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ORIGINAL ARTICLE

Safety, tolerability, pharmacokinetics, pharmacodynamics, bioavailability and food effect of single doses of soticlestat in healthy subjects

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Aims: Soticlestat is a first-in-class selective inhibitor of cholesterol 24-hydroxylase, the enzyme that converts brain cholesterol to 24*S*-hydroxycholesterol (24HC), a positive allosteric modulator of *N*-methyl-D-aspartate receptors. Soticlestat is under development as treatment for rare developmental and epileptic encephalopathies.

Methods: In this first-in-human study, 48 healthy men and women received single ascending doses of soticlestat oral solution or placebo. Subsequently, nine healthy subjects received soticlestat tablets under fed and fasting conditions to assess the relative oral bioavailability and effects of food. Serial blood and urine samples were collected for pharmacokinetic and pharmacodynamic assessments.

Results: Soticlestat appeared to be well tolerated up to a single dose of 1350 mg. Adverse events (AEs) were mild in intensity, and dose-dependent increase in AE prevalence was not apparent. Soticlestat administered via oral solution was rapidly absorbed (median time to maximum plasma concentration [C_{max}] 0.250–0.520 h). Mean C_{max} and area under plasma concentration–time curve from zero to infinity increased by 183- and 581-fold, respectively, over a 90-fold dose increase. Mean terminal elimination half-life was 0.820–7.16 hours across doses. Renal excretion was negligible. Administration of soticlestat tablets, and with food, lowered C_{max} but did not affect overall exposure. Plasma 24HC concentrations generally decreased with increasing dose.

Conclusions: Soticlestat appeared to be well tolerated after a single oral administration of up to 1350 mg and dose-dependently reduced plasma 24HC concentrations. Systemic exposure increased in a greater than dose-proportional manner over the dose range evaluated but was not affected by formulation or administration with food.

KEYWORDS
24*S*-hydroxycholesterol, cholesterol 24-hydroxylase inhibitor, epilepsy, NMDA signalling, pharmacokinetics

1 | INTRODUCTION

Approximately 50 million people worldwide have epilepsy.[1,2] In the United States of America, epilepsy affects nearly 3.4 million Americans of all ages[3] at an estimated annual cost of $17.6 billion in direct and indirect costs.[4] Between 49 and 139 new cases of epilepsy are diagnosed annually per 100 000 population.[1] Nearly a third of patients are refractory to available medications,[5] and the National Institute of Neurological Diseases and Stroke has identified "an urgent need for novel chemical entities that will provide a greater likelihood of complete control of seizures in patients who are currently treatment resistant".[6]

Cholesterol 24-hydroxylase (CH24H), also known as CYP46A1, is a brain-specific enzyme responsible for cholesterol catabolism.[7–9] Although CH24H is predominantly expressed in neurones in a normal state, non-clinical and human post-mortem studies have shown that neurodegeneration and brain insults lead to induction of CH24H expression in astrocytes and microglia.[10,11] In particular, **24*S*-hydroxycholesterol** (24HC), the end-product of the reaction catalysed by CH24H, may have a neurotoxic liability, such as oxidative stress and necroptosis.[12–14]

Importantly, 24HC is a positive allosteric modulator of ***N*-methyl-D-aspartate** (NMDA) receptor activity,[15] suggesting that CH24H may play a role in disorders, such as some epilepsies, that are characterized by central glutamatergic overactivation. In particular, plasma 24HC levels are higher in children and adolescents than in adults,[16] suggesting that 24HC could be a potential therapeutic target in children experiencing generalized tonic–clonic seizures observed in some developmental epileptic encephalopathies (DEE).[17] This suggestion is based on experimental findings in rodents showing that blocking NMDA receptors using **NR2B** inhibitors would restrict the spatiotemporal spread of activity in the cortex.[18]

Soticlestat is a novel small-molecule compound in development for epilepsy; it inhibits CH24H, reducing 24HC.[19] It is known that 24HC binds to NR2B subunit of the NMDA receptor to enhance its functions cooperatively,[15] so soticlestat was studied in several rodent models of epilepsy, showing its anti-epileptic effects mostly in non-focal epilepsy tests. In a mouse pentylenetetrazol (PTZ)-induced kindling development model, dose-dependent effects of soticlestat in reducing the generalized seizure frequency were observed, showing a strong correlation with 24HC lowering.[20] Furthermore, in a series of preclinical studies, soticlestat suppressed spontaneous convulsions in young APP/PS1-transgenic mice,[21] reduced kainic-acid-induced neurodegeneration in the hippocampus,[22,23] attenuated convulsions in PTZ-exposed rats[20] and blocked audiogenic seizures in genetically susceptible young mice (unpublished data). Subsequently, non-clinical pharmacokinetic (PK), genotoxicity and toxicological studies of soticlestat were successfully performed in rats and dogs. Accordingly, soticlestat was identified as a promising therapeutic candidate with the potential to control seizures in difficult-to-treat patients with developmental epileptic encephalopathies.[24]

This article describes two first-in-human (FIH), single, escalating-dose studies conducted to evaluate the safety, tolerability, PK, bioavailability, food effect and pharmacodynamics (PD) of soticlestat in healthy subjects.

What is already known about this subject

- Cholesterol 24-hydroxylase (CH24H) produces 24*S*-hydroxycholesterol (24HC), a positive allosteric modulator of *N*-methyl-D-aspartate receptors.
- Soticlestat, a first-in-class inhibitor of CH24H, reduces 24HC levels and has demonstrated efficacy in several distinct rodent models of epilepsy.

What this study adds

- Single oral doses of soticlestat up to 1350 mg appeared to be well tolerated by healthy subjects; all adverse events were mild in intensity.
- Tablet formulation and food do not significantly affect the bioavailability of soticlestat or overall drug exposure.
- Soticlestat decreases plasma 24HC levels in humans.

2 | METHODS

For each study, the clinical study protocol, protocol amendments and informed consent forms were reviewed and approved by an Institutional Review Board. Both studies were performed in accordance with the principles of the Declaration of Helsinki and in compliance with the International Council on Harmonisation Guidelines for Good Clinical Practice at Celerion (Lincoln, NE, USA; Study TAK-935-101; NCT02201056) and Parexel (Glendale, CA, USA; Study TAK-935-1005; NCT02906813). All participants provided written informed consent before participation. Both studies were prospectively registered on the ClinicalTrials.gov database prior to enrolling the first subjects in each study.

