Received: 28 July 2021 | Revised: 5 November 2021 | Accepted: 10 November 2021

DOI: 10.1111/cts.13204

ARTICLE

Effect of cenobamate on the single-dose pharmacokinetics of multiple cytochrome P450 probes using a cocktail approach in healthy subjects

Stephen A. Greene | Charles Kwak | Marc Kamin | Laurent Vernillet | Kelli J. Glenn | Lana Gabriel | Hong Wook Kim

SK Life Science, Inc., Paramus, New Jersey, USA

Correspondence
Stephen A. Greene, Clinical Pharmacology and Pharmacometrics, Moderna, Inc., 200 Technology Square, Cambridge, MA 02139, USA.
Email: steve.a.greene@gmail.com

Present address
Stephen A. Greene, Clinical Pharmacology and Pharmacometrics, Moderna Therapeutics, Cambridge, Massachusetts, USA

Laurent Vernillet, Clinical Development Sciences, BioCryst Pharmaceuticals Inc., Durham, North Carolina, USA

Funding information
This study was funded by SK Life Science, Inc. (Paramus, NJ).

Abstract

This study was designed to evaluate the effects of cenobamate, an antiseizure medication for focal seizures, on the pharmacokinetics of cytochrome P450 probes (bupropion, CYP2B6; midazolam, CYP3A4/5; warfarin, CYP2C9; and omeprazole, CYP2C19) in healthy subjects. Probes were administered alone on days 1 (bupropion) and 7 (midazolam/warfarin/omeprazole), and with cenobamate 100 mg/day on day 69 (midazolam) and cenobamate 200 mg/day on days 99 (bupropion) and 105 (midazolam/warfarin/omeprazole). No significant interaction was concluded if 90% confidence intervals (CIs) for geometric mean ratios (GMRs) for area under the curve (AUC) and maximum concentration of CYP substrates and/or their metabolites were within the no-effect interval (0.80–1.25). When co-administered with cenobamate 100 mg/day, AUC from time of administration up to the time of the last quantifiable concentration (AUC_{0-last}) GMR (90% CIs) for midazolam was 0.734 (0.647–0.832). When co-administered with cenobamate 200 mg/day, AUC_{0-last} GMRs (90% CI) for midazolam, bupropion, S-warfarin, and omeprazole were 0.277 (0.238–0.323), 0.615 (0.522–0.724), 1.14 (1.10–1.18), and 2.07 (1.44–2.98), respectively. Co-administration of cenobamate with midazolam and bupropion probes led to values that were outside and below the no effect boundary, indicating that cenobamate induces the CYP3A4/5 and CYP2B6 enzymes. Co-administration of cenobamate led to omeprazole values which were outside and above the no-effect boundary, but with high variability, suggesting that cenobamate may moderately inhibit CYP2C19 activity. No effect on CYP2C9 was observed with the cenobamate and warfarin combination. Co-administration of cenobamate with these probes drugs was well-tolerated. In this study, 200 mg/day cenobamate moderately induced CYP3A4/5

SK Life Science, Inc. was the affiliation at the time work was done for both the authors Stephen A. Greene and Laurent Vernillet.

(dose-dependently; 100 mg/day was a weak inducer), was a weak inducer of CYP2B6, moderately inhibited CYP2C19, and had a negligible effect on CYP2C9.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Drug-drug interactions are a challenging aspect of managing epilepsy because many antiseizure medications (ASMs) induce or inhibit CYP450 enzymes, which are commonly involved in drug metabolism of many ASMs. Previous studies suggest that cenobamate, a US Food and Drug Administration (FDA)-approved ASM for the treatment of adults with focal seizures, may affect the activity of certain CYP450 enzymes.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study was designed to determine the effects of cenobamate on the pharmacokinetics of drugs known to affect the activity of these CYP450 enzymes, known as probe drugs. These probe drugs include bupropion, (CYP2B6), midazolam (CYP3A4/5), warfarin (CYP2C9), and omeprazole (CYP2C19).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The results of this study indicate that cenobamate induces CYP2B6 activity, exhibits a dose-dependent induction of CYP3A4/5 activity, inhibits CYP2C19 activity, and has a negligible effect on CYP2C9 activity.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These findings suggest that dose adjustments may be required when agents metabolized through these CYP450 pathways are used in conjunction with cenobamate.

INTRODUCTION

Drug-drug interactions (DDIs) are a challenging aspect of treatment with antiseizure medications (ASMs), in that many can induce or inhibit enzymes involved in drug metabolism, such as cytochrome P450 (CYP) isoenzymes.[1,2] Most ASMs have narrow therapeutic indices, and changes in their pharmacokinetic (PK) profile may result in suboptimal responses or safety concerns.[1] Consequently, it is important to examine the effects that a new ASM may have on the PK of medications that may be co-administered to treat epilepsy and its comorbidities.

Cenobamate (YKP3089) is an ASM that has recently been approved by the US Food and Drug Administration (FDA) for the treatment of adults with focal (partial-onset) seizures.[3] The precise mechanism by which cenobamate exerts its therapeutic effects in patients with focal seizures is not fully known; however, cenobamate has been shown to reduce repetitive neuronal firing by inhibiting voltage-gated sodium channels, and is also a positive allosteric modulator of the γ-aminobutyric acid ($GABA_A$) ion channel by binding to non-benzodiazepine $GABA_A$ receptor sites.[4,5]

Characterization of potential DDIs is critical in development of new medications. The FDA and the European Medicines Agency (EMA) recommend the evaluation of investigational drugs thought to be inhibitors or inducers of CYP enzymes in combination with specific probe drugs.[6,7] These probes are recommended as investigational tools because of a large body of research regarding the relative contribution of specific enzymatic pathways on their overall elimination, appropriate dosing, safety profiles, and anticipated effects when co-administered with agents known to induce or inhibit the specific pathways in question.[6,7]

In vitro study data indicated that cenobamate may interact with substrates of cytochrome P450 (CYP)2B6, CYP2C9, CYP2C19, and CYP3A4/5. In one or more human hepatocyte cultures up to 600 μM, some concentration-dependent increases were observed (>2.0-fold change and >20% of the positive control) in CYP2B6, CYP2C19, and CYP3A4/5 activity, and in CYP2B6 and CYP3A4 mRNA concentrations following treatment with cenobamate (data on file). Human microsomal studies revealed that cenobamate directly inhibited CYP2B6 (Ki = 82 μM), CYP2C19 (Ki = 110 μM), and CYP3A4/5 (half-maximal inhibitory concentration [IC_{50}] = 720–890 μM; data on

file). Previous phase I DDI studies have also suggested that cenobamate may affect the activity of the CYP2B6, CYP3A4/5, CYP2C9, and CYP2C19 enzymes.[8] Probe drugs recommended by the FDA and EMA to examine effects on the CYP enzymes hypothesized to be affected by cenobamate include bupropion (CYP2B6 probe), midazolam (CYP3A4/5 probe), warfarin (CYP2C9 probe), and omeprazole (CYP2C19 probe).[6,7] The metabolic pathways for cenobamate and each of the recommended probes examined here, including the CYP enzymes for which they are sensitive index substrates, are outlined in Figure 1.

The primary objective of this study was to assess the effect of steady-state cenobamate on the PK of the FDA- and EMA-recommended CYP probe drugs[6,7]: bupropion/S-bupropion (hydroxylation) for CYP2B6, midazolam/1-hydroxymidazolam (hydroxylation) for CYP3A4/5, warfarin/S-warfarin (hydroxylation) for CYP2C9, and omeprazole/5′-hydroxyomeprazole for CYP2C19. The secondary objective was to evaluate the safety of cenobamate when co-administered with these probe drugs.

