Evaluation of Pharmacokinetic Interaction between PA-824 and Midazolam in Healthy Adult Subjects

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ABSTRACT

This study assessed the safety, tolerability, and pharmacokinetic interaction between PA-824, a novel antitubercular nitroimidazo-oxazine, and midazolam, a CYP3A4 substrate, in 14 healthy adult male and female subjects. The study followed up on observations in vitro that PA-824 caused weak and time-dependent inhibition of CYP3A4. Subjects received a single oral dose of midazolam (2 mg) followed by a 2-day washout. After the washout, all subjects received PA-824 (400 mg) once daily for 14 consecutive days. On the 14th day, all subjects received the final PA-824 dose coadministered with a 2 mg oral dose of midazolam. The pharmacokinetic endpoints AUC(0-t), AUC(0-inf), and Cmax for midazolam and 1-hydroxy midazolam were compared between midazolam administered alone versus midazolam coadministered with PA-824. Statistical analysis demonstrated that the mean midazolam values of Cmax, AUC(0-t), and AUC(0-inf) parameters were reduced by approximately 16%, 15% and 15%, respectively, when PA-824 was coadministered with midazolam. The total exposure (AUC) of 1-hydroxy midazolam was 13-14% greater when coadministered with PA-824 as compared with midazolam administered alone. The Cmax of 1-hydroxy midazolam was similar between treatments. Based on these results, PA-824 does not inhibit or induce CYP3A4 to a clinically meaningful extent and is not likely to markedly affect the pharmacokinetics of CYP3A4 metabolized drugs.

INTRODUCTION

PA-824 is an antitubercular nitroimidazo-oxazine that possesses significant activity against replicating and nonreplicating/persistent Mycobacterium tuberculosis (Mtb) via a complex mechanism of action distinct from that of any currently marketed drugs for the
treatment of tuberculosis (1, 2). Its mechanism of action is believed similar to that of Delamanid, a drug currently under review for market approval by the regulatory authorities (3). PA-824 interferes with Mtb cell wall biosynthesis by inhibiting the oxidation of hydroxymycolate to ketomycolate. A deazaflavin (F420)-dependent nitroreductase has also been identified whose activity in Mtb cells is involved in PA-824 activation and activity (2). Reduction of PA-824 to its des-nitroimidazole metabolite by this nitroreductase is associated with generation of reactive nitrogen species, including nitric oxide. PA-824 is active against Mtb isolates resistant to single or multiple antituberculous drugs and has proven effective in shortening treatment time of drug-sensitive Mtb in a murine model of tuberculosis (TB) as part of novel drug regimens (4).

Moreover, PA-824 was highly active as monotherapy in a 14-day dose ranging early bactericidal activity (EBA) study in humans where similar efficacy profiles were observed across all doses assessed (200–1200 mg/day) (5). In EBA studies, the rate of change over time in the number of Mtb colony forming units per mL of sputum in an overnight sputum collection is used to compare different dosing and treatment regimens. In a follow up study exploring a lower dose range (50-200 mg/day), a dose-response trend was detected with doses 100mg/day and above showing similar efficacy profiles (6). In addition, in a 14-day EBA study of novel antituberculosis drug combinations, the combination of PA-824 (200 mg/day), moxifloxacin (400 mg/day) and pyrazinamide (25 mg/kg-day) demonstrated EBA activity comparable to the current WHO recommended gold standard therapy of rifampicin, isoniazid, pyrazinamide and ethambutol (7). Based on the results of these EBA studies, the expected therapeutic doses of 100 mg/day and 200 mg/day are being evaluated in longer term trials in patients with pulmonary TB.
The current treatment of TB is greatly complicated by the human immunodeficiency virus (HIV) co-epidemic. In 2011, 13% of the estimated 8.7 million new cases of TB, were co-infected with HIV and of the 1.4 million deaths from TB, 430,000 deaths were in patients who were also HIV-positive (8). The treatment of TB alone requires the use of multiple antibiotics to prevent the development of drug resistance. The use of multiple antiretroviral agents to treat HIV infection greatly increases the likelihood of drug-drug interactions for patients with both infections. Many important drugs used to treat HIV infection such as the protease inhibitors nevirapine, lopinavir, and ritonavir are substrates of CYP3A4, and/or p-glycoprotein and have interactions with TB drugs such as rifampicin, that are inducers of hepatic enzymes (9). In vitro data indicate that PA-824 is not an inducer, but is a weak substrate and potential weak inhibitor of CYP3A4 (data not shown). An assessment of PA824’s drug-drug interaction potential is critical to determine its place in future TB therapy.

