Clinical Pharmacology in Drug Development
2019, 8(4) 529–540

DOI: 10.1002/cpdd.660

A Cocktail Interaction Study Evaluating the Drug-Drug Interaction Potential of the Perpetrator Drug ASP8477 at Multiple Ascending Dose Levels

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Abstract

ASP8477 (molecular weight 325.36 g/mol) is a fatty acid amide hydrolase inhibitor intended for the treatment of neuropathic pain. Results from in vitro studies indicated that ASP8477 is a direct inhibitor of cytochrome P450 (CYP) 2C8, 2C9, 2C19, 2D6, and 3A4 enzymes at expected efficacious concentrations, with the strongest effect on CYP2C19; a phase 1 study confirmed ASP8477 to be a CYP2C19 inhibitor. To further evaluate the interaction potential of ASP8477, a cocktail interaction study was performed using the probe substrates of the validated Inje cocktail containing losartan (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), and midazolam (CYP3A4). Because ASP8477 shows nonlinear pharmacokinetics, 3 doses (20, 60, and 100 mg) were evaluated. This study revealed changes in exposure (area under the concentration-time curve) of the probe substrates after treatment with 20, 60, and 100 mg ASP8477, respectively, compared with substrates alone with geometric mean ratios of: midazolam, 119%, 151%, and 158%; losartan, 107%, 144%, and 190%; omeprazole, 213%, 456%, and 610%; and dextromethorphan, 138%, 340%, and 555% (with increasing doses, respectively). Overall, ASP8477 was a weak inhibitor for CYP3A4 and CYP2C9, a moderate to strong inhibitor for CYP2C19, and a weak to strong inhibitor for CYP2D6, with doses from 20 to 100 mg. This study confirmed that the Inje cocktail approach was able to detect relevant drug-drug interactions impacting further development of ASP8477 and future therapeutic use. With the approach used here, the inhibiting effect of a perpetrator drug on different CYP enzymes can be evaluated, and at different doses, thereby supporting dose recommendations for potential interactions.

Keywords

drug-drug interaction, cytochrome P450, ASP8477, inhibition, nonlinear pharmacokinetics

ASP8477 is a novel, potent, and selective fatty acid amide hydrolase (FAAH) inhibitor intended for the treatment of neuropathic pain, but development has since been discontinued. FAAH is an enzyme responsible for the breakdown of endogenous cannabinoids such as N-arachidonoyl ethanolamine (anandamide; AEA). AEA exerts anti-inflammatory and antihyperalgesic effects by binding to and activating the cannabinoid receptors (CB1 and CB2) and noncannabinoid receptors (TRPV1, PPAR, and opioid receptors) within the central nervous system and the periphery.[1] The actions of AEA are short in duration due to rapid catabolism by the FAAH enzyme, and therefore, the inhibition of FAAH would lead to increased levels of AEA. ASP8477 acts both centrally and peripherally by increasing AEA levels and has shown analgesic effects in nonclinical models for neuropathic pain without untoward behavioral side effects.[2]

A large proportion of the target population for ASP8477 was expected to consist of elderly patients, who commonly use concomitant medication. Therefore, it was essential to investigate the potential for drug-drug interactions (DDIs).

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Submitted for publication 25 April 2018; accepted 4 January 2019.

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Table 1. Results of In Vitro CYP Inhibition Studies in Human Liver Microsomes Using Probe Substrates and Corresponding R Values as Defined by the FDA and the EMA

CYP		K_i (μmol/L)	ASP8477 20 mg QD	ASP8477 60 mg QD	ASP8477 100 mg QD
2C8	...	15	1.074[a]/0.011[b]	1.385[a]/0.06[b]	1.785[a]/0.12[b]
2C9	Losartan	8	1.140[a]/0.021[b]	1.723[a]/0.11[b]	2.472[a]/0.22[b]
2C19	Omeprazole	1	2.116[a]/0.167[b]	6.781[a]/0.87[b]	12.775[a]/1.77[b]
2D6	Dextromethorphan	9.8	1.114[a]/0.017[b]	1.590[a]/0.09[b]	2.202[a]/0.18[b]
3A4	Midazolam	18	1.062[a]/0.009[b]	1.321[a]/0.05[b]	1.654[a]/0.10[b]

Calculations were based on the following converted values (molecular weight of ASP8477 is 325.36 g/mol) observed in the MAD study after 10 days of dosing in healthy female subjects:
At 20 mg QD, $C_{max,total}$ 1.116 μmol/L; $C_{max,unbound}$ 0.1674 μmol/L.
At 60 mg QD, $C_{max,total}$ 5.781 μmol/L; $C_{max,unbound}$ 0.86715 μmol/L.
At 100 mg QD, $C_{max,total}$ 11.775 μmol/L; $C_{max,unbound}$ 1.76625 μmol/L.
C_{max} indicates maximum blood concentration; CYP, cytochrome P450; EMA, European Medicines Agency; FDA, US Food and Drug Administration; IC_{50}, half-maximal inhibitory concentration; K_i, inhibitory constant; MAD, multiple ascending dose; QD, once daily.
[a]FDA definition is $R \geq 1.1$ where $R = 1 +$ (total C_{max}/K_i).
[b]EMA definition is $R \geq 0.02$ where $R =$ unbound C_{max}/K_i.

The proposed in vitro metabolism of ASP8477, or 3-pyridyl 4-(phenylcarbamoyl)piperidine-1-carboxylate,[2] in human hepatocytes involved hydroxylation and subsequent glucuronidation and sulfation of the benzene ring, oxidation of the pyridine ring, and cleavage between the piperidine ring and the carboxyl group with subsequent hydroxylation of the benzene ring. CYP2C8, CYP2C9, and CYP3A4 were considered to be involved in the metabolism of ASP8477 based on CYP identification studies; hence, ASP8477 is not expected to be a sensitive substrate for any particular CYP enzyme. In vitro CYP isozyme inhibition studies showed that ASP8477 caused direct inhibitory effects on CYP3A4, 2C8, 2C9, 2C19, and 2D6 enzyme activities (but not CYP1A2 and CYP2B6), with inhibitory constant (K_i) values of 18 μmol/L, 15 μmol/L, 8.0 μmol/L, 1.0 μmol/L, and 9.8 μmol/L, respectively. In addition, in a first-in-human study, a DDI arm was included to investigate the inhibitory effect of ASP8477 on CYP2C19, which according to in vitro data should show the strongest interaction. The study was a drug-interaction, open-label, randomized, 2-period crossover design with administration of a single oral 20 mg dose of omeprazole (CYP2C19 substrate) alone or in combination with a 100 mg dose of ASP8477 in female subjects. ASP8477 strongly inhibited the metabolism of omeprazole, increasing the maximum concentration (C_{max}) and area under the concentration-time curve from time 0 to infinity (AUC_{inf}) by 3.5-fold and 7-fold, respectively. To investigate the inhibitory effects of ASP8477 on CYP enzymes that exhibited an interaction in vitro, the present cocktail study was designed. No clinically relevant effect was observed on the induction of CYP enzymes in vitro.

