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ORIGINAL RESEARCH ARTICLE

Absorption, Distribution, Metabolism, and Excretion of [^{14}C]-Volixibat in Healthy Men: Phase 1 Open-Label Study

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Abstract

Background and Objectives Volixibat is a potent inhibitor of the apical sodium-dependent bile acid transporter in development for the treatment of nonalcoholic steatohepatitis. This phase 1, open-label study investigated the absorption, distribution, metabolism, and excretion of [^{14}C]-volixibat in heathy men.

Methods Eligible men ($n = 8$) aged 18–50 years (body mass index 18.0–30.0 kg/m^2; weight >50 kg) received a single oral dose of [^{14}C]-volixibat 50 mg containing ~5.95 µCi radioactivity. The primary objectives were to assess the pharmacokinetics of [^{14}C]-volixibat and to determine the total radioactivity in whole blood, plasma, urine, and feces at pre-selected time points over 6 days. The secondary objectives were to characterize metabolites and to assess the safety and tolerability.

Results Low concentrations of volixibat (range 0–0.179 ng/mL) were detected in plasma up to 8 h following administration; the pharmacokinetic parameters could not be calculated. No radioactivity was observed in plasma or whole blood. The percentage (mean ± standard deviation) of total radioactivity in urine was 0.01 ± 0.007%. The vast majority (92.3 ± 5.25%) of volixibat was recovered in feces (69.2 ± 33.1% within 24 h). Unchanged volixibat was the only radioactive component detected in feces. Adverse events were mild in severity and mostly gastrointestinal. Changes in laboratory values were not clinically meaningful.

Conclusions Following oral administration, [^{14}C]-volixibat was excreted unchanged from the parent compound almost exclusively via fecal excretion, indicating that the drug is minimally absorbed. Consistent with other studies, adverse events were primarily gastrointestinal in nature. ClinicalTrials.gov identifier NCT02571192.

Key Points

Volixibat interrupts enterohepatic recirculation of bile acids to the liver and is being evaluated in a phase 2 study as a potential pharmacotherapy for nonalcoholic steatohepatitis.

This phase 1 study indicates that volixibat is minimally absorbed after oral administration, is not metabolized, and is eliminated from the body almost exclusively via fecal excretion.

Diarrhea and associated gastrointestinal adverse events are consistent with a drug that is minimally absorbed and that increases the proportion of bile acids reaching the colon.

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1 Introduction

Nonalcoholic steatohepatitis (NASH) is a severe form of nonalcoholic fatty liver disease (NAFLD) that is characterized histologically by the accumulation of excessive fat in the liver (steatosis) coupled with inflammation and

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features of hepatocyte injury (ballooning), which can progress to fibrosis and cirrhosis [1–3]. Prospective, long-term, histological follow-up studies involving patients with NASH have reported that liver fibrosis develops in 27–43% of individuals, with progression to cirrhosis in up to 22% of patients [4–8]. Progression of NASH-related cirrhosis is associated with poor long-term prognosis [9], with complications including hepatocellular carcinoma [10] and the need for liver transplant [11, 12].

The prevalence of NASH is uncertain because the majority of patients are asymptomatic [13], liver-related blood tests may be totally normal [14, 15], and a definitive diagnosis requires a liver biopsy to characterize liver histology [1, 16]. It was estimated that, in 2014, approximately 6 million people in the USA had NASH, of whom approximately 600,000 had associated cirrhosis [17]. There are several well-established metabolic risk factors for NASH, including type 2 diabetes mellitus [18–20], central or visceral obesity [21], dyslipidemia [22, 23], and hypertension [24]. In 2014, NASH surpassed hepatitis C virus infection as the leading etiology of chronic liver disease among adults under the age of 50 years requiring liver transplant in the USA [11]. Overall, the prevalence of NAFLD, and therefore of NASH, is growing at an epidemic rate, in parallel with that of obesity [25].