2.1 | Single rising-dose study

A phase 1, randomized, double-blind, placebo-controlled, single-dose study was performed on six cohorts of eight subjects each. Six subjects were randomly assigned to a single dose of soticlestat (oral solution; 15, 50, 200, 600, 900 or 1350 mg) and two subjects to placebo after fasting overnight. The starting dose of 15 mg was based on the no-observed-adverse-effect level (NOAEL) in dogs (considered the more sensitive species) and the projected pharmacologically active dose/exposures in humans from the epileptic mouse model, per regulatory guidance.[25] Using allometric scaling principles, applying a

10-fold safety factor, and adjusting for cross-species differences in protein binding and oral drug bioavailability, the selected 15 mg single dose was associated with a 44-fold safety margin from the dog NOAEL. The initially proposed maximal dose of 600 mg was selected to be several times the predicted pharmacologically active dose to identify doses that are safe to administer to humans, without exceeding the dog NOAEL exposure limits. Based on the favourable emerging safety and tolerability profile, and lower than predicted plasma exposure of soticlestat measured up to 600 mg, two additional doses of 900 mg and 1350 mg were studied.

A sentinel dosing approach was also used in this study, whereby initially only two subjects received soticlestat and one received placebo in each cohort. After the 24-hour postdose safety and tolerability data for these three subjects were reviewed and considered acceptable, the remaining five subjects in each cohort were administered study drug. Dose escalation decisions occurred after full review of the blinded safety, tolerability and PK data from the preceding cohort. After the first dose, dose escalation was limited to an escalated dose that was predicted to result in no greater than a three-fold increase in either the C_{max} or AUC of the immediate prior dose level. Soticlestat or placebo was administered on the morning of Day 1 after a fast of approximately 10 hours. Subjects in all cohorts then continued to fast for an additional 4 hours after dosing. Subjects could consume water ad libitum, except during the period 1 hour before and 1 hour after dosing. Dose escalation only occurred after investigator review of emerging safety and tolerability, and available PK data from all subjects in the previous cohort(s).

Eligible subjects were healthy male and female subjects, aged 19–55 years, with body mass index between 18 kg m^{-2} and 30 kg m^{-2}, weight of at least 45 kg and no medical history relevant to the study as defined by the following exclusion criteria. Subjects were excluded if they: had history of seizure or convulsion (lifetime), any uncontrolled illness, a positive urine drug screen, or history of drug or alcohol abuse; were pregnant; had current or recent (in the past 6 months) gastrointestinal disease that would have been expected to influence the absorption of drugs, a positive result for hepatitis B surface antigen or hepatitis C virus antibody, or known history of human immunodeficiency virus infection; or used nicotine-containing products.

A 4 mL blood sample was collected pre-dose on Day 1 (in the 30 minutes before dosing) and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72 and 96 hours post-dose for quantitation of plasma concentrations of soticlestat and its metabolite M-I. Additional 8 mL blood samples (for time-matched quantitation of plasma 24HC levels) were collected pre-dose (in the 30 minutes before dosing) and 0.5, 1, 2, 4, 6, 8, 12 and 16 hours post-dose on Day 1 and at the same time points at baseline on Day −1.

Urine samples for quantitation of soticlestat and its metabolite M-I were collected −12 to 0 (pre-dose on Day 1), 0 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 72 and 72 to 96 hours post-dose. Urine volume was recorded within 2 hours of the end of the collection period.

2.2 | Relative bioavailability and food-effect study

A phase 1, randomized, open-label, single-dose, three-way crossover study assessed the oral bioavailability of soticlestat 300 mg (administered as three 100 mg tablets) relative to a soticlestat 300 mg oral solution and assessed the effect of food on the bioavailability of soticlestat 300 mg when administered as tablets in healthy male and female adult subjects. A single dose of 300 mg was selected on the basis that it is expected to fall within the efficacious dose range, while providing sufficient safety margins for evaluating the effects of formulation or food on soticlestat PK. Eligible subjects ($N = 9$) were randomized in a 1:1:1 ratio to one of three sequences (ABC, BCA or CAB). Regimen A consisted of soticlestat 300 mg in tablet formulation (3 × 100 mg tablets) administered 30 minutes after starting ingestion of a high-fat meal; regimen B consisted of soticlestat 300 mg in tablet formulation (3 × 100 mg tablets) after a 10-hour fast; and regimen C consisted of a single 300 mg dose of soticlestat oral solution (10 mg/mL in sterile water with citric acid and polysorbate 80) administered after a 10-hour fast. For all regimens (A, B and C), no food was allowed within 4 hours postdose. Each treatment regimen was administered after a washout period of at least 3 days.

Blood samples (one 4 mL sample per scheduled time) for PK analysis of soticlestat and its metabolite M-I were collected at pre-dose (in the 30 minutes before dose administration) and at 0.17, 0.25, 0.33, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours after Day 1 dosing in each period.

2.3 | Safety evaluation

Safety was evaluated throughout both studies by monitoring for adverse events (AEs) and by performing clinical laboratory tests, vital sign measurements, 12-lead electrocardiograms (ECGs) and physical examinations.

Samples for clinical laboratory tests were collected after subjects had fasted for at least 10 hours overnight and in accordance with acceptable laboratory procedures. Vital signs included oral temperature, respiration rate, heart rate and blood pressure.

2.4 | Pharmacokinetic and pharmacodynamic analyses

Concentrations of soticlestat and its metabolite M-I were measured in plasma and urine by high-performance liquid chromatography with tandem mass spectrometry detection (HPLC-MS/MS). The analytes and their internal standards were extracted from human plasma or urine (50 μL) by a solid phase extraction procedure or by dilution, respectively, and detected by multiple reaction monitoring. Both assays were validated with a concentration range of 1.00 ng/mL (lower limit of quantitation [LLOQ]) to 2000 ng/mL (upper limit of quantitation [ULOQ]) for both soticlestat and its metabolite M-I.