In clinical studies ASP8477 AUC and C_{max} increased more than dose proportionally in the dose range of 20-100 mg. The mean terminal half-life ($t_{½}$) increased with increasing dose and slightly increased over time from day 1 to day 10 for each dose. In general, the total clearance of ASP8477 from plasma after oral administration, and the volume of distribution after nonintravenous administration decreased with dose and also decreased from first to last dosing. Mean trough concentration-time profiles indicated that steady state was achieved on day 1 with ASP8477 20 mg once daily, on day 3 with 60 mg once daily, and on day 10 with 100 mg once daily. Approximately 1% of the ASP8477 dose is excreted in urine as unchanged drug.

Based on the in vitro K_i values and the ASP8477 plasma concentrations (converted to molar units with a molecular weight of 325.36 g/mol) observed in the clinical phase 1 multiple ascending dose study performed with ASP8477 in healthy female subjects, the R values calculated, as outlined by US Food and Drug Administration (FDA)[3] and the European Medicines Agency (EMA),[4] suggest a weak to strong likelihood of an in vivo inhibition of CYP2C8, 2C9, 2C19, 2D6, and 3A4 at different ASP8477 exposure levels (Table 1). To evaluate the in vivo inhibition potential of ASP8477 as observed in vitro, the Inje cocktail was used in an open-label, 1-sequence, multiple ascending dose study in healthy female subjects. The validated Inje cocktail contains 5 probe substrates for 5 CYP isozymes that do not interfere with their respective metabolism; these are caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), and midazolam (CYP3A4).[5,6] All probe substrates were orally administered. Caffeine was administered because it was seen as a fixed part of the validated cocktail; however, because an interaction with 1A2 was not expected, caffeine concentrations were not measured. For CYP2C8,

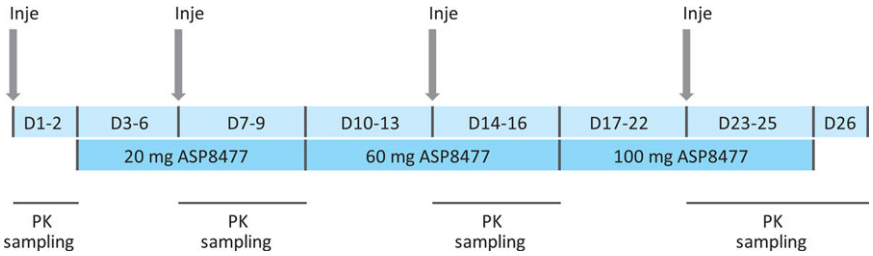

Figure 1. Study design. Inje cocktail includes 5 model substrates for 5 CYP isoenzymes: caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), and midazolam (CYP3A4).[6] CYP indicates cytochrome P450; D, day; PK, pharmacokinetic.

no suitable probe substrate for inclusion in a cocktail was available.

Because the pharmacokinetic (PK) properties of ASP8477 are nonlinear, 3 multiple-dose steady-state levels of ASP8477, covering the expected efficacious dose range of 20-100 mg, were used to evaluate the relative inhibitory potential of ASP8477 on these 4 CYP enzymes. To prevent tolerability issues occurring at the 100 mg dose, a dose titration was applied using a fixed sequence design.

Methods

Ethics

The original study protocol and the amendment were reviewed by the Independent Ethics Committee of Berlin, Germany, and approved. An Independent Ethics Committee–approved written informed consent was obtained from each subject before initiation of any study-specific procedures. The study was conducted in accordance with ethical principles that have their origins in the Declaration of Helsinki, Good Clinical Practice, the International Conference on Harmonisation guidelines, and applicable laws and regulations. The DDI study was conducted at a single center at PAREXEL (Berlin, Germany).

Subjects

Healthy, nonsmoking female subjects aged 18-65 years with a body mass index between 18 kg/m^2 and 30 kg/m^2 and weighing at least 50 kg were eligible for enrollment. The study was performed in women only because an effect of ASP8477 on the human male reproductive system could not be excluded by preclinical studies. All participants were to be in good health, determined by medical history, physical examination, routine laboratory screening, blood pressure measurements, and examination of electrocardiogram (ECG) recordings, and were to be in compliance with the inclusion criteria of the study. Subjects with any history or evidence of any clinically significant disease or malignancy that would influence the results of the study were excluded. Subjects were also excluded if any liver function tests were above the upper limit of normal or if the subject had any clinically significant abnormality following the investigator's review of her physical examination, blood pressure, ECG, and clinical laboratory tests at screening. Subjects whose genotype indicated that they may be poor metabolizers for CYP2C9, CYP2C19, and/or CYP2D6 were excluded.

Use of prescribed or over-the-counter drugs was not permitted for 4 weeks before admission to the clinical unit, except for acetaminophen (up to 2 g/day). Use of products containing grapefruit, marmalade, star fruit, and Seville orange (juice) was not permitted from 1 week before admission to the clinical unit until the end-of-study visit. Regular use of any inducer of metabolism (eg, barbiturates, rifampin) within 3 months before admission to the clinical unit was not permitted. Subjects with a history of smoking more than 10 cigarettes a day within the 2 months preceding clinical unit admission as well as those with a history of drinking over 14 units of alcohol per week within the 3 months before clinical unit admission were excluded (1 unit = approximately 10 g of pure alcohol). Consumption of beverages containing caffeine was not allowed during the residential period.

Study Design

This trial was a single-center, open-label, 1-sequence, multiple ascending dose DDI study in 14 healthy female subjects. Subjects received a single oral dose of the Inje cocktail (100 mg caffeine, 25 mg losartan, 20 mg omeprazole, 30 mg dextromethorphan, and 2 mg midazolam) on day 1 (Figure 1). Caffeine was administered because it was regarded as a fixed part of the validated cocktail; however, caffeine concentrations were not measured because no interaction with CYP1A2 was indicated. Thereafter, subjects received the Inje cocktail on 3 separate occasions under multiple dosing conditions of ASP8477, dosed orally in ascending order, on day 7 (20 mg ASP8477 once daily from day 3 to day 9), on day 14 (60 mg ASP8477 once daily from day 10 to day 16), and on day 23 (100 mg ASP8477 once daily from day 17 to day 25). The Inje

cocktail and ASP8477 were dosed orally at the same time under fasting conditions. The subjects stayed in the clinical unit from day –1 until day 26 and received standard meals and beverages during their stay.

Blood Sampling

Serial blood samples were collected in 5-mL tubes containing heparin as anticoagulant on days 1, 7, 14, and 23 for the analyses of plasma concentrations of losartan and its metabolite EXP-3174, omeprazole and its metabolite 5-hydroxyomeprazole, dextromethorphan and its metabolite dextrorphan, and midazolam and its metabolite 1-hydroxymidazolam. Blood samples were collected predose (before cocktail administration) and 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours after the cocktail administration on day 1 and predose, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, and 72 hours after cocktail administration on days 7, 14, and 23. Samples were cooled in an ice-water bath immediately after collection.

During the period of ASP8477 dosing, blood samples for the determination of ASP8477 minimum concentration (C_{trough}) were drawn into heparin-containing tubes immediately before the time of drug intake on days 4-6, 11-13, and 20-22. In addition, serial blood sampling for ASP8477 was performed predose on days 7, 14, and 23, and 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose in relation to ASP8477 administration to obtain a full steady-state ASP8477 PK profile.

