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ORIGINAL ARTICLE

Pharmacokinetics, pharmacodynamics and safety assessment of multiple doses of soticlestat in healthy volunteers

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Aims: Soticlestat, a first-in-class inhibitor of cholesterol 24-hydroxylase (also known as cytochrome P450 46A1), is currently in development for the treatment of developmental and epileptic encephalopathies. Here, we report safety, tolerability, pharmacokinetic and pharmacodynamic outcomes from a phase I, randomized, double-blind, placebo-controlled, multiple-rising-dose study of soticlestat in healthy adults.

Methods: Five cohorts of healthy subjects (n = 8 each, randomized 6:2 soticlestat: placebo) received oral soticlestat 100-600 mg once daily (QD) or 300 mg twice daily (BID) for 10-14 days. Serial blood and urine samples were obtained on days 1, 7 (blood only) and 14.

Results: Soticlestat in the dose range 100-400 mg/day for up to 14 days was generally well tolerated. In total, 45 treatment-emergent adverse events (TEAEs) were reported; most (91%) were transient and mild in intensity. Two subjects experienced TEAEs leading to discontinuation: one receiving soticlestat 600 mg QD reported a severe event of acute psychosis; another receiving 300 mg BID reported a mild event of confusional state. Steady-state exposure to soticlestat increased in a slightly greater than dose-proportional manner across the dose range 100-400 mg QD. Peak plasma concentrations were reached within 0.33-0.5 hour, and soticlestat elimination half-life was approximately 4 hours. Renal excretion of soticlestat was negligible. Soticlestat 100-400 mg QD reduced 24*S*-hydroxycholesterol levels by 46.8 (coefficient of variation [CV%] −9.2) to −62.7% (CV% −7.3) at steady state; values of enzymatic inhibition were compatible with antiepileptic effects observed in preclinical models.

Conclusion: The pharmacokinetic and pharmacodynamic profiles of soticlestat characterized here provided a data-driven rationale for clinical trial dose selection.

KEYWORDS
24*S*-hydroxycholesterol, brain, cholesterol 24-hydroxylase, epilepsy, N-methyl-D-aspartate (NMDA) receptor

I and the authors of this manuscript confirm that the Principal Investigator for this paper was myself, Dr. Theresa Pham M.D., and that I had direct clinical responsibility for its patients. I further state that my name was not provided for authorship on this manuscript.

Grace Chen, Emilio Merlo Pich and John Affinito were Takeda employees at the time the study was conducted.

1 | INTRODUCTION

Epilepsy is relatively common, affecting an estimated 65-70 million people worldwide,[1,2] with a prevalence of 0.4-1.0%, accounting for approximately 5% of the global burden of neurological diseases.[3,4] Developmental and epileptic encephalopathies (DEEs) comprise an important group of epilepsies associated with severe cognitive and developmental impairments, including Dravet syndrome and Lennox-Gastaut syndrome, with symptoms that typically manifest during infancy and childhood.[5] Traditional antiseizure medications (ASMs) often fail to control seizures in DEEs and minimally impact the cognitive and behavioural impairments associated with the progression of the disorders.[6] The recognition of this situation has prompted several new lines of research addressing molecular mechanisms that were not fully explored previously.

Glutamate, the major excitatory neurotransmitter in the central nervous system, is known to play an important role in neuronal excitability, synaptic plasticity, memory, learning and other cognitive functions.[7,8] Abnormal glutamate signalling leading to overstimulation of glutamate receptors can lead to increased neuronal excitability and cellular degeneration via excitotoxicity, a well-known mechanism involved in epileptogenesis.[7,8]

Studies in patients with status epilepticus suggest that antagonizing or modulating the N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptors (tetrameric structures comprising the NR1, NR2A and NR2B subunits) can have an antiepileptogenic effect and may be disease-modifying, reducing the risk of developing epilepsy-related cognitive impairment due to the excitotoxicity damage.[8,9] Furthermore, patients with focal epilepsies have been shown to have increased NMDA receptor-mediated signalling throughout the brain.[10]

Felbamate is currently the only ASM with a clinically relevant action at both NMDA and gamma-aminobutyric acid (GABA) receptors[8] that is used in children with one of the DEE syndromes (Lennox-Gastaut syndrome). Of note, the development of novel ASMs with a mechanism of action mediated via NMDA-receptor blockade has been limited by the potential risk of psychotomimetic behavioural effects and neurotoxicity observed in preclinical safety studies.[8,11,12] A consistent set of observations indicated that this limitation could be circumvented by exerting a negative allosteric modulation of the NMDA-receptor function rather than antagonism or blockade, and by identifying the proper exposure to optimize effectiveness and safety.[8,9,13] In the normal brain, endogenous positive modulatory agents are present in the extracellular fluid, contributing to the regulation of the NMDA-receptor activity, augmenting the synaptic glutamate effect as neurotransmitter. One of them, 24S-hydroxycholesterol (24HC),[14] is synthesized by the enzyme cholesterol 24-hydroxylase (CH24H, also known as cytochrome P450 46A1), with expression almost completely restricted to the brain.[13,14] The brain-produced 24HC acts locally and then diffuses into the circulatory system to be metabolized in the liver. In healthy children, the circulating 24HC levels are higher than in adults,[15] therefore it is possible that in young subjects with DEEs, the high levels of 24HC may contribute to the enhanced activity of central NMDA receptors. This could partially explain the higher frequency of seizures observed in children compared with in adults. This hypothesis is consistent with the experimental findings in young rodents, showing that the antiepileptic and neuroprotective effects of soticlestat, a novel brain-penetrant CH24H enzyme inhibitor, were associated with a dose-dependent reduction in circulating levels of 24HC.[16,17]