Plasma 24HC levels were measured by HPLC-MS/MS within a validated concentration range of 2.00 ng/mL (LLOQ) to 100 ng/mL (ULOQ). Following incubation in sodium hydroxide and methanol, the analyte and its internal standard were extracted from human plasma (500 μL) by a liquid–liquid extraction procedure and detected by multiple reaction monitoring.

For all above-mentioned assays, intra-assay accuracy and precision were evaluated for each plasma (or urine) quality control (QC) pool by multiple analyses ($n = 6$) of the pool in one run. Acceptance criteria were met with accuracy within ±15% (±20% for LLOQ) of the nominal value for each QC level and %CV ≤ 15% for each QC level (≤ 20% for LLOQ). Similarly, inter-assay accuracy and precision were achieved from the plasma (or urine) QC pools ($n = 6$) in three validation runs over three separate days.

The PK and PD parameters were estimated from plasma or urine concentration–time data using standard non-compartmental analysis methods with Phoenix WinNonlin™ version 6.3 (Certara, Princeton, NJ, USA). Plasma PK parameters included area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration ($AUC_{(0\text{-}t)}$), area under plasma concentration–time curve from zero to infinity (AUC_{∞}), maximum plasma concentration (C_{max}), apparent plasma clearance of drug after extravascular administration (CL/F; for soticlestat only), terminal elimination half-life ($t_{1/2z}$), time to C_{max} (t_{max}), apparent volume of distribution during the terminal phase after extravascular administration (V_z/F; for soticlestat only) and metabolic ratio (MR; calculated as [AUC_{∞} (M-I) × soticlestat molecular weight] [AUC_{∞} (soticlestat) × M-I molecular weight]$^{-1}$). Urine PK parameters included cumulative amount of drug excreted unchanged in urine from zero to 96 hours (Ae_{96}), fraction of dose excreted unchanged into urine (f_e; for soticlestat only) and renal clearance of drug (CL_R). Plasma PD parameters included area under the effect–time curve from zero to 24 hours ($AUEC_{24}$; baseline and post-dose), area under the effect–time curve from zero to 96 hours ($AUEC_{96}$; post-dose), observed effect at 24 hours (E_{24}; baseline and post-dose), percent change from time-matched baseline for $AUEC_{24}$ and percent change from time-matched baseline for E_{24}.

2.5 | Statistical analysis

All data analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). Data for PK parameters for soticlestat and its metabolite M-I, and PD parameters for plasma 24HC are presented using descriptive statistics throughout. Dose proportionality for C_{max} and AUC was assessed using the Power model. Increases in C_{max}, $AUC_{(0\text{-}t)}$ and AUC_{∞} were considered to be dose-proportional if the 90% confidence interval (CI) for the slope of the log-transformed dose and PK parameter was within the range of 0.95–1.05. The effect of dose on t_{max} and terminal rate constant (λ_z) was assessed using an analysis of variance (ANOVA) model with dose level as a fixed factor.

In the bioavailability study, ANOVA was performed on natural logarithms of soticlestat C_{max} and AUC with factors of sequence, subject nested within sequence, period and regimen. The factor of subject nested within sequence was a random effect, and other factors are fixed effects. Within the framework of ANOVA, pairwise comparisons were performed to assess the relative bioavailability of soticlestat and M-I for the tablet formulation relative to the oral solution formulation via point estimates of the central value ratios of two formulations and their 90% CIs for C_{max} and AUC. The effect of food on the bioavailability of soticlestat tablet formulation (after a high-fat meal *vs* in a fasted state) were evaluated via point estimates of the central value ratios of fed/fasting conditions and their 90% CIs for C_{max} and AUC for soticlestat and M-I.

AEs were summarized as the number and percentage of subjects who experienced at least one AE.

2.6 | Nomenclature of targets and ligands

All molecular target nomenclature conforms to the IUPHAR/BPS guide to pharmacology nomenclature classification. Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[26,27]

3 | RESULTS

In total, 48 subjects were enrolled in the single rising-dose study in a total of six cohorts and were randomly assigned to treatment or placebo. Nine subjects were enrolled in the relative bioavailability and food-effect study. All subjects completed the studies. Subject demographics are presented in Table 1.

3.1 | Safety and tolerability of soticlestat

Single doses of soticlestat ranging from 15 mg to 1350 mg administered as an oral solution appeared to be well tolerated. Five (41.7%) of the 12 subjects who received placebo and 14 (38.9%) of the 36 who received soticlestat reported a total of 13 and 29 treatment-emergent AEs (TEAEs), respectively. The incidence of individual TEAEs in subjects who received soticlestat did not appear to be dose-dependent.

The most frequently reported TEAEs were headache (placebo, $n = 2$; soticlestat, $n = 3$), ECG electrode application-site dermatitis (soticlestat, $n = 2$) and nausea (soticlestat, $n = 2$). All TEAEs were considered by the investigator to be mild in intensity. No deaths, other serious or severe AEs or AEs leading to study drug or study visit discontinuation were reported. No clinically significant abnormalities in clinical laboratory tests (including hormone levels), or changes from baseline in vital signs, physical examinations, comprehensive eye examinations or ECG measurements were observed during the study at all doses.

TABLE 1 Subject demographics and baseline characteristics

	SRD			rBA/FE
Characteristic	Placebo ($n = 12$)	Soticlestat (all arms) ($n = 36$)	Total ($N = 48$)	Total ($N = 9$)
Age, mean (SD), years	35.9 (10.41)	32.8 (10.10)	33.6 (10.15)	36.7 (8.00)
Sex, *n* (%)				
Male	7 (58.3)	19 (52.8)	26 (54.2)	7 (77.8)
Female	5 (41.7)	17 (47.2)	22 (45.8)	2 (22.2)
Race, *n* (%)				
White	10 (83.3)	29 (80.6)	39 (81.3)	3 (33.3)
Black or African American	2 (16.7)	6 (16.7)	8 (16.7)	5 (55.6)
Multiracial	0	1 (2.8)	1 (2.1)	1 (11.1)
Ethnicity, *n* (%)				
Hispanic or Latino	1 (8.3)	3 (8.3)	4 (8.3)	2 (22.2)
Non-Hispanic or -Latino	11 (91.7)	33 (91.7)	44 (91.7)	7 (77.8)
Height, mean (SD), cm	170.8 (5.99)	169.9 (9.16)	170.1 (8.43)	171.1 (3.44)
Weight, mean (SD), kg	70.2 (10.48)	73.9 (12.70)	73.0 (12.18)	77.6 (9.04)
BMI, mean (SD), kg m^{-2}	24.0 (2.53)	25.5 (3.16)	25.1 (3.06)	26.5 (2.43)

Abbreviations: BMI, body mass index; rBA/FE, relative bioavailability and food-effect study; SD, standard deviation; SRD, single rising-dose study.

