EFFECT OF PROPIVERINE ON CYTOCHROME P450 ENZYMES: A COCKTAIL INTERACTION STUDY IN HEALTHY VOLUNTEERS


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ABSTRACT:

The present study was conducted to assess a possible in vivo effect of propiverine, an anticholinergic drug to treat urinary incontinence and related disorders, on the activity of intestinal CYP3A4 and of hepatic CYP3A4, CYP2C9, CYP2C19, and CYP1A2. The activity of the respective cytochromes P450 was measured using the following metrics of selective substrates given as a tailored low-dose phenotyping cocktail: intestinal availability of midazolam (2 mg orally), clearance of midazolam (1 mg i.v.), apparent clearance of tolbutamide (125 mg orally), urinary excretion of 4′-hydroxyphenytoin 0 to 8 h postdose (50 mg of mephentyoin orally), and the paraxanthine/caffeine plasma ratio 6 h postdose (150 mg of caffeine orally). These metrics were determined in 16 healthy young men at the end of 7 days of treatment with 15 mg of propiverine (test) or placebo (reference) twice daily. All phenotyping drugs were quantified by liquid chromatography-tandem mass spectrometry. Chronic propiverine treatment reduced hepatic and intestinal CYP3A4 activity slightly to 0.89-fold and 0.80-fold, respectively [90% confidence interval (CI) for test/reference ratios 0.85–0.93 and 0.72–0.89], with the combined effect resulting in a 1.46-fold increase in area under the curve of oral midazolam (90% CI 1.36–1.57). Propiverine had no relevant effect on CYP2C9, CYP2C19, and CYP1A2 (90% CI for test/reference ratios 0.93–1.00, 0.84–0.96, and 0.97–1.07, respectively). All study drugs were well tolerated. In conclusion, propiverine has a minor potential to cause drug-drug interactions.

Propiverine hydrochloride, described below as propiverine, is indicated for the treatment of urinary incontinence, as well as urinary urgency and frequency in patients who have either idiopathic detrusor overactivity (overactive bladder) or neurogenic detrusor overactivity (detrusor hyperreflexia) from spinal cord injuries, e.g., transverse lesion paraplegia. It exhibits antagonistic effects toward muscarinic acetylcholine receptors and calcium channel-modulating properties (Siegmund et al., 1990; Yono et al., 1999; Madersbacher and Mürtz, 2001).

After oral administration, propiverine is rapidly and almost completely absorbed from the gastrointestinal tract. The maximal serum concentration is reached approximately 90 min after a single dose of 15 mg. Propiverine undergoes extensive presystemic metabolism via N-oxidation to propiverine-oxide with involvement of cytochrome P450 enzymes. Mean elimination half-life after chronic administration of propiverine is about 15 h. The major fraction of propiverine and its metabolites is eliminated in the urine (Haustein and Hüller, 1988; Siepmann et al., 1998). Information on the enzymes mediating phase I metabolism of propiverine has been obtained in several in vitro systems (APOGEPHA, data on file). The primary metabolic route involves the oxidation of the piperidyl-N and is mediated by CYP3A4 and flavin-containing monooxygenases 1 and 3, and leads to the formation of the much less active N-oxide. CYP2C9 and CYP2C19 may also mediate a fraction of overall propiverine elimination, whereas other enzymes (e.g., CYP1A2 and CYP2D6) were involved to a minor extent.

Several studies concerning possible drug-drug interactions of propiverine have been published. Müller et al. (1993) demonstrated that there is no effect of the CYP2D6 genotype on propiverine biotransformation in humans, so that no effect of CYP2D6 inhibitors on propiverine pharmacokinetics is expected. Results of a study performed in rats provided no evidence for propiverine to cause relevant drug–drug interactions (Borchert et al., 1986). Studies on the effect of propiverine on drug-metabolizing enzymes in human and rat hepatocyte cultures demonstrated an increase in CYP3A4 mRNA and
protein expression, although the corresponding enzyme activities were inhibited (APOGEPHA, data on file). In preliminary studies in human liver microsomes, propiverine showed competitive inhibition of CYP2D6 with Ki values more than 30-fold above propiverine plasma peak concentrations (APOGEPHA, data on file). Recently, Walter et al. (2003) reported that the drug administered in rats in doses about 100 times above the therapeutic doses in humans is a phenobarbital-type enzyme inducer. In summary, propiverine appears to interact with P450s mainly as a substrate. The available, mainly preclinical data suggest that any clinically relevant interaction of propiverine with cytochrome P450 enzymes as a substrate, inhibitor, and/or inducer would mainly concern CYP3A4 and possibly also CYP2C9 or CYP2C19, but data with respect to induction or inhibition of P450s are incomplete.

Whereas in vitro studies using human liver microsomes or expressed enzymes provide only preliminary information about how the test drug might interfere with the respective P450s in patients (Blakey et al., 2004), Phenotyping, i.e., estimation of enzyme activity by administration of a selective substrate for this enzyme and subsequent determination of pharmacokinetic parameters reflecting its activity, is considered to be the most favorable method for assessment of actual metabolic capacities resulting from both intrinsic and extrinsic factors (Streetman et al., 2000a). A major shortcoming of this procedure is the necessity of performing several phenotyping studies, often in sequential hierarchy, to characterize the effect of a test drug on the activity of all important cytochrome P450 enzymes. Here, this problem was overcome by simultaneous administration of low dosages of validated probe drugs in a tailored “cocktail” approach, providing information on several metabolic pathways in a single experimental session and minimizing the confounding influence of inasubject variability over time (Frye et al., 1997; Tanaka et al., 2003).

