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# Duration of Pleconaril Effect on Cytochrome P450 3A Activity in Healthy Adults Using the Oral Biomarker Midazolam

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Abbreviations: CYP, cytochrome P450; ANOVA, analyses of variance; LS-GMR, least squares geometric mean ratios; 90% CI, ninety percent confidence interval; LS/MS/MS, liquid chromatography/mass spectrometry/mass spectrometry; $C_{max}$, maximum plasma concentration; $AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; $AUC_{0-last}$, area under the concentration-time curve extrapolated to the last measurable concentration; CL, clearance; F, bioavailability; V, volume of distribution; $t_{1/2}$, elimination half-life.

## ABSTRACT

The objective of this study was to evaluate the duration of oral pleconaril (a picornavirus inhibitor) effect on intestinal and hepatic cytochrome P450 (CYP) 3A activity as assessed by oral midazolam. Healthy adults received oral midazolam 0.075 mg/kg on Days 1 (baseline), 7, 9, 13, 20, 27, and 34. Oral pleconaril 400 mg three times daily for 15 doses was administered on Days 2 through 7. Blood samples were collected during each day of midazolam dosing to determine plasma midazolam concentrations. On Days 5, 6, and 7, blood samples were collected to determine plasma pleconaril concentrations. Midazolam pharmacokinetics were determined by noncompartmental analyses, with bioequivalence assessed by least squares geometric mean ratios (LS-GMR) and 90% confidence intervals (90% CI). Eighteen subjects completed the study. Midazolam $C_{max}$ (LS-GMR; 90% CI) decreased 24% on Day 7 (0.76; 0.66 – 0.87). Midazolam oral clearance increased 53% on Day 7 (1.53; 1.38 – 1.69). Midazolam oral clearance remained different on Days 9 (1.38; 1.25 – 1.52) and 13 (1.19; 1.07 – 1.31) versus Day 1. Midazolam volume of distribution (1.82; 1.57 – 2.11) and elimination half-life (1.19; 1.03 – 1.38) were also different on Day 7 in comparison to Day 1. Oral pleconaril increased intestinal and hepatic CYP3A activity. The duration of increased CYP3A activity by pleconaril was at least 6 days (but no longer than 13 days) after pleconaril discontinuation.

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Pleconaril is a novel agent under evaluation for treatment of infections caused by rhinoviruses and enteroviruses, which cause the common cold, acute exacerbation of asthma and chronic obstructive pulmonary disease, viral meningitis, and encephalitis (Makela et al., 1998; Chidekel et al., 1997; Rotbart, 1995; Rotbart, 1994). Pleconaril antiviral activity is attributed to the disruption of viral replication and subsequent attenuation of viral attachment to the ICAM-1 cellular receptor (Billich, 2000; Florea et al., 2003).

*In vitro* and animal studies showed a lack of cytochrome P450 (CYP) 3A inhibition and induction by pleconaril (data on file, ViroPharma, Inc.; Rhodes et al., 2001a). In healthy adults, oral pleconaril increases hepatic CYP3A activity after intravenous midazolam administration (Ma et al., 2006). Due to the prolonged half-life (~180 hrs) of pleconaril (Florea et al., 2003, Rhodes et al., 2001b, Rhodes et al., 2001c), the duration of increased CYP3A activity may be an important parameter in evaluating potential drug interactions with pleconaril. The purpose of this study was to evaluate the duration and extent of increased hepatic and intestinal CYP3A activity by pleconaril in healthy adults as assessed by oral midazolam.

**MATERIALS AND METHODS**

**Subjects.** This study was approved by the Institutional Review Board of Bassett Healthcare, Cooperstown, NY, with written informed consent obtained from each subject. All subjects underwent a medical history, physical examination, electrocardiogram, and blood and urine laboratory tests. Subjects were 18 to 55 years of age, healthy, nonsmokers (for at least 6 months) and not on any medications known to affect CYP3A

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activity.  Subjects refrained from consuming grapefruit and grapefruit juice for 7 days,

and caffeine-containing foods for 2 days before study start until study completion.

Subjects with clinically significant findings by medical history, physical examination,

laboratory safety testing, history of alcohol or drug abuse, history of medication or skin

allergy, or intolerance to benzodiazepines were excluded.