Plasma was generated from each sample and stored at –70°C. Samples were shipped to the laboratories of SGS Céphac (Saint-Benoit, France) for the determination of ASP8477 concentration and to Pharmaceutical Product Development Inc (Middleton, Wisconsin, and Richmond, Virginia) for the determination of the concentration of the Inje cocktail substrates and metabolites.

Bioanalytical Methods

Concentrations of ASP8477, cocktail substrates, and their metabolites were determined using the liquid chromatography–tandem mass spectrometry methods that were validated in accordance with the FDA and EMA guidelines for bioanalytical method validation[7,8] including selectivity tests (Inje cocktail substrates and ASP8477). Stable isotope-labeled internal standards were used.

ASP8477 and its internal standard ASP8477-d_5 were extracted from plasma by solid-phase extraction on an HLB μElution Plate (Waters, Milford, Massachusetts). Extracts were injected on an XBridge 2.5 μm C_{18}, 2.1 mm × 50 mm analytical column (Waters) and eluted with a mobile phase consisting of a water-acetonitrile gradient with a fixed concentration of 1 mmol/L ammonium bicarbonate, before detection by an API4000 triple quadrupole mass spectrometer (AB Sciex, Framingham Massachusetts) in negative-ion mode. Single reaction monitoring (transition from m/z 324.3 to 94.0) was used for ASP8477 detection. The calibration range was 1-1000 ng/mL, and the intrarun accuracy, as determined during method validation, was between –1.0% and 6.7% with an intrarun precision of between 1.5% and 11.2%.

Following protein precipitation and solid-phase extraction, losartan was determined by the transition m/z 432.2 to 207.0, and its metabolite losartan acid (EXP-3174) by m/z of 437.2 to 235.1; the peak area ratio to the internal standard (losartan-d_9 and losartan acid-d_9, respectively) was used for quantification. The calibration range for both analytes was from 0.5 to 500 ng/mL; intrarun accuracy was between –2.0% and 2.5%, and the intrarun precision was between 3.0% and 7.6%. Following protein precipitation, omeprazole was determined by the transition m/z 364.3 to 198.3, and 5-OH omeprazole by the transition m/z 362.3 to 214.4; omeprazole-d_3 and 5-hydroxyomeprazole-d_3 were used as internal standards. The calibration range was 1-1000 ng/mL, the intrarun accuracy was between –3.9% and 6.0%, and the intrarun precision between 2.4% and 8.3%. Following liquid-liquid extraction, dextromethorphan was determined by the transition m/z 272.1 to 215.1, and its metabolite dextrorphan by the transition m/z 258.1 to 199.1, using dextromethorphan-d_3 and dextrorphan-d_3 as internal standards. The calibration range was 0.05-50 ng/mL for dextromethorphan and 0.8-800 ng/mL for dextrorphan. Intrarun accuracies were between –3.6% and 4.8% and intrarun precisions between 2.4% and 9.6%. After liquid-liquid extraction, midazolam was determined by the transition m/z 326.1 to 291.3, and its metabolite 1-OH midazolam by the transition m/z 342.2 to 324.2; midazolam-d_4 and 1-OH-midazolam-d_4 were used as internal standards. The calibration range was 0.1-100 ng/mL, and intrarun accuracy was between –3.2% and 8.2% and intrarun precision between 2.0% and 11.6%.

Sample Size Calculation

Based on literature data for midazolam and omeprazole,[9] losartan,[10] and dextromethorphan,[11] the expected intrasubject coefficients of variation (CVs) for AUC and C_{max} of these substrates were losartan 33% to 47%, midazolam 30%, omeprazole 38% to 58%, and dextromethorphan 108% to 110%. From these numbers, 12 subjects provided adequate precision to assess the effect of 20, 60, and 100 mg ASP8477 on the estimated AUC_{inf} and C_{max} ratios between the cocktail administered alone and in combination with ASP8477, for each substrate of the cocktail, assuming a range of intrasubject CVs from 30% (midazolam) to 110% (dextromethorphan). To compensate for a potential dropout rate of up to 15%

during the study, 14 subjects were enrolled to ensure that there were approximately 12 subjects with complete data sets.

Data Analysis

Standard graphical displays were generated for plasma concentrations of all cocktail probe substrates and each one's major metabolite, and summary statistics were calculated. Noncompartmental PK data analysis was performed using Phoenix software version 6.2.1 (Pharsight Cooperation, Mountain View, California). The following plasma PK parameters were assessed and tabulated for each individual cocktail probe and its metabolite: AUC_{inf}, AUC from time 0 to time of last measurable concentration, C_{max}, $t_{½}$, lag time, time to maximum plasma concentration, CL/F (parent only), and ratio $AUC_{inf\ (metabolite)}/AUC_{inf\ (parent)}$. For ASP8477 in plasma, AUC for a dosing interval, C_{max}, C_{trough}, peak-trough ratio, and time to maximum plasma concentration were assessed. Summary statistics included number of subjects, mean, SD, median, minimum, maximum, and %CV for plasma concentration data and continuous PK parameters. Additionally, the geometric mean was calculated for C_{max} and AUC_{inf}. Summaries were provided by assessment day and by dose level of ASP8477.

For each cocktail probe substrate and its metabolite, the drug interaction between the substrates and ASP8477 at doses of 20, 60, and 100 mg was assessed by providing a 90% CI for the geometric mean ratio (GMR) estimated for the cocktail in combination with ASP8477 versus cocktail alone. This was based on a mixed-effects model of the log-transformed ratio of PK parameters (AUC and C_{max}) with the presence/absence of ASP8477 as a fixed effect and subject as a random effect. No adjustments for multiplicity were made in the statistical analyses. Predefined limits around the 90% CIs were not established, but a difference between treatments was concluded in case the CI fell outside of the 80% to 125% range.

Safety and Tolerability

All safety and tolerability data were summarized using descriptive statistics and were listed and summarized in tabular and/or graphical form by treatment and assessment day. An adverse event (AE) starting or worsening after the first study drug intake was considered a treatment-emergent AE (TEAE). The incidence and number of TEAEs were summarized by treatment, as applicable. Furthermore, the number of subjects with TEAEs was summarized by the *Medical Dictionary for Regulatory Activities* (version 14.0) system organ class and preferred terms. Similar summaries were presented for treatment-related TEAEs, serious TEAEs, and non-TEAEs. Vital signs and laboratory measurements were summarized by treatment and assessment day, as applicable, including change from baseline. ECG and physical examination data were summarized as appropriate.

The psychiatric tests (Bond-Lader and Bowdle visual analog scales [VAS] as well as Physician Withdrawal Checklist-20 questionnaires) were summarized by treatment and assessment day. The Bond-Lader VAS results were analyzed and grouped by "mood," "calmness," and "alertness." For the Bowdle VAS results, the factors "internal perception" and "external perception" were derived.

Results

Subjects

Fourteen subjects were enrolled in the study and received study drugs. All enrolled subjects were included in the safety analysis set and PK analysis set. Thirteen randomized subjects completed the study, and 1 subject discontinued the study on day 15 due to an AE of moderate severity (emotional lability). The emotional lability was considered to be possibly related to ASP8477 but not related to the Inje cocktail. For this subject, the last dose of ASP8477 was administered on day 12, and the last dose of cocktail was administered on day 7.