Thus, the present study was conducted to evaluate a possible in vivo effect of propiverine on the activity of human P450s, using a two-arm crossover cocktail phenotyping approach. The primary objective was to obtain information about the effects on intestinal CYP3A4 and on hepatic CYP3A4, CYP2C9, and CYP2C19. Secondly, effects on CYP1A2 and CYP2D6 were to be investigated.

Materials and Methods

The study protocol was reviewed and approved the Ethics Committee of the Faculty of Medicine of the University of Cologne. Seventeen male white volunteers participated in the trial after having provided written informed consent. One early dropout, unrelated to the study medication, occurred. In the 16 subjects who completed the study, the respective means and ranges for age and body mass index were 29 (23–42) years and 23.5 (21.4–26.6) kg/m2. All subjects were nonsmokers and healthy as confirmed on the basis of an extensive prestudy examination.

Study Design. The study had a single-center, controlled, open, crossover design with period-balanced randomly allocated sequences reference-test, test-reference. Between the two periods, there was a 7-day washout interval.

According to the primary objective to obtain information about the effects of propiverine on intestinal CYP3A4 and on hepatic CYP3A4, CYP2C9, and CYP2C19, the evaluation of the respective metrics (Table 1) was mandatory. If the study showed pronounced inhibition of these enzymes, indicating high propiverine concentrations at hepatic P450 binding sites, which might result in relevant in vivo effects on enzymes for which propiverine had only a minor effect in vitro, effects on CYP1A2 and CYP2D6 activity were to be evaluated additionally. Although there was no such pronounced inhibition, propiverine effects on CYP1A2 were also evaluated, prompted by recent additional data (Walter et al., 2003). We also decided to evaluate the effects on CYP2D6, but these results will be reported separately because extensive additional evaluations are required to assess the validity of dextromethorphan metabolic ratios in urine and plasma for the quantification of drug-drug interactions (Streetman et al., 2000a; Özdemir et al., 2004; Borges et al., 2005).

In the test period, 15 mg of propiverine hydrochloride (1 tablet of Mictonorm, APOGEPHA Arzneimittel GmbH, Dresden, Germany) were administered orally in the morning and in the evening for 7 days at approximately 7:00 AM and 7:00 PM, respectively. In the reference period, placebo tablets were administered instead. For each drug intake, the volunteers reported to the ward. They were instructed to abstain from fluid and food intake from 1 h before each dosing until 1 h thereafter.

For phenotyping with a six-probe metabolic cocktail, the volunteers were hospitalized from 13 h before cocktail administration on study day 7 in each study period until 24 h thereafter. Phenotyping drugs were administered in the morning on day 7 (profiling day) of each period as follows: 125 mg of tolbutamide (a quarter of a tablet of Tolbutamin RAN), 50 mg of mefenpytoin (half a tablet of Epilan Gerot Tabletten), 150 mg of caffeine (three tablets of Percodafrienol), and 30 mg of dextromethorphan-HBr (one capsule of Hustenstillrer, ratiopharm) were administered together with the propiverine or placebo dose for evaluation of CYP2C9, CYP2C19, CYP1A2, and CYP2D6, respectively. One minute thereafter, 2 mg of midazolam (Dormicum) were given orally for intestinal CYP3A4.

Finally, 1 mg of midazolam was administered intravenously 4 h after the oral propiverine/placebo administration for hepatic CYP3A4. Table 1 shows an overview of the cocktail drugs and the respective phenotyping metrics used to assess the in vivo activity of P450s. Study participants abstained from alcohol, methylxanthines, and grapefruit from 72 h before the first dosing until 48 h after the last dosing. Food and fluid were standardized during hospitalization. The subjects were fasted from 9 h before until 6 h after cocktail dosing and adhered to a relaxed recumbent position during the 6 h after the cocktail administration.

In the test period, blood for the subsequent determination of plasma concentrations of propiverine and its main metabolite was sampled 3 min before the administration of the morning dose on day 7 and 2, 3.57, 8, and 11.57 h thereafter to demonstrate exposure. In each study period, blood samples for phenotyping were collected 3 min before dosing in the morning of day 7 and