**Study design.**  An open label, single sequence study design was used due to the

prolonged elimination half-life of pleconaril (Florea et al., 2003).  After an overnight fast,

subjects were administered oral midazolam 0.075 mg/kg on the morning of Day 1

(baseline).  Oral pleconaril 400 mg (Picovir[TM], 200 mg tablet, ViroPharma Inc., Exton,

PA) was administered three times daily for 15 doses.  This dosing regimen was based on

the expected clinical use for treatment of viral infections.  The first pleconaril dose began

in the afternoon of Day 2.  The last pleconaril dose was concurrently administered with

midazolam on the morning of Day 7.  All pleconaril doses, except for the morning Day 7

dose, were administered within 5 minutes after completion of a meal or snack since

pleconaril bioavailability is increased with food (Abdel-Rahman and Kearns, 1998).

Midazolam dosing was repeated on Days 7, 9, 13, 20, 27, and 34.  Venous blood samples

were collected at predose and 5, 30, 60, 90, 120, 180, 240, 300, 360, and 480 minutes

after each midazolam dose to determine plasma midazolam concentrations and on Days

5, 6, and 7 before the morning pleconaril dose to determine plasma pleconaril

concentrations.

**Analytical Procedures.**  Plasma midazolam concentrations were analyzed by a

LC/MS/MS assay developed by Prevalere Life Sciences, Whitesboro, NY.  Details of this

procedure are described elsewhere (Kashuba et al., 1998).  The linear range of the assay

was 25 to 10,000 pg/mL. Intra- and interday percent coefficients of variation for

midazolam were $\leq 9.4\%$ at quality control samples of 75, 750, and 7500 pg/mL. For

midazolam detection with mass spectrometry, the transition $m/z$ 326→291 was selected.

Plasma pleconaril was analyzed by a LC/MS/MS assay developed by MDS Pharma

Services, Montreal, Quebec, Canada. Plasma pleconaril (0.1 mL) was isolated by a

liquid-liquid extraction with addition of internal standard (VP 64027, 750 ng/mL) and 5

mL of hexanes. After mixing, samples were evaporated to dryness and reconstituted in

100 mL of mobile phase (85:15 acetonitrile/water). A BDZ-Hypersil $C_{18}$ column was

coupled to a Sciex API 3000 or API III MS system (Perkin-Elmer Sciex, Rochester, NH)

and an Alliance 2690 autosampler (Waters Corporation, Milford, MA). The linear range

of the assay was 5 to 500 ng/mL, with intra- and interday percent coefficients of variation

$\leq 10\%$ at quality control samples of 5, 15, 150, and 350 ng/mL. For pleconaril detection

with mass spectrometry, the transition $m/z$ 382→298 was selected.

**Pharmacokinetic analysis.** Midazolam pharmacokinetics were determined by

noncompartmental analysis using Kinetica version 2.0.1 (InnaPhase Corporation,

Philadelphia, PA). The area under the concentration-time curve (AUC) from time zero to

the last measurable concentration ($AUC_{0\text{-last}}$) was calculated by the log-linear trapezoidal

method. The AUC from time zero to infinity ($AUC_{0\text{-}\infty}$) was the sum of $AUC_{0\text{-last}}$ plus the

ratio of the last measurable concentration and the elimination rate constant ($k_e$).

Elimination half-life ($t_{1/2}$) was estimated by linear regression. Oral clearance (CL/F) was

calculated as Dose/$AUC_{0\text{-}\infty}$.

**Statistical analyses.** Based on previous data (Ma et al., 2006), using $\alpha = 0.05$ and $\beta = $

0.20 (power of 80%), an estimated sample size of 20 would detect a 25% difference in

midazolam $AUC_{0-\infty}$. Bioequivalence testing was done, and results are presented as least

squares geometric mean ratios (LS-GMR) with 90% confidence intervals (90% CI). A

lack of bioequivalence (i.e., a drug interaction) was determined if the 90% CI were

outside of the 0.80 to 1.25 interval (FDA Guidance for Industry, 2001). Statistical

analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC).

## RESULTS

Nineteen subjects were enrolled and eighteen subjects (9 males) completed the

study. Median age was 31 years (range: 21 – 49 years). Weight (mean ± SD) was 76.5 ±

13.1 kg. One subject developed an upper respiratory infection and Bell's palsy that was

determined to be unrelated to pleconaril or midazolam, and was removed from the study

on Day 13. Data for this subject were used only for evaluation of plasma midazolam

concentrations (Figure 1) on Days 1, 7, 9, and 13 and plasma pleconaril concentrations on

Days 5, 6, and 7. Mild sedation was noted with midazolam in all subjects. No significant

adverse events were reported.

Plasma midazolam concentration versus time profiles are presented in Figure 1.