In spite of this alarming prevalence, there are no pharmacological treatments for NASH with demonstrated long-term efficacy and safety. Management guidelines for patients with NASH recommend the treatment of associated metabolic comorbidities, as well as weight reduction through dietary changes and increased physical exercise [26–28]. However, these lifestyle interventions are difficult to implement successfully or durably in most patients owing to poor long-term compliance or physical disability [29–32]. Overall, there is an urgent and growing unmet medical need for effective pharmacological treatments for this disease, which is associated with increased liver-related mortality compared with an age- and sex-matched general population [5] or with individuals with NAFLD [33].

Volixibat (SHP626; formerly LUM002) is a highly potent, minimally absorbed, competitive inhibitor of the apical sodium-dependent bile acid transporter (ASBT) [34], a transmembrane protein localized on the luminal surface of ileal enterocytes. Inhibition of bile acid (BA) reabsorption via ASBTs in the terminal ileum increases fecal BA excretion and reduces recirculation of BA back to the liver via the hepatic portal vein [35]. This interruption of the enterohepatic circulation is thought to stimulate de novo synthesis of BA from cholesterol in the liver, which maintains a constant BA pool [36, 37]. Supporting this, increases in serum levels of 7α-hydroxy-4-cholesten-3-one (C4, a biomarker of BA synthesis) are observed following

inhibition of the ASBT [38]. It is hypothesized that, by increasing the synthesis of BA in the liver, inhibition of the ASBT may increase elimination of hepatotoxic free cholesterol [39]. Accumulation of free cholesterol is implicated in liver injury associated with NASH [37, 40]. These events may also have positive metabolic, anti-inflammatory, anti-steatotic, and anti-fibrotic effects, supporting the idea of luminally targeted inhibition of ASBTs as a therapeutic approach for patients with NASH. Evidence from nonclinical studies also lends credence to this approach [39].

This paper presents results from a phase 1, open-label study to characterize the mass balance and absorption, distribution, metabolism, and excretion (ADME) profile of [14C]-volixibat following administration of a single oral dose in healthy male adults. To the authors' knowledge this is the first ADME study of a radiolabeled ASBT inhibitor.

2 Methods

This phase 1, open-label, single-arm, ADME study of [14C]-volixibat (SHP626-102; ClinicalTrials.gov identifier NCT02571192) was conducted between September 8, 2015 and October 16, 2015 at a single site in North America (Covance Clinical Research Unit, Madison, WI, USA). The study was performed in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline for Good Clinical Practice, the principles of the Declaration of Helsinki, and other applicable local ethical and legal requirements. The study protocol and patient documents were approved by an institutional review board before initiating the study (MidLands Independent Review Board, Kansas City, KS, USA). The study drug was manufactured by Sanofi, Montpellier, France. All participants provided written informed consent before commencing any study-related procedures.

2.1 Study Participants

Eligible participants were healthy men (aged 18–50 years) with a body mass index in the range 18.0–30.0 kg/m^2, a body weight exceeding 50 kg, and a minimum of one bowel movement per day. Healthy status was determined by the absence of evidence for any active or chronic disease following a detailed medical and surgical history and physical examination, including vital signs, 12-lead electrocardiogram (ECG), hematology, thyroid panel, blood chemistry, coagulation, and urinalysis. Key exclusion criteria were: history of any hematological, hepatic, respiratory, cardiovascular, renal, neurological, or psychiatric disease; gall bladder removal; gastric bypass surgery; ileal

resection; any small intestinal resection; or current or recurrent disease that could have affected the action, absorption, or disposition of the study drug or clinical or laboratory assessments. Full inclusion and exclusion criteria are provided (Online Resource: Appendix S1).

2.2 Study Design

Participants were screened for up to 28 days before administration of the study drug and eligible individuals were admitted to the clinical research center on the day before dosing (day 1). A single dose of [14C]-volixibat 50 mg containing approximately 5.95 μCi radioactivity was administered orally as a capsule 30 min before breakfast on the morning of day 1 with 240 mL of water. All participants followed a standardized meal schedule while at the center; the total daily nutritional composition was approximately 50% carbohydrate, 35% fat, and 15% protein (caloric intake limit of approximately 3200 kcal). Participants had to refrain from consuming grapefruit, Seville oranges, or products containing these items from 7 days before administration of the study drug until discharge from the center on days 7–10. Participants also had to avoid alcohol and products containing caffeine or xanthine for 48 h before admission to the center until discharge from the center, and tobacco or products containing nicotine for 30 days before admission until discharge. Participants had to refrain from taking any medication for 14 days before administration of study drug until discharge, including over-the-counter multivitamins and herbal or homeopathic preparations, apart from occasional use of ibuprofen and acetaminophen. Participants were excluded if they had taken an investigational medicinal product within 30 days or within the pharmacokinetic equivalent of five half-lives before administration of the study drug. At the discretion of the investigator, the occasional dose of over-the-counter nonsteroidal anti-inflammatory drug or acetaminophen could be administered during the study.

