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

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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 ADVAN5 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:

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

where Cl is midazolam clearance; t, time relative to the start of blood sampling; \( \theta_{\text{average}} \) the average clearance; \( \theta_{\text{amplitude}} \) the amplitude of the cosine function; and \( \theta_{\text{phase shift}} \) 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>
<thead>
<tr>
<th>Parameter [unit]</th>
<th>Lower limit of 95% CI</th>
<th>Point estimate</th>
<th>Upper limit of 95% CI</th>
<th>Between subject variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance of midazolam [lh]</td>
<td>19.5</td>
<td>22.4</td>
<td>25.3</td>
<td>34%</td>
</tr>
<tr>
<td>Clearance of 1-OH-midazolam [lh]</td>
<td>136.3</td>
<td>153</td>
<td>169.7</td>
<td>33%</td>
</tr>
<tr>
<td>Volume of distribution of midazolam [l]</td>
<td>100.2</td>
<td>120</td>
<td>139.8</td>
<td>28%</td>
</tr>
<tr>
<td>Volume of distribution of 1-OH-midazolam [l]</td>
<td>12.2</td>
<td>35.7</td>
<td>59.2</td>
<td>190%</td>
</tr>
<tr>
<td>Amplitude (magnitude) of cosine function [lh]</td>
<td>2.2</td>
<td>3.0</td>
<td>3.7</td>
<td>not included in the model</td>
</tr>
<tr>
<td>Phase shift of cosine function [-]</td>
<td>1.5</td>
<td>1.7</td>
<td>1.9</td>
<td>not included in the model</td>
</tr>
</tbody>
</table>

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.