The present study was designed to ascertain whether PA-824 affected the pharmacokinetics (PK) of midazolam, a sensitive probe substrate and representative compound for drugs metabolized by CYP3A4. Midazolam is one of the most commonly used in vivo and in vitro CYP3A4 probe substrates for drug-drug interaction studies.

**MATERIALS AND METHODS**

**Study design.** A Phase I study was conducted to evaluate the effects of the multiple-dose administration of PA-824 on the PK of orally administered midazolam and to evaluate the safety and tolerability of PA-824 when coadministered with midazolam. The study was an open-label, multidose, fixed-sequence design performed in keeping with the United States Food and Drug Administration’s industry guidance, “Drug Interaction
The clinical study was conducted by Celerion Inc. (formerly MDS Pharma Services Inc.) in Lincoln, NE, under the sponsorship of the Global Alliance for TB Drug Development. Subjects were enrolled into the study and housed in the clinical facility from the evening of Day 1 (at least 15 hours before dosing) through the morning of Day 18. The total study duration for each subject from check-in through study termination (including a 2-day washout) spanned 18 nights/19 days, with follow up evaluation over 3 months. All subjects received an initial single oral dose of 2 mg midazolam CIV hydrochloride syrup (Boehringer Ingelheim/Roxane Laboratories) administered with 240 mL of water followed by a 2-day washout. Following the washout, all subjects received 400 mg of PA-824 (2 X 200 mg tablets) administered with 240 mL of water once daily for 14 consecutive days. On the 14th day of PA-824 dosing, a single oral dose of 2 mg midazolam was coadministered with 400 mg PA-824 with 240 mL of water. Subjects fasted for a minimum of 8 hours prior to and 4 hours following dosing. Water was permitted ad libitum one hour prior to and then again one hour following dosing. Subjects remained in an upright position (seated or standing) for 4 hours following dosing. Dietary restrictions included caffeine, alcohol, poppy seeds and grapefruit products prior to and throughout the study. No medication was permitted without sponsor approval except acetaminophen.

**Subject inclusion/exclusion criteria.** Subjects were included if they were healthy non-smokers aged 19-50, with a BMI 18 to 29 inclusive with negative tests for alcohol and drugs of abuse.
Subjects were excluded if they had any clinically significant history or presence of a cardiovascular, pulmonary, hepatic, renal, hematologic, gastrointestinal, endocrine, immunologic, dermatologic, or neurologic disease/disorder. Subjects also were excluded if they had a psychological, psychiatric, or metabolic disorder (including eating disorders) or if they had any acute illness within 4 weeks. Female subjects were excluded if they were pregnant (positive test for serum human chorionic gonadotropin at screening or check-in), breastfeeding, or planning to conceive a child within 30 days of cessation of treatment. Male subjects were excluded if they were planning to father a child within 12 weeks of cessation of treatment. Finally, subjects were excluded if they had a lens opacity or evidence of lens opacity on slit lamp ophthalmologic examination to follow up on findings that some rats developed cataracts in toxicology studies at high doses over 3 to 6 months of dosing.

All subjects provided written informed consent prior to participation in the study. Study protocols and consent forms were reviewed and approved by Celerion’s Institutional Review Board, and the study was conducted in accordance with U.S. Code of Federal Regulations (21 CFR Part 50, 56, and 312) principles and requirements and International Conference on Harmonisation guidelines (ICH E6).