3.2 | Pharmacokinetics of soticlestat

Mean plasma soticlestat C_{max} ranged from 43.5 ng/mL for the 15 mg dose to 7950 ng/mL for the 1350 mg dose (Table 2). Median t_{max} was between 0.25 hours and 0.52 hours for all soticlestat doses (15–1350 mg) (Table 2). A second smaller peak was observed at approximately 8 hours post-dose for all doses (Figure 1).

Mean AUC_{∞} (% coefficient of variation [%CV]) ranged from 23.3 (28.8) ng·h/mL for the 15 mg dose to 13 500 (42.6) ng·h/mL for the 1350 mg dose (Table 2). Similar results were observed for $AUC_{(0\text{-}t)}$ (33.3 [86.9] ng·h/mL and 13 500 [42.8] ng·h/mL, respectively). Mean $t_{1/2z}$ for soticlestat ranged from 0.820 hours to 7.16 hours (Table 2), although $t_{1/2z}$ estimates at the lower doses of 15 mg and 50 mg must be viewed with caution because soticlestat levels rapidly dropped below the LLOQ. Mean CL/F for soticlestat decreased with increasing soticlestat dose from 689 L/h with 15 mg dosing to 112 L/h with 1350 mg dosing (Table 2). Mean V_z/F ranged from 732 to 2240 L, but no dose-related trend was apparent (Table 2).

The estimated slopes (90% CI) for the relationship between log-transformed soticlestat PK parameters and log-transformed soticlestat doses were 1.22 (1.11, 1.32), 1.38 (1.28, 1.48) and 1.42 (1.31, 1.53) for C_{max}, $AUC_{(0\text{-}t)}$ and AUC_{∞}, respectively. All estimated slopes failed to meet the pre-specified criteria for dose proportionality and were significantly higher than 1 ($P < .001$ for all).

Mean plasma M-I concentrations increased with increasing soticlestat dose with a C_{max} at approximately 0.5–1 hours after soticlestat administration, followed by a relatively fast decline (Table 3).

Renal excretion of soticlestat was negligible. Only approximately 0.05–0.6% of each soticlestat dose was excreted in urine, and CL_R was only 0.230–0.620 L/h (Table 2). In contrast, CL_R of M-I was higher at 3.23–4.76 L/h (Table 3).

The oral solution and tablet formulations for soticlestat offered similar plasma concentration–time profiles with a 300 mg dose. Plasma soticlestat concentrations for the tablet formulation were only slightly lower than for the oral solution 16 hours post-dose. A secondary peak in plasma soticlestat levels was observed approximately 10 hours post-dose with both the oral solution and tablet formulations.

Soticlestat C_{max} was 36.9% lower with the tablet than with the oral solution formulation, but AUC_{∞} was only 15.8% lower (Table 4). Median t_{max} was also delayed from 0.35 hours with the oral solution to 0.53 hours with the tablet. No difference in $t_{1/2z}$ was observed between the oral solution and oral tablet formulations (mean [%CV]: 4.29 [33.5] h and 4.13 [38.5] h, respectively).

In the food-effect study, after reaching C_{max}, plasma concentrations of soticlestat tablet under fed conditions were slightly higher than the levels reported under fasting conditions up to 10 hours post-dose. Plasma concentrations were comparable between fasting and fed conditions from 10 hours post-dose (Figure 2). A secondary peak in plasma soticlestat concentration was observed approximately 10 hours post-dose under both fasting and fed conditions but was slightly more apparent under fasting conditions. AUC_{∞} for soticlestat when administered as an oral tablet under fed conditions was only 10.6% lower than that under fasting conditions (Table 4). Compared with under fasting conditions, median t_{max} under fed conditions was delayed from 0.53 hours to 2.00 hours, and the corresponding soticlestat C_{max} decreased by 59.7% under fed conditions (Table 4). After a single-dose administration of 300 mg soticlestat oral tablets, mean (%CV) soticlestat $t_{1/2z}$ values were similar under fasting or fed conditions (4.13 [38.5] hours and 5.00 [61.7] hours, respectively).

TABLE 2 Summary of plasma and urine PK parameters of soticlestat after administration of a single oral dose in healthy subjects

	Soticlestat 15 mg ($n = 6$)	Soticlestat 50 mg ($n = 6$)	Soticlestat 200 mg ($n = 6$)	Soticlestat 600 mg ($n = 6$)	Soticlestat 900 mg ($n = 6$)	Soticlestat 1350 mg ($n = 6$)
Soticlestat plasma PK parameters						
C_{max}, mean (%CV), ng/mL	43.5 (94.7)	210 (72.8)	571 (45.1)	4552 (56.5)	5080 (35.5)	7953 (27.0)
t_{max}, median (range), h	0.25 (0.25–0.25)	0.25 (0.25–0.50)	0.25 (0.23–0.50)	0.52 (0.25–1.00)	0.50 (0.50–1.48)	0.50 (0.50–1.00)
$AUC_{(0\text{-}t)}$, mean (%CV), ng·h/mL	33.3 (86.9)	169 (52.4)	563 (35.0)	5790 (59.2)	7620 (25.3)	13 500 (42.8)
AUC_{∞}, mean (%CV), ng·h/mL	23.3 (28.8)[a]	172 (62.1)[b]	621 (38.7)[a]	5810 (59.0)	7660 (25.6)	13 500 (42.6)
$t_{1/2z}$, mean (%CV), h	0.820 (82.8)[a]	2.99 (68.8)[b]	4.39 (43.3)[a]	4.77 (53.8)	7.16 (34.3)	5.31 (21.3)
V_z/F, mean (%CV), L	732 (62.3)[a]	1500 (49.6)[b]	2240 (50.1)[a]	993 (69.7)	1230 (26.3)	852 (34.0)
CL/F, mean (%CV), L/h	689 (33.9)[a]	549 (112.4)[b]	355 (37.4)[a]	145 (64.3)	124 (25.4)	112 (34.7)
Soticlestat urine PK parameters						
CL_R, mean (%CV), L/h	0.280 (33.3)	0.230 (35.4)	0.360 (27.0)	0.320 (39.1)	0.440 (38.0)	0.620 (27.5)
f_e (%), mean (%CV)	0.0500 (39.2)	0.0700 (46.6)	0.100 (27.4)	0.360 (91.8)	0.400 (57.8)	0.600 (42.2)

Values rounded to three significant figures, except for t_{max} presented as two decimal places.