The biodistribution and target engagement of soticlestat has been assessed in mice, with CH24H-knockout mice exhibiting a substantially lower level of soticlestat distribution in the brain than wild-type controls.[17] In addition, single oral administration of soticlestat in mice was associated with a dose-dependent reduction in 24HC levels in the brain, confirming that soticlestat is brain penetrable.[18] The brain accounts for nearly all of the production of 24HC, which demonstrates unidirectional excretion. Approximately 99% of the total excretion of 24HC by the brain occurs across the blood-brain barrier directly into the bloodstream,[19] therefore plasma 24HC levels can be used as a pharmacodynamic maker reflecting the level of CH24H inhibition by soticlestat in the brain.

What is already known about this subject

- Soticlestat, a first-in-class inhibitor of cholesterol 24-hydroxylase, has demonstrated efficacy in several distinct rodent models of epilepsy.
- Soticlestat single doses were generally well tolerated in healthy subjects and dose-dependently reduced plasma 24*S*-hydroxycholesterol concentrations, indicative of central target engagement and downstream pharmacodynamic effects.
- Soticlestat could provide a new treatment option for developmental and epileptic encephalopathies such as Dravet syndrome and Lennox-Gastaut syndrome via a different mechanism of action from those of currently available antiseizure medications.

What this study adds

- Soticlestat showed slightly greater than dose-proportional steady-state exposure after daily dosing for 14 days.
- Soticlestat daily doses inhibited cholesterol 24-hydroxylase activity, resulting in a dose-dependent decrease in 24*S*-hydroxycholesterol levels thought to be associated with antiseizure effects seen in preclinical models.
- This study informed dosing in subsequent clinical trials in paediatric patients with developmental and epileptic encephalopathies.

A recent phase I, first-in-human, single-rising-dose study has found soticlestat to be well tolerated in healthy subjects in doses up to 1350 mg.[20] In the same study, a dose-dependent reduction of circulating levels of 24HC was observed, in keeping with the preclinical findings. The aim of the study reported here was to further investigate the safety and tolerability of multiple escalating daily doses of soticlestat in healthy subjects, and the pharmacokinetics (PK) and pharmacodynamics (PD) of soticlestat over 14 days of administration. This study also aimed to guide dosing in subsequent clinical studies that included paediatric and adult patients with DEEs.

2 | METHODS

2.1 | Study description and treatment

This was a phase I, randomized, double-blind, placebo-controlled, multiple-rising-dose study conducted at a single centre in the United States. The main objectives were to characterize the safety, tolerability and PK profiles of soticlestat when administered as an oral solution at escalating daily dose levels. Additional exploratory objectives were to characterize the PK of the *N*-oxide metabolite (M-I) of soticlestat and explore the PD effect of multiple doses of soticlestat on plasma 24HC levels.

This study was conducted in compliance with the Institutional Review Board regulations stated in Title 21 of the United States Code of Federal Regulations, Part 56, Good Clinical Practice (GCP) regulations and guidelines, and all applicable local regulations. It was conducted according to the ethical principles that have their origin in the Declaration of Helsinki and the International Conference on Harmonisation Tripartite Guideline for GCP. All subjects provided written informed consent prior to enrolment. This study was registered on **ClinicalTrials.gov** prior to enrolment of the first study subject (**ClinicalTrials.gov** identifier: NCT02539134). All molecular target nomenclature conforms to the IUPHAR/BPS Guide to PHARMACOLOGY nomenclature classification.[21]

2.2 | Study eligibility criteria

Study participants were healthy male and female adults, 18-55 years old, with a body mass index (BMI) of 18-30 kg/m^2. All eligible subjects were required to use adequate contraception from the time of providing informed consent throughout the duration of the study, and for 30 days after the last dose. All female subjects undertook a serum pregnancy test 3 days before study drug administration. Any subject with a significant history of uncontrolled clinically significant neurological (including seizure disorders), psychiatric or other disorder, disease or abnormality that may have impacted his/her ability to participate in the study or potentially confound the study results was excluded.

Eligible subjects were confined to the study site for 2 days prior to dosing and for the duration of the dosing period (from days −3 through 15) and for 1 day of follow-up. Subjects were fed three standard meals (breakfast, lunch and dinner) and an evening snack daily, collectively containing 30% fat. Dosing in the morning on days 1-14 occurred after a fast of at least 8 hours, and subjects remained fasted until 2 hours post dosing, after which time breakfast was served. The meals served on PK assessment days (days 1 and 14) were identical across cohorts.

2.3 | Study cohorts

A total of five cohorts (eight subjects each) received multiple doses of soticlestat or placebo given as an oral solution for a planned duration of 14 days. After each cohort, the decision to escalate to the next dose level for the subsequent cohort was made after a full review of the blinded safety and tolerability, and available PK data from the preceding cohorts.

Each cohort consisted of six subjects randomized to active drug and two subjects randomized to placebo. Cohorts 1 and 2 received oral soticlestat 100 and 300 mg, respectively, once daily (QD). Cohorts 3 and 4 received 300 mg twice daily (BID) and 600 mg QD, respectively. Given that treatment-emergent neurological and psychiatric adverse events (AEs) were observed in cohorts 3 and 4, cohort 5 proceeded with a de-escalated dose of 400 mg QD. The doses for cohort 1 were selected based on the results of a single rising-dose study in healthy adults. The doses for the subsequent cohorts were based on the data from previous multiple rising-dose cohorts, and were predicted not to exceed the maximum plasma concentration (C_{max}) observed at the no-observed-adverse-effect level (NOAEL) in dogs.