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**Table 1**

| Cytochrome P450 Enzymes | Probe Substrate | Dose and Administration Route | Matrix Sampled | Pharmacokinetic Parameters Determined
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Hepatic CYP3A4</td>
<td>Midazolam</td>
<td>1 mg intravenously</td>
<td>Plasma</td>
<td>Au/Cmax, t1/2, clearance of i.v. midazolam, F&lt;sub&gt;hep&lt;/sub&gt;</td>
</tr>
<tr>
<td>Intestinal CYP3A4</td>
<td>Midazolam</td>
<td>2 mg orally</td>
<td>Plasma</td>
<td>F&lt;sub&gt;intestina&lt;/sub&gt;a,b, AU&lt;sub&gt;Cmax&lt;/sub&gt;, C&lt;sub&gt;max&lt;/sub&gt;, t&lt;sub&gt;max&lt;/sub&gt; at 11.57 h, t&lt;sub&gt;1/2&lt;/sub&gt;, F&lt;sub&gt;L/L&lt;/sub&gt;</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Tolbutamide</td>
<td>125 mg orally</td>
<td>Urine and plasma</td>
<td>UB&lt;sub&gt;6.12.3&lt;/sub&gt; (OH-tolbut.) + carboxytolbut./tolbut., AU&lt;sub&gt;Cmax&lt;/sub&gt;, C&lt;sub&gt;max&lt;/sub&gt;, t&lt;sub&gt;max&lt;/sub&gt;, t&lt;sub&gt;1/2&lt;/sub&gt;, F&lt;sub&gt;L/L&lt;/sub&gt;</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Mephenytoin</td>
<td>50 mg orally</td>
<td>Urine</td>
<td>CYP2C19, 4′-OH-mephenytoin</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>Caffeine</td>
<td>150 mg orally</td>
<td>Plasma</td>
<td>Paraxanthine/caffeine plasma ratio 6 h postdose</td>
</tr>
</tbody>
</table>

<sup>a</sup> The main phenotyping metrics are printed in bold.

<sup>b</sup> For F<sub>intestinal</sub> (intestinal availability of midazolam), to enable a better comparison, in the text, changes in intestinal CYP3A4 activity were calculated as the inverse of changes in F<sub>intestinal</sub>-
and from 50 to 50,000 ng/ml for 4-hydroxytolbutamide and 4-carboxytolbutamide were fitted over a concentration range from 12.5 to 50,000 ng/ml for tolbutamide. In urine, linear calibration curves could be obtained. Method for the quantification of tolbutamide and its metabolites 4-hydroxytolbutamide, 4-carboxytolbutamide, and 4-hydroxyethyltheophylline was published (Jetter et al., 2004). In urine, linear calibration curves were fitted over a concentration range from 15 to 10,000 ng/ml was expressed in correlation coefficients (LLOQ). Standard curves were weighted with the factor 1/concentration2. In all cases, coefficient of correlation of the intra- and interday precision was between 0.7% and 8.4%. The lowest concentration of the standard curves was set as the LLOQ. Standard curves were weighted with the factor 1/concentration2 weighting. In all cases, coefficient of correlation of the intra- and interday precision was between 0.7% and 8.4%. The lowest concentration of the standard curves was set as the LLOQ. The accuracy [percentage bias] ranged from +1.6% to +3.3% and +10.4% for all analytes. For both intra- and interday calculations, precision was found to be between 0.8% and 12.6% for tolbutamide, 4-hydroxytolbutamide, and 4-carboxytolbutamide. Intra- and interday accuracy was within −8.8% and +14.0% for all analytes. For both intra- and interday calculations, precision was found to be between 0.7% and 8.4%. In both assays, chlorpropamide was used as internal standard. The calibration curves were calculated from the peak area ratios of analyte/internal standard and the nominal analyte concentrations using linear regression with 1/concentration2 weighting. In all cases, coefficient of correlation of the weighted standard curves was set as the LLOQ. The 4'-hydroxymephenytoin was used for validation. The lowest points of the standard curves were set as the lower limit of quantification (LLOQ). Standard curves were weighted with the factor 1/concentration2 and showed correlation coefficients >0.998. All concentration data were calibrated externally. The accuracy [percentage bias] for propiverine and propiverine-N-oxide ranged from −3.5% to +7.4%. Precision [% CV] was found to be between 0.8% and 5.5% for propiverine and between 1.1% and 11.7% for propiverine-N-oxide. As for all assays, precision of accuracy were calculated as the mean coefficient of variation (CV) of all samples used for validation and the mean relative deviation of validation samples from their nominal values, respectively.

Tolbutamide, 4-Hydroxytolbutamide, and 4-Carboxytolbutamide. The method for the quantification of tolbutamide and its metabolites 4-hydroxytolbutamide and 4-carboxytolbutamide in urine and plasma by LC-MS/MS has been published (Jetter et al., 2004). In urine, linear calibration curves could be fitted over a concentration range from 12.5 to 50,000 ng/ml for tolbutamide, and from 50 to 50,000 ng/ml for 4-hydroxytolbutamide, and 4-carboxytolbutamide. Intra- and interday accuracy was within −8.8% and +14.0% for all analytes. For both intra- and interday calculations, precision was found to be between 0.6% and 12.6% for tolbutamide, 4-hydroxytolbutamide, and 4-carboxytolbutamide. Additionally, up to 20-fold dilution of samples with blank human urine was tested during method validation.

In plasma, calibration curves were linear between 15 and 15,000 ng/ml for tolbutamide. Intra- and interday accuracy ranged from +1.6% to +3.3% and +10.4% for all analytes. For both intra- and interday calculations, precision was found to be between 0.7% and 8.4%. In both assays, chlorpropamide was used as internal standard. The calibration curves were calculated from the peak area ratios of analyte/internal standard and the nominal analyte concentrations using linear regression with 1/concentration2 weighting. In all cases, coefficient of correlation of the weighted standard curves was set as the LLOQ. The accuracy [percentage bias] ranged from +1.6% to +3.3% and +14.0% for all analytes. For both intra- and interday calculations, precision was found to be between 0.7% and 8.4%.

