UNIKLINIK KÖLN
Department of Pharmacology
University Hospital of Cologne

Evaluation of Circadian Rhythms in Hepatic CYP3A4 Activity Using Population Pharmacokinetics of Midazolam

Dorota Tomalik-Scharte, Daria Kunz, Dennis Rokitta, Paola Di Gion, Christian Queckenberg and Uwe Fuhr

Department of Pharmacology, Clinical Pharmacology, University of Cologne

Introduction

Chronopharmacology deals with the impact of circadian rhythms on the pharmacokinetics and pharmacodynamics of different drugs. Diurnal changes in the activity of drug metabolising enzymes may be an important factor affecting the variability in drug disposition. The aim of this project was to evaluate the role of circadian rhythms in the activity of hepatic CYP3A4, metabolizing nearly 50% of currently prescribed drugs.

Methods

Clinical study: Sixteen healthy subjects, 8 males and 8 females, were recruited to participate in this open-label, one-period study and were given a continuous intravenous infusion with low-dosed midazolam (commercial Dormicum® V 5 mg/5 ml), a well established model substrate of CYP3A4 activity. Plasma levels of midazolam and its metabolite 1-OH-midazolam were hourly determined over a period of 24 hours during the infusion at a rate of 0.004 mg midazolam/kg b.w./hour following achievement of a steady state. To achieve a steady state drug level, a continuous intravenous infusion at a rate of 0.004 mg midazolam/kg b.w./hour was administered for 6 hours following an i.v. bolus infusion of 0.01 mg midazolam/kg b.w.

Quantification of midazolam and 1-OH-midazolam:

The measurement of midazolam and its metabolite was performed by a specific and sensitive LC-MS/MS method; The lower limit of quantification was 0.0006 µmol/l (0.2ng/ml).

Pharmacostatistical modeling:

Population pharmacokinetic analysis was performed using the nonlinear mixed-effects software in NONMEM, version VI (GloboMax, Hanover, MD). PLT Tools (A Graphical Interface for the NONMEM System, Version 3.5.0, unlicensed version) was used for executing the NONMEM analysis. Plasma concentrations of midazolam and 1-OH-midazolam were fitted simultaneously by a two-compartment base model (parent and metabolite) using the NONMEM subroutine ADVANA5 TRANS1 and first order conditional estimation. The fraction of parent drug which was metabolized was assumed to be 100%. Interindividual variability in pharmacokinetic parameters and residual intraindividual variability were modeled using, respectively, an exponential and additive error.

To evaluate circadian changes in CYP3A4 hepatic activity, the variability in the steady-state clearance of midazolam was modeled by a cosine function with a 24-h period as follows:

$$Cl = \theta_{average} + \theta_{amplitude} \cdot \cos\left(\frac{2\pi}{24} \cdot t + \theta_{phase\ shift}\right)$$

where Cl is midazolam clearance; t, time relative to the start of blood sampling; $\theta_{average}$, the average clearance; $\theta_{amplitude}$, the amplitude of the cosine function; and $\theta_{phase\ shift}$, phase shift of the cosine function

Finally, covariates such as, body weight, gender and food intake were evaluated for their impact on the pharmacokinetics of midazolam.

Results

The average age and body mass index (±SD) of the study population were, respectively, 30 years (±4.5) and 23.2 kg/m2 (±2.6). Midazolam infusion was well tolerated by all subjects. The circadian model yielded an improvement of 92 points in the NONMEM objective function over the base model. Population pharmacokinetic parameters are displayed in the table below.

Table: Summary of population pharmacokinetic parameters estimated with the circadian model

Parameter [unit]	Lower limit of 95% CI	Point estimate	Upper limit of 95% CI	Between subject variability
Clearance of midazolam [l/h]	**19.5**	**22.4**	**25.3**	**34%**
Clearance of 1-OH-midazolam [l/h]	**136.3**	**153**	**169.7**	**33%**
Volume of distribution of midazolam [l]	**100.2**	**120**	**139.8**	**28%**
Volume of distribution of 1-OH-midazolam [l]	**12.2**	**35.7**	**59.2**	**190%**
Amplitude (magnitude) of cosine function [l/h]	**2.2**	**3.0**	**3.7**	**not included in the model**
Phase shift of cosine function [-]	**1.5**	**1.7**	**1.9**	**not included in the model**

The mean amplitude of the cosine function (2.97L/h) results in a circadian variability in the midazolam clearance of about ±13%. The estimated phase shift of the cosine function (1.5) corresponds to the time of the maximal clearance value (acrophase) at about 12:30.