METHODS

Study design

This phase I, single-center, open-label, within-group comparison study evaluated the PK of cenobamate administered in one fixed treatment sequence in healthy subjects in the United States (ClinicalTrials.gov identifier NCT03234699). The study was conducted from March through July 2017 and consisted of a screening period, three in-house periods of confinement, 11 ambulatory visits, an end-of-study (EOS) visit, and a follow-up visit (Figure 2). Subjects who successfully completed the 28-day screening period returned to the clinical site and remained there from day 1 through day 14, day 54 through day 62, and day 68 through day 111. Between these periods of confinement, subjects returned to the clinical site twice a week (≥2 days apart) for a total of 11 clinical visits. Subjects also participated in an EOS visit on day 124 for a safety assessment and a follow-up visit on day 138.

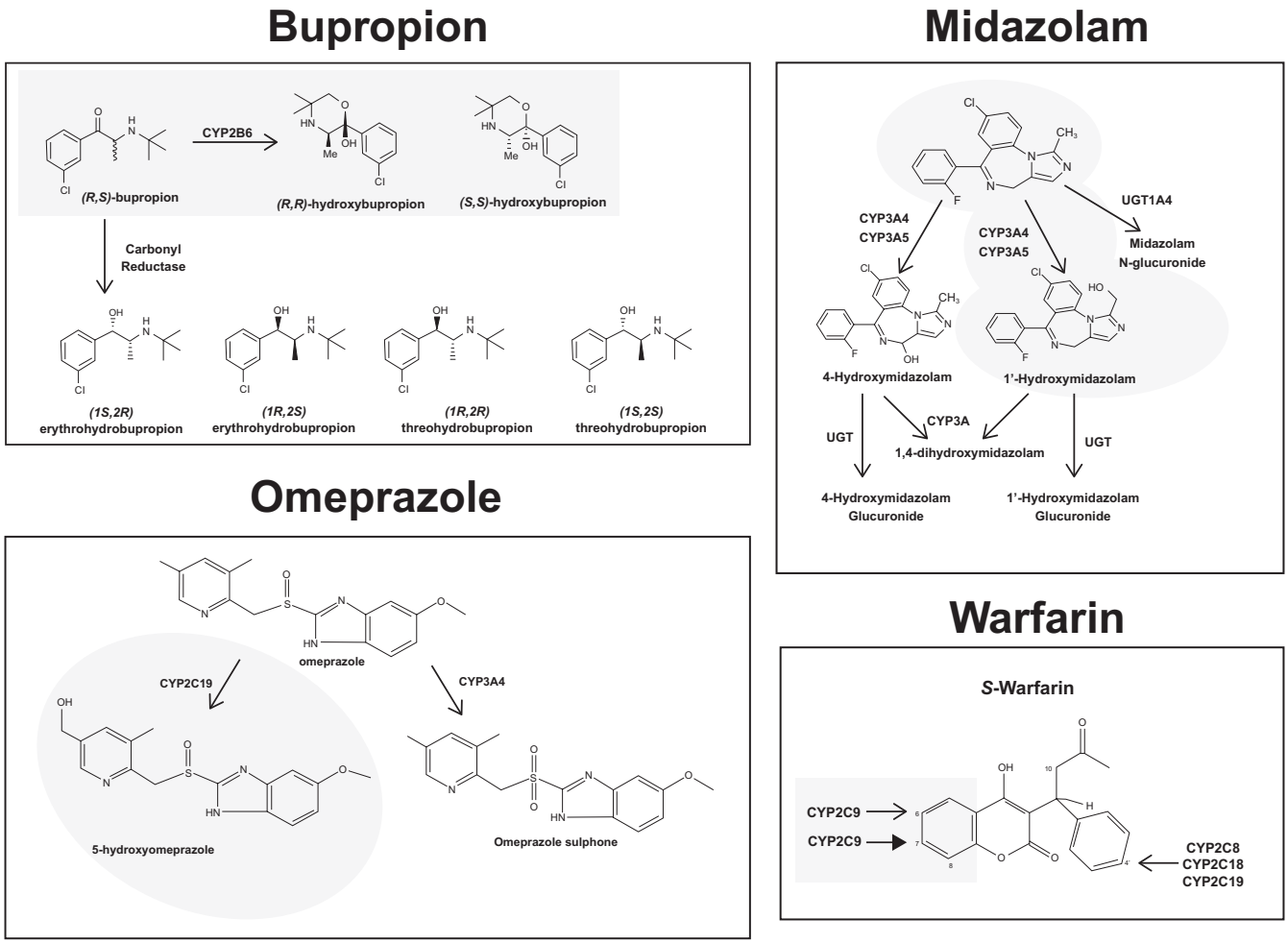

FIGURE 1 Main metabolic pathways of bupropion, midazolam, warfarin and omeprazole.[16–19] Cenobamate metabolic scheme has previously been published[20]

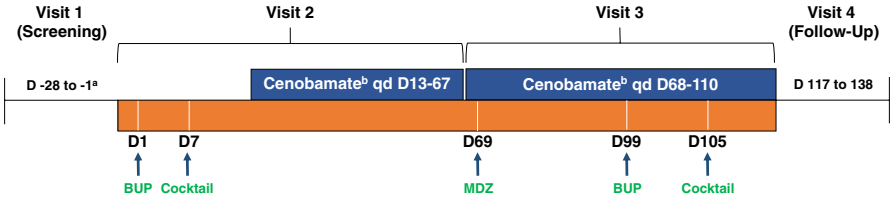

FIGURE 2 Study design. Probe drugs were administered in the morning following an overnight fast of at least 10 h during in-clinic visits on days 1, 7, 69, 99, and 105. Subjects continued to fast for at least 4 h after the administration of the probe drugs. [a]Subjects were in-house at the clinical site from days −1 to 14, from days 54 to 62, and from days 68 to 111. [b]Subjects received increasing cenobamate doses of 12.5 mg (days 13–26), 25 mg (days 27–40), 50 mg (days 41–54), 100 mg (days 55–70), 150 mg (days 71–84), and 200 mg (days 85–110). Cenobamate was self-administered when subjects were outside the period of confinement. BUP, bupropion 150 mg; cocktail: midazolam 2-mg oral syrup, omeprazole 20-mg delayed-release tablet, and warfarin 5-mg tablet; D, day; MDZ, midazolam 2-mg oral syrup; q.d., once daily

The study was conducted in accordance with the principles of the Declaration of Helsinki Guideline for Good Clinical Practice. The protocol was approved by MidLands Independent Review Board (Overland Park, KS, USA), and a written informed consent was obtained from each subject before any study-related procedures were performed.

Study population

Healthy male and female subjects between 18 and 50 years of age who had a body mass index (BMI) between 19.0 and 29.9 kg/m^2 were included in the trial. Subjects with any history of drug-related hypersensitivity reactions, severe hypersensitivity reactions (such as angioedema), or drug rash with eosinophilia and systemic symptoms syndrome to any drugs were excluded. All female subjects of childbearing potential and all male subjects must have agreed to use an accepted contraceptive regimen during the study. An accepted contraceptive regimen had to be used for 30 days (women) and 90 days (men) after the last dose of the study drug.