4'-Hydroxymephenytoin. The method for the quantification of 4'-hydroxymephenytoin with 4'-methoxymephenytoin as internal standard in urine by LC-MS/MS has been published in detail (Klaassen et al., 2004). Linearity of the weighted (1/concentration) calibration curves over a concentration range from 15 to 10,000 ng/ml was expressed in correlation coefficients >0.999. The LLOQ for 4'-hydroxymephenytoin, corresponding to the lowest concentration of quality control samples, was 20 ng/ml. Accuracy [percentage bias] ranged from −1.6% to +2.3% and precision [% CV] was between 4.4% and 8.6%.

Midazolam. The method for the quantification of midazolam in plasma by LC-MS/MS has been reported (Kasel et al., 2002). A standard curve using weighted quadratic polynomial regression with weights of 1/concentration2 was fitted over a concentration range from 0.09 to 186 ng/ml with coefficients of correlation higher than 0.998. All concentration data were calibrated externally. The LLOQ for midazolam, corresponding to the lowest concentration of quality control samples, was 0.44 ng/ml. Intra- and interday accuracy [percentage bias] ranged from +3.0% to +11.4%. Intra- and interday precision [% CV] was within 5.2% and 14.1%, respectively.

Caffeine and Paraxanthine. After addition of internal standard [7-(β-hydroxyethyl)theophylline] to 150 μl of plasma, plasma proteins were precipitated and the clear supernatant was evaporated to dryness. Thereafter, the pellets were redissolved and analyzed by LC-MS/MS (TSQ Quantum; Thermo Electron Corporation, Waltham, MA) in a validated assay. Chromatography was performed in 4.5 min using a 50 × 2.1 mm, 5-μm Hypersil Gold column (Thermo Electron Corporation, Runcorn, UK) and a linear gradient of methanol and formic acid (0.1%). Caffeine and paraxanthine were detected by positive electrospray ionization in the selected reaction monitoring mode with following ion transitions [m/z]: 195.23→138.16 for caffeine and 181.19→124.15 for paraxanthine. Area peaks were used for quantification of caffeine and peak height for that of paraxanthine, respectively.

Genotyping. DNA was extracted from 5-ml EDTA blood samples using the MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Mannheim, Germany), and purified DNA was eluted with a low salt buffer and stored at 4°C. For *CYP2C19* genotyping, the *2* allele was determined using polymerase chain reaction-restriction fragment length polymorphism as described earlier (De Morais et al., 1994).

Pharmacokinetic Analysis. Pharmacokinetic calculations were performed using Excel 97 (Microsoft, Redmond, WA) and WinNonlin, Version 2.1 (Pharsight, Mountain View, CA) in the noncompartmental mode. All metabolic ratios were calculated using molar concentrations and/or doses. The respective phenotypic metrics used for determination of the activity of the cytochrome P450 enzymes and the additional pharmacokinetic parameters of the phenotyping substrates are shown in Table 1.

CYP3A4 activities were calculated by noncompartmental analysis of midazolam plasma concentrations (Fig. 1) and checked by an independent compartmental analysis. For the noncompartmental evaluation, the AUC attributable to the oral midazolam dose was defined as the AUC after oral administration until the point of time just before intravenous administration, plus the AUC from the point of time when the concentration just before intravenous administration was reached again after intravenous administration to infinity. The point of time when the concentration just before intravenous administration was reached after intravenous administration was obtained by log-linear extrapolation from the two adjacent points of the concentration versus time profile. The AUC attributable to the intravenous dose was calculated as overall AUC minus the AUC attributable to the oral dose.

Hepatic availability of i.v. administered midazolam was...
to be sufficient to allow rejection of the respective null hypotheses “clinically up to 30% for the phenotyping metrics, a sample size of

Thus, the test versus reference comparison is a ratio for all parameters. On the basis of the anticipated intrasubject multiplicative coefficients of variation of

and for the calculation of analyses of variance, BioQ software, version 1.2.2 to check whether they having reached steady-state concentrations on

dence intervals. Propiverine trough values before the last morning dose were usually does not fully show for therapeutic drugs. A statistically significant

bioequivalence range of 0.8 to 1.25 because the elimination of most drugs is

intervals (CIs) for test/reference ratios of the phenotyping metrics were within

The extent of the reduction of hepatic and intestinal CYP3A4 activity propiverine reduced clearance and increased intestinal availability.

Comparative pharmacokinetics of midazolam were assessed by population pharmacokinetic analysis using the NONMEM software (V version 1.1; NONMEM Project Group, University of California at San Francisco, 1998). The most simple model, i.e., a one-compartment model with no intraindividual variation in any parameter, was stepwise expanded to more complex models if these were superior as evaluated by the plausibility of the parameter estimates and their 95% confidence intervals, by goodness-of-fit plots, and by a significant change (p < 0.05) of the objective function value provided by NONMEM. Fitting was performed with the “first order conditional estimates” algorithm, taking interactions between the parameters into account. The apparent clearance of tolbutamide was calculated as CL/F = dose/aur/AUC0–12 h.