Abbreviations: AUC_{∞}, area under plasma concentration–time curve from zero to infinity; $AUC_{(0\text{-}t)}$, area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration; CL/F, apparent plasma clearance of drug after extravascular administration; CL_R, renal clearance of drug; C_{max}, maximum plasma concentration; CV, coefficient of variation; f_e, fraction of dose excreted unchanged into urine; PK, pharmacokinetic; $t_{1/2z}$, terminal elimination half-life; t_{max}, time to maximum plasma concentration; V_z/F, apparent volume of distribution during the terminal phase after extravascular administration.

[a]$N = 3$;

[b]$N = 4$.

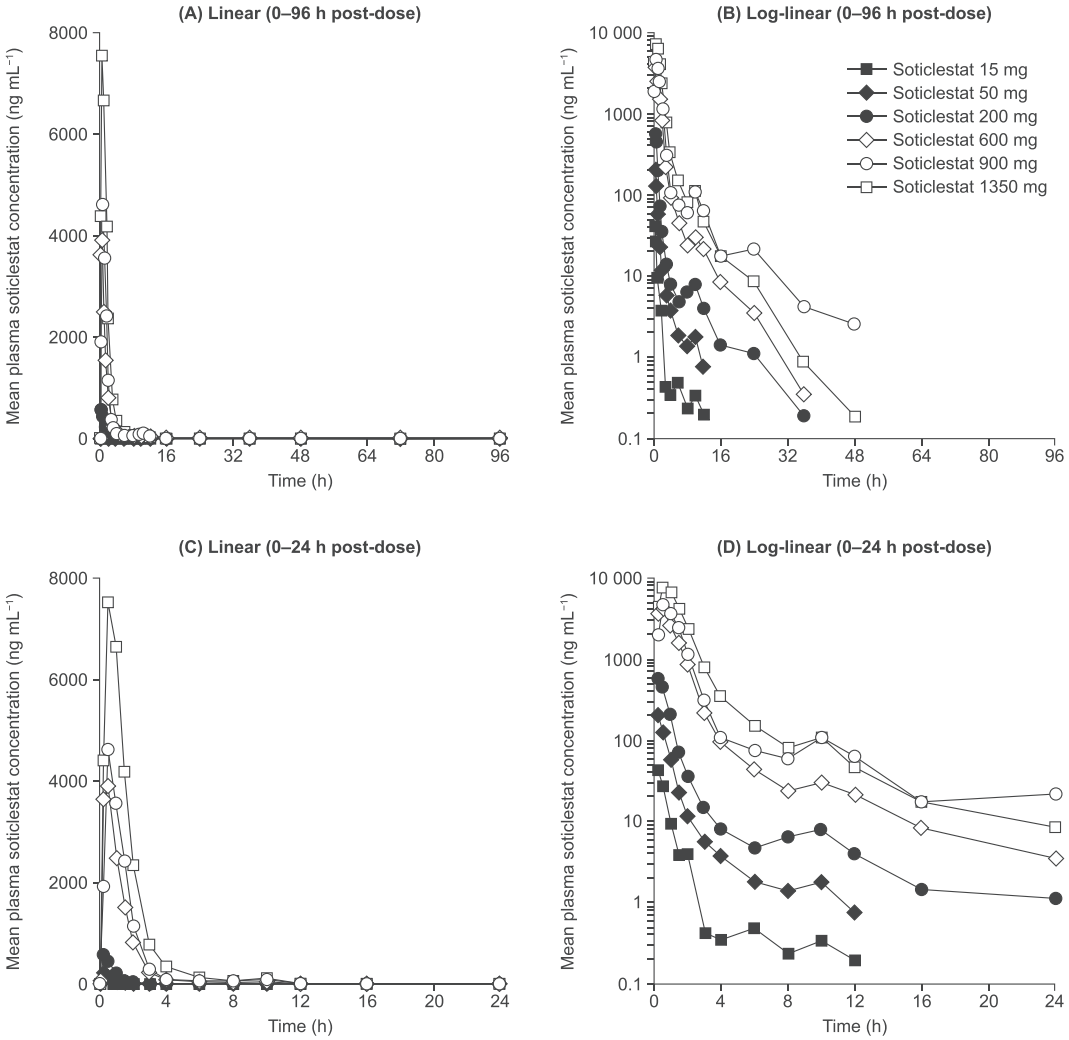

FIGURE 1 Mean plasma concentration–time profiles of soticlestat after administration of a single oral dose

3.3 | Pharmacodynamics of soticlestat

At baseline (Day −1), mean plasma 24HC concentrations generally fluctuated from 40 ng/mL to 55 ng/mL across the placebo and soticlestat groups (Figure 3). No difference in mean plasma 24HC concentrations was apparent between the placebo group and soticlestat 15 mg group, but mean plasma 24HC concentrations trended lower in the soticlestat 50–1350 mg groups (Figure 3). The magnitude of any decrease in 24HC levels from Days 1 to 4 post-dose appeared to be greater with increasing dose (Figure 3C).

For the 50, 200 and 600 mg groups, maximum reductions in 24HC levels were observed approximately 16 hours post-dose, and approximately 48 hours post-dose in the 900 and 1350 mg groups (Figure 3A and 3C). 24HC levels returned to baseline levels by 96 hours post-dose in all groups (Figure 3C). Time-matched analyses over the first 24 hours following soticlestat administration also showed a maximum mean decrease in plasma 24HC concentration of approximately 23% at 16 hours for the 900 mg group (Figure 3B).