Treatments administered

Probe drugs were administered in the morning following an overnight fast of at least 10 h during in-clinic visits on days 1, 7, 69, 99, and 105 (Figure 2). Subjects continued to fast for at least 4 h after the administration of the probe drugs. The probe drugs were administered as follows: bupropion 150 mg was administered in tablet form on days 1 and 99; midazolam 2 mg was administered as oral syrup on days 7, 69, and 105; warfarin 5 mg was administered in tablet form on days 7 and 105; and omeprazole 20 mg was administered as 1 delayed-release tablet on days 7 and 105. Cenobamate was administered daily on days 13 through 110. The initial cenobamate dose was 12.5 mg/day and the dose was titrated every 2 weeks to 25 mg/day on day 27, 50 mg/day on day 41, 100 mg/day on day 55, 150 mg/day on day 71, and up to a final dose of 200 mg/day on days 85 through 110. Cenobamate 200 mg/day was chosen because it is the target therapeutic dose for cenobamate and may be titrated to 400 mg/day only if the benefits outweigh the risks.[3] It is anticipated that the majority of the target population will achieve appropriate efficacy and tolerability at the 200 mg/day dose. Cenobamate was self-administered when subjects were outside the period of confinement, and subjects were required to complete a daily dosing diary during these administrations.

Assessments

Blood samples for the measurement of plasma concentrations of each administered medication were collected at scheduled times throughout the study. With the administration of bupropion (days 1 and 99), blood samples were collected at predose, 1, 2, 3, 4, 5, 6, 8, 10,12, 24, 47, 71, 95, and 119 h postdose. With the administration of midazolam, warfarin, and omeprazole as a drug cocktail (days 7 and 105), blood samples were collected at predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdose. Blood samples were also collected beyond 24 h for warfarin at 47, 71, 95, 119, and 142 h postdose. When cenobamate was administered with midazolam alone (outside of the drug cocktail administration) on day 69, blood samples were collected at predose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdose. Cenobamate trough blood samples were also collected within 1 h of cenobamate administration on day 13 and within 1 h before the administration of the next dose on days 20, 27, 34, 41, 48, 55, 62, 69, 70, 71, 78, 85, 92, 99, 100, 105, 106, and 111 (no cenobamate dosing on day 111).

Plasma samples were assayed using validated methods with high-performance liquid chromatography-tandem mass spectrometry (Worldwide Clinical Trials for cenobamate and PPD Laboratories for all probes), for cenobamate, *S*-bupropion and its metabolites (*R*-bupropion, *R*,*R*-hydroxybupropion, *S*,*S*-hydroxybupropion, and threohydrobupropion), midazolam and 1′-hydroxymidazolam, *S*- and *R*-warfarin, and omeprazole and 5′-hydroxyomeprazole. For all assays, all

sample analysis runs met acceptance criteria and valid results were achieved for all samples submitted for analysis. Total bupropion and total hydroxybupropion were calculated by the addition of their respective *R*- and *S*-isomers.

Primary end points (area under the curve from time of administration up to the time of the last quantifiable concentration [AUC_{0-last}] and maximum concentration [C_{max}]) were analyzed for probe substrates and their metabolites when administered without cenobamate versus when co-administered with cenobamate at steady-state. Additional PK parameters evaluated included time to C_{max} (T_{max}), terminal half-life ($t_{1/2}$), and AUC extrapolated to infinity ($AUC_{0-\infty}$).

Safety was assessed through reporting of adverse events (AEs), laboratory and physical examinations, vital signs, and electrocardiograms (ECGs) throughout the study. Treatment-emergent AEs (TEAEs) were assigned by the last treatment taken (i.e., date/time of last treatment dosing on or before the start of the event), irrespective of any washouts between the start and end of the TEAE. If there were greater than one treatment taken at the same time, the TEAE was assigned to both treatments. Any TEAEs that started during follow-up were assigned to the last study treatment(s) that the subject received.

Statistical analyses

A population of 24 subjects was deemed to be sufficient to achieve the objectives of the study. The PK analysis population included all subjects with a complete profile who took a dose of a CYP probe or cenobamate without major protocol deviations that would affect the PK evaluation (outlined in Figure 3). The safety population included all subjects who received at least one dose of a probe (e.g., bupropion on day 1; $N = 24$). All safety analyses were conducted using SAS software, version 9.4 (SAS Institute Inc.) and all PK analyses were conducted using Phoenix WinNonlin version 6.3 and Phoenix Connect version 1.3.1.

The plasma PK parameters were estimated using a noncompartmental approach with a log-linear terminal phase assumption. The linear trapezoidal method was used to estimate AUC parameters.

Relative bioavailability was estimated by the geometric mean ratios (GMRs) for AUC_{0-last} and C_{max} for the CYP probe drugs bupropion, midazolam, warfarin, and omeprazole (and their respective measured metabolites) administered with and without cenobamate at steady-state. A mixed effects model on the logarithmic scale was used with treatment received as a fixed effect and subject as a random effect. A lack of significant differences in PK interaction was determined if the 90% confidence intervals (CIs) of the GMRs were within the accepted limits of 0.80 and 1.25. Safety results were analyzed descriptively. Molecular-weight-adjusted metabolite-to-parent AUC_{0-last} ratios were calculated based on respective molecular weights of the drug and the drug metabolite.

RESULTS

Subject disposition and baseline characteristics

A total of 24 subjects were randomized, and 21 completed the study (Figure 3). Two subjects discontinued the study

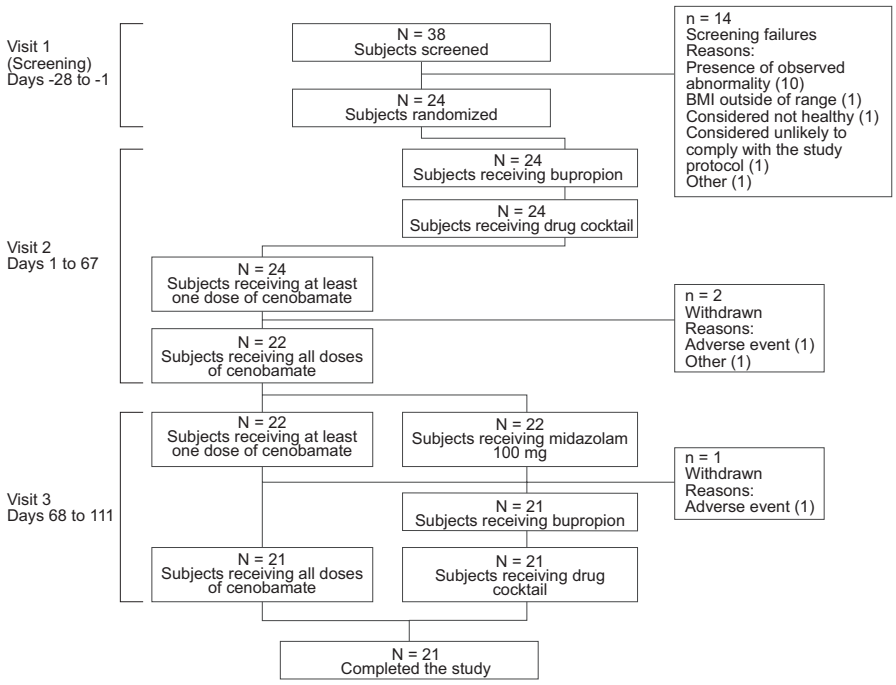

FIGURE 3 Subject disposition. BMI, body mass index

due to a TEAE and one subject was withdrawn due to a protocol deviation (positive alcohol test day 54). Subjects ranged in age from 23 to 50 years (mean age, 35.3 years), with BMIs ranging from 19.9 to 29.7 kg/m^2. Thirteen subjects were men (54.2%), 11 were women (45.8%), 16 were African American (66.7%), and 8 were White (33.3%; Table S1).