2.4 | Pharmacokinetic and pharmacodynamic assessments

Serial blood sampling (one 4-mL sample) was performed for PK analysis of soticlestat and M-I on days 1 and 14 (pre dose [<30 minutes before dosing], at 10, 15, 20 and 30 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16 and 24 hours post dose), with trough sampling on days 7, 11, 12 and 13 (<30 minutes before dosing). Additional blood samples for PK analysis were collected on day 7 for the 400 mg QD cohort at 15 and 30 minutes, and 1, 2, 4 and 8 hours post dose.

Urine samples were also collected for PK analysis on days 1 and 14 (pre dose [−12 to 0 hours for day 1 and −2 to 0 hours for day 14] and at intervals of 0-6, 6-12 and 12-24 hours post dose). For subjects who received BID dosing, blood and urine samples were collected up to 12 hours after morning dosing.

Serial blood sampling (one 8-mL sample) for PD analysis of plasma levels of the 24HC biomarker were collected at baseline (day −1, pre dose [< 30 minutes before dosing], and at 30 minutes and 1, 2, 4, 8, 12, 16 and 24 hours post dose) and on days 1 and 14 at time points matching day −1 sample collection. Additional trough samples were taken < 30 minutes before dosing on days 7, 12 and 13.

Plasma and urine soticlestat and M-I, and plasma 24HC concentrations were measured using high-performance liquid chromatography with tandem mass spectrometry detection. Assays were validated with a concentration range of 1.00-2000 ng/mL for both plasma or urine soticlestat and M-I and 2.00-100 ng/mL for plasma 24HC. Further details of these bioanalytical methods have been published previously.[15]

PK parameters for soticlestat and M-I, and PD parameters for 24HC were derived using standard noncompartmental analysis methods with Phoenix WinNonlin, Version 6.3 (Certara, Princeton, NJ, USA).

2.5 | Safety assessments

Safety assessments were performed daily on days 1-15 and at a follow-up assessment on day 28 ± 2 days. Evaluations included recording and assessment of AEs, physical examinations, BMI, vital signs and clinical laboratory tests.

2.6 | Statistical analyses

The safety analysis set consisted of all subjects who enrolled and received at least one dose of study drug. The PK set comprised all subjects in the safety set who had at least one measurable plasma or urine concentration. The PD set consisted of all subjects who were in the safety set and had at least one measurable 24HC plasma concentration.

The primary endpoints for this study were the percentage of subjects who had at least one treatment-emergent AE (TEAE), markedly abnormal criteria for safety laboratory tests, vital signs or electrocardiographic measurements at least once after dosing.

For subjects who received QD administration of study drug, dose proportionality was assessed using the Power model for day 1 and day 14 plasma exposures (C_{max} and area under the plasma concentration curve [AUC] AUC_{∞} on day 1 and AUC_{τ} on day 14). An analysis of variance (ANOVA) was also performed to assess time dependency between day 1 and day 14 for C_{max} and AUC (AUC_{∞} on day 1/AUC_{τ} on day 14) for QD cohorts. Incomplete or missing data were not imputed.

2.7 | 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, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.[22,23]

3 | RESULTS

3.1 | Study population

Of the 176 subjects screened, 40 (aged 19-54 years) were randomly assigned to treatment (30 received soticlestat and 10 received placebo) and entered the treatment period; 24 randomized subjects (60%) completed the study and 16 (40%) prematurely discontinued the study drug (including 14 subjects in the two ongoing cohorts of 600 mg per day and 300 mg BID, withdrawn by the sponsor as a precautionary safety measure). The 30 subjects who received soticlestat were included in the PK set, and all 40 subjects (including those administered placebo) were included in the PD set.

The majority of subjects in the study were white (31 subjects [77.5%]) and non-Hispanic or non-Latino (21 subjects [52.5%]) (Table 1). At least half of the subjects in each dose group were male. Mean age in each group ranged from 27 to 40 years, mean body weight from 67.7 to 79.4 kg and mean BMI from 24.2 to 26.7 kg/m^2 (Table 1).

3.2 | Pharmacokinetics

Soticlestat C_{max} was reached rapidly, with a median time to reach C_{max} (t_{max}) 0.33-0.5 hours after a single oral or multiple oral administration(s) of the solution across the dose range studied (Table 2). Over the 6-fold dose range of 100-600 mg, single-dose mean soticlestat C_{max} and AUC_{∞} on day 1 increased 6.55-fold and 9.35-fold, respectively (Table 2).

The cohorts receiving the 600 mg/day (300 mg BID or 600 mg QD) dosing regimens were not included in the multiple-dose exposure analyses; their treatment course was discontinued before planned intensive PK on day 14 because of the emergence of AEs (see section 3.4). For the other cohorts, concentration-time profiles for soticlestat were similar across dose ranges and appeared to be similar on days 1 and 14. Over the 4-fold dose range (100-400 mg QD), mean soticlestat C_{max} and AUC_{τ} on day 14 was 6.08-fold and 6.12-fold higher, respectively (Table 2).