None of the subjects reported intake of previous medication from 4 weeks before admission. Based on the results of genotyping, none of the subjects participating in the study was predicted to be a poor metabolizer for CYP2C9, 2C19, and/or 2D6.[12–16] No subjects screened positive for drugs of abuse or alcohol or were pregnant. All recorded past or ongoing conditions and surgical procedures were not considered by the study investigators to influence study results.

The mean age of the subjects was 44.6 years (range 26-61 years), mean body weight was 61.6 kg, and the mean body mass index was 22.5 kg/m^2. All subjects were white except for 1 subject allocated to the combined group of white and Asian.

During the follow-up period, 1 subject received ophthalmic ofloxacin for the treatment of acute conjunctivitis from days 32 to 38. This was approximately 9 days after receiving the last dose of drug cocktail and approximately 7 days after receiving the last dose of ASP8477. This concomitant therapy was not expected to influence the study results. No other concomitant therapy was recorded.

Midazolam and 1-OH-Midazolam PK: Assessment of CYP3A4 Activity

The C_{max} and AUC_{inf} of midazolam increased after administration of the drug cocktail in the presence of 20, 60, and 100 mg ASP8477 compared with administration of the drug cocktail alone, with GMRs

of 118% and 119%, 145% and 151%, and 152% and 158%, respectively (Table 2 and Figure 2).

For 1-OH-midazolam, C_{max} decreased after administration of the drug cocktail in the presence of once daily 20, 60, or 100 mg ASP8477 compared with administration of the drug cocktail alone, with GMRs of 87%, 86%, and 83%, respectively. AUC_{inf} also decreased in the presence of 20 and 100 mg ASP8477 with GMRs of 88% and 87%, whereas AUC_{inf} was comparable after administration of the drug cocktail in the presence and absence of 60 mg ASP8477 (Table 2).

The mean metabolite-to-parent ratio (1-OH-midazolam/midazolam) based on AUC_{inf} decreased from 0.3370 (drug cocktail alone) to 0.2336, 0.1909, and 0.1759 in the presence of multiple doses of 20, 60, and 100 mg of ASP8477, respectively.

Losartan and EXP-3174 PK: Assessment of CYP2C9 Activity

The C_{max} of losartan increased after administration of drug cocktail in the presence of 20, 60, and 100 mg ASP8477 compared with administration of the drug cocktail alone, with GMRs of 120%, 120%, and 116%, respectively. AUC_{inf} of losartan in the presence of 20 mg ASP8477 was comparable to the AUC_{inf} of losartan alone, whereas AUC_{inf} increased in the presence of 60 and 100 mg ASP8477, with GMRs of 144% and 190%, respectively (Table 2 and Figure 2).

For EXP-3174, C_{max} and AUC_{inf} were comparable after administration of drug cocktail alone or in the presence of 20 mg ASP8477. Compared to administration of drug cocktail alone, C_{max} of EXP-3174 decreased with GMRs of 54% and 34%, and AUC_{inf} decreased with GMRs of 85% and 67% in the presence of ASP8477 60 and 100 mg, respectively (Table 2).

The metabolite-to-parent ratios (EXP-3174/losartan) based on AUC_{inf} were comparable in the absence (5.9) and presence (5.5) of 20 mg ASP8477, whereas the metabolite-to-parent ratios decreased in the presence of 60 and 100 mg ASP8477 to 3.6 and 2.3, respectively.

Omeprazole and 5-OH-Omeprazole PK: Assessment of CYP2C19 Activity

Compared to administration of drug cocktail alone, the C_{max} and AUC_{inf} of omeprazole increased in the presence of 20, 60, and 100 mg ASP8477 with GMRs of 207% and 213%, 296%, and 456%, and 367%, and 610%, respectively (Table 2 and Figure 2).

The C_{max} and AUC_{inf} of 5-OH-omeprazole were comparable based on the GMRs after administration of 20 mg omeprazole alone, and in the presence of 20 mg ASP8477. By contrast, the C_{max} of 5-OH-omeprazole decreased in the presence of 60 and 100 mg ASP8477 with GMRs of 65% and 52%, respectively, while the AUC_{inf} of 5-OH-omeprazole remained unchanged (Table 2).

The metabolite-to-parent ratio (5-OH-omeprazole/omeprazole), based on the AUC_{inf}, decreased with increasing ASP8477 dose from 1.053 (alone) to 0.1600 (in combination with 100 mg ASP8477 once daily). This change in metabolite-to-parent ratio is due to the increase in AUC_{inf} of omeprazole; the AUC_{inf} of 5-OH-omeprazole remained unchanged.

Dextromethorphan and Dextrorphan PK: Assessment of CYP2D6 Activity

Compared to 30 mg dextromethorphan administered alone, the C_{max} and AUC_{inf} of dextromethorphan increased in the presence of 20, 60, and 100 mg ASP8477, with GMRs of 121% and 138%, 253% and 340%, and 377% and 555%, respectively (Table 2 and Figure 2).

The C_{max} and AUC_{inf} of dextrorphan were comparable after administration of 30 mg dextromethorphan alone or in the presence of 20 and 60 mg ASP8477 (90% CI of the GMRs within 80.00% to 125.00%). However, in the presence of 100 mg ASP8477, the C_{max} of dextrorphan decreased, whereas AUC_{inf} increased with GMRs of 82% and 123% (Table 2).

The metabolite-to-parent ratios (dextrorphan/dextromethorphan) of AUC_{inf} were comparable in the presence (274.0) and absence (329.9) of 20 mg ASP8477, whereas the metabolite-to-parent ratios decreased in combination with 60 and 100 mg ASP8477 to 116.9 and 77.57, respectively.

ASP8477 PK

The PK parameters of ASP8477 (C_{max} and AUC_{tau}) increased in a more than dose-proportional manner (Table 3), in line with previously obtained clinical studies. Based on C_{trough} concentrations, steady-state conditions were reached before the cocktail dosing on days 14 and 23 for 60 and 100 mg ASP8477 (Figure 3). For 20 mg ASP8477 accumulation could not be observed, as most C_{trough} values of ASP8477 from days 4 to 8 were below the lower limit of quantification (Figure 4).