Plasma concentration-time profiles and effects on probe drugs

Midazolam (CYP3A4/5 probe)

Single-dose midazolam (and associated metabolites) PK was assessed alone (day 7) and in combination with cenobamate (day 69) following 56 days (8 weeks) of daily cenobamate dosing for the 100 mg/day dose assessment and in combination with cenobamate (day 105) following 98 days (14 weeks) of cenobamate daily dosing for the 200 mg/day dose assessment. The additional assessment for midazolam at 100 mg/day cenobamate was performed because a previous DDI study was conducted at this cenobamate dose, for which CYP3A4/5 was a major component in the interpretation of the PK data (data on file). Because there are multiple pathways by which midazolam is metabolized, the primary metabolite of midazolam, 1′-hydroxymidazolam, was also assessed to provide a more complete examination of the CYP3A4/5 pathway. Following a 2 mg dose of midazolam, both midazolam and 1′-hydroxymidazolam reached peak concentrations at ~0.5–1.0 h postdose (range of 0.5–2.0 h) in the presence or absence of cenobamate 100 or 200 mg/day (Figure 4). When midazolam was co-administered with cenobamate 100 mg/day, GMR values for AUC_{0-last} and C_{max} both decreased by 27%; whereas with co-administration of midazolam and cenobamate 200 mg/day, the GMR decreased by 72% for AUC_{0-last} and 61% for C_{max} (Figure 5 and Table 1). Conversely, both AUC_{0-last} and C_{max} for 1′-hydroxymidazolam increased in the presence of either dose of cenobamate, with greater increases observed with the 200 mg/day dose (100 mg/day cenobamate, 29% and 13% increase; 200 mg/day cenobamate, 46% and 53% increase, respectively). Compared to midazolam alone, the ratio of AUC_{0-last} for 1′-hydroxymidazolam over midazolam increased by 1.8-fold with co-administration of 100 mg/day cenobamate and 5.2-fold with 200 mg/day of cenobamate (ratios of 0.43, 0.77, and 2.25, respectively), suggesting a potential dose-dependent induction of the CYP3A4/5-mediated hydroxylation pathway for midazolam by cenobamate.

The 90% CIs of the GMR values for AUC_{0-last} and C_{max} of midazolam were outside and below the no-effect boundaries (80%–125%), indicating that cenobamate induces the CYP3A4/5 isoenzymes. Although the values with both doses of cenobamate were outside of the no-effect boundaries, the effect of the 200 mg/day dose of cenobamate was more pronounced than the 100 mg/day dose, further indicating a dose-dependent induction.

Bupropion (CYP2B6 probe)

Single-dose bupropion (and associated metabolites) PK was assessed alone (day 1) and in combination with cenobamate (day 99) following 86 days (~12 weeks) of cenobamate daily dosing. Bupropion has competing metabolic pathways (Figure 1); thus, in addition

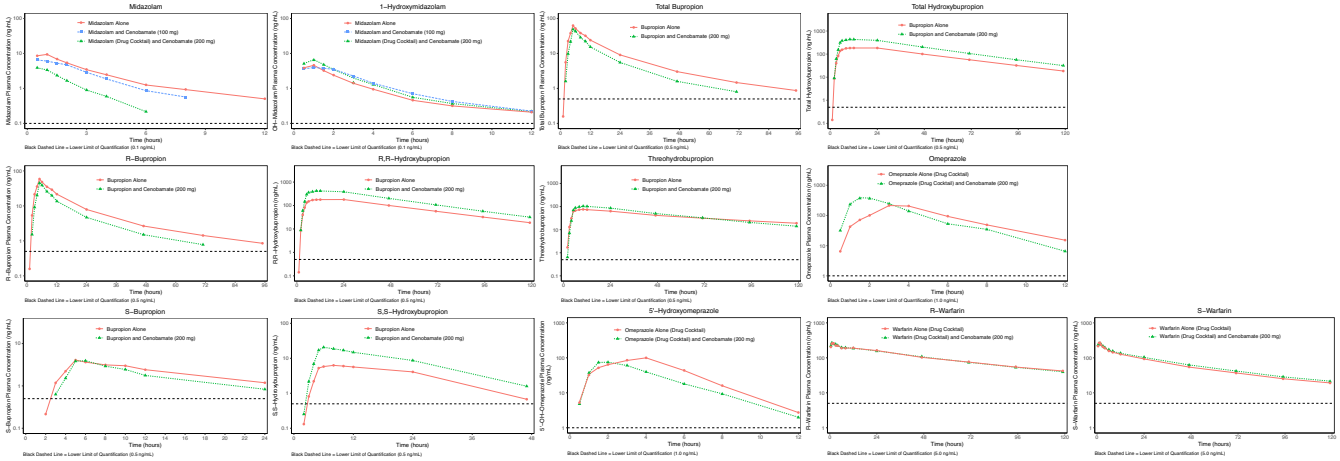

FIGURE 4 Mean plasma concentration-time profiles (semi-log scale) of midazolam, 1′-hydroxymidazolam, *R*-bupropion, *R*,*R*-hydroxybupropion, *S*-bupropion, *S*,*S*-hydroxybupropion, total bupropion, total hydroxybupropion, threohydrobupropion, omeprazole (linear scale), 5′-hydroxyomeprazole (linear scale), *R*-warfarin, and *S*-warfarin. Black dotted line = lower limit of quantification (LLOQ). Any missing data points fell below the LLOQ. Drug cocktail: midazolam 2 mg oral syrup, omeprazole 20-mg delayed-release tablet, and warfarin 5-mg tablet

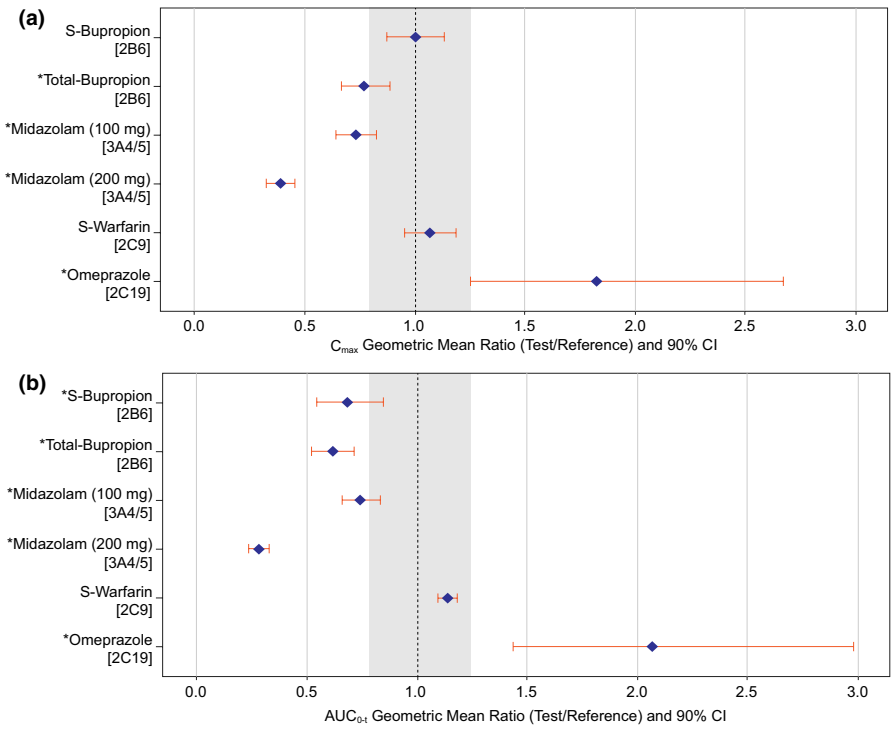

FIGURE 5 Effects of cenobamate on (a) C_{max} and (b) AUC_{0-last} of co-administered cytochrome P450 probes. Shaded region = 0.8–1.25 reference range. *Effect of cenobamate. AUC_{0-last}, area under the curve from time of administration up to the time of the last quantifiable concentration; CI, confidence interval; C_{max}, maximum concentration