The mean terminal elimination half-life ($t_{½z}$) for soticlestat was similar on day 1 and day 14, ranging from 3.49 to 4.83 hours (Table 2). Once-daily dosing of 100 or 400 mg soticlestat for 14 days did not appear to result in exposure accumulation on day 14 compared with day 1, whereas 300 mg QD dosing for 14 days resulted in approximately 1.74-fold and 1.42-fold increases in C_{max} and AUC_{τ} at steady state, respectively (Figure 1).

Renal elimination was negligible, with 0.08-0.25% of soticlestat dose excreted unchanged in urine. Mean renal clearance (CL_R) values of soticlestat were similar from day 1 to day 14, ranging from 0.20 to 0.38 L/h, with a trend of increased CL_R with increasing soticlestat dose.

Peak plasma concentrations of the circulating metabolite M-I were observed at a median t_{max} of approximately 0.5-1.0 hours post dose, shortly after soticlestat t_{max} (Table 2). Exposure to M-I (AUC_{∞} and AUC_{τ}) was comparable from day 1 to day 14 after QD dosing of

TABLE 1 Summary of subject demographics and baseline characteristics

Characteristic	Placebo, n = 10	Soticlestat dose					Total, N = 40
		100 mg QD, n = 6	300 mg QD, n = 6	400 mg QD, n = 6	300 mg BID, n = 6	600 mg QD, n = 6	
Age, mean, years (SD)	34.8 (8.51)	33.8 (4.62)	33.7 (10.71)	37.7 (13.08)	26.7 (4.50)	40.3 (11.55)	34.5 (9.60)
Sex, n (%)							
Male	7 (70.0)	5 (83.3)	3 (50.0)	4 (66.7)	5 (83.2)	6 (100.0)	30 (75.0)
Female	3 (30.0)	1 (16.7)	3 (50.0)	2 (33.3)	1 (16.7)	0	10 (25.0)
Ethnicity, n (%)							
Hispanic or Latino	5 (50.0)	0	4 (66.7)	5 (83.3)	4 (66.7)	1 (16.7)	19 (47.5)
Non-Hispanic or non-Latino	5 (50.0)	6 (100.0)	2 (33.3)	1 (16.7)	2 (33.3)	5 (83.3)	21 (52.5)
Race, n (%)							
White	7 (70.0)	5 (83.3)	4 (66.7)	5 (83.3)	5 (83.3)	5 (83.3)	31 (77.5)
Black or African American	3 (30.0)	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	9 (22.5)
Height, mean, cm (SD)	172.7 (10.02)	175.5 (5.65)	165.5 (6.16)	167.5 (7.29)	174.8 (8.57)	175.7 (5.43)	172.0 (8.19)
Weight, mean, kg (SD)	78.90 (13.136)	79.37 (8.586)	67.68 (10.860)	75.28 (12.367)	73.55 (6.227)	78.25 (7.936)	75.85 (10.647)
BMI, mean, kg/m^2 (SD)	26.30 (2.756)	25.76 (2.386)	24.66 (3.476)	26.67 (2.971)	24.16 (2.629)	25.31 (1.491)	25.56 (2.666)

Abbreviations: BID, twice daily; BMI, body mass index; QD, once daily; SD, standard deviation.

soticlestat 100, 300 or 400 mg (Table 2 and Figure 1). Mean metabolic ratio, based on AUC, generally decreased with increasing dose, ranging from 0.56 to 0.31 after a single dose (100-600 mg) on day 1, and from 0.44 to 0.26 after multiple doses (100-400 mg QD) on day 14.

Mean $t_{½z}$ values for M-I ranged from 2.32 to 3.88 hours (Table 2). After single-dose and multiple-dose administrations of soticlestat 100 mg QD, 300 mg QD, 400 mg QD, 300 mg BID and 600 mg QD, mean CL_R values for M-I were similar across all dose groups, ranging from 2.42 to 4.10 L/h.

3.3 | Pharmacodynamics

A dose-dependent decrease in plasma 24HC concentrations was generally observed following multiple QD doses of soticlestat for 14 days, approaching maximal response by day 7 (Figure 2). Similarly, a dose-dependently decreasing trend on day 14 was also observed, with time-matched percentage change in 24HC area under the effect-time curve from time 0 to 24 hours ($AUEC_{24}$) and observed effect at 24 hours (E_{24}); $AUEC_{24}$ ranged from 46.82% to 62.66% and E_{24} ranged from 49.11% to 65.59% across soticlestat 100-400 mg QD doses. A summary of time-matched percentage change from baseline in 24HC parameters on days 1 and 14 after single and multiple doses of soticlestat is provided in Table 3.

3.4 | Safety

In this 14-day multiple-rising-dose study, soticlestat was well tolerated in most healthy subjects up to 400 mg daily. In total, 45 TEAEs were reported by 14 subjects (46.7%) administered soticlestat, of which 31 events in 13 subjects were considered to be related to soticlestat (Table 4). The majority of TEAEs (91%) were transient and mild in intensity. Two subjects discontinued study drug due to a TEAE: one with acute psychosis (cohort 4: soticlestat 600 mg QD) that was reported as severe intensity, and one with mild confusional state (cohort 3: soticlestat 300 mg BID) occurring on study days 10 and 9, respectively. Both events were assessed as related to soticlestat by the investigator and no additional treatment was required beyond cessation of study drug. All TEAEs reported during the study were reversible and resolved without treatment and before study exit on day 15. No serious AEs were reported.

No clinically significant findings for clinical laboratory (including hormones), vital signs, physical examination, eye examination or electrocardiographic measurements were observed during the study, and no subjects had clinically meaningful changes from baseline in these safety measurements. No subjects had abnormal liver function test results.