**Sampling.** PK blood samples for midazolam (1 x 6 mL) were collected prior to dosing and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, and 24 hours after each 2 mg oral dose of midazolam. PK blood samples for PA-824 (1 x 6 mL) were collected predose on the 12th and 13th days of consecutive PA-824 dosing and predose on the 14th day prior to coadministration.
Bioanalytical methods. Blood samples were collected and centrifuged, and plasma was separated and stored at −20°C. Plasma samples were analyzed for PA-824, midazolam, and the midazolam metabolite 1-hydroxy midazolam using separate validated liquid chromatography/mass spectrometry methods developed at Covance Laboratories, Inc. PA-824 and the internal standard (ISTD; added during sample processing), triazolam, were extracted from human plasma samples using liquid-liquid extraction. After evaporation under nitrogen, the residue was reconstituted and analyzed using liquid chromatography with tandem mass spectrometric detection. The PA-824 calibration range for quantitation was 10 to 10000 ng/mL. Accuracy ranged from 96.3 to 100% and precision (RSD) ranged from 1.7% to 4.4%.

Midazolam, 1-hydroxy midazolam, and the ISTD (added during sample processing) alpha-hydroxytriazolam were extracted from human plasma samples using liquid-liquid extraction. After evaporation under nitrogen, the residue was reconstituted and analyzed using liquid chromatography with tandem mass spectrometric detection using a validated method proprietary to Covance Laboratories, Inc. The midazolam and 1-hydroxy midazolam calibration range for quantitation was 0.1 to 100 ng/mL. Accuracy ranged from 102.0% to 102.7% and 102% to 103.3% for midazolam and 1-hydroxy midazolam, respectively. Precision (RSD) ranged from 4.5% to 6.3% and 3.3% to 4.7% for midazolam and 1-hydroxy midazolam, respectively.

Pharmacokinetic analysis. Midazolam and 1-hydroxy midazolam plasma PK parameters were calculated for each subject when midazolam was dosed alone and in combination with PA-824 by applying a noncompartmental approach using WinNonlin®.
Professional Version 5.0.1 (Pharsight Corp., Mountain View, California). The key PK parameters calculated for midazolam and 1-hydroxy midazolam included C\textsubscript{max} (maximum observed concentration), T\textsubscript{max} (time at which C\textsubscript{max} occurs), K\textsubscript{el} (terminal elimination rate constant), t\textsubscript{1/2} (elimination half-life), AUC\textsubscript{(0-t)} (area under the concentration-time curve during the dosing interval), and AUC\textsubscript{(0-\infty)} (area under concentration-time curve extrapolated to infinity).

Plasma concentrations below the limit of quantitation (BLQ) were handled in the following manner: The lower limit of quantitation for midazolam and 1-hydroxy midazolam bioanalysis was 0.1 ng/mL. PK parameters were not calculated for subjects for whom there was insufficient data. For the calculation of the PK parameters, plasma concentrations that were BLQ prior to the first quantifiable concentration were set to zero and plasma concentrations BLQ after the first quantifiable concentration were treated as missing. For each subject, the elimination rate constant (K\textsubscript{el}) was estimated by unweighted log-linear regression using at least 3 data points from the last portion of the plasma concentration profile according to the least-squares approach ending with the last concentration prior to the first assay that was below the limit of quantification.

Elimination half-life (t\textsubscript{1/2}) was calculated from K\textsubscript{el}, using the formula ln(2)/K\textsubscript{el}. The K\textsubscript{el} was not assigned if the terminal elimination phase was not apparent, if C\textsubscript{max} was one of the 3 last data points, or if the R\textsuperscript{2} value was less than 80%. In cases where the K\textsubscript{el} interval was not assigned, the values of K\textsubscript{el}, AUC\textsubscript{(0-inf)}, and t\textsubscript{1/2} were considered not calculable and were not reported. When the resulting t\textsubscript{1/2} was more than half as long as the sampling interval, the K\textsubscript{el} values and associated parameters [t\textsubscript{1/2} and AUC\textsubscript{(0-inf)}] were not presented. AUCs were calculated using linear trapezoidal summation from time zero.
to the specified timepoint (either the last available timepoint, or by extrapolation to infinity).

