration, whereas this effect is minimal after intravenous administration. Midazolam concentrations after oral administration are higher in the morning and afternoon compared to concentrations after administration in the evening and night. In addition, the time to maximum concentration (Tmax) is shorter after oral administration at 14:00. In the clinic, midazolam is mainly given as an intravenous dose, for example as pre-medication or for induction of anesthesia, upon which the time of administration will have no clinical impact. However, midazolam is also prescribed as a hypnotic to patients with insomnia. For these patients, who take an oral dose in the evening, lower serum concentrations should be anticipated.

In conclusion, this study shows that oral bioavailability of midazolam is subject to 24-

hour variation and that absorption rate constant is increased at 14:00 in the afternoon. The clearance of midazolam is also subject to 24-hour variation, although its magnitude is small and without clinical significance. As a result, the 24-hour variation in oral bioavailability results in higher serum concentrations during the day compared to the night upon oral midazolam dosing, while the concentration-time profiles are hardly affected by time of administration after intravenous dosing. Future research should elucidate the specific processes that contribute to the 24-hour variation in the pharmacokinetics of midazolam, and of other drugs with similar physicochemical properties, for example by using markers for intestinal motility or blood flow.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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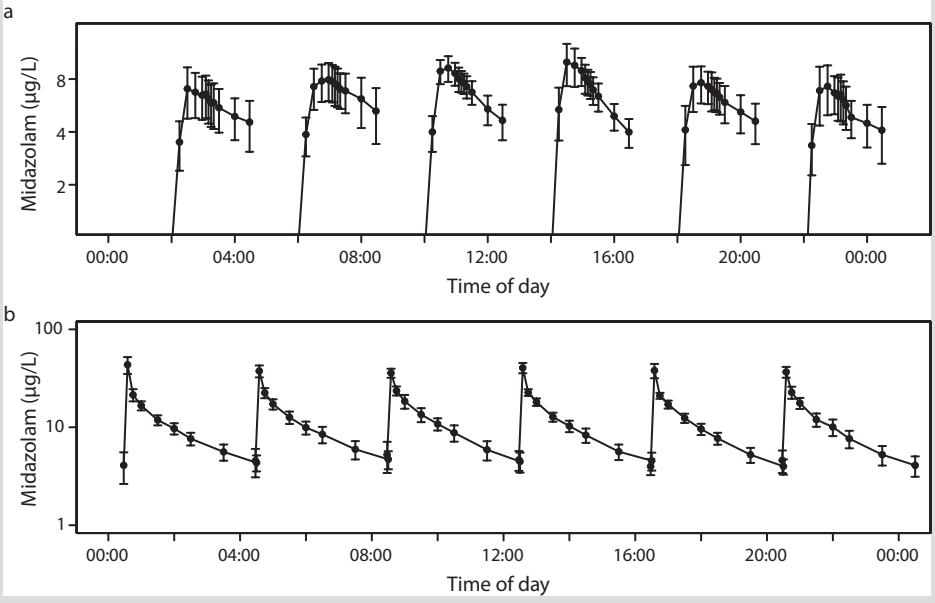
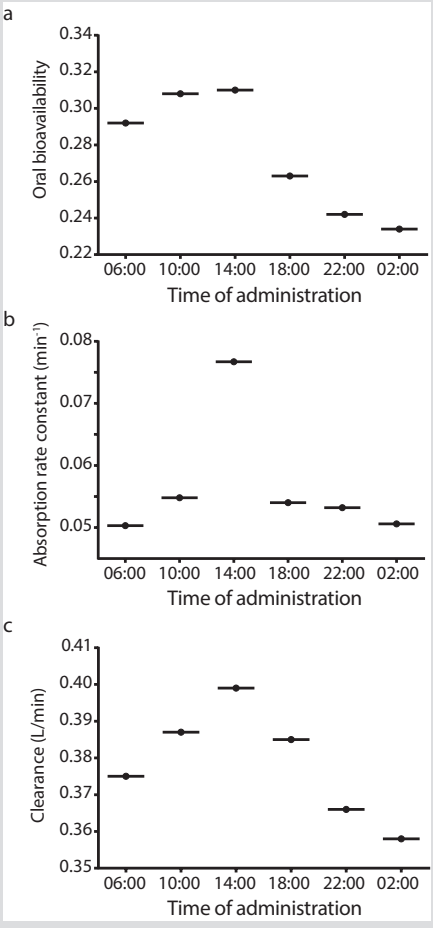
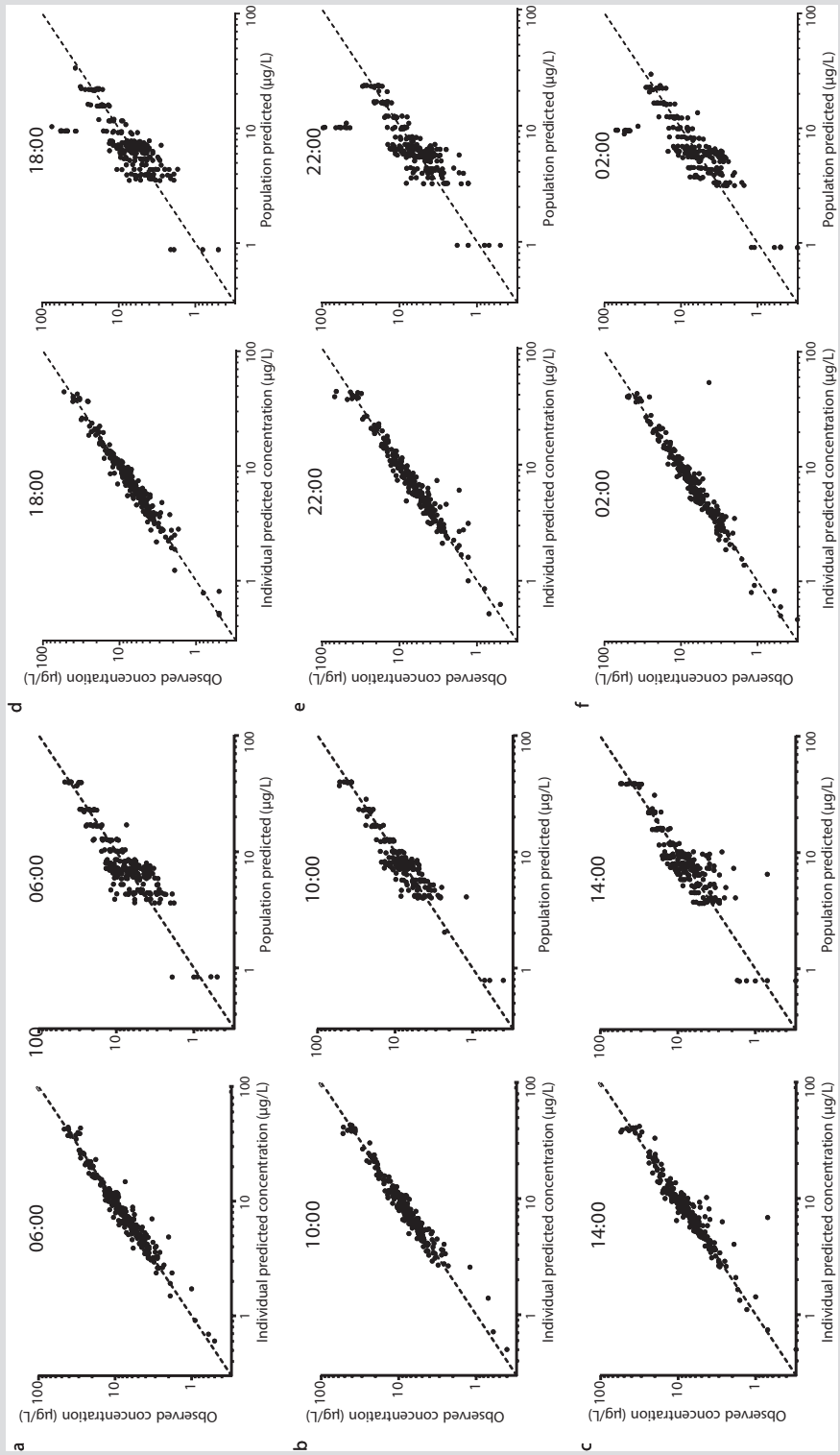