Pleconaril decreased midazolam $C_{max}$ 24% on Day 7 compared to Day 1 and this decrease

was maintained on Day 9 (Table 1). Midazolam $C_{max}$ values returned to baseline by Day

13 and remained steady until Day 34 when it increased slightly. Midazolam $AUC_{0-\infty}$

decreased 16 to 35% over Day 7 to 13, and returned to baseline by Day 20. Midazolam

CL/F increased 19 to 53% over Day 7 to 13 and returned to baseline by Day 20. The

observed results in midazolam CL/F and V/F were similar after weight normalization

(data not shown). Midazolam $t_{1/2}$ increased on Day 7, but decreased on Days 13 and 20

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(Table 1). Plasma pleconaril concentrations (mean ± SD) increased on Days 5, 6, and 7 (697.5 ± 339.2, 787.7 ± 302.9, and 968.2 ± 366.8 ng/mL).

## DISCUSSION

This study evaluated the duration of increased intestinal and hepatic CYP3A activity by pleconaril in healthy adults. The increase in midazolam CL/F continued to Day 13 and returned to baseline by Day 20. These results are surprising as we expected the duration of increased CYP3A activity to be sustained until complete elimination of pleconaril (23-30 days).

Induction and heterotropic cooperativity are two possible explanations for increased CYP3A activity by pleconaril. The molecular mechanisms underlying CYP induction are not completely understood, but generally result in a quantitative increase in CYP3A enzyme (Lin and Lu, 1998). The duration of CYP3A induction may be exponential and reflective of the degradation rate of CYP3A (Thummel et al., 1998). Based on an irreversible enzyme inhibition model, the half-life of CYP3A is reported to be ~8 hours (Takanaga et al., 1999). However, there are limited and contrasting *in vivo* CYP3A data to support this hypothesis (Lee et al., 1993; Ohnhaus et al., 1989). The other explanation may be a qualitative change in existing CYP3A enzyme, termed heterotropic cooperativity. This has been reported primarily from *in vitro* experiments (Ekins et al., 1998; Tang and Stearns, 2001). Distinct binding sites in the active site of CYP3A or changes in CYP3A enzyme conformation during pleconaril and midazolam co-administration are possible but remain theoretical. In addition, a change of the kinetic characteristics of pleconaril (e.g., increase in $V_{max}$, decrease in $K_m$, or a change in both)

may also be involved. Additional evidence to suggest CYP3A cooperativity comes from two, short-term pleconaril administration studies (data on file, ViroPharma Inc.). In one study, a pleconaril 800 mg loading dose, followed by 400 mg every 6 hours for 3 doses significantly decreased oral midazolam $AUC_{0-\infty}$ by 36%. In another, the $AUC_{0-\infty}$ of ethinyl estradiol (a CYP3A substrate) significantly decreased by 17% after administration of a pleconaril 800 mg loading dose, followed by 400 mg every 4 hours for four doses. Heterotropic cooperativity may explain the discordance between pleconaril elimination half-life and the duration of increased CYP3A activity.

Midazolam V/F increased on Day 7. This may be due to decreased midazolam oral bioavailability as a result of increased intestinal CYP3A activity by pleconaril. Midazolam $t_{1/2}$ also increased on Day 7. Because $t_{1/2}$ is a dependent variable, changes in either the V/F or oral clearance may affect elimination half-life. The increase in V/F, to a greater extent that oral clearance, may account for the transient increase in the midazolam $t_{1/2}$ on Day 7.

Pleconaril elimination half-life has been reported to be 6.7-34 hours (Abdel-Rahman and Kearns, 1998, Abdel-Rahman and Kearns, 1999), which is in contrast to the half-life of ~180 hours (Florea et al., 2003, Rhodes et al., 2001b, Rhodes et al., 2001c). However, the shorter half-lives reported in the literature were determined from a truncated sample collection period (24-36 hours). This did not allow for an accurate estimation of the terminal elimination half-life of the drug (Gilbaldi and Weintraub, 1971). The reported half-life of ~180 hours was based on a sample collection period that extended to 11 days after pleconaril administration and utilized analytical methods with increased sensitivity (Rhodes et al., 2001b, Rhodes et al., 2001c).