**Statistical analysis.** All descriptive and inferential statistics were calculated in SAS Version 9.1.3. Plasma concentration values for midazolam, 1-hydroxy midazolam, and PA-824 were listed and summarized using descriptive statistics. Descriptive statistics were calculated for PK parameters. The PK endpoints $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and $C_{\text{max}}$ for midazolam and 1-hydroxy midazolam were compared between midazolam alone versus midazolam with PA-824 using an analysis of variance (ANOVA) model. The ANOVA model using SAS® PROC Mixed procedure included treatment, period, and sequence as fixed effects, and subject-within-sequence as a random effect. For this study, a potentially clinically important effect of PA-824 on midazolam pharmacokinetics, as specified in the protocol, would be concluded if the 90% confidence interval (CI) for the geometric mean ratios (GMRs) of $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, and $C_{\text{max}}$ were not contained within the prespecified “no-effect” 50%–200% CI limit. If the GMRs fell below 50% or above 200%, then a clinically important effect would be concluded. For the purposes of this study, a clinically important effect was considered to be a drug interaction of sufficient magnitude to require dose adjustment of drugs predominantly metabolized by CYP3A when co-administered with PA-824. If the GMRs were within the 50%–200% limit, then sequentially narrower criteria would be tested i.e. 70%–143%. If these narrower criteria were met, then the standard “no-effect” or bioequivalence criteria, 80%–125%, were to be assessed. The intended sample size was based on the known variability in AUC and $C_{\text{max}}$ associated with midazolam. Sample size calculations were based on a study by Stoch, et al. (2009) (11) which reported 90% CI’s for geometric
mean ratio (GMR) of midazolam AUC’s based on within-subject AUC variability and midazolam 2-mg oral doses in the presence of ketoconazole. Natural log-scale SD’s computed from those CI’s were 0.22 for AUC and 0.31 for Cmax. Twelve subjects provided greater than 96% power of yielding a 90% CI for AUC or Cmax within 0.5 to 2.0 bounds if the true underlying GMR was 1.00. Midazolam and 1-hydroxy midazolam T1/2 and Tmax were compared between midazolam alone versus midazolam with PA-824 using the t-test and Wilcoxon ranked sign test. If T1/2 and Tmax data failed tests for normality, only the Wilcoxon ranked sign test results were reported.

RESULTS

A total of 14 healthy male (n=10) and female (n=4) subjects participated in this clinical study to assess the safety, tolerability, and PK parameters of midazolam when coadministered with PA-824. All subjects were aged 19 to 46 (mean 27.2 years), had a body mass index of 18 to 29 (mean 25.4 kg/m²), and were medically healthy as determined by the Principal Investigator based on their medical history, clinical laboratory results, 12-lead electrocardiograms (ECGs), and physical examination. At both screening and check-in, subjects had negative urine test results for alcohol and other drugs of abuse, such as amphetamines, cannabinoids, and cocaine metabolites. Study participants represented a racially diverse group consisting of one Asian, one black, two Hispanics and ten white subjects. All 14 subjects who were enrolled in the study, completed all study procedures and there were no dropouts.

Pharmacokinetics. Mean plasma concentrations for midazolam and 1-hydroxy midazolam are shown in Figures 1 and 2, respectively. Pharmacokinetic (PK) parameters for midazolam and 1-hydroxy midazolam are provided in Table 1. The significant
presence of BLQ plasma concentrations resulted in the loss of AUC(0-inf) and t1/2 data for
the midazolam data of one subject and 1-hydroxy midazolam data for four subjects. Pre-
dose PA-824 plasma concentrations (mean (SD)) on the 12th, 13th and 14th day of once-
daily administration of 400 mg PA-824 were 1637 (610) ng/mL, 1581 (539) ng/mL and
1607 (556) ng/mL, respectively. PA-824 plasma concentrations showed no sign of
additional accumulation, indicating that steady-state had been achieved for PA-824 prior
to the dosing of midazolam. These PA-824 plasma concentrations are similar to those
found in earlier multiple-dose studies using similar doses.