Blood, urine, and stool samples were collected during study days 1–6 and participants were discharged from the center after completing day 7 study assessments and procedures if radioactivity had reached either of the following threshold values: 90% of the dose had been recovered; or urine total radioactivity and fecal total radioactivity had both reached under 1% of the administered dose for two consecutive 24-h intervals. Participants who had not met the discharge criteria on day 7 were reassessed on a daily basis up to day 10, when they were discharged regardless of radioactivity measurements. All patients had a follow-up telephone call 7 ± 2 days after discharge or early termination to assess safety, including any ongoing adverse events (AEs) or changes in medications; follow-up continued until the investigator was satisfied that there was no longer any concern about safety.

Based on the results of previous phase 1 dose-finding studies (data on file), the 50 mg dose of volixibat was chosen for the following reasons: maximal inhibition of bile acid reabsorption (the mechanism by which the drug may have a therapeutic effect) occurs at doses of 20 mg or higher; it is a high but well-tolerated dose (no serious AEs have been reported at this dose); the likelihood of detecting this minimally absorbed compound and any metabolites in blood and urine increases with dose.

A sensitive liquid chromatography tandem mass spectrometry method for detection of [14C]-volixibat was used to minimize radioactive exposure to the participants. Participants could be discharged on day 10 irrespective of radioactivity measurements, because the dose used in this study (5.95 μCi) was considered to pose a negligible risk to participants or those around them; the dose was well below the maximum exposure limits set by the US Food and Drug Administration [41], considerably lower than that typically administered in ADME studies (100 μCi), and is associated with exposure to a lower dose of radioactivity than a single X-ray.

2.3 Outcome Measures

The primary objectives of this study were to assess the pharmacokinetics of a single oral dose of [14C]-volixibat and to determine the resulting total radioactivity in whole blood, plasma, urine, and feces. The secondary objectives were to characterize and identify, if present, the metabolites of [14C]-volixibat in plasma, urine, and feces, and assess the safety and tolerability of [14C]-volixibat.

2.4 Pharmacokinetic Parameters

Blood samples (K2EDTA anticoagulant) were collected for pharmacokinetic analysis of volixibat concentrations in plasma, analysis of [14C]-volixibat radioactivity levels in blood and plasma, and metabolite profiling and identification. Urine samples for pharmacokinetic analysis of volixibat, and urine and stool samples for analysis of [14C] radioactivity and metabolite identification and profiling, were also collected. Blood samples (5 mL) were obtained 45 min before dosing, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h after dosing on day 1. Urine samples (approximately 5 mL) were collected 45 min before dosing and then at 4, 8, and 12 h after dosing on day 1. Blood and urine samples were also collected 24 and 36 h (day 2), 48 h (day 3), 72 h (day 4), 96 h (day 5), 120 h (day 6), and 144 h (day 7) after dosing. If any participant experienced emesis within 4 h following dosing, vomitus was collected and stored for possible radioanalysis. Details of the timings of all study assessments are provided in Online Resource: Appendices S2 and S3.

Plasma and urine samples were stored at −80 °C. Volixibat concentrations in plasma and urine were assessed using validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods. Validation parameters included linearity, selectivity, sensitivity, carryover, precision, accuracy, matrix effects, recovery, reinjection reproducibility, hyperlipidemia and hemolysis effect (plasma only), and stability.