to assessing the effects with racemic bupropion, both *R*,*R*- and *S*,*S*-hydroxybupropion (2R, 3R-, and 2S, 3S-hydroxybupropion) were measured to evaluate any changes in the hydroxylation pathway via CYP2B6. Threohydrobupropion was also assessed to examine the effects on the aminoketone group metabolic pathway (11β-hydroxysteroid dehydrogenase type 1 [11β-HSD1]). Mean plasma concentration–time profiles for bupropion and metabolites following administration of a 150 mg dose of bupropion with and without 200 mg/day cenobamate at steady-state are shown in Figure 4. Regardless of cenobamate co-administration, maximum concentrations of total bupropion and of *R*- and *S*-bupropion were reached at ~5 h postdose (range of 3–10 h), at 12 h (range of 6–24 h) postdose for *R*,*R*-hydroxybupropion and total hydroxybupropion, and at 10 h postdose for threohydrobupropion. Peak concentrations of *S*,*S*-hydroxybupropion occurred at 8 h (range of 5–10 h) and 6 h (range of 5–10 h) in the absence and presence of cenobamate, respectively.

In the presence of cenobamate, GMR values for AUC_{0-last} for *R*-bupropion, *S*-bupropion, and total bupropion decreased by 39%, 31%, and 39%, respectively, compared with administration of bupropion alone (Figure 5 and Table 1). GMR AUC_{0-last} values for *R*,*R*-hydroxybupropion, *S*,*S*-hydroxybupropion, total hydroxybupropion, and threohydrobupropion increased by 122%, 163%, 123%, and 29%, respectively. Mean C_{max} values for *S*-bupropion were independent of cenobamate coadministration. In the presence of cenobamate, C_{max} decreased by 24% and 23% for *R*-bupropion and total bupropion, and increased by 128%, 187%, 129%, and 49% for *R*,*R*-, *S*,*S*-hydroxybupropion, total hydroxybupropion, and threohydrobupropion, respectively.

For both *R*,*R*-hydroxybupropion and *S*,*S*-hydroxybupropion, a four-fold increase in the metabolite-to-parent ratio was observed when bupropion was co-administered with cenobamate, suggesting an induction of the bupropion hydroxylation pathway through CYP2B6 by cenobamate. Co-administration of cenobamate also resulted in an effect on the aminoketone group pathway with a 29% increase in threohydrobupropion exposure (AUC_{0-last}). These results suggest an induction of 11β-HSD1 by cenobamate. The 90% CIs of the GMR values for *R*-, *S*-, and total bupropion AUC_{0-last}; for *R*-bupropion and total bupropion C_{max}; and for *R*,*R*-, *S*,*S*-hydroxybupropion, total hydroxybupropion, and threohydrobupropion AUC_{0-last} and C_{max} were outside of the no-effect boundaries (80%–125%), indicating that cenobamate induced both CYP2B6-mediated and 11β-HSD1-mediated metabolism of bupropion.

Omeprazole (CYP2C19 probe)

Single-dose omeprazole (and associated metabolites) PK was assessed alone (day 7) and in combination with cenobamate (day 105) following 98 days (14 weeks) of cenobamate daily dosing. Because there are two competing pathways for metabolism of omeprazole, 5′-hydroxyomeprazole was also measured in order to better differentiate between effects on the CYP2C19 versus CYP3A4/5 isoenzymes (Figure 1).

TABLE 1 GMRs and 90% CIs of PK parameters for probe drugs

Parameter	Geometric LS means: Drug + cenobamate 200 mg[a]	Geometric LS means: Drug alone	GMR[b] (%)	90% CIs for GMRs
Midazolam + 100 mg cenobamate[a] ($n = 21$; drug alone $n = 23$)				
C_{max}[c]	7.3030	9.9480	73.414	64.720–83.275
AUC_{0-last}[d]	19.857	27.1230	73.210	64.998–82.460
Midazolam ($n = 20$; drug alone $n = 23$)				
C_{max}	3.8350	9.9480	38.551	32.874–45.207
AUC_{0-last}	7.5230	27.1230	27.735	23.831–32.279
1′-hydroxymidazolam + 100 mg cenobamate[a] ($n = 21$; drug alone $n = 23$)				
C_{max}	5.2640	4.6500	113.19	96.390–132.92
AUC_{0-last}	15.024	11.627	129.21	116.11–143.79
1′-hydroxymidazolam ($n = 20$; drug alone $n = 23$)				
C_{max}	7.0940	4.6500	152.54	128.68–180.82
AUC_{0-last}	16.956	11.627	145.83	127.45–166.86
R-bupropion ($n = 21$; drug alone $n = 24$)				
C_{max}	45.303	59.595	76.017	66.074–87.457
AUC_{0-last}	407.65	671.44	60.712	51.575–71.469
R,*R*-hydroxybupropion ($n = 21$; drug alone $n = 24$)				
C_{max}	383.89	168.19	228.26	207.78–250.75
AUC_{0-last}	18805	8464.8	222.05	198.86–247.96
S-bupropion ($n = 21$; drug alone $n = 24$)				
C_{max}	4.2730	4.2690	100.08	87.740–114.16
AUC_{0-last}	39.710	57.844	68.651	55.659–84.675
S,*S*-hydroxybupropion ($n = 21$; drug alone $n = 22$)				
C_{max}	17.569	6.1190	287.14	256.55–321.38
AUC_{0-last}	295.44	112.40	262.84	228.31–302.59
Total bupropion ($n = 21$; drug alone $n = 24$)				
C_{max}	49.316	63.840	77.249	67.234–88.757
AUC_{0-last}	453.30	737.42	61.471	52.168–72.433
Total hydroxybupropion ($n = 21$; drug alone $n = 22$)				
C_{max}	397.96	173.71	229.10	208.71–251.47
AUC_{0-last}	19163	8601.9	222.78	199.53– 248.74
Threohydrobupropion ($n = 21$; drug alone $n = 24$)				
C_{max}	108.09	72.461	149.16	126.02–176.55
AUC_{0-last}	4996.8	3887.5	128.54	107.21–154.10
Omeprazole ($n = 21$; drug alone $n = 23$)				
C_{max}	464.49	253.26	183.41	125.97–267.04
AUC_{0-last}	1156.0	558.49	206.99	143.87–297.82
5′-hydroxyomeprazole ($n = 20$; drug alone $n = 24$)				
C_{max}	94.658	155.35	60.933	46.595–79.683
AUC_{0-last}	309.01	418.37	73.860	65.779–82.934
R-warfarin ($n = 20$; drug alone $n = 23$)				
C_{max}	301.45	285.60	105.55	96.486–115.47
AUC_{0-last}	13079	12677	103.17	99.080–107.44

TABLE 1 (Continued)

Parameter	Geometric LS means: Drug + cenobamate 200 mg[a]	Geometric LS means: Drug alone	GMR[b] (%)	90% CIs for GMRs
S-warfarin ($n = 20$; drug alone $n = 23$)				
C_{max}	305.03	285.81	106.73	95.896–118.78
$AUC_{0–last}$	8154.0	7169.8	113.73	110.06–117.51

Abbreviations: $AUC_{0–last}$, area under the curve from time of administration up to the time of the last quantifiable concentration; CI, confidence interval; C_{max}, maximum concentration; GMR, geometric mean ratio; LS, least squares; PK, pharmacokinetic.