None of the tested covariates was determined to be significant for midazolam pharmacokinetics.

Fig. 1 Circadian variability in midazolam concentrations in each individual subject (circles: observed, blue line: population, red line: individual prediction).

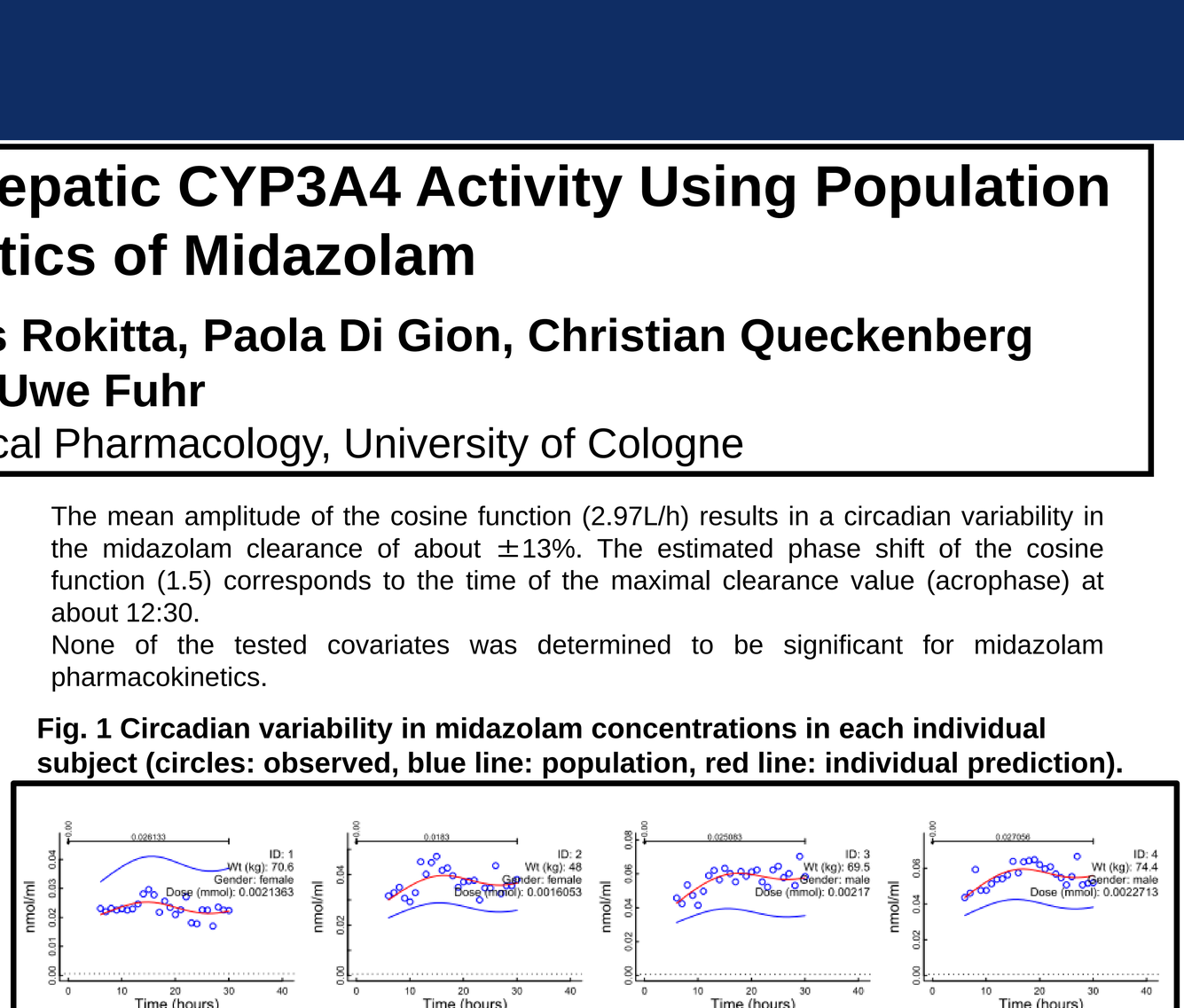
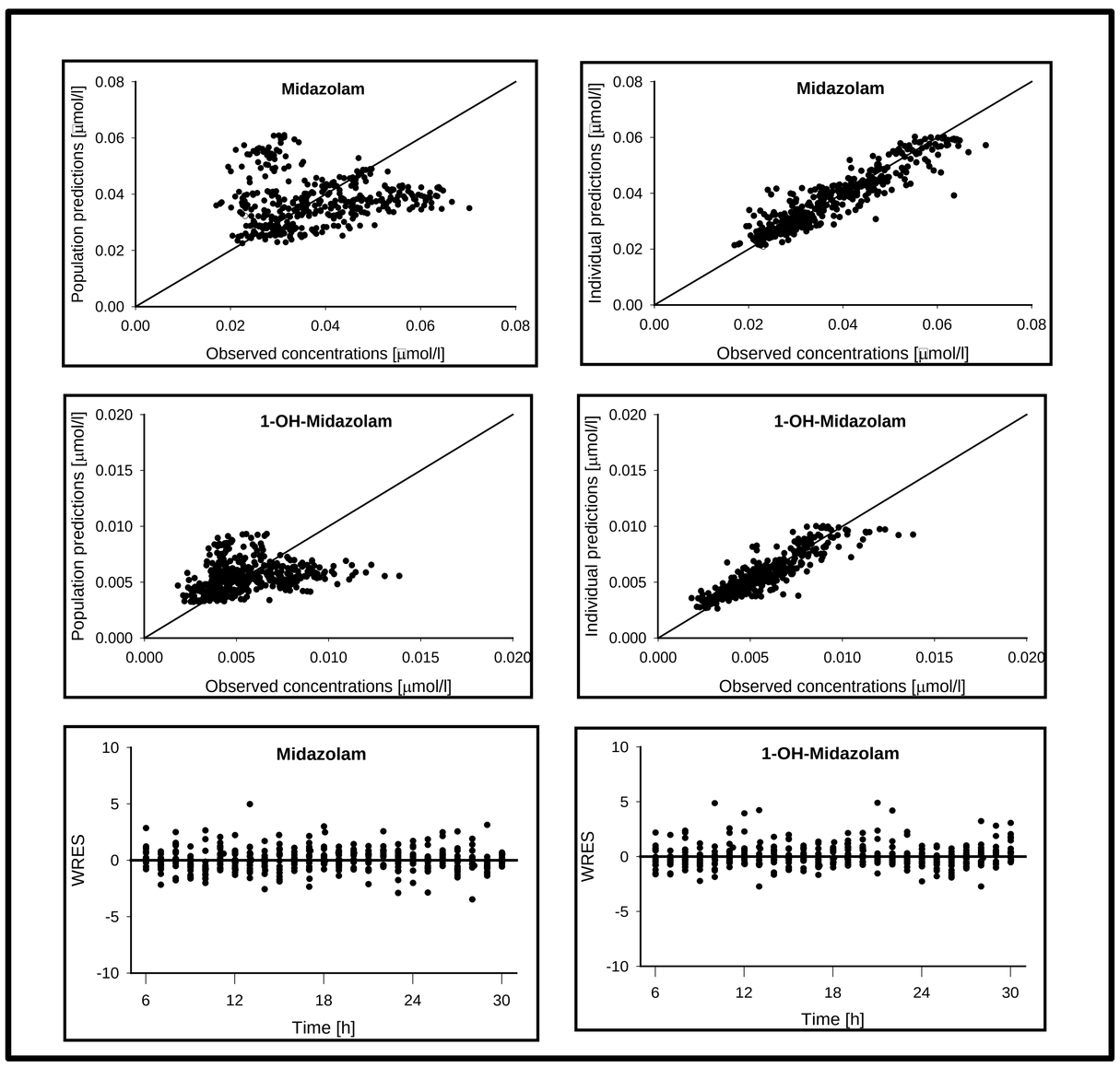


Fig. 2 Goodness of fit plots for the circadian model



Conclusions

The results of this pilot study provide evidence for a circadian variability in hepatic CYP3A4 activity, however, its effect seems to be moderate. Further population studies are needed to explore the clinical relevance of circadian rhythms in CYP3A4 activity for the treatment with drugs metabolized via this enzyme.