Statistical Analysis. Testing for a pharmacokinetic interaction was handled as a bioequivalence problem (Steinijans et al., 1991). Test and reference treatments were compared with respect to the phenotyping metrics.

Lack of a clinically relevant interaction was assumed if 90% confidence intervals (CIs) for test/reference ratios of the phenotyping metrics were within the range 0.7 to 1.43. This range was used instead of the more narrow standard bioequivalence range of 0.8 to 1.25 because the elimination of most drugs is not limited exclusively to a single pathway; thus, inhibition of a single enzyme

A statistically significant difference, however, was considered if unity was not included in the confidence intervals. Propiverine trough values before the last morning dose were compared with propiverine trough values before the previous dose by the same procedure to check whether they have reached steady-state concentrations on profiling days. For bioequivalence testing of the pharmacokinetic parameters and for the calculation of analyses of variance, BioQ software, version 1.2.2 for Windows (E. Diletti, Byk Gulden Pharmaceuticals, Konstanz, Germany) was used.

For all primary phenotyping metrics, a multiplicative model was assumed. Thus, the test versus reference comparison is a ratio for all parameters. On the basis of the anticipated intrasubject multiplicative coefficients of variation of up to 30% for the phenotyping metrics, a sample size of n = 16 was considered to be sufficient to allow rejection of the respective null hypotheses “clinically relevant interaction present” with α = 0.05 and a power of at least 80% for all parameters if the true p/a reference ratios are within the 0.95 to 1.05 range (Diletti et al., 1991, 1992).

Results

Tables 2 and 3 show the mean values of the phenotypic metrics (see Table 1) and additional pharmacokinetic parameters for model substrates determined in test and reference periods. Table 4 displays the selected comparisons, i.e., point estimates and 90% confidence intervals for test/reference ratios. Figures 2 to 5 show the values of the important pharmacokinetic metrics in all subjects in test and reference periods.

In the noncompartmental analysis of midazolam pharmacokinetics, propiverine reduced clearance and increased intestinal availability. The extent of the reduction of hepatic and intestinal CYP3A4 activity was to 0.89-fold and to 0.80-fold, respectively, of the reference period (90% CI for test/reference ratios 0.85–0.93 and 0.72–0.89), with the combined effect resulting in a 1.46-fold increase in AUC of oral midazolam (90% CI 1.36–1.57) (Table 4; Figs. 1 and 2). For the compartmental analysis of midazolam pharmacokinetics used to check the results of the noncompartmental evaluation, the best fit was obtained by a two-compartment model with first-order absorption and intraindividual variation in each parameter. The presence of comedication with propiverine had a major effect on the values of midazolam clearance and bioavailability (changes in NONMEM objective function by −32, p < 0.005, and −91, p < 0.005, respectively). In this evaluation, clearance decreased to 0.90-fold (95% CI 0.85–0.96) in the presence of comedication with propiverine, the point estimates for clearance were 24.4 and 27.0 l/h with and without comedication, respectively. Intestinal midazolam availability and oral bioavailability increased by a factor of 1.29 (90% CI 1.15–1.39) and 1.32 (95% CI 1.22–1.42), respectively, upon comedication with propiverine. The point estimates for oral bioavailability were 0.34 and 0.45, and mean values for intestinal availability were 0.57 and 0.71 without and with comedication, respectively. Furthermore, body weight was a signifi-