4 | DISCUSSION

In this study, multiple daily doses of soticlestat in the dose range 100-400 mg for up to 14 days were generally well tolerated in healthy

TABLE 2 Estimates of pharmacokinetic parameters of soticlestat and M-I after single (day 1) and multiple (day 14) doses of soticlestat

	Soticlestat dose							
	100 mg QD		300 mg QD		400 mg QD		300 mg BID[a,b]	600 mg QD[a]
	Day 1, n = 6	Day 14, n = 6	Day 1, n = 6	Day 14, n = 6	Day 1, n = 6	Day 14, n = 6	Day 1, n = 6	Day 1, n = 6
Soticlestat, plasma								
C_{max}, mean, ng/mL (CV%)	468 (50.0)	481 (25.3)	2020 (61.7)	3100 (41.1)	2630 (50.6)	2930 (40.3)	1330 (32.5)	3060 (70.2)
t_{max}, median, h (range)	0.42 (0.33-0.53)	0.42 (0.25-0.50)	0.42 (0.33-1.05)	0.33 (0.17-0.33)	0.33 (0.25-0.50)	0.33 (0.33-0.50)	0.44 (0.33-1.00)	0.50 (0.50-1.00)
AUC_{∞}, mean, h*ng/mL (CV%)	451 (36.7)[c]	509 (24.8)[c]	2040 (55.3)	2840 (40.1)[d]	2520 (48.1)	3090 (32.8)[d]	1510 (48.0)[e]	4220 (59.4)
AUC_{τ},[f] mean, h*ng/mL (CV%)	446 (37.1)[c]	458 (28.4)	2030 (55.7)	2690 (40.0)	2500 (48.8)	2800 (40.3)	1460 (49.7)[e]	4180 (59.6)
$t_{½z}$, mean, h (CV%)	3.91 (20.7)[c]	3.83 (14.2)[c]	4.44 (19.8)	4.22 (23.4)[d]	4.67 (38.1)	3.66 (42.4)[d]	3.49 (59.2)[e]	4.83 (20.9)
CL/*F*, mean, L/h (CV%)	248 (40.4)[c]	234 (29.6)	185 (47.6)	130 (43.9)	187 (38.8)	166 (44.4)	232 (47.3)[e]	194 (57.5)
V_z/F, mean, L (CV%)	1320 (21.1)[c]	1140 (24.2)[c]	1180 (48.1)	732 (43.3)[d]	1330 (56.5)	713 (38.0)[d]	1360 (101)[e]	1320 (61.4)
Soticlestat, urine								
Ae_t,[g] mean, mg (CV%)	0.0900 (25.6)	0.0800 (30.0)	0.660 (84.3)	0.56 (83.4)	0.920 (47.7)	1.01 (31.9)	0.490 (75.9)	1.440 (74.4)
CL_R, mean, L/h (CV%)	0.220 (40.4)	0.200 (47.9)	0.300 (36.4)	0.220 (69.1)	0.370 (15.0)	0.380 (23.8)	0.350 (36.8)	0.340 (51.1)
f_e, mean, % (CV%)	0.0900 (25.6)	0.0800 (30.0)	0.220 (84.3)	0.190 (83.4)	0.230 (47.7)	0.250 (31.9)	0.160 (75.9)	0.240 (74.4)
M-I, plasma								
C_{max}, mean, ng/mL (CV%)	105 (16.7)	106 (23.5)	398 (35.2)	449 (33.9)	369 (28.3)	383 (24.8)	302 (18.8)	407 (17.4)
t_{max}, median, h (range)	0.51 (0.33-1.00)	0.50 (0.33-0.53)	0.50 (0.50-1.05)	0.50 (0.33-0.50)	0.50 (0.33-1.00)	0.50 (0.33-0.50)	0.50 (0.37-1.00)	1.00 (0.50-1.50)
AUC_{∞}, mean, h*ng/mL (CV%)	258 (18.6)[d]	245 (19.8)	788 (35.3)	829 (41.9)[d]	731 (31.8)	795 (27.6)[d]	601 (29.4)	1180 (23.8)
AUC_{τ},[f] mean, h*ng/mL	255 (18.5)[d]	243 (19.7)	784 (35.5)	858 (37.3)	726 (32.1)	741 (31.8)	590 (29.2)	1170 (23.7)
$t_{½z}$, mean, h (CV%)	2.34 (23.3)[d]	2.32 (5.3)	3.15 (44.4)	2.50 (28.5)[d]	3.43 (53.1)	2.42 (21.2)[d]	2.40 (15.8)	3.88 (32.1)
MR (based on AUC_{∞}), mean, (CV%)	0.560 (40.9)	0.440 (17.7)	0.410 (22.8)	0.280 (14.9)	0.310 (35.1)	0.260 (39.6)	0.410 (17.3)	0.330 (47.0)
M-I, urine								
Ae_t,[g] mean, mg (CV%)	0.620 (31.1)	0.600 (34.4)	3.08 (51.5)	1.97 (82.7)	2.70 (28.1)	2.99 (32.0)	2.28 (39.7)	4.48 (31.4)
CL_R, mean, L/h (CV%)	2.55 (22.5)	2.45 (22.1)	3.83 (17.9)	2.42 (61.8)	3.88 (29.5)	4.10 (19.5)	3.87 (23.0)	3.81 (18.5)

Note: Values rounded to three significant figures except for t_{max} and $t_{½z}$ presented as two decimal places.