Supplementary Figure 1 Concentration time profiles of midazolam after **(a)** oral and **(b)** intravenous administration at six different clock times. Data are presented as mean ± 95% confidence intervals



Supplementary Figure 2 Oral bioavailability **(a)**, absorption rate constant **(b)** and clearance **(c)** versus time from models in which variation in parameters were estimated with different multiplication factors for each of the different administration times



Supplementary Figure 3 Observed versus individual-predicted concentrations (left panel) and observed versus population-predicted concentrations (right panel) of all 6 administration times (occasions) of the final model.

Supplementary Table 1: Summary of key model building steps and associated changes in objective function, interindividual variability, interoccasion variability, η-shrinkage and residual error

Model	OFV	#		IIV (%)	η-shrink. IIV (%)[a]	IOV (%)	η-shrink. IOV (%)[a]	Residual error (%)	
								Oral	IV
Simple model	2807	12	F	23.6				25.9	16.1
			Ka	18.7					
			CL	16					
IOV F	2459	13	F	23.1		20	33	19	16
			Ka	21.4					
			CL	16					
IOV Ka	2548	13	F	25		36	55	20.1	16.2
			Ka	15.8	26				
			CL	15.4					
IOV CL	2714	13	F	23.7				24.9	14.7
			Ka	19.2					
			CL	15.8		8.2	14		
IOV F + COS F	2431	15	F	23.1		15.4	18	19	16.1
			Ka	21					
			CL	16					
+ IOV Ka	2087	16	F	24.7		14.8	10	13.6	16
			Ka	14.5	22	30.8	60		
			CL	15.8					
IOV F + COS F + IOV Ka + MF Ka 14:00	2078	17	F	24.6		14.9	11	13.6	15.9
			Ka	15.3	18	28.3	55		
			CL	15.5					
+ MF Ka 14:00 (IOV Ka closed)	2345	16	F	23.7		15.0	14	18.1	16.0
			Ka	19.4					
			CL	15.9					
+ IOV CL	2284	17	F	24.4		15.0	20	17.7	14.6
			Ka	19.2					
			CL	23.4		8.0	25		
IOV F + COS F + MF Ka 14:00 + IOV CL + COS CL	2258	19	F	23.4		14.1	18	17.7	14.4
			Ka	18.9					
			CL	15.9		6.9	20		
+ COS CL (IOV CL closed) (Final model)	2299	18	F	23.3		14.8	12	18.0	15.4
			Ka	19.1					
			CL	16.2					

a. Only shrinkage values of ≥ 10% are reported.

= number of parameters; shrink. = shrinkage; CL= clearance; COS= cosine function; F= oral bioavailability, IIV= interindividual variability; IOV= interoccasion variability; IV= intravenous; Ka= oral absorption rate; MF= multiplication factor