Safety and Tolerability

TEAEs occurred in 1 subject (7.1%) following administration of the drug cocktail alone, in 12 subjects (85.7%) following administration of 20 and 60 mg ASP8477, and in 13 subjects (100%) following administration of 100 mg ASP8477 (Supplementary Table 1); note that the observation periods were different (ie, 2 days with Inje cocktail alone, 5 days of 20 mg ASP8477, 5 days of 60 mg ASP8477, and 7 days of 100 mg ASP8477). AEs considered possibly or probably related to study drug by the investigator occurred in 11 subjects (78.6%)

Table 2. Summary Statistics of PK Parameters of Drug Cocktail Probes and Statistical Results of the Effect of 20, 60, and 100 mg ASP8477 Once Daily on a Single Dose of Drug Cocktail Probes on Days 7, 14, and 23, Respectively

Probe	n	C_{max} (ng/mL)	AUC_{inf} (h·ng/mL)	t_{max} (median [min-max]) (h)	$t_{½}$ (h)	CL/F (L/h)	C_{max} LSM Ratio (90% CI)	AUC_{inf} LSM Ratio (90% CI)
CYP3A4								
Midazolam alone	14	14 ± 5.3	32 ± 8.3	0.52 (0.48-1.0)	5.2 ± 1.6	66 ± 15		
Midazolam + 20 mg ASP8477 (day 7)	14	16 ± 5.7	38 ± 11	0.52 (0.50-1.0)	5.0 ± 0.93	56 ± 14	118 (103-135)	119 (111-128)
Midazolam + 60 mg ASP8477 (day 14)	13	20 ± 5.1	49 ± 15	0.52 (0.50-1.0)	5.1 ± 0.95	44 ± 13	145 (126-166)	151 (140-163)
Midazolam + 100 mg ASP8477 (day 23)	13	21 ± 5.1	51 ± 15	0.50 (0.50-1.0)	5.1 ± 1.2	42 ± 11	152 (132-174)	158 (147-170)
1-OH-Midazolam alone	14	5.5 ± 3.0	12 ± 6.1	0.52 (0.48-1.0)	7.8 ± 13			
1-OH-Midazolam + 20 mg ASP8477 (day 7)	14	4.4 ± 1.6	8.8 ± 1.9	0.52 (0.50-1.0)	3.4 ± 2.7		87 (74-103)	88 (78-99)
1-OH-Midazolam + 60 mg ASP8477 (day 14)	13[a]	4.4 ± 1.8	9.2 ± 2.0	0.52 (0.50-1.0)	4.9 ± 3.7		86 (72-102)	92 (81-105)
1-OH-Midazolam + 100 mg ASP8477 (day 23)	13[b]	4.3 ± 1.5	8.9 ± 2.7	0.50 (0.50-1.1)	4.0 ± 2.6		83 (70-99)	87 (76-100)
CYP2C9								
Losartan alone	14	64 ± 33	220 ± 100	1.0 (0.48-2.0)	3.7 ± 2.5	140 ± 61		
Losartan + 20 mg ASP8477 (day 7)	14	78 ± 37	230 ± 100	0.52 (0.50-2.0)	4.0 ± 2.2	130 ± 46	120 (98-147)	107 (99-117)
Losartan + 60 mg ASP8477 (day 14)	13	81 ± 59	320 ± 140	0.52 (0.50-2.0)	5.0 ± 2.2	96 ± 44	120 (97-148)	144 (132-158)
Losartan + 100 mg ASP8477 (day 23)	13	80 ± 64	410 ± 180	0.50 (0.50-2.0)	7.6 ± 3.3	72 ± 34	116 (94-142)	190 (174-208)
EXP-3174 alone	14[c]	100 ± 65	1100 ± 370	4.0 (4.0-8.1)	8.0 ± 0.64			
EXP-3174 + 20 mg ASP8477 (day 7)	14[c]	96 ± 48	1100 ± 280	5.0 (4.0-8.0)	8.8 ± 3.5		96 (82-112)	100 (88-113)
EXP-3174 + 60 mg ASP8477 (day 14)	13[d]	54 ± 26	960 ± 240	8.0 (4.0-12)	13 ± 3.7		54 (46-63)	85 (75-96)
EXP-3174 + 100 mg ASP8477 (day 23)	13[d]	35 ± 19	780 ± 250	8.10 (8.0-12)	12 ± 2.5		34 (29-40)	67 (59-76)
CYP2C19								
Omeprazole alone	14[a]	320 ± 150	630 ± 300	2.0 (0.98-4.0)	0.79 ± 0.17	40 ± 20		
Omeprazole + 20 mg ASP8477 (day 7)	14[d]	640 ± 190	1200 ± 520	2.0 (1.0-4.0)	0.77 ± 0.20	20 ± 10	207 (181-237)	213 (184-248)
Omeprazole + 60 mg ASP8477 (day 14)	13	910 ± 260	2500 ± 680	3.0 (1.1-4.0)	1.1 ± 0.25	8.8 ± 2.8	296 (257-339)	456 (394-527)
Omeprazole + 100 mg ASP8477 (day 23)	13	1100 ± 250	3300 ± 1100	3.0 (1.0-4.0)	1.5 ± 0.30	6.7 ± 2.4	367 (320-421)	610 (527-705)
5-OH-Omeprazole alone	14[c]	230 ± 76	550 ± 130	2.0 (0.98-4.0)	1.4 ± 0.56			
5-OH-Omeprazole + 20 mg ASP8477 (day 7)	14	230 ± 72	550 ± 87	2.5 (2.0-4.0)	1.1 ± 0.21		101 (89-114)	102 (94-108)
5-OH-Omeprazole + 60 mg ASP8477 (day 14)	13	150 ± 51	540 ± 94	3.1 (2.0-4.0)	1.6 ± 0.30		65 (57-74)	96 (90-103)
5-OH-Omeprazole + 100 mg ASP8477 (day 23)	13[d]	120 ± 34	520 ± 97	3.0 (1.0-4.0)	1.9 ± 0.32		52 (45-59)	93 (86-100)
CYP2D6								
Dextromethorphan alone	14[c]	1.3 ± 0.72	12 ± 7.9	2.0 (1.0-6.0)	8.0 ± 2.5	4000 ± 3200		
Dextromethorphan + 20 mg ASP8477 (day 7)	14	1.8 ± 1.2	16 ± 12	3.0 (2.0-6.0)	7.7 ± 2.1	3300 ± 2900	121 (99-148)	138 (115-166)
Dextromethorphan + 60 mg ASP8477 (day 14)	13	3.8 ± 2.3	39 ± 25	3.1 (2.0-6.1)	9.3 ± 1.4	1200 ± 1100	253 (207-309)	340 (282-412)
Dextromethorphan + 100 mg ASP8477 (day 23)	13	5.6 ± 3.1	65 ± 44	3.0 (2.0-6.0)	9.9 ± 1.5	720 ± 630	377 (309-460)	555 (459-671)
Dextrorphan alone	14	490 ± 133	2400 ± 450	2.0 (1.0-4.0)	5.8 ± 1.2			
Dextrorphan + 20 mg ASP8477 (day 7)	14	470 ± 110	2400 ± 420	2.0 (2.0-3.0)	6.5 ± 1.7		96 (90-101)	100 (97-104)
Dextrorphan + 60 mg ASP8477 (day 14)	13	430 ± 110	2700 ± 510	3.1 (2.0-4.0)	8.8 ± 2.1		88 (83-93)	113 (109-117)
Dextrorphan + 100 mg ASP8477 (day 23)	13	400 ± 100	3000 ± 520	3.0 (2.0-4.0)	9.9 ± 2.4		82 (78-87)	123 (118-127)

Data are mean ± SD unless otherwise specified. AUC_{inf} indicates area under the plasma concentration-time curve from time 0 to infinity; CL/F, total clearance of drug from plasma after oral administration; C_{max}, maximum blood concentrations; CYP, cytochrome P450; LSM, least-squares mean; PK, pharmacokinetic; $t_{½}$, elimination half-life; t_{max}, time to reach the maximum plasma concentration.

[a] n = 10 for AUC_{inf} and $t_{½}$.

[b] n = 9 for AUC_{inf} and $t_{½}$.

[c] n = 13 for AUC_{inf} and $t_{½}$.