Abbreviations: Ae_t, amount of drug excreted in urine from time 0 to time t; AUC_{12}, area under the effect-time curve from time 0 to 12 hours; AUC_{24}, area under the effect-time curve from time 0 to 24 hours; AUC_{∞}, area under the plasma concentration-time curve from time 0 to infinity; BID, twice daily; C_{max}, maximum observed plasma concentration; CL_R, renal clearance; CL/*F*, apparent clearance after extravascular administration; CV%, percentage coefficient of variation; f_e, fraction of drug excreted in urine; M-I, *N*-oxide metabolite; MR, metabolic ratio; PK, pharmacokinetics; QD, once daily; $t_{½z}$, terminal elimination half-life; t_{max}, time to reach C_{max}; V_z/F, apparent volume of distribution during the terminal disposition phase after extravascular administration.

[a]Dosing for 300 mg BID and 600 mg QD groups was discontinued from day 11 to day 14, therefore PK parameters on day 14 were not available for the 300 mg BID and 600 mg QD groups.

[b]TAK-935 PK parameters were derived using plasma concentration data after the first dose on day 1.

[c]n = 4.

[d]n = 5.

[e]n = 3.

[f]$AUC_{\tau} = AUC_{24}$ for QD dosing, $AUC_{\tau} = AUC_{12}$ for BID dosing.

[g]$Ae_{\tau} = Ae_{24}$ for QD dosing, $Ae_{\tau} = Ae_{12}$ for BID dosing.

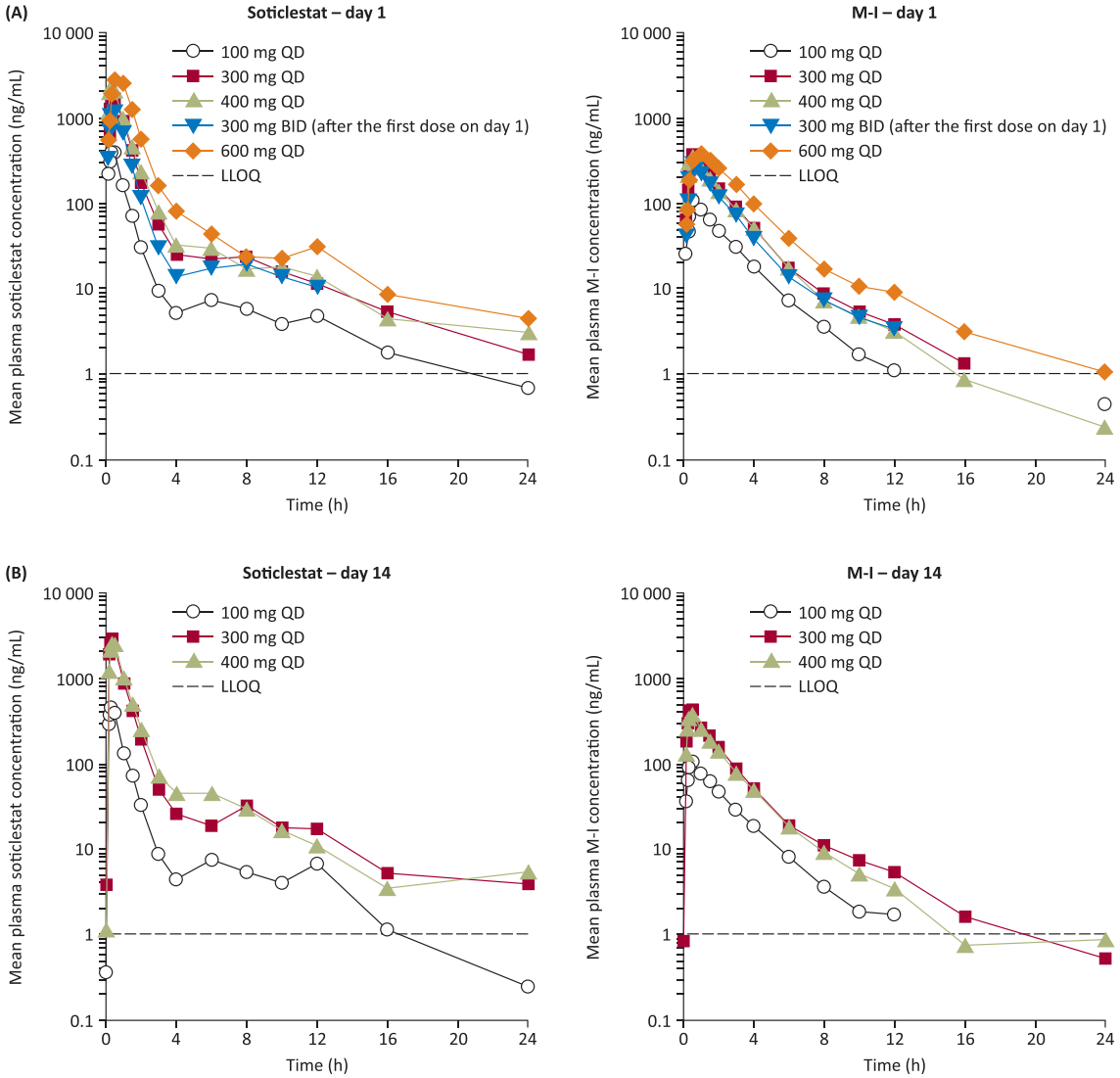

FIGURE 1 Mean plasma PK profiles of soticlestat and M-I after single and multiple oral dosing of soticlestat on (a) day 1 and (b) day 14. BID, twice daily; LLOQ, lower limit of quantification; M-I, N-oxide metabolite; PK, pharmacokinetics; QD, once daily