Dose-dependent decreases in E_{24}, $AUEC_{24}$ and $AUEC_{96}$ estimates were also observed, with an estimated maximum decrease in mean $AUEC_{96}$ of approximately 28.7% *vs* placebo observed with the 900 mg dose. The time-matched maximum mean change was approximately 21% for E_{24} and 14% for $AUEC_{24}$.

4 | DISCUSSION

Single oral doses of soticlestat ranging from 15 mg to 1350 mg appeared to be generally well tolerated when administered as an oral solution or tablet to healthy subjects in a fasting or fed state. All AEs were mild in intensity, no dose-limiting toxicities were observed and no subjects experienced AEs leading to study drug or study visit discontinuation. Accordingly, the full planned dose range of this study was investigated, and the PK and PD profiles of soticlestat were characterized. Furthermore, compared with the observed exposure (area under the plasma concentration–time curve from zero to 24 hours [AUC_{24}] = 700 ng·h/mL) at the minimal

TABLE 3 Summary of plasma and urine PK parameters of the soticlestat metabolite M-I after administration of a single oral dose in healthy subjects

	Soticlestat 15 mg ($n = 6$)	Soticlestat 50 mg ($n = 6$)	Soticlestat 200 mg ($n = 6$)	Soticlestat 600 mg ($n = 6$)	Soticlestat 900 mg ($n = 6$)	Soticlestat 1350 mg ($n = 6$)
M-I plasma PK parameters						
C_{max}, mean (%CV), ng/mL	19.3 (34.4)	56.4 (66.1)	151 (35.9)	542 (23.6)	833 (13.2)	900 (19.8)
t_{max}, median (range), h	0.50 (0.25–1.98)	0.38 (0.25–0.50)	0.50 (0.48–0.53)	0.53 (0.50–1.00)	1.00 (0.50–1.50)	1.25 (0.98–1.50)
$AUC_{(0\text{-}t)}$, mean (%CV), ng·h/mL	39.1 (43.5)	114 (55.1)	293 (26.5)	1490 (31.8)	2710 (17.1)	3110 (18.3)
AUC_{∞}, mean (%CV), ng·h/mL	45.8 (37.0)[a]	118 (53.6)	299 (25.9)	1500 (31.7)	2780 (18.0)[a]	3120 (18.2)
$t_{1/2z}$, mean (%CV), h	1.59 (29.2)[a]	1.44 (10.8)	2.92 (32.3)	3.64 (45.7)	7.82 (52.6)[a]	4.45 (47.7)
MR (based on AUC_{∞}),[b] mean (%CV)	1.84 (42.9)[c]	0.750 (30.0)[d]	0.580 (24.4)[c]	0.300 (37.6)	0.340 (22.8)[a]	0.240 (32.6)
M-I urine PK parameters						
CL_R, mean (%CV), L/h	3.66 (32.4)	3.23 (23.4)	3.87 (24.9)	3.51 (41.8)	3.52 (14.0)	4.76 (14.8)

Values rounded to three significant figures except for t_{max} presented as two decimal places.
Abbreviations: AUC_{∞}, area under plasma concentration–time curve from zero to infinity; $AUC_{(0\text{-}t)}$, area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration; CL_R, renal clearance of drug; C_{max}, maximum plasma concentration; MR, metabolic ratio; PK, pharmacokinetic; $t_{1/2z}$, terminal elimination half-life; t_{max}, time to maximum plasma concentration.
[a]$N = 5$;
[b]MR = (AUC_{∞} of M-I) × (soticlestat molecular weight)/(AUC_{∞} of soticlestat) × (M-I molecular weight)$^{-1}$;
[c]$N = 3$;
[d]$N = 4$.

TABLE 4 Plasma PK parameters after administration of a single 300 mg soticlestat dose (oral tablet *vs* oral solution [fasted], and oral tablet [fed] *vs* oral tablet [fasted])

	Tablet *vs* solution formulation					Food effect				
	LS mean (natural logarithmic scale)					LS mean (natural logarithmic scale)				
Parameter	*n*	Regimen B: Tablet, fasted (test)	n	Regimen C: Solution, fasted (reference)	Point estimate (90% CI)[a]	*n*	Regimen A: Tablet, fed (test)	*n*	Regimen B: Tablet, fasted (reference)	Point estimate (90% CI)[a]
C_{max}, ng/mL	9	6.903	9	7.363	0.631 (0.411, 0.969)	9	5.993	9	6.903	0.403 (0.262, 0.618)
$AUC_{(0\text{-}t)}$, ng.h/mL	9	7.139	9	7.301	0.850 (0.706, 1.024)	9	7.020	9	7.139	0.889 (0.738, 1.070)
AUC_{∞}, ng.h/mL	8	7.144	8	7.315	0.842 (0.676, 1.050)	9	7.031	8	7.144	0.894 (0.726, 1.100)

Abbreviations: AUC_{∞}, area under plasma concentration–time curve from zero to infinity; $AUC_{(0\text{-}t)}$, area under the plasma concentration–time curve from zero to the time of the last quantifiable concentration; CI, confidence interval; C_{max}, maximum plasma concentration; LS, least-squares; PK, pharmacokinetic.
Regimen A: three 100 mg soticlestat tablets (total dose 300 mg) on day 1, administered orally 30 minutes after starting ingestion of a high-fat meal.
Regimen B: three 100 mg soticlestat tablets (total dose 300 mg) on day 1, administered orally after a 10-hour fast.
Regimen C: a single 300 mg dose of soticlestat solution on day 1, administered orally after a 10-hour fast.
[a]Obtained by taking exponentiation of the differences in natural-log scale.

efficacious dose in an epileptic mouse model (unpublished data), similar mean soticlestat exposure (AUC_{∞}) was reached with a 200 mg dose of soticlestat (621 ng·h/mL) and was approximately 19-fold higher than that with the 1350 mg dose (13 500 ng·h/mL), suggesting that therapeutic dosing can be achieved with an acceptable safety and tolerability profile.