[d] n = 12 for AUC_{inf} and $t_{½}$.

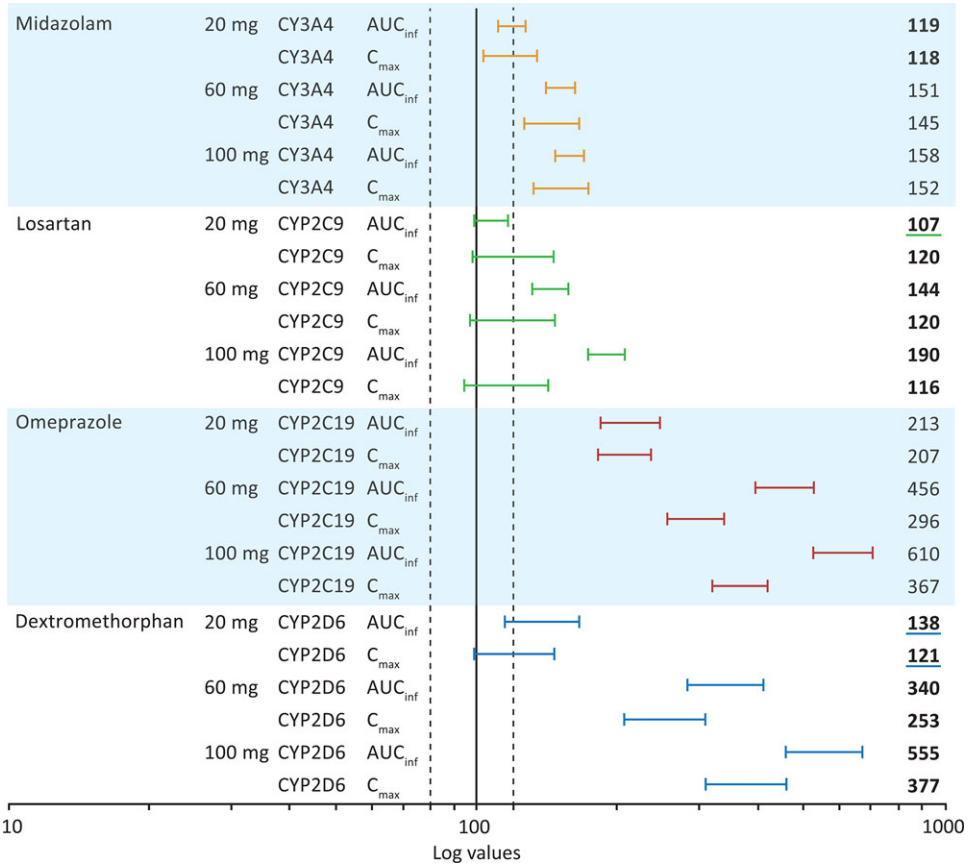

Figure 2. The effect of ASP8477 on drug cocktail probes after administration of 20, 60, and 100 mg once daily in combination with a single dose of drug cocktail on days 7, 14, and 23, respectively. Values on the right side represent the LSM ratios (%), horizontal lines represent the 90% CIs, and the dashed lines represent the 80% and 125% boundaries. Bolded values are not predicted by the FDA guidelines ($R \geq 1.1$). Underlined values are not predicted by the EMA guidelines ($R \geq 0.02$). AUC_{inf} indicates area under the plasma concentration-time curve from time 0 to infinity; C_{max} maximum blood concentration; EMA, European Medicines Agency; FDA, US Food and Drug Administration; LSM, least-squares mean.

Table 3. Summary Statistics of PK Parameters of ASP8477 After Multiple Administrations of 20 mg, 60 mg, and 100 mg ASP8477 Once Daily on Days 7, 14, and 23, Respectively

	n	C_{max} (ng/mL)	AUC_{tau} (h·ng/mL)	t_{max} (median [min-max]) (h)	C_{trough} (ng/mL)
Day 7, 20 mg ASP8477 qd	14	400 ± 120	1600 ± 710	1.0 (0.52-2.0)	0.27 ± NA
Day 14, 60 mg ASP8477 qd	14	2000 ± 490	17 000 ± 6400	2.0 (0.55-3.1)	91 ± 110
Day 23, 100 mg ASP8477 qd	13	4200 ± 1200	47 000 ± 17 000	2.0 (0.50-4.0)	740 ± 640

Data are mean ± SD unless otherwise specified. Molecular weight of ASP8477 is 325.36 g/mol. AUC_{tau}, area under the plasma concentration-time curve from time 0 to 24 hours; C_{max}, maximum blood concentrations; C_{trough}, trough blood concentrations; NA, not applicable; PK, pharmacokinetic; qd, once daily; t_{max}, time to reach the maximum plasma concentration.

following 20 and 60 mg ASP8477 and in 13 subjects (100%) following 100 mg ASP8477. No serious AEs or deaths were observed during this study. One subject experienced an AE leading to study discontinuation (emotional lability possibly related to ASP8477 but not related to the drug cocktail).

The most common AEs were related to nervous system and gastrointestinal disorders including dizziness, disturbance in attention, fatigue, feeling drunk, headache, lethargy, asthenia, dry mouth, nausea, anxiety, and decreased appetite (Supplementary Table 1). Dose-dependent increases in AEs (following 20, 60, and 100 mg ASP8477) were observed for dizziness, disturbance in attention, and feeling drunk. Fatigue occurred at all dose levels and was the most frequent moderate AE, occurring in 5 subjects, and was assessed