The %GMRs (90% CI) when midazolam was coadministered with PA-824 as
compared to when midazolam was administered alone were Cmax = 83.63% (75.11% –
93.11%), AUC(0-t) = 84.61% (74.21% – 96.47%) and AUC(0-inf) = 84.45% (73.79% –
96.64%). The 90% CIs for the %GMRs of AUCs and Cmax were all within the pre-
defined 50-200% and 70%-143% “no-effect” limit, however the upper limit of the CIs
was less than 100%. The 90% CIs for the %GMRs of the AUCs and Cmax were not within
the “no-effect” limit of 80%-125%. The total extent (AUC) and peak (Cmax) exposure of
midazolam decreased by approximately 15-16% when midazolam was coadministered
with PA-824 as compared with midazolam administered alone. Based on the
nonparametric Wilcoxon ranked sign test, midazolam Tmax (p= 0.891) and t1/2 (p= 0.893)
were not different between both treatments.

As seen in Table 2, a statistical analysis of 1-hydroxy midazolam PK parameters
demonstrated that the %GMRs (90% CI) when midazolam was coadministered with PA-
824 as compared to when midazolam was administered alone were $C_{\text{max}} = 105.23\%$
(93.13\% – 118.9\%), $AUC(0-t) = 113.98\%$ (105.53\% – 123.09\%) and $AUC(0-\text{inf}) = 112.68\%$
(103.07\% – 123.18\%). The lower bound of the 90% CIs for the %GMRs of the AUCs
was greater than 100%, but all were within the “no-effect” limit of 80%–125%. Based on
the nonparametric Wilcoxon ranked sign test, 1-hydroxy midazolam $T_{\text{max}} (p= 0.988)$ and
t1/2 (p= 1.00) were not different between both treatments.

**Safety and tolerability.** PA-824 was well tolerated throughout the study and
when coadministered with midazolam. No serious adverse events occurred. The only
adverse events reported by more than one subject during any treatment period were
headache, nausea, abdominal discomfort, and diarrhea. These events were reported more
commonly by subjects during the periods when PA-824 was administered alone or in
combination with midazolam. There was a trend towards increased serum creatinine
centations during dosing with PA-824, consistent with an earlier study that
determined higher doses of PA-824 do not affect renal function but may increase serum
creatinine by inhibiting renal tubular secretion of creatinine, a clinically benign effect that
has been seen with several marketed drugs (12). Mean (SD) serum creatinine
concentrations were 0.785 (0.173) at check-in, 0.811 (0.184) when midazolam was dosed
alone, 0.916 (0.175) following seven days of once daily dosing of PA-824 and 0.955
(0.189) following 14 days of once daily dosing of PA-824 with co-administration of
midazolam on the 14th day. All other mean laboratory parameters for serum chemistry,
hematology, and urinalysis remained within reference range. There were no remarkable
findings in the vital signs, ECGs, physical examinations, visual acuity tests, and slit lamp
examinations in this study.
When this study was designed, the clinical dose range in patients with TB had not been determined. The 400 mg PA-824 dose used in this study was chosen as the highest dose that might be used in future clinical studies. The 400 mg PA-824 dose was expected to produce the maximum change in midazolam exposure relevant to the clinic. Any effects observed at the 400 mg PA-824 dose level could be extrapolated to lower doses of PA-824, potentially requiring additional studies to appropriately characterize the drug interaction at lower clinical doses. The 0.5 to 2.0 limit for the 90% CI was chosen as a “no-effect” criterion based on a clinical assessment that if dose-adjustment of important drugs metabolized by CYP3A were required during co-administration of PA-824 then continued development of PA-824 as a therapy for TB would not be warranted. The 2 mg oral midazolam dose is at the lower range of that typically used in drug-drug interaction studies, but was chosen due to concerns over the possible magnitude of CYP3A time-dependent inhibition following 14 days dosing of PA-824.