[a]All comparisons are with cenobamate 200 mg except when indicated as 100 mg cenobamate.

[b]Ratio of drug + cenobamate/drug alone.

[c]C_{max} in ng/ml throughout.

[d]$AUC_{0–last}$ in ng h/ml throughout.

Following a 20-mg dose of delayed-release omeprazole, both omeprazole and its metabolite, 5′-hydroxyomeprazole, reached peak concentrations at ~3 h (range of 1–8 h) postdose (Figure 4). Following multiple doses of cenobamate, time to C_{max} decreased to 2 h (range 1–8 h) for both omeprazole and 5′-hydroxyomeprazole. When administered with cenobamate, omeprazole GMR values for $AUC_{0–last}$ and C_{max} increased by 107% and 83%, respectively (Figure 5 and Table 1), whereas these values decreased by 26% and 39% for 5′-hydroxyomeprazole. Compared to omeprazole alone, the 5′-hydroxyomeprazole-to-omeprazole ratio for $AUC_{0–last}$ decreased by 4.5-fold when omeprazole was co-administered with cenobamate, suggesting a possible inhibition by cenobamate of the CYP2C19-mediated metabolic pathway of omeprazole. Moreover, the 90% CIs of the GMR values for $AUC_{0–last}$ and C_{max} were above the no-effect boundaries, further confirming that cenobamate inhibited the CYP2C19-mediated metabolic pathway.

S-warfarin (CYP2C9 probe)

Single-dose warfarin (and associated metabolites) PK was assessed alone (day 7) and in combination with cenobamate (day 105) following 98 days (14 weeks) of cenobamate daily dosing. Following a 5-mg dose of warfarin, *R*-warfarin and *S*-warfarin reached C_{max} at ~1.0 h (range of 0.5–5.0 h) postdose in either the presence or absence of cenobamate 200 mg/day (Figure 4). Slight increases in $AUC_{0–last}$ and C_{max} (range: 3%–14%) were observed for *R*-warfarin and *S*-warfarin with concomitant cenobamate administration. However, the 90% CIs of the GMR values for C_{max} and $AUC_{0–last}$ of *R*- and *S*-warfarin remained within the no-effect boundaries of 80%–125%, regardless of cenobamate co-administration. These results indicate that cenobamate had no significant effect on the primary metabolic pathway for *S*-warfarin, via CYP2C9 (Figure 5 and Table 1).

Cenobamate

During the development of cenobamate, rash and hypersensitivity reactions were reported, some of which resulted in hospitalization and/or discontinuation. An analysis of the rates of these reactions in both healthy subjects and patients with epilepsy was performed; it indicated that a lower starting dose of cenobamate and slower rates of dose up-titration were associated with lower risks of rash/hypersensitivity reactions (data on file). Data from this analysis on hypersensitivity reactions led to the chosen starting dose and titration schedule for cenobamate in the current study (see Methods section). Figure 6 illustrates individual trough plasma concentration-time profiles of cenobamate by dose level. The median trough cenobamate concentrations increased dose-dependently, with values ranging from 0.521 μg/ml with the 12.5-mg dose to 18.4 μg/ml for the 200-mg dose. Decreases in cenobamate concentrations were observed in some patients, most of which occurred for doses above 100 mg. Additionally, variability in individual trough concentrations were increasingly observed toward the end of the study.

Safety and tolerability

A total of 20 of 24 subjects (83.3%) experienced 59 TEAEs during the study (Table 2). The frequency of TEAEs was higher following administration of multiple doses of cenobamate alone (62.7%; days 13–98) than when a single dose of the midazolam, warfarin, and omeprazole cocktail was administered after cenobamate 200 mg/day (22%; day 105), or following single dose administration of midazolam alone after cenobamate 100 mg/day (3.4%; day 69). Subjects who received a single dose of the probe drug cocktail (midazolam, warfarin, and omeprazole; day 7) alone reported no TEAEs and four (6.7%) TEAEs were reported in three subjects who received

a single dose of bupropion alone on day 1. More than half of TEAEs were considered not related to the study treatment (32/59) and were mild in severity (49/59). The most commonly reported TEAE by preferred term was somnolence, with five events experienced by five subjects (20.8%) who received cenobamate alone. The next most common TEAE was elevated alanine aminotransferase, with six events experienced by three subjects (12.5%) who received cenobamate alone, and in one subject (4.8%) who received cenobamate followed

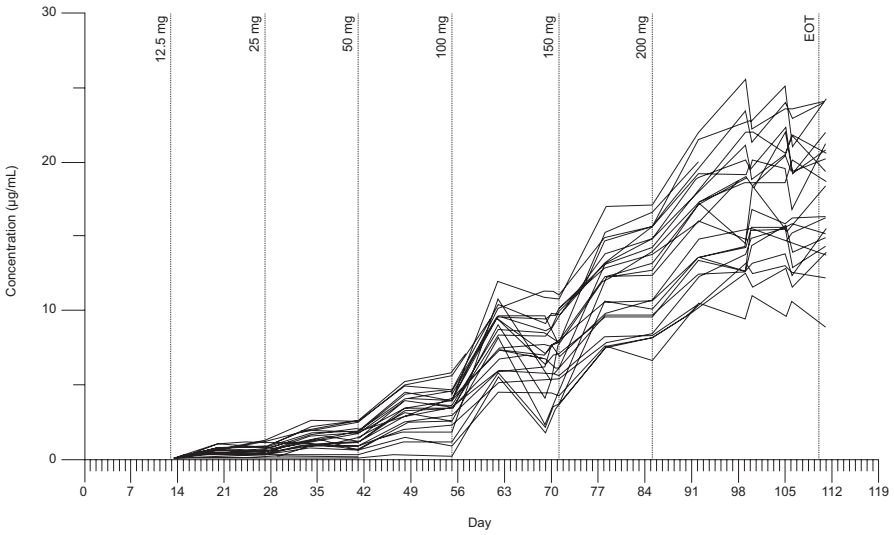

FIGURE 6 Individual trough plasma cenobamate concentration-time profiles by dose level. Reference line indicates change in the dose that occurred after the pharmacokinetic sampling on that same day. EOT, end of trial

TABLE 2 Safety summary and TEAEs occurring in greater than or equal to two (8%) subjects per group

n (%)	Bupropion alone (*n* = 24)	CYP cocktail (*n* = 24)	Cenobamate alone (*n* = 24)	Midazolam + cenobamate 100 mg (*n* = 22)	Bupropion + cenobamate 200 mg (*n* = 21)	CYP cocktail + cenobamate 200 mg (*n* = 21)
Total *N* of TEAEs[a]	4 (6.7)	0 (0.0)	37 (62.7)	2 (3.4)	3 (5.1)	13 (22.0)
Subjects with ≥1 TEAE[b]	3 (12.5)	0 (0.0)	18 (75.0)	1 (4.5)	3 (14.3)	9 (42.9)
Subjects with ≥1 TEAE of special interest[b]	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with TEAE leading to discontinuation[b]	0 (0.0)	0 (0.0)	2 (8.3)	0 (0.0)	0 (0.0)	0 (0.0)
TEAEs by SOC preferred term, *n* (%)[b]						
Investigations	0 (0.0)	0 (0.0)	5 (20.8)	0 (0.0)	1 (4.8)	5 (23.8)
Alanine aminotransferase increased	0 (0.0)	0 (0.0)	3 (12.5)	0 (0.0)	0 (0.0)	1 (4.8)
Blood creatine phosphokinase increased	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	3 (14.3)
Nervous system disorders	2 (8.3)	0 (0.0)	5 (20.8)	0 (0.0)	0 (0.0)	0 (0.0)
Somnolence	0 (0.0)	0 (0.0)	5 (20.8)	0 (0.0)	0 (0.0)	0 (0.0)
Headache	2 (8.3)	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
Gastrointestinal disorders	1 (4.2)	0 (0.0)	3 (12.5)	1 (4.5)	0 (0.0)	1 (4.8)
Nausea	0 (0.0)	0 (0.0)	2 (8.3)	0 (0.0)	0 (0.0)	1 (4.8)
Skin and subcutaneous disorders	0 (0.0)	0 (0.0)	4 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Ecchymosis	0 (0.0)	0 (0.0)	3 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)

Note: Each TEAE was counted only once for each subject within each SOC and Medical Dictionary for Regulatory Activities (MedDRA) preferred team.