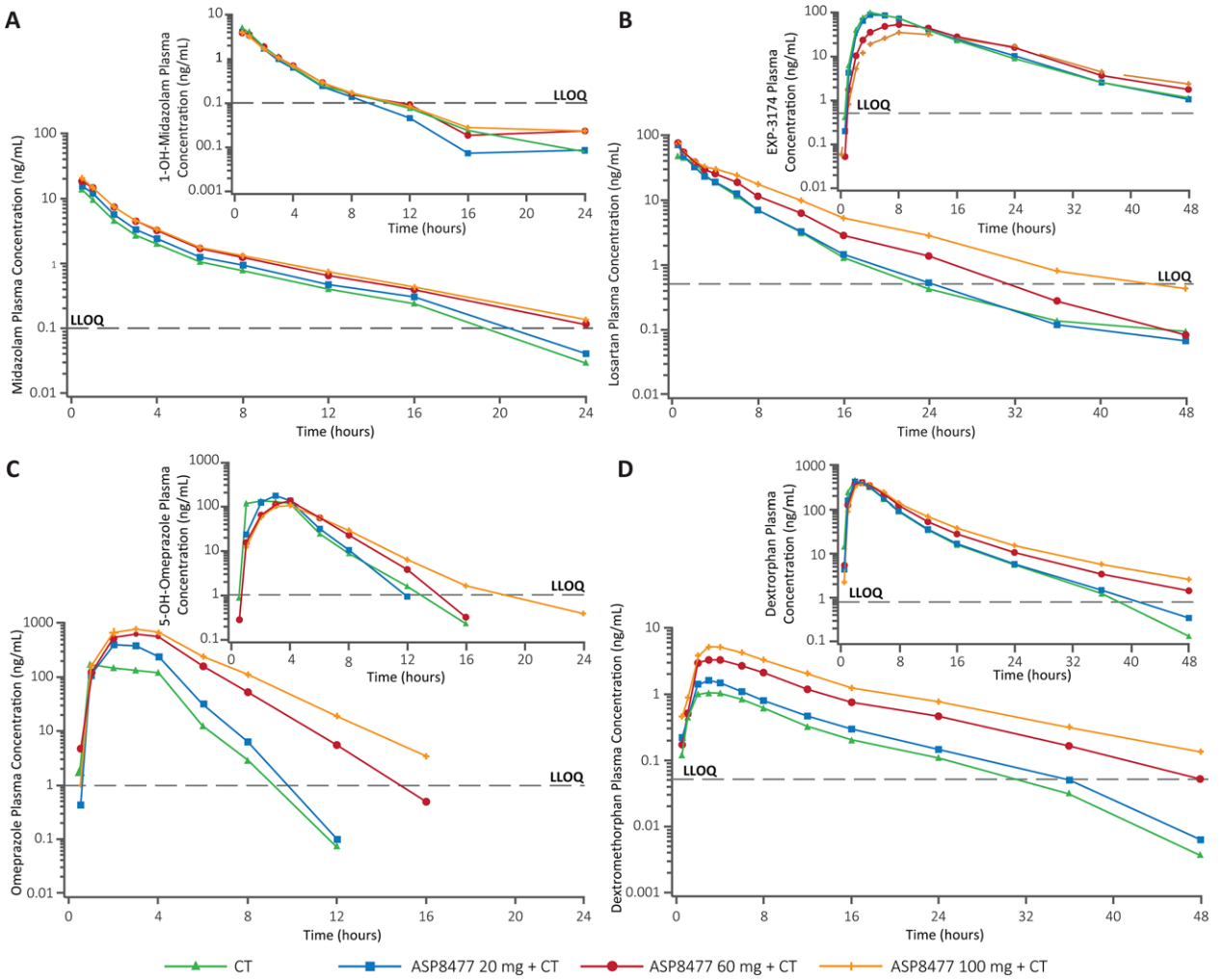

Figure 3. Mean (±SD) plasma concentration-time curves after administration of the drug cocktail alone on day 1 and in combination with multiple doses of 20, 60, and 100 mg ASP8477 on days 7, 14, and 23, respectively. A, Midazolam and 1-OH-midazolam (CYP3A4), (B) losartan and EXP-3174 (CYP2C9), (C) omeprazole and 5-OH-omeprazole (CYP2C19), and (D) dextromethorphan and dextrorphan (CYP2D6). LLOQ of midazolam and 1-OH midazolam is 0.1 ng/mL. LLOQ of losartan and EXP-3174 is 0.5 ng/mL. LLOQ of omeprazole and 5-OH-omeprazole is 1 ng/mL. LLOQ of dextromethorphan is 0.05 ng/mL, and LLOQ of dextrorphan is 0.8 ng/mL. CT, cocktail; LLOQ, lower limit of quantification.

as possibly related to ASP8477 but not related to the cocktail. Most AEs were mild in intensity. No AE of severe intensity was reported.

Safety laboratory data did not reveal any clinically significant aberrant values. The psychiatric tests showed no relevant changes over time; however, a slight increase of the VAS score was seen for some of the items in the Bond-Lader test (Supplementary Table 2) and the Bowdle VAS (Supplementary Table 3). Vital signs (blood pressure and body temperature) and ECG parameters did not show any clinically relevant changes.

Discussion

For target indications for which the use of concomitant medication by patients is likely, it is essential to investigate the potential for DDIs at the therapeutic exposure range because it may potentially result in overexposure or underexposure of the victim drug. CYP enzymes are often involved in such DDIs because many drugs are metabolized by isoforms of these enzymes.

Whether the DDI potential of a drug should be evaluated in a clinical setting is based on results of in vitro DDI studies in combination with clinical PK data (C_{max} at the therapeutic exposure range), as recommended by the FDA and EMA.[3,4] The FDA states that an interaction is possible if the ratio of (direct) inhibitor $[I]/K_i > 0.1$ and the R value is greater than 1.1 ($R = 1 + [\text{total } C_{max}/K_i]$), whereas the EMA states that this cutoff value is defined as R = unbound C_{max}/K_i, and further clinical testing is recommended when $R \geq 0.02$. Therefore, the difference between

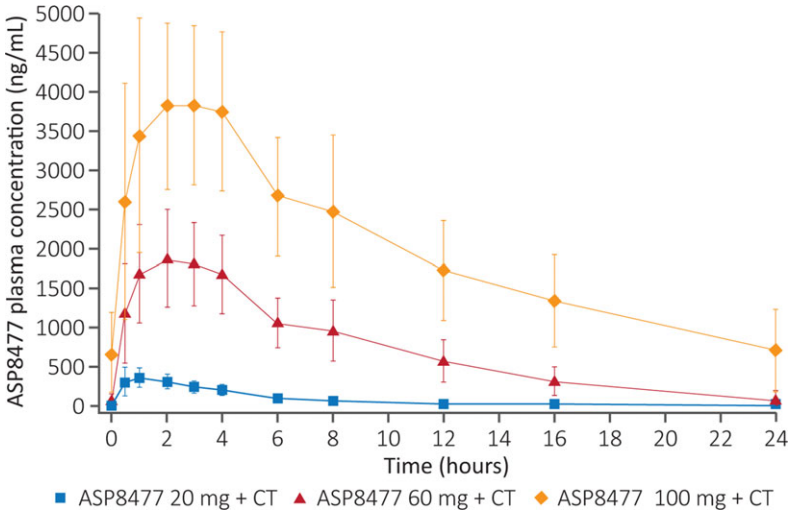

Figure 4. Mean (±SD) plasma concentration-time curves after multiple administrations of ASP8477 of 20 mg, 60 mg, and 100 mg ASP8477 once daily on days 7, 14, and 23, respectively. LLOQ of ASP8477 is 1 ng/mL. Molecular weight of ASP8477 325.36 g/mol. CT, cocktail; LLOQ, lower limit of quantification.

the recommendations of these 2 agencies is largely dependent on the fraction unbound.

For ASP8477, the results of the in vitro studies using human liver microsomes indicated a potential to directly inhibit the CYP enzymes CYP2C19, CYP2C9, CYP2D6, CYP2C8, and CYP3A4 with K_i values of 1.0, 8.0, 9.8, 15.0, and 18 μmol/L, respectively. At doses of 20, 60, and 100 mg ASP8477, inhibiting effects were expected based on C_{max} plasma concentrations converted to molar units (molecular weight of ASP8477 = 325.36 g/mol), except for CYP2C8, CYP2D6 (according to the EMA only) and CYP3A4, all at the lowest dose of 20 mg ASP8477 (Table 1). The fraction of unbound ASP8477 in humans was estimated to be 15%, independent of the clinical concentration range.