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter (Unit)</th>
<th>Phenotyping Metric for</th>
<th>Mean (% CV) in Test Period</th>
<th>Mean (% CV) in Reference Period</th>
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<tbody>
<tr>
<td>Clearance of intravenous midazolam (l/h)</td>
<td>Fintertial Fintertial Fintertial</td>
<td>26.0 (20.3)</td>
<td>29.2 (20.1)</td>
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<td>Intestinal CYP3A4</td>
<td></td>
<td></td>
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<tr>
<td>Fintertial</td>
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<td>Fintertial</td>
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<td>Fintertial</td>
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<tr>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>39.9 (30.0)</td>
<td>27.3 (23.8)</td>
</tr>
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<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>38.2 (20.1)</td>
<td>34.1 (20.2)</td>
</tr>
<tr>
<td>AUC0–12 h midazolam (ng/ml)</td>
<td>AUC0–12 h midazolam (ng/ml)</td>
<td>14.2 (23.1)</td>
<td>10.5 (26.9)</td>
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<tr>
<td>Cmax oral midazolam (ng/ml)</td>
<td>Cmax oral midazolam (ng/ml)</td>
<td>3.02 (23.3)</td>
<td>2.98 (29.6)</td>
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<td>t1/2 oral midazolam (h)</td>
<td>t1/2 oral midazolam (h)</td>
<td>0.630 (27.2)</td>
<td>0.661 (34.2)</td>
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<td>t1/2 oral midazolam (h)</td>
<td>t1/2 oral midazolam (h)</td>
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*Geometric means (geometric CV) are given for all parameters except for t1/2oral, where arithmetic mean (arithmetic CV) is given. For pharmacokinetic parameters, see footnotes to Table 1.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter (Unit)</th>
<th>Phenotyping Metric for</th>
<th>Mean (% CV) in Test Period</th>
<th>Mean (% CV) in Reference Period</th>
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<tr>
<td>Mean pharmacokinetic parameters of tolbutamide in urine and plasma, mephenytoin in urine, and caffeine in plasma for test and reference period</td>
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<tr>
<td>URT ≥32 h (OH-tolbut.-carboxytol.)/tolbut.</td>
<td>CYP2C9</td>
<td>525 (42.6)</td>
<td>664 (33.9)</td>
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<tr>
<td>AUC0–12 h tolbutamide (µg/ml · h)</td>
<td>CYP2C9</td>
<td>160.9 (16.3)</td>
<td>154.6 (15.1)</td>
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<tr>
<td>CL/F tolbutamide (ml/min)</td>
<td>CYP2C9</td>
<td>12.93 (16.3)</td>
<td>13.44 (15.1)</td>
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<td>Cmax tolbutamide (µg/ml)</td>
<td>CYP2C9</td>
<td>11.7 (11.1)</td>
<td>12.3 (12.3)</td>
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<td>t1/2ka tolbutamide (h)</td>
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<td>3.99 (4.5)</td>
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<td>Tolfbutamide plasma concentration 24 h postdose (µg/ml)</td>
<td>CYP2C9</td>
<td>20.6 (27.4)</td>
<td>18.2 (30.9)</td>
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<td>AUC0–12 h OH-mephenytoin (% of dose)</td>
<td>CYP2C9</td>
<td>18.2 (195)</td>
<td>19.3 (228)</td>
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<td>Paraxanthine/caffeine plasma ratio 6 h postdose</td>
<td>CYP1A2</td>
<td>0.49 (37)</td>
<td>0.51 (39)</td>
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</tbody>
</table>

*Geometric means (geometric CV) are given for all parameters except for t1/2oral, where arithmetic mean (arithmetic CV) is given. For pharmacokinetic parameters, see footnotes to Table 1.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter (Unit)</th>
<th>Phenotyping Metric for</th>
<th>Mean (% CV) in Test Period</th>
<th>Mean (% CV) in Reference Period</th>
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</thead>
<tbody>
<tr>
<td>Mean pharmacokinetic parameters of midazolam in plasma for test and reference periods (noncompartmental evaluation)</td>
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<tr>
<td>Hepatic CYP3A4</td>
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<td>Intestinal CYP3A4</td>
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<td>Fintertial</td>
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<tr>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>39.9 (30.0)</td>
<td>27.3 (23.8)</td>
</tr>
<tr>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>AUC0–12 h midazolam (ng/ml · h)</td>
<td>38.2 (20.1)</td>
<td>34.1 (20.2)</td>
</tr>
<tr>
<td>AUC0–12 h midazolam (ng/ml)</td>
<td>AUC0–12 h midazolam (ng/ml)</td>
<td>14.2 (23.1)</td>
<td>10.5 (26.9)</td>
</tr>
<tr>
<td>Cmax oral midazolam (ng/ml)</td>
<td>Cmax oral midazolam (ng/ml)</td>
<td>3.02 (23.3)</td>
<td>2.98 (29.6)</td>
</tr>
<tr>
<td>t1/2 oral midazolam (h)</td>
<td>t1/2 oral midazolam (h)</td>
<td>0.630 (27.2)</td>
<td>0.661 (34.2)</td>
</tr>
<tr>
<td>t1/2 oral midazolam (h)</td>
<td>t1/2 oral midazolam (h)</td>
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</table>
cant covariate for clearance (increase of 0.85% per kg of body weight) and for both central and peripheral volume of distribution (increase of 0.73% per kg of body weight). In summary, these results fully confirmed those obtained by the noncompartmental analysis.

Propiverine had no relevant effect on CYP2C9 (Fig. 3), CYP2C19 (Fig. 4), and CYP1A2 (Fig. 5). The respective 90% CI values for test/reference ratios were 0.93 to 1.00, 0.84 to 0.96, and 0.97 to 1.07 (Table 4).

The exposure of propiverine and its major metabolite propiverine-N-oxide at steady state was similar between treatments: mean ± S.D. in test and reference were 3466 ± 1268 ml and 2744 ± 966, respectively, within 24 h. The mean urinary pH ranged between 6.7 and 7.3 during daytime and between 6.2 and 6.5 at night, respectively, in both treatments.

Figure 6 depicts mean concentrations of propiverine and its metabolite propiverine-N-oxide in steady state, measured after the last morning administration in the test period on study day 7. Since the 90% confidence interval of the ratio of trough evening propiverine concentration and its trough morning concentration on study day 7 in the test period was 0.85 to 1.07 (point estimate was 0.95), it was assumed that steady state was reached.