Abbreviations: SOC, system organ class; TEAEs, treatment-emergent adverse events.

[a]Of a total of 59 TEAEs reported across all treatment periods.

[b]Of a total of 24 subjects in the safety population.

by administration of probe drugs (midazolam, warfarin, and omeprazole).

Eleven subjects (45.8%) had clinically significant changes in laboratory parameters that were associated with TEAEs. These laboratory changes included increases in blood creatine phosphokinase, alanine aminotransferase, gamma-glutamyltransferase, and aspartate aminotransferase, leukopenia, leukocytosis, and laboratory signs of urinary tract infection (Table S2), all of which resolved by the end of the study. However, no subjects discontinued due to laboratory changes. Six subjects (25.0%) had clinically significant findings on physical examination that were considered TEAEs. These included rash, subcutaneous abscess, ecchymosis, laceration, and skin abrasion, all of which resolved by study end.

No deaths or serious AEs occurred, and two subjects were discontinued due to TEAEs. One subject was withdrawn due to a bilateral axillary rash of mild severity that developed ~23 h after administration of cenobamate 12.5 mg on day 16. Results of the laboratory assessment for this hypersensitivity reaction were within reference range and judged not to be clinically significant, and the rash resolved within 8 days of onset. One subject was also withdrawn due to the subcutaneous abscess on the left axilla identified upon physical examination, this was moderate in severity and resolved within 17 days of onset.

DISCUSSION

The current study was designed to assess the effects of cenobamate on the PK of CYP probe substrates (bupropion, midazolam, warfarin, and omeprazole) as a means of predicting possible DDIs. The PK end points AUC_{0-last} and C_{max} were used to assess the magnitude of change in drug exposure for each substrate and metabolite(s). Results were interpreted based on whether the 90% CIs of these PK parameters were within the "no-effect boundaries" or the interval within which a change in systemic exposure is considered not clinically relevant.[9]

The data indicated that daily administration of cenobamate in healthy subjects resulted in induction of CYP3A4/5 activity at both 100 and 200 mg/day doses, following 14 days of treatment with each cenobamate dose. This induction was demonstrated by the 90% CIs of the GMR for AUC_{0-last} and C_{max} for midazolam falling outside and below the no-effect boundaries (0.80–1.25) when co-administered with cenobamate. When midazolam was administered with cenobamate 100 mg/day, the inductive effect seen in midazolam PK parameters was lower than that observed after administration of cenobamate 200 mg/day, which indicated that the induction was dose dependent. Specifically, midazolam C_{max} and AUC were reduced by 27% when co-administered with cenobamate 100 mg/day and by 61% and 72% when co-administered with the 200 mg/day dose. As the midazolam exposure decreased with the increased dose of cenobamate, 1′-hydroxymidazolam (a CYP3A4/5 specific metabolite of midazolam) showed increases in C_{max} and AUC with the higher dose of cenobamate. Because of a potential for reduced efficacy, drugs that are substrates for CYP3A4/5 may require dose increases when administered with cenobamate.

Results for bupropion were also outside and below the no-effect boundary, indicating that cenobamate induced CYP2B6-mediated metabolism of bupropion. S-bupropion AUC and total bupropion AUC were reduced by 31% and 39% when co-administered with 200 mg/day cenobamate (14 days of 200 mg/day treatment). This suggests reduced efficacy and that dosages of CYP2B6 substrates may need to be increased as needed when used concomitantly with cenobamate. However, it should be noted that concentrations of the primary active metabolite of bupropion, hydroxybupropion (produced through CYP2B6), increased, which may reduce the need for bupropion dose adjustments.

Results following co-administration of omeprazole and cenobamate (20 days of 200 mg/day treatment) indicated that peak omeprazole plasma concentrations and exposure were outside and above the no-effect boundaries, but with high variability (Figure 5), indicating that cenobamate was a moderate inhibitor of CYP2C19 activity. Based on in vitro CYP inhibition studies in human liver microsomes (data on file), cenobamate inhibited CYP2C19 with a Ki of 110 μM, which is near the 200 mg/day steady state exposure level (~89 μM). The reduction observed for the 5′-hydroxyomeprazole metabolite in the presence of cenobamate also supports that cenobamate inhibits CYP2C19, because that metabolite of omeprazole is primarily formed through the CYP2C19 pathway. Because of a potential for an increase in the risk of adverse reactions due to an increase in parent drug exposure, a dose reduction may be necessary for CYP2C19 substrates as clinically appropriate when used concomitantly with cenobamate.

When cenobamate was administered along with the warfarin probe (20 days of 200 mg/day cenobamate treatment), results remained within the no-effect boundaries for warfarin, indicating that cenobamate had no significant effect on the CYP2C9 isoenzyme. Overall, administration of cenobamate with these various probe drugs appeared to be generally safe and well-tolerated in the healthy subjects included in this study.

Individual trough cenobamate plasma concentrations observed in the current study are similar to those seen in a previous PK study of cenobamate in healthy subjects.[10] The median trough concentration in the current trial was 18.4 μg/ml and, in an ascending dose PK study, following multiple

doses of 200 mg/day cenobamate, the mean trough cenobamate plasma concentration was 19.0 μg/ml. Further, in a phase III trial in patients with uncontrolled focal seizures, mean steady-state concentrations after cenobamate 200 mg/day were generally similar to the median trough concentrations observed here with the 200 mg/day dose (phase III study: 15.5 μg/ml; current study, range: 15.5–18.4 μg/ml).[11] The increase in variability in individual trough concentrations observed toward the end of this study has also been observed in another study in healthy subjects, for which a low-to-moderate intrasubject variability was observed both for total plasma exposure and peak plasma concentrations (coefficient of variation: 4–5% and 14%, respectively).[12]

A limitation of the current study is that no assessments of transporters were conducted. As more evidence is collected on the characteristics of cenobamate, additional clinical studies may be warranted to assess possible drug/transporter interactions. Another limitation is the use of healthy subjects rather than patients with epilepsy and other special populations within that group. The population of patients who will receive cenobamate in a clinical setting will be more heterogeneous and the magnitude of drug interactions will be more variable and complex than examined here. Further studies in larger populations of patients with focal epilepsy should broaden our understanding of drug interactions with adjunctive cenobamate.