subjects. The emergence of events of neurological and psychiatric relevance with daily doses of 600 mg (given BID or QD) leading to dosing discontinuation suggested that the maximum tolerated dose following repeat dosing had been reached. This is in partial contrast to the previous results from the first-in-human study, in which single doses of soticlestat as high as 1350 mg appeared to be well tolerated in healthy subjects.[20] Dose titration was included in the subsequent studies in DEE subjects to achieve maximum benefit while ensuring adequate safety and tolerability.[24] In the multiple-rising-dose study reported here, over 90% of AEs were transient and mild in severity. Furthermore, all TEAEs reported in this study were reversible and resolved without treatment and before study exit on day 15.

The two AEs of acute psychosis and confusional state that resulted in discontinuation of two subjects who received soticlestat 600 mg per day without initial dose titration were likely related to reduced glutamatergic activity in the brain, owing to lower levels of the NMDA positive allosteric modulator 24HC.[14] Subsequent clinical studies included a titration scheme up to 300 mg BID to maximize tolerability.

The concentration-time profile of soticlestat was similar across the soticlestat doses, with maximum plasma concentrations observed at 0.33-0.5 hours post dose (median t_{max}), suggesting rapid absorption after oral administration of the soticlestat solution. Soticlestat appeared to be rapidly cleared from plasma across the 100-600 mg dose range and, consistent with this, soticlestat plasma trough concentrations showed minimal exposure accumulation from the previous dose.

The mean half-life of soticlestat is relatively short (approximately 4 hours at 300 mg QD and 400 mg QD), therefore little accumulation is expected with QD dosing. The slight difference observed in accumulation ratio based on AUC between 300 and 400 mg QD (1.42 *vs* 1.20) could be due to moderate inter-individual variability observed in the PK parameters for soticlestat (Table 2), which may be explained by expected random variation. For example, the mean AUCτ (percentage coefficient of variation) for 300 mg QD and 400 mg QD was

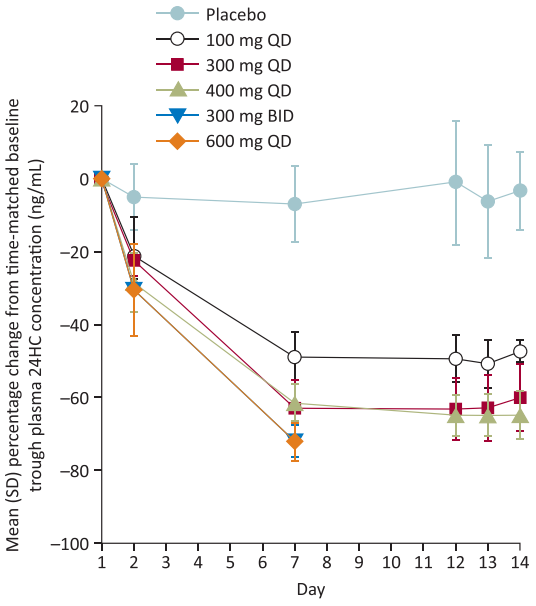

FIGURE 2 Mean time-matched percentage change from baseline in trough plasma 24HC concentrations over time after single and multiple oral doses of soticlestat administration or placebo. Dosing for the 300 mg BID and 600 mg QD groups was discontinued from day 11, therefore trough plasma 24HC concentrations on days 12, 13 and 14 were not available for the 300 mg BID and 600 mg QD groups. 24HC, 24*S*-hydroxycholesterol; BID, twice daily; QD, once daily; SD, standard deviation

2690 (40.0) h*ng/mL and 2800 (40.3) h*ng/mL after day 14 dosing, respectively. Cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT) polymorphisms are unlikely to explain the observed variability because direct glucuronidation by UGT1A9 and 2B4 is the predominant clearance pathway for soticlestat, and metabolism via CYP3A4 appears to be a minor route of elimination. The present study was included in a population PK analysis of healthy volunteer and patient studies (manuscript in preparation), but the identified covariates that impact PK parameters do not explain the variability seen here.

Following single doses of 100-600 mg QD, systemic exposure to soticlestat increased in a slightly greater than dose-proportional manner, at 6.55-fold and 9.35-fold for C_{max} and AUC_{∞}, respectively. This level of nonlinearity could be explained by moderate PK variability, but not by apparent changes in terminal half-life. The PK variability was moderate across doses, with C_{max} ranging from 25.3% to 70.2% and AUC_{τ} ranging from 28.4% to 59.6% across all doses.

A time-dependent effect of soticlestat PK on repeat dosing was not apparent. Furthermore, the very low levels of soticlestat excreted unchanged in the urine suggest hepatic metabolism as the main elimination route. A human radiolabelled mass balance study is planned to characterize the metabolism and clearance mechanisms of soticlestat.