Safety Results. Forty-one adverse events occurred during the study. Most of them, like dry mouth or accommodation problems, were clearly explained as antagonistic effects of propiverine toward muscarinic acetylcholine receptors. On the profiling days, the most frequent adverse events were dizziness, tiredness, and sedation, which were closely temporally related to the intravenous administration of midazolam. Forty adverse events were classified as mild, whereas one adverse event only was moderate in severity (dizziness in one subject). All adverse events were transient and resolved shortly after the administration of the cocktail, when related to the phenotyping drugs, or after the cessation of propiverine therapy, when dependent on its anticholinergic properties. Notably, an extensive monitoring of sedation and hypoglycemic symptoms on profiling days did not reveal any relevant abnormalities. Clinical examinations, vital signs, subjects’ well being, and clinical laboratory data did not provide evidence for health impairment caused by the study drugs. Together, all study drugs were well tolerated.

Discussion

The aim of this study was to evaluate a possible effect of propiverine hydrochloride intake at therapeutic doses on the activity of important human cytochrome P450 enzymes. Our results indicate that propiverine has a modest inhibitory activity on CYP3A4 and negligible effects on other P450s.

The exposure of propiverine and its main metabolite, propiverine-N-oxide, at steady state during the test period is in agreement with published data (Siepmann et al., 1998). The selected dosing scheme has been shown to be therapeutically effective and is used clinically (Alloussi et al., 2005). This allowed the characterization of eventual changes in the activity of the respective metabolic enzymes under conditions similar to those in clinical practice.

The cocktail approach, which is proposed as a screening method for assessment of drug-drug interactions in vivo, has been used for almost two decades (Schellens et al., 1988; Streetman et al., 2000a; Tanaka et al., 2003). Unfortunately, no general agreement has been reached on the applicability of the cocktail approach in clinical investigations.
and during drug development (Zhou et al., 2004). Here, the low-dose cocktail strategy could be shown to be an effective tool to assess the drug-drug interactions profile of propiverine in vivo. All probe substrates used in this cocktail approach have been previously shown to be enzyme-selective substrates without relevant mutual interaction in vivo (Endres et al., 1996; Frye et al., 1997; Streetman et al., 2000b; Bruce et al., 2001; Palmer et al., 2001; Wang et al., 2001; Zhu et al., 2001; Blakey et al., 2004). The composition of the cocktail used here was tailored primarily with respect to the previous information obtained in in vitro studies, suggesting that any clinically relevant interaction of propiverine with cytochrome P450 enzymes as a substrate, inhibitor, and/or inducer would mainly concern CYP3A4 and possibly also CYP2C9 and/or CYP2C19. Nevertheless, the inclusion of optional cocktail components allowed a further evaluation of propiverine effects. Phenotyping for CYP3A4 activity with midazolam is a well-established method, although it is elaborate due to the requirement of multiple blood collections (e.g., Streetman et al., 2000a). A problem is the independent regulation of intestinal and hepatic CYP3A4 (Gorski et al., 1998; Tsunoda et al., 1999; Lee et al., 2002). For this reason, two different metrics have to be used for estimation of both activities. In the present study, for the evaluation of hepatic and intestinal CYP3A4 activity, a sequential administration of midazolam, first orally and then intravenously, was conducted. Application of the oral midazolam dose concomitantly with the morning dose of propiverine was considered to be adequate for assessment of potential interaction between both drugs during absorption when the concentration at enzyme site is maximal. The time interval of 4 h between the oral and intravenous midazolam administration allowed absorption and distribution of the oral midazolam to be completed. Therefore, it was possible to attribute the two components of the obtained midazolam AUC to the respective midazolam doses (see Fig. 1). Lee et al. (2002) have shown that such phenotyping results obtained with semisimultaneously administered midazolam were not different from those for administration of midazolam on two separate occasions. The validity of the procedure is supported by the similar results obtained in this
study by both noncompartmental and compartmental methods. Both midazolam doses, 2 mg orally and 1 mg intravenously, were chosen to obtain similar concentrations after the two administrations, taking into account the mean oral bioavailability of midazolam of approximately 40%. For phenotyping with midazolam, dosage considerations are very important because of safety reasons. Due to low dosages of the drug in our study, the adverse events observed in the subjects were very mild. Based on the clearance of intravenously administered midazolam and intestinal availability of orally administered midazolam, which were the phenotypic metrics for hepatic and intestinal CYP3A4 activity, respectively, chronic treatment with propiverine had some inhibitory effect on CYP3A4. For hepatic and intestinal CYP3A4 activity in the test period, we observed a mean reduction to 0.89-fold and to 0.80-fold, respectively, of the activity observed in the reference period. The extent of these effects taken separately was considered not to be clinically relevant, since the 90% confidence interval for test/reference ratios of the metrics were within the predefined limits of 0.7 to 1.43. However, the combined effect on intestinal and hepatic CYP3A4 resulted in a 1.46-fold increase in AUC of oral midazolam. This effect of chronic therapy with propiverine would be considered as relevant, even taking inherent variation in midazolam pharmacokinetic parameters into account. The intrasubject coefficients of variation observed in our study were 6.8%, 17.6%, and 11.6%, respectively, for clearance of i.v. administered midazolam, intestinal availability, and AUC of oral midazolam. These results are similar to those published by Kharasch et al. (1999), who demonstrated an interday coefficient of variation in midazolam clearance of 19% ± 12%.