In the present clinical study a cocktail consisting of 5 model substrates—caffeine (CYP1A2), losartan (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6), and midazolam (CYP3A4)—was used to test the inhibiting potential of ASP8477 on these enzymes. The 3 dose levels (20, 60, and 100 mg) were selected because of the unknown therapeutic dose range at this point in ASP8477 development and the nonlinearity observed in exposure in the first-in-human study. Due to the relative poor tolerability of acute dosing of 100 mg ASP8477 (blood pressure–related AEs and psychotropic events), titration of multiple doses from 20 to 100 mg was used, and consequently no randomization for sequence was possible (single sequence design). The dosing scheme (5 days of 20 mg, 5 days of 60 mg, and 7 days of 100 mg) before cocktail dosing resulted in steady-state levels for the different dose levels at the time of cocktail dosing, as expected.

As expected from the in vitro K_i of 18 μmol/L for CYP3A4, slight increases in midazolam (CYP3A4) exposure with GMRs for AUC_{inf} of 119%, 151%, and 158% for 20, 60, and 100 mg ASP8477 once daily, respectively, were observed in this study. However, $t_{½}$ did not change, indicating that this increase in exposure was primarily due to a decreased first-pass effect. As indicated by in vitro results, a much more pronounced increase was seen with dextromethorphan (CYP2D6 in vitro K_i was 9.8 μmol/L) with GMRs for an AUC_{inf} of 138%, 340%, and 555% for 20, 60, and 100 mg ASP8477, respectively. For CYP2C9, a comparable in vitro K_i (8 μmol/L) value was observed; however, the increase in exposure of losartan was smaller compared with dextromethorphan, with GMRs for AUC_{inf} of 144% and 190% for 60 and 100 mg ASP8477, respectively, although no increase in exposure was observed for 20 mg ASP8477. The underlying mechanism for this difference in in vitro and clinical outcome is not known; however, a potential cause might be the formation of 1 or several metabolites of ASP8477 after multiple dosing in the clinical study, which may have an inhibitory potential on CYP2D6; such an effect would not be observed by an in vitro study. As predicted from in vitro data (in vitro K_i of 1 μmol/L), the effect of ASP8477 on CYP2C19 was high with GMRs for omeprazole exposures of 213%, 456%, and 610% for 20, 60, and 100 mg ASP8477. The results on omeprazole inhibition were in line with the outcome of the first-in-human study (8477-CL-0001), where a 7-fold increase of the omeprazole AUC_{inf} was observed after coadministration of 20 mg omeprazole with a single dose of 100 mg ASP8477. The increase in $t_{½}$ observed for losartan, omeprazole, and dextrorphan when administered with increasing doses of ASP8477 indicated that the increase in exposure was at least partly caused by inhibition of hepatic clearance.

In this study, not only the parent probe substrates but also the main metabolite of each substrate formed by the specific CYP enzyme (eg, 1-OH-midazolam and midazolam for CYP3A4) was analyzed. Effects of ASP8477 on metabolite exposure were as expected for 1-OH-midazolam (CYP3A4) and EXP-3174 (CYP2C9) based on the effect on parent exposure, for which no or a slight decrease in exposure was observed. Metabolite-to-parent ratios were also as expected; however, no effects were observed on metabolite exposure for 5-OH-omeprazole (CYP2C19) and dextrorphan (CYP2D6), whereas weak to strong effects were observed for parent exposure. At 60 and 100 mg ASP8477 once daily, a slight increase in AUC_{inf} was observed for dextrorphan instead of the expected decrease. These results should be interpreted with caution, as the influence of a potential perpetrator on the clearance of a metabolite cannot easily be determined. In addition, a similar AUC for the metabolite could also be due to a slower formation of metabolite when the substrate is administered together with an inhibitor. The metabolite-to-parent ratios of all probe substrates showed a decrease at all dose levels, as expected, but this was merely due to the increase in exposure of the parent and not to a decrease in exposure of the metabolite.

Trough concentrations confirmed that steady-state conditions for ASP8477 were achieved, and thus, maximal inhibition at each dose level was expected. The PK results of ASP8477 derived from this study were in line with steady-state PK results observed in other phase 1 clinical studies, including a multiple ascending dose study and a phase 1 study in which the analgesic/antihyperalgesic effect of ASP8477 was evaluated.[17] Both studies were performed in healthy female subjects; therefore, it can be concluded that the cocktail probe substrates had no effect on the PK of ASP8477.

According to the FDA guidance on DDIs, in vivo CYP inhibition can be classified as weak, moderate, and strong with values of 1.25- to <2-fold, 2- to <5-fold, and ≥5-fold increase in exposure, respectively, based on the AUC of the probe substrate (parent). Therefore, the inhibiting effect of ASP8477 on the 4 investigated CYP enzymes can be classified as no effect for CYP2C9 at the 20 mg dose level; as weak for CYP3A4 at the 20, 60, and 100 mg dose levels, for CYP2C9 at the 60 and 100 mg dose levels, and for CYP2D6 at the 20 mg dose level; as moderate for CYP2D6 at the 60 mg dose level, and for CYP2C19 at the 20 and 60 mg dose levels; and as strong for CYP2D6 and CYP2C19 at the 100 mg dose level. These results indicate that medications that are almost exclusively metabolized by CYP2C19 and/or CYP2D6 should not be taken concomitantly with ASP8477, depending on the ASP8477 dose. This exclusion criterion was applied in a phase 2a study in which ASP8477 was dosed at 30 mg twice a day for 21 days.[18] If ASP8477 had not been discontinued for development, more specific drug-drug interaction studies should have been required using the intended therapeutic dose of ASP8477 with drugs that are expected to be relevant to the target population. Alternatively these interactions could have been predicted using pharmacologically based PK modeling. The results of such studies and/or pharmacologically based PK modeling would have been included in the label.

ASP8477 administration in conjunction with cocktail probes was generally well tolerated in these healthy female subjects. One subject was withdrawn at the 60 mg level due to a moderate central nervous system–related AE, which was attributed by the investigators to the intake of ASP8477.

Conclusions

In conclusion, the degrees of inhibition of ASP8477 on the probe substrates of the Inje cocktail were dependent on ASP8477 exposure levels; inhibition increased with increasing ASP8477 dosing. The results of the clinical study were mostly in line with predictions based on in vitro studies conducted in accordance with recommendations of the FDA and the EMA, with the exception of CYP2D6, where the actual inhibition was higher than expected based on the in vitro K_i, indicating that some caution is indicated with predictions made based on in vitro results. With the approach used in this study, the inhibiting effect of a perpetrator drug on different CYP enzymes can be evaluated, and it can be evaluated at different dose levels, thereby supporting dose recommendations for many potential interactions.

Declaration of Conflict of Interest and Financial Disclosure

This study was funded by Astellas Pharma Global Development, Inc.

N. Treijtel, C. Collins, M. van Bruijnsvoort, E. Ernault, S. Gangaram-Panday, and P. Passier were employed by Astellas Pharma Europe BV at the time of the study. P. Passier is now employed by Galapagos SV. R. Fuhr was employed by PAREXEL who were contracted by Astellas Pharma Global Development, Inc., to provide support for this study.

Minor editorial assistance was provided by Succinct-Choice Medical Communications, London, UK, funded by Astellas Pharma Global Development, Inc.

Acknowledgments

The authors would like to thank the study subjects, study center staff, and Astellas study personnel. The authors are grateful to Michiel de Vries and Walter Krauwinkel for their input

on the manuscript. The authors are also grateful to Alice Paul and Donna Kowalski for their statistical contribution.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.