Given that seizures occur in patients with a variety of concomitant disorders, and many patients with epilepsy are on more than one ASM, the rationale for prescribing a new ASM requires careful consideration of the factors known to affect absorption, distribution, and metabolism of the drug in question and any concomitant agents.[13] Several DDI studies have evaluated the PK effects of combination treatment with cenobamate and three commonly used ASMs: phenobarbital, carbamazepine, phenytoin, or divalproex sodium.[3,8] These agents are metabolized, at least in part, through the CYP3A4/5, CYP2C9, and CYP2C19 pathways, which can lead to changes in plasma exposure of these ASMs when taking adjunctive cenobamate.[14] Thus, the cenobamate-mediated effects on CYP enzymes observed here may result in the need for dose adjustments to other ASMs, which are commonly taken together. Combination treatment with the current probe drugs and cenobamate were generally safe and well-tolerated throughout.[3,8] Data from a post hoc analysis of a long-term, phase III, open-label study with adjunctive cenobamate (C021) has provided more specific insights into how dose adjustments should be made to commonly used concomitant ASMs.[15]

Due to the effects of cenobamate on the CYP3A4/5, CYP2B6, and CYP2C19 enzymes observed here, cenobamate may reduce plasma concentrations of drugs metabolized by CYP3A4/5 and CYP2B6 and may increase plasma concentrations of drugs metabolized by CYP2C19.[8] Based on clinical trials in patients with epilepsy, guidance on management of specific concomitant ASMs with adjunctive cenobamate has been provided in the US prescribing information.[3] The current data provide valuable insights on how cenobamate affects CYP450-mediated metabolism of concomitantly administered drugs, which helps to predict and understand possible DDIs with cenobamate.

ACKNOWLEDGEMENTS

These studies were supported by SK Life Science (Paramus, NJ). Medical editing and medical writing assistance were provided by Don Fallon, ELS, and Debika Chatterjea, PhD, of MedVal Scientific Information Services, LLC (Princeton, NJ), and were funded by SK Life Science, Inc. (Paramus, NJ). This manuscript was prepared according to the International Society for Medical Publication Professionals' "Good Publication Practice for Communicating Company-Sponsored Medical Research: GPP3."

CONFLICT OF INTEREST

C.K., M.K., K.J.G., L.G., and H.W.K. are current employees of SK Life Science, Inc. S.A.G. and L.V. are former employees of SK Life Science, Inc.

AUTHOR CONTRIBUTIONS

S.A.G., C.K., M.K., L.V., K.J.G., L.G., and H.W.K. wrote the manuscript. L.V., C.K., M.K., K.J.G., L.G., and H.W.K. designed the research. L.V., K.J.G., and H.W.K. performed the research. S.A.G., C.K., L.G., and H.W.K. analyzed the data.

PREVIOUS PRESENTATION

Greene S, et al. The effect of cenobamate on the single dose pharmacokinetics of multiple cytochrome P450 probes using a cocktail approach in healthy subjects. Presented at American Society for Clinical Pharmacology & Therapeutics Annual Meeting, March 13–16, 2019, Washington, DC. Published in abstract form as Greene S, et al. *Clin Pharmacol Ther*. 2019;105(Suppl 1):S97.

REFERENCES

1. Perucca E. Clinically relevant drug interactions with antiepileptic drugs. *Br J Clin Pharmacol*. 2006;61:246-255.
2. Patsalos PN, Perucca E. Clinically important drug interactions in epilepsy: general features and interactions between antiepileptic drugs. *Lancet Neurol*. 2003;2:347-356.
3. XCOPRI® (cenobamate tablets), for oral use, CV [prescribing information]. SK Life Science. Inc.; April 2021.
4. Nakamura M, Cho JH, Shin HS, Jang IS. Effects of cenobamate (YKP3089), a newly developed anti-epileptic drug, on voltage-gated sodium channels in rat hippocampal CA3 neurons. *Eur J Pharmacol*. 2019;855:175-182.

5. Sharma R, Nakamura M, Neupane C, et al. Positive allosteric modulation of $GABA_A$ receptors by a novel antiepileptic drug cenobamate. *Eur J Pharmacol*. 2020;879:173117.
6. US Food and Drug Administration. Guidance for industry. Drug interaction studies — study design, data analysis, implications for dosing, and labeling recommendations; 2012. Accessed August 29, 2019. https://pdfs.semanticscholar.org/3aa6/c5fa064816cb8525d967be25bd92dfe3444d.pdf?_ga=2.24434686.577539498.1567088324-73214531.1567088324
7. European Medicines Agency. Guideline on the investigation of drug interactions; 2012. Accessed August 29, 2019. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-drug-interactions_en.pdf
8. Vernillet L, Kamin M. Drug–drug interactions between cenobamate and other antiepileptic drugs: results from phase I studies with carbamazepine, phenobarbital, phenytoin, and divalproex sodium [poster]. Presented at American Society for Clinical Pharmacology & Therapeutics Annual Meeting, March 21–24, 2018, Orlando, FL.
9. US Food and Drug Administration. Clinical drug interaction studies — study design, data analysis, and clinical implications. Guidance for industry; 2017. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf. Accessed July 15, 2020.
10. Vernillet L, Greene SA, Kamin M. Pharmacokinetics of cenobamate: results from single and multiple oral ascending-dose studies in healthy subjects. *Clin Pharmacol Drug Dev*. 2020;9:428-443.
11. Krauss GL, Klein P, Brandt C, et al. Safety and efficacy of adjunctive cenobamate (YKP3089) in patients with uncontrolled focal seizures: a multicentre, double-blind, randomised, placebo-controlled, dose-response trial. *Lancet Neurol*. 2020;19:38-48.
12. Greene SA, Orlinski L, Glenn K, Vernillet L. Relative bioavailability, food effect and intra-subject variability of cenobamate, a newly-approved antiepileptic drug for the treatment of focal (partial-onset) seizures [poster]. Presented at American College of Clinical Pharmacology Virtual Meeting, September 21–23, 2020.
13. van Dijkman SC, Rauwé WM, Danhof M, Della Pasqua O. Pharmacokinetic interactions and dosing rationale for antiepileptic drugs in adults and children. *Br J Clin Pharmacol*. 2018;84:97-111.
14. Marvanova M. Pharmacokinetic characteristics of antiepileptic drugs (AEDs). *Ment Health Clin*. 2016;8:8-20.
15. Rosenfeld WE, Nisman A, Ferrari L. Efficacy of adjunctive cenobamate based on number of concomitant antiseizure medications, seizure frequency, and epilepsy duration at baseline: a post-hoc analysis of a randomized clinical study. *Epilepsy Res*. 2021;172:106592.
16. Kaminsky LS, Zhang ZY. Human P450 metabolism of warfarin. *Pharmacol Ther*. 1997;73:67-74.
17. Linden R, Ziulkoski AL, Wingert M, Tonello P, Souto AA. Simultaneous determination of omeprazole, hydroxyomeprazole and omeprazole sulphone in human plasma by isocratic HPLC-DAD: application to the phenotyping of CYP2C19 and CYP3A4 in Brazilian volunteers. *J Braz Chem Soc*. 2007;18:733-740.
18. Teitelbaum AM, Flaker AM, Kharasch ED. Development and validation of a high-throughput stereoselective LC-MS/MS assay for bupropion, hydroxybupropion, erythrohydrobupropion, and threohydrobupropion in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2016;1017–1018:101-113.
19. Nguyen HQ, Kimoto E, Callegari E, Obach RS. Mechanistic modeling to predict midazolam metabolite exposure from in vitro data. *Drug Metab Dispos*. 2016;44:781-791.
20. Vernillet L, Greene SA, Kim HW, Melnick SM, Glenn K. Mass balance, metabolism, and excretion of cenobamate, a new antiepileptic drug, after a single oral administration in healthy male subjects. *Eur J Drug Metab Pharmacokinet*. 2020;45:513-522.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Greene SA, Kwak C, Kamin M, et al. Effect of cenobamate on the single-dose pharmacokinetics of multiple cytochrome P450 probes using a cocktail approach in healthy subjects. *Clin Transl Sci*. 2022;15:899-911. doi:10.1111/cts.13204