The total extent (AUC) and peak (C_{max}) exposure of midazolam decreased by approximately 16% and 15%, respectively, with the concomitant coadministration of midazolam and PA-824 400 mg as compared with the administration of midazolam alone. The total extent (AUC) and peak (C_{max}) exposure of 1-hydroxy midazolam increased 14 and 5%, respectively, with the concomitant co-administration of midazolam and PA-824 as compared with the administration of midazolam alone. The 90% CI for the GMRs of AUC_{(0-\infty)}, AUC_{(0-\infty)}, and C_{max} for midazolam and 1-hydroxy midazolam were all within the prespecified “no-effect” 50–200% limit and within the narrower prespecified 70–143% limit, whereas only the 1-hydroxy midazolam GMRs...
also fell within the default bioequivalence 80-125% limit. The relatively small decrease in midazolam exposure together with the small increase in 1-hydroxy midazolam exposure observed when midazolam is co-administered appears consistent with a small inductive effect by PA-824 on CYP3A. This result appears inconsistent with in-vitro data indicating that PA-824 is a weak inhibitor of CYP3A4 and exhibits metabolism-dependent inhibition. However, our in-vitro metabolism data are too limited to indicate the likely mechanism. It has been reported that some metabolism-dependent inhibitors can form relatively stable PA-824 metabolite enzyme complexes resulting in decreased degradation of the enzyme itself and ultimately higher steady-state levels of active enzyme. Midazolam is known to exhibit allosteric activation of CYP3A mediated metabolism with some drugs. Allosteric activation involves the binding of an ‘activator’ to the enzyme resulting in an increased rate of metabolism. 1-hydroxy midazolam forms a glucuronide metabolite. Glucuronide metabolites can be excreted by transporters in the kidney and other tissues. Increased serum creatinine concentrations were reported in this study. It is possible that PA-824 inhibition of a common transporter for creatinine and 1-hydroxy midazolam might have led to the increased 1-hydroxy midazolam exposure observed here. However, the decrease in midazolam exposure seen in this study is not consistent with inhibition of a renal transporter. This study demonstrates that PA-824 at the 400 mg dose level does not produce changes in midazolam exposure that would require dose-adjustment. Any changes in midazolam exposure that occur for the 100 mg and 200 mg PA-824 doses are likely to be even less than that observed for the 400 mg dose. Therefore, this study indicates that PA-824 is not likely to affect the pharmacokinetics of CYP3A metabolized drugs to a clinically
meaningful extent. Most importantly, concomitant use of PA-824 is not expected to produce significant drug interactions with anti-retroviral drugs that are substrates of CYP3A4, such as the protease inhibitors. Overall, single doses of oral midazolam (2 mg) administered alone and then following multiple doses of PA-824 (400 mg daily for 14 days) with a single oral dose of midazolam (2 mg on Day 14 of PA-824 dosing) were well tolerated by the subjects in this study.

ACKNOWLEDGEMENTS

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REFERENCES


FIGURE LEGEND

FIG. 1. Mean (+/- SD) plasma midazolam concentrations by time following 2 mg oral dose.