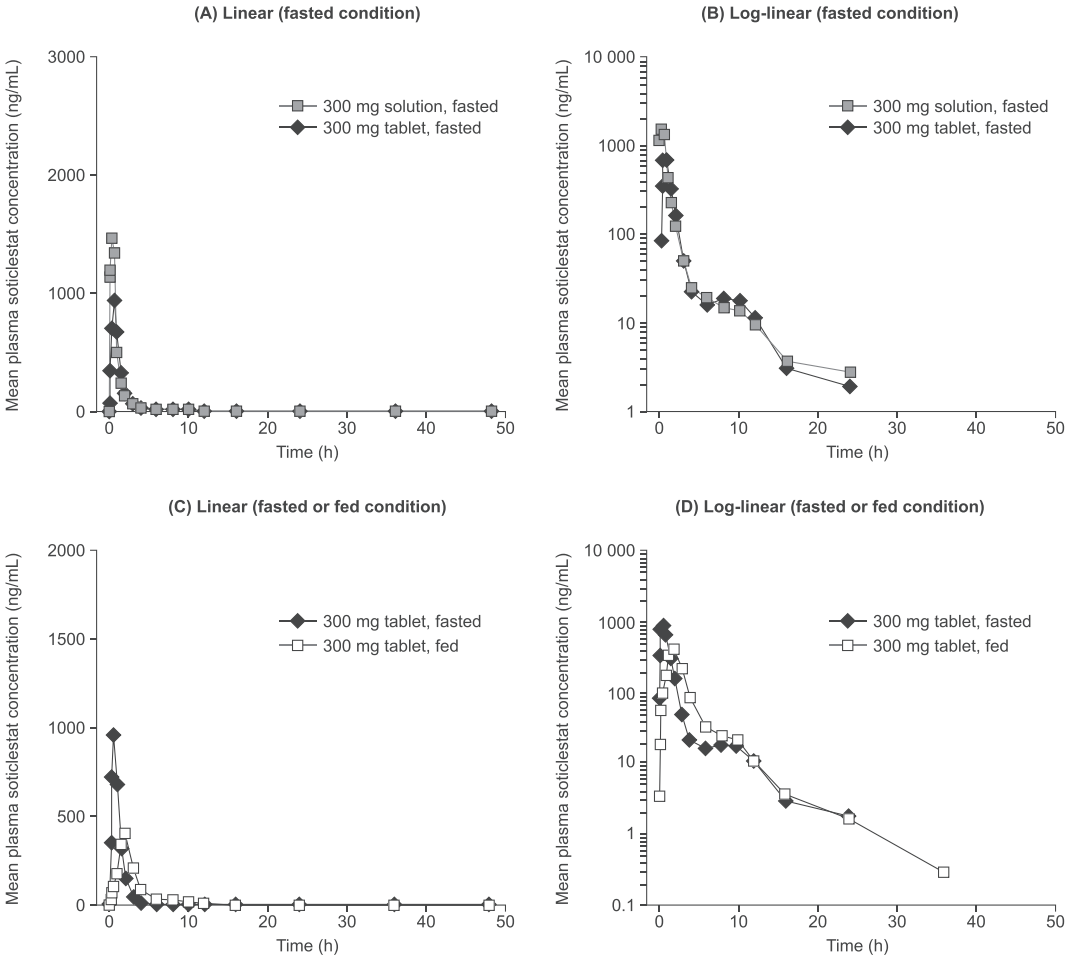

FIGURE 2 Mean plasma concentration–time profiles of soticlestat after single oral administration of the solution under fasted conditions, and tablet under fasted or fed conditions

Soticlestat oral solution was rapidly absorbed with a median t_{max} of 0.25–0.52 hours post-dose, which was soon followed by the plasma C_{max} for M-I at 0.5–1 hours. Soticlestat was eliminated from plasma with a mean $t_{1/2z}$ of approximately 4–7 hours, although the accuracy of $t_{1/2z}$ could not be adequately characterized at the lower doses of soticlestat because plasma levels rapidly dropped below the LLOQ. Exposure to soticlestat increased in a greater than dose-proportional manner over the 15–1350 mg dose range, indicating a nonlinear PK profile that may warrant attention when multiple-dose studies are performed. It was noted that the apparent CL/F values of soticlestat decreased as dose increased, although V_z/F did not show dose-related trends, suggesting that drug absorption (i.e., bioavailability) rather than drug disposition (i.e., metabolism and excretion) might be affected by dose.

Renal excretion of the parent drug soticlestat was limited, with no more than 0.6% of soticlestat being recovered in urine, suggesting a minor contribution to soticlestat elimination. This is consistent with the metabolite profiling from animal and in vitro studies, indicating that the major route of elimination of soticlestat is via hepatic metabolism. Glucuronidation is the major metabolic pathway for soticlestat in human hepatocytes, with oxidation playing a minor role.

The circulating N-oxide metabolite of soticlestat (M-I) identified in preclinical studies was measured in human plasma with a mean metabolic ratio (based on AUC_{∞}) of 1.84 to 0.240, generally decreasing over the soticlestat dose range of 15–1350 mg, which is consistent with the observed greater than dose-proportional increase in soticlestat plasma exposures. Given the weak potency of M-I, as shown by an approximate 200-fold higher IC_{50} value than soticlestat in an in vitro assay on human CH24H activity, M-I is expected to contribute negligibly to the PD effects of soticlestat.