The time-matched plasma 24HC measurements allowed the evaluation of the drug effect on 24HC concentration, taking into account potential intrinsic influences, such as circadian rhythm. After soticlestat multiple-dose administration, a dose-dependent decrease

TABLE 3 Summary of percentage (%) change from baseline (day −1) in 24HC parameters on day 1 and day 14 after single and multiple doses of soticlestat

PD parameters (percentage change from baseline)	Soticlestat dose									
	100 mg QD		300 mg QD		400 mg QD		300 mg BID	600 mg QD
	Day 1, n = 6[a,b]	Day 14, n = 6[c,d,e]	Day 1, n = 6[a,b]	Day 14, n = 6[c,d,e]	Day 1, n = 6[a,b]	Day 14, n = 6[c,d,e]	Day 1, n = 6[a,b]	Day 1, n = 6[a,b]
$AUEC_{\tau}$, mean, h*ng/mL (CV%)	−8.15 (−45.4)	−46.8 (−9.2)	−12.7 (−36.5)	−61.9 (−17.0)	−11.7 (−34.5)	−62.7 (−7.3)	−6.90 (−75.9)	−14.0 (−27.9)
E_t, mean, ng/mL (CV%)	−20.9 (−49.0)	−49.1 (−11.9)	−22.4 (−19.5)	−63.8 (−19.3)	−28.4 (−28.1)	−65.6 (−6.0)	−10.5 (−63.8)	−30.6 (−41.0)

Note: Values rounded to three significant figures.

Abbreviations: AUECτ, area under the effect-time curve during a dosing interval; $AUEC_{12}$, area under the effect-time curve from time 0 to 12 hours; $AUEC_{24}$, area under the effect-time curve from time 0 to 24 hours; BID, twice daily; CV%, percentage coefficient of variation; E_t, observed effect at time *t*; E_{12}, observed effect at 12 hours; E_{24}, observed effect at 24 hours; PD, pharmacodynamic; QD, once daily.

[a]$AUEC_{\tau} = AUEC_{24}$ for QD dosing and $AUEC_{\tau} = AUEC_{12}$ for BID dosing.

[b]$E_t = E_{24}$ for QD dosing and $E_t = E_{12}$ for BID dosing.

[c]Dosing for 300 mg BID and 600 mg QD groups was discontinued from day 11 to day 14, therefore PD parameters on day 14 were not available for 300 mg BID and 600 mg QD groups.

[d]$AUEC_{\tau} = AUEC_{24}$.

[e]$E_t = E_{24}$.

TABLE 4 Overview of treatment-emergent adverse events

Number of subjects (%)[a]	Placebo, n = 10	Soticlestat 100 mg QD, n = 6	Soticlestat 300 mg QD, n = 6	Soticlestat 300 mg BID, n = 6	Soticlestat 400 mg QD, n = 6	Soticlestat 600 mg QD, n = 6	Soticlestat total, n = 30
TEAEs[b,c]	4 (40.0)	0	1 (16.7)	5 (83.3)	5 (83.3)	3 (50.0)	14 (46.7)
Related	2 (20.0)	0	1 (16.7)	5 (83.3)	4 (66.7)	3 (50.0)	13 (43.3)
Not related	2 (20.0)	0	0	0	1 (16.7)	0	1 (3.3)
Mild	4 (40.0)	0	1 (16.7)	5 (83.3)	4 (66.7)	2 (33.3)	12 (40.0)
Moderate	0	0	0	0	1 (16.7)	0	1 (3.3)
Severe	0	0	0	0	0	1 (16.7)	1 (3.3)
Leading to discontinuation	0	0	0	1 (16.7)	0	1 (16.7)	2 (6.7)
SAEs	0	0	0	0	0	0	0
Deaths	0	0	0	0	0	0	0

Note: Percentages rounded to one decimal place.

Abbreviations: AE, adverse event; BID, twice daily; QD, once daily; SAEs, serious adverse events; TEAEs, treatment-emergent adverse events.

[a]A subject with two or more different AEs within the same levels of the MedDRA term and treatment is counted only once in that level using the most related or severe incident.

[b]A TEAE is defined as an AE or SAE that occurs or gets worse after receiving the first dose of study drug and within 30 days after the last dose of study drug.

[c]MedDRA Dictionary (Version 18.0) was used for coding AEs.

in plasma 24HC concentrations was observed, with more profound decreases at higher doses. According to concentration data at trough time points, plasma 24HC reduction appeared to approach steady state on day 7 after multiple-dose treatment. On day 7, the time-matched percentage change from baseline in trough 24HC concentration (day 7 *vs* day −1) showed a maximum decrease of about 70% for the 300 mg BID and 600 mg QD doses. In the dose range 100-400 mg QD, time-matched percentage of change from baseline on day 14 showed a maximum decrease of 62.66% for $AUEC_{24}$ and 65.59% for E_{24} for the 400 mg dose. The degree of 24HC reduction on day 14 was not notably different between the 300 mg QD and 400 mg QD groups, which was consistent with similar soticlestat exposure in plasma. The PK and PD information generated in this study helped inform the rationale for the paediatric dosing regimen, using quantitative modelling approaches[25] in patient clinical trials, where efficacy and safety were further investigated. Full details of a population PK/PD modelling analysis will be published separately and will describe the relationships between dose and exposures of soticlestat, positron emission tomography-derived CH24H enzyme occupancy in the brain and reduction in 24HC plasma levels using pooled data from multiple clinical studies.

5 | CONCLUSION

This study provides valuable information regarding the clinical safety, tolerability, PK and PD profiles of soticlestat, which may offer a potential treatment for DEEs such as Dravet syndrome and Lennox-Gastaut syndrome.

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CONFLICT OF INTEREST

All authors are employees or former employees of the study sponsor and manufacturer of the study drug, Takeda Development Center Americas, Inc.

CONTRIBUTORS

All authors contributed to the study conception and design. Data analysis was performed by Clinical Pharmacology and Biostatistics. 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 individual participants' data supporting the results reported in this article, will be made available within 3 months from initial 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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