FIG. 2. Mean (+/- SD) plasma 1-hydroxy midazolam concentrations by time following a 2 mg oral dose of midazolam.
TABLE 1. Midazolam and 1-Hydroxy midazolam pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Treatment A (Reference)</th>
<th>Treatment B (Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Midazolam alone</td>
<td>Midazolam + PA-824</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD (N)</td>
<td>Mean ± SD (N)</td>
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<tr>
<td>Midazolam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>11.9 ± 5.46 (14)</td>
<td>9.64 ± 3.43 (14)</td>
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<tr>
<td>AUC&lt;sub&gt;(0-t)&lt;/sub&gt; (ng*hr/mL)</td>
<td>30.7 ± 15.3 (14)</td>
<td>25.3 ± 10.50 (14)</td>
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<td>AUC&lt;sub&gt;(0-inf)&lt;/sub&gt; (ng*hr/mL)</td>
<td>32.1 ± 15.7 (14)</td>
<td>25.0 ± 9.56 (13)</td>
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<td>CL/F (L/hr)</td>
<td>75.8 ± 32.0 (14)</td>
<td>91.5 ± 36.2</td>
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<td>t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>0.505 (0.499, 1.00) (14)</td>
<td>1.00 (0.499, 1.00) (14)</td>
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<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</td>
<td>5.69 ± 2.14 (14)</td>
<td>5.44 ± 2.4400 (13)</td>
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<td>1-Hydroxy midazolam</td>
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<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>5.32 ± 2.48 (14)</td>
<td>5.42 ± 2.17 (14)</td>
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<tr>
<td>AUC&lt;sub&gt;(0-t)&lt;/sub&gt; (ng*hr/mL)</td>
<td>12.0 ± 5.23 (14)</td>
<td>13.7 ± 6.24 (14)</td>
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<td>AUC&lt;sub&gt;(0-inf)&lt;/sub&gt; (ng*hr/mL)</td>
<td>12.9 ± 5.82 (13)</td>
<td>14.6 ± 7.73 (10)</td>
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<td>t&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>0.505 (0.499, 1.00) (14)</td>
<td>1.00 (0.499, 1.00) (14)</td>
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<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt; (hr)</td>
<td>4.09 ± 2.16 (13)</td>
<td>4.45 ± 2.87 (10)</td>
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</table>

*<sup>T</sup>max is presented as median and range (Minimum, Maximum). Other values are arithmetic means ± standard deviations.

Note. N= number of subjects contributing data; C<sub>max</sub> = maximum observed concentration; T<sub>max</sub> = time at which C<sub>max</sub> occurs; t<sub>1/2</sub> = elimination half-life; AUC(0-t) = area under the concentration-time curve during the dosing interval; AUC(0-inf) = area under concentration-time curve extrapolated to infinity.
TABLE 2. Statistical comparisons of Midazolam and 1-Hydroxy midazolam pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Treatment A (Reference)</th>
<th>Treatment B (Test)</th>
<th>% Geometric Mean Ratio (Test: Reference)</th>
<th>Confidence Interval (90% Confidence)</th>
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<td></td>
<td>Midazolam alone</td>
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<td></td>
<td>Geometric LS Mean (N)</td>
<td>Geometric LS Mean (N)</td>
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<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>10.82 (14)</td>
<td>9.05 (14)</td>
<td>83.63</td>
<td>75.11-93.11</td>
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<td>AUC&lt;sub&gt;(0-t)&lt;/sub&gt; (ng*hr/mL)</td>
<td>27.63 (14)</td>
<td>23.38 (14)</td>
<td>84.61</td>
<td>74.21-96.47</td>
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<tr>
<td>AUC&lt;sub&gt;(0-inf)&lt;/sub&gt; (ng*hr/mL)</td>
<td>28.97 (13)</td>
<td>24.47 (13)</td>
<td>84.45</td>
<td>73.79-96.64</td>
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<tr>
<td>1-Hydroxy midazolam</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>4.76 (14)</td>
<td>5.00 (14)</td>
<td>105.23</td>
<td>93.13-118.9</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-t)&lt;/sub&gt; (ng*hr/mL)</td>
<td>10.86 (14)</td>
<td>12.38 (14)</td>
<td>113.98</td>
<td>105.53-123.09</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;(0-inf)&lt;/sub&gt; (ng*hr/mL)</td>
<td>11.57 (10)</td>
<td>13.04 (10)</td>
<td>112.68</td>
<td>103.07-123.18</td>
</tr>
</tbody>
</table>

Note. N = number of subjects contributing data; C<sub>max</sub> = maximum observed concentration; AUC(0-t) = area under the concentration-time curve during the dosing interval; AUC(0-inf) = area under concentration-time curve extrapolated to infinity; LS Mean = least-squares mean.
Midazolam + PA-824

Time (hours)

1-Hydroxy midazolam plasma concentration (ng/mL)

- Midazolam
- Midazolam + PA-824