The AUC for the oral tablet formulation was comparable to that of the oral solution for a single 300 mg dose of soticlestat, whereas C_{max} was approximately 37% lower, as may be expected when absorption is slowed by a tablet versus solution formulation. Coadministration with food also had little impact on the AUC of the soticlestat tablet but further delayed oral absorption (median t_{max} 2.00 h *vs* 0.500 h) and lowered the C_{max} by nearly 60%. The lower C_{max} with the tablet formulation, and when administered with food, may need to be considered when selecting dosages for further studies to ensure that potentially therapeutic concentrations are being achieved and maintained, while potentially avoiding the risk of greater than dose-proportional drug accumulation with high doses. However,

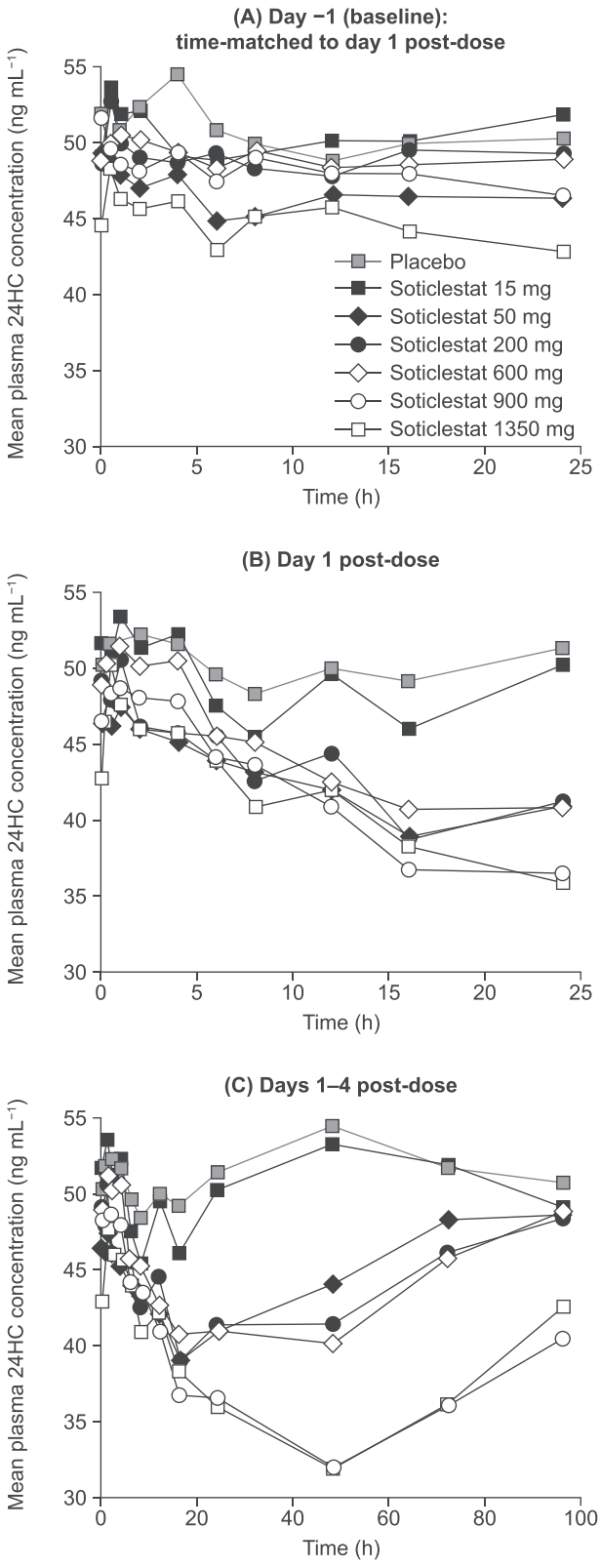

FIGURE 3 Plasma concentration–time profiles of 24HC at baseline and after administration of a single oral dose of soticlestat. Abbreviation: 24HC, 24-hydroxycholesterol

as total systemic exposure (AUC) remained nearly unchanged, the effectiveness of soticlestat is not expected to be compromised. Thus, it has been concluded that administration of soticlestat oral tablets independently of meals is feasible.

After soticlestat single-dose administration, plasma 24HC concentrations decreased in a dose-dependent manner, indicating that soticlestat likely reached and interacted with the target enzyme, CH24H, in brain tissue. Time-matched percentage change from baseline in 24HC plasma concentrations over a 24-hour interval, controlled for the potential influence of circadian rhythm, showed a maximum decrease of 23% at 16 hours post-dose after a single 900 mg dose of soticlestat. The corresponding maximum decrease in E_{24} and $AUEC_{24}$ was approximately 21% and 14%, respectively. Given that the nadir following this relatively high dose was observed as late as 48 hours post-dose, which is considerably delayed compared with the median t_{max} of 0.25–0.52 hours for soticlestat in plasma, these time-matched estimates from a 24-hour sampling period should be interpreted with some caution. However, the observed lag between C_{max} and a PD response is typical of drugs that act through an indirect mechanism of action, in which time to maximal response is dose-dependent and delayed at larger doses.[28–30]

These initial observations after single-dose treatment are to be investigated further following multiple daily dosing to optimize soticlestat dosing regimen. Of note, while cerebrospinal fluid samples were not collected in this FIH study, positron emission tomography (PET) imaging conducted in healthy subjects demonstrated that single, oral doses of 50 to 600 mg of soticlestat were centrally penetrant and led to specific CH24H enzyme occupancy in a dose- and time-dependent manner, thus establishing the strong relationship between soticlestat exposure and target engagement in humans.[31] Taking these data together, a population PK/PD modelling effort is currently underway to describe the relationships between dose and exposures of soticlestat, PET-derived CH24H enzyme occupancy in the brain, and reduction in 24HC plasma levels using pooled data from multiple clinical studies to support rational dose selection for subsequent studies in patients following chronic treatment with soticlestat.[32]

In conclusion, soticlestat was well tolerated after a single oral administration of up to 1350 mg in healthy subjects with greater than dose-proportional increase in systemic exposure observed over the dose range evaluated and a dose-dependent decrease in the plasma 24HC concentrations, indicative of central target engagement and downstream PD effects. Administration of soticlestat as a tablet formulation, or in combination with food, decreased C_{max} but did not affect overall drug exposure compared with administration as an oral solution.

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COMPETING INTERESTS

S.W., M.C. and H.F. are employees of Takeda Pharmaceutical Limited Company and own stock or stock options. G.C., E.M.P. and J.A. are former employees of Takeda and own stock or stock options.

CONTRIBUTORS

All authors contributed to the study conception and design. Data (PK and PD) analyses were performed by the Quantitative Clinical Pharmacology group at Takeda. All authors were involved in manuscript preparation and review. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The datasets, including the redacted study protocol, redacted statistical analysis plan, and de-identified individual participants data supporting the results reported in this article, will be available 3 months after the submission of a request, to researchers who provide a methodologically sound proposal. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection and requirements for consent and anonymization.

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