For the assessment of the CYP2C9 activity, both urinary ratio (4'-hydroxytolbutamide + carboxytolbutamide)/tolbutamide 6 to 12 h postdose, which was proposed as a convenient phenotyping metric (Veronese et al., 1990), and tolbutamide plasma data were determined. Based on the urinary metabolic ratio of tolbutamide, there was a statistically significant and clinically relevant inhibition of the CYP2C9 activity due to the chronic treatment with propiverine since the confidence interval of the ratio test/reference exceeded the tolerance zone of 0.70 to 1.43. In contrast, using plasma data (i.e., oral tolbutamide clearance) as well as tolbutamide plasma concentration 24 h postdose, a slight statistically significant inhibition of CYP2C9 that was observed was without clinical significance according to the definition used. In a recent paper by Jetter et al. (2004), no correlation could be found between the proposed urinary metabolic ratio and the tolbutamide plasma clearance in 26 healthy volunteers following administration of 125 mg of the drug. On the other hand, the authors observed a close correlation between tolbutamide plasma clearance and 24-h plasma tolbutamide concentration, and thus, they proposed the latter parameter as a simple metric for determining CYP2C9 activity. Moreover, a close relationship between tolbutamide plasma pharmacokinetics and genetic CYP2C9 polymorphisms was confirmed following administration of 125 mg of tolbutamide (Jetter et al., 2004) as well as 500 mg of tolbutamide (Kirchheiner et al., 2002; Lee et al., 2003). For that reason, we assumed the plasma-based CYP2C9 metric to be more reliable and do not expect any pronounced interaction between propiverine and drugs metabolized by CYP2C9.

CYP2C9 phenotyping with a standard tolbutamide dose i.e., 500 mg, was reported to be possibly accompanied by hypoglycemia (Streetman et al., 2000a). This problem, however, could be minimized by coadministration of oral glucose (Lee et al., 2003) or, as in our study, avoided by using a very low tolbutamide dose of 125 mg.

Mephenytoin as a probe drug for CYP3A4 is well established and used in epidemiological studies as well as in clinical pharmacological research (Tammenga et al., 2001). Urinary recovery of 4'-hydroxymephenytoin in an 8-h urine sample has been shown to discriminate precisely between poor and extensive metabolizers of mephenytoin (Wedlund et al., 1984, 1985). In our study, a statistically significant small difference between excreted amount of 4'-hydroxymephenytoin in test and reference periods did not reach the magnitude of expected clinical relevance; thus, we conclude that there is no clinically relevant effect of chronic treatment with propiverine on the activity of CYP2C9. The high interindividual coefficient of variation of the CYP2C9 metric was the result of the participation of a poor metabolizer (CYP2C9*2/*2) for mephenytoin in the study who showed minimal urine concentrations of 4'-hydroxymephenytoin as compared with the other participants. For safety reasons, the study subjects were administered a low dose of mephenytoin, i.e., 50 mg, so that sedation, which is a frequently reported adverse reaction of mephenytoin, was not observed.

Based on the paraxanthine/caffeine plasma ratio 6 h postdose, which is extensively validated for assessment of CYP1A2 activity (Fuhr et al., 1996; Streetman et al., 2000a), we found no inducing (or inhibitory) effect of chronic treatment with propiverine on the CYP1A2 function, indicating that the characterization of propiverine as a phenobarbital-type inducer at high doses in animals (Walter et al., 2003) is not predictive for an effect of therapeutic propiverine doses on CYP1A2 in humans.

In contrast to other existing cocktails (e.g., Frye et al., 1997; Palmer et al., 2001; Blakey et al., 2004), the cocktail used here has several useful peculiarities, including semisequential midazolam administration, allowing the assessment of hepatic and intestinal CYP3A4 activity within 1 day; low doses for all substances, minimizing the risk for adverse reactions; the inclusion of optional cocktail components; and the use of fully validated metrics for all P450s included.

Finally, since the intraindividual coefficients of variation for all phenotyping metrics did not exceed 30% and were clearly below this margin for most substances, the sample size proved to be sufficient (Diletti et al., 1991, 1992) and may even be decreased further in such studies, depending on the enzymes to be assessed.

Our results suggest that propiverine may cause relevantly increased concentrations of drugs only if they undergo extensive first-pass metabolism by both intestinal and hepatic CYP3A4. Examples of such drugs include verapamil, saquinavir, and felodipine. However, a very pronounced concentration increase for such drugs is not expected since the influence of chronic treatment with propiverine seems to be
small compared with classical enzyme inhibitors such as ketoconazole or grapefruit juice. There was no relevant effect of chronic treatment with propivipine on CYP2C9, CYP2C19, and CYP1A2, so that no interaction between propivipine and drugs metabolized by these cytochrome P450 enzymes is expected.

In summary, propivipine has a minor potential to cause drug-drug interactions. The application of this tailored cocktail is an effective and well tolerated method for assessment of drug-drug interaction in healthy volunteers.

References


Diletti E, Haushcke D, and Stejanins VW (1992) Sample size determination: extended tables for the multiplicative model and bioequivalence ranges of 0.9 to 1.1 and 0.7 to 1.43. Int J Clin Pharmacol Ther 30 (Suppl 1):59–62.


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