The increases in intestinal and hepatic CYP3A activity are consistent with findings from another study of healthy adults, where oral pleconaril (400 mg three times daily for 16 doses) increased hepatic CYP3A activity after intravenous midazolam administration (Ma et al., 2006). Geometric mean Day 1 and Day 7 midazolam $AUC_{0-\infty}$ (ng min/mL) were 3093.6 and 2222.4, respectively. Midazolam $AUC_{0-\infty}$ decreased modestly (28%, [LS-GMR; 90%CI, 0.718; 0.674 - 0.765]). In comparison, rifampin (a potent CYP3A inducer) has been reported to decrease midazolam $AUC_{0-\infty}$ by 96% (Beckman et al., 1996).

In summary, oral pleconaril increases intestinal and hepatic CYP3A activity. The duration was at least 6 days (but no longer than 13 days) after pleconaril discontinuation. The effect of pleconaril on CYP3A activity may be CYP3A induction, heterotropic cooperativity, or both. Duration and extent of increased CYP3A activity need to be considered when evaluating the clinical significance and potential for drug interactions upon co-administration of pleconaril and other drugs primarily metabolized by CYP3A.

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 **FOOTNOTES**

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**FIGURE LEGENDS**

Figure 1. Mean plasma midazolam concentration versus time in healthy adults following

oral administration of midazolam 0.075 mg/kg on Days 1 (baseline), 7, 9, 13, 20, 27, and

34.  Data on Days 1, 7, 9, and 13 represent 19 subjects.  Data on Days 20, 27, and 34

represent 18 subjects.

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Table 1. Midazolam Pharmacokinetic Parameters in 18 Healthy Adults on Days 1 (baseline), 7, 9, 13, 20, 27, and 34.[a]

| Parameter (Units) | Day | Geometric Mean | LS-GMR (Day n/Day 1) | 90% CI |
|---|---|---|---|---|
| $C_{max}$ | 1 | 19.7 | | |
| (ng/mL) | 7 | 14.9 | 0.76 | 0.66 – 0.87* |
| | 9 | 16.6 | 0.84 | 0.73 – 0.97* |
| | 13 | 20.5 | 1.04 | 0.91 – 1.20 |
| | 20 | 20.5 | 1.04 | 0.91 – 1.20 |
| | 27 | 21.0 | 1.07 | 0.93 – 1.22 |
| | 34 | 22.5 | 1.14 | 1.00 – 1.31* |
| $AUC_{0-\infty}$ | 1 | 2620.4 | | |
| (ng min/mL) | 7 | 1714.9 | 0.65 | 0.59 – 0.72* |
| | 9 | 1900.6 | 0.73 | 0.66 – 0.80* |
| | 13 | 2210.6 | 0.84 | 0.76 – 0.93* |
| | 20 | 2525.1 | 0.96 | 0.87 – 1.07 |
| | 27 | 2699.2 | 1.03 | 0.93 – 1.14 |
| | 34 | 2941.1 | 1.12 | 1.02 – 1.24 |
| CL/F | 1 | 2164.2 | | |
| (mL/min) | 7 | 3306.9 | 1.53 | 1.38 – 1.69* |
| | 9 | 2983.8 | 1.38 | 1.25 – 1.52* |

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|---|---|---|---|---|
| | 13 | 2565.4 | 1.19 | 1.07 – 1.31* |
| | 20 | 2245.9 | 1.04 | 0.94 – 1.15 |
| | 27 | 2101.0 | 0.97 | 0.88 – 1.07 |
| | 34 | 1928.2 | 0.89 | 0.81 – 0.99 |
| V/F | 1 | 515.6 | | |
| (L) | 7 | 939.7 | 1.82 | 1.57 – 2.11* |
| | 9 | 663.9 | 1.29 | 1.11 – 1.49* |
| | 13 | 505.6 | 0.98 | 0.85 – 1.14 |
| | 20 | 472.0 | 0.92 | 0.79 – 1.06 |
| | 27 | 508.3 | 0.99 | 0.85 – 1.14 |
| | 34 | 493.9 | 0.96 | 0.83 – 1.11 |
| $t_{1/2}$ | 1 | 165.1 | | |
| (min) | 7 | 197.0 | 1.19 | 1.03 – 1.38* |
| | 9 | 154.2 | 0.93 | 0.81 – 1.08 |
| | 13 | 136.6 | 0.83 | 0.72 – 0.96* |
| | 20 | 145.7 | 0.88 | 0.76 – 1.02* |
| | 27 | 167.7 | 1.02 | 0.88 – 1.18 |
| | 34 | 177.6 | 1.08 | 0.93 – 1.24 |

[a]Data are expressed as geometric means and least squares geometric mean ratios (LS-

GMR) with 90% confidence intervals (90% CI).

*90% CI does not satisfy the 0.80 – 1.25 bioequivalence criteria.