Plasma (100 μL) samples were subjected to a solid phase extraction procedure, using a SOLAμ WAX (2 mg, Thermo Scientific, CA, USA) material. Urine samples were treated with the non-ionic surfactant Tween 80 (0.2%; Sigma-Aldrich, MO, USA). Urine samples (200 μL) were extracted using a Waters Oasis Prime 96 HLB uElution plate (3 mg; Waters UK, Hertfordshire, UK). A stable-labeled internal standard ([13C6]-volixibat) was added to all standards and quality control and study samples before extraction.

LC–MS/MS analysis of plasma and urine extracts was performed with reversed-phase LC combined with a triple-quadrupole mass spectrometry and electrospray ionization in the positive ion mode. Sciex API-5000 and API-3000 mass spectrometers (AB Sciex, MA, USA) were used for plasma and urine analyses, respectively. The autosampler temperature was maintained at 4 °C. Chromatographic separation was achieved with a Pursuit 3 μm C18, 2.0 × 50 mm column (Agilent Technologies, CA, USA) with a mobile phase gradient. The gradient was generated with varying concentrations of mobile phase A (0.1% formic acid in type 1 H2O; Fisher Scientific, PA, USA) and mobile phase B (acetonitrile; Sigma-Aldrich). Mass spectrometric data were acquired with the following ESI-MS parameters for plasma analysis: ion spray voltage 5000 V; curtain gas setting 35 L/min; collision gas setting 7 L/min; source temperature 550 °C. For urine analysis, the following ESI-MS parameters were used: ion spray voltage 5000 V; curtain gas setting 10 L/min; collision gas setting 4 L/min; source temperature 400 °C. Both Q1 and Q3 were set at unit resolution. The selected reaction monitoring transition was 806.4 m/z → 726.5 m/z and 812.4 m/z → 732.5 m/z for the internal standard ([^{13}C6]-volixibat). Data acquisition and peak integration were made in Analyst Software version 1.4.2 (Applied Biosystems, CA, USA) and peak–area ratios were calculated using Watson LIMS 7.2.0.02 (Thermo Scientific). The lower and upper limits of quantification for volixibat were 0.0500 and 50.0 ng/mL, respectively, in human plasma and 0.250 and 100 ng/mL, respectively, in human urine. Volixibat calibration standards were prepared in human plasma at nominal concentrations of 0.0500, 0.100, 0.250, 1.00, 5.00, 25.00, 40.00, and 50.00 ng/mL, and in urine (treated with Tween 80) at nominal concentrations of 0.250, 0.500, 1.25, 5.00, 20.00, 50.00, 80.00, and 100.00 ng/mL. Volixibat

quality control (QC) samples were prepared at low (0.150 ng/mL for plasma and 0.750 ng/mL for urine), medium (15.0 ng/mL for plasma and urine) and high (37.5 ng/mL for plasma and 74.9 ng/mL for urine) concentrations for analysis alongside the study samples.

The inter-assay coefficient of variation values for sample analysis runs ranged from 2.5 to 6.0% for human plasma QC samples and 2.3–4.9% for human urine QC samples. Accuracy values (expressed as relative error) ranged from 1.3 to 6.0% for plasma QC samples and from 0.0 to 3.3% for human urine QC samples (accuracy and precision data were calculated from two bioanalytical runs for plasma and from three bioanalytical runs for urine). Incurred sample reproducibility was not performed because, with the exception of one sample, all were below the lower limit of quantification. Pharmacokinetic parameters were determined from plasma and blood concentration–time data for total radioactivity and from plasma concentration–time data for volixibat by non-compartmental analysis. Assessed pharmacokinetic parameters included: maximum plasma drug concentration (C_{max}); time to maximum plasma concentration during a dosing interval (t_{max}); area under the curve from the time of dosing to the last measurable concentration ($AUC_{0–t}$); the first-order rate constant estimated from log-linear regression analysis of the terminal elimination phase of the concentration–time profile (λ_z); the mean elimination half-life ($t_{1/2}$); the total body clearance for extravascular administration divided by the fraction of dose absorbed (CL/F); and the volume of distribution associated with the terminal slope following extravascular administration divided by the fraction of dose absorbed (V_z/F). When possible, the following pharmacokinetic parameters were calculated for volixibat concentrations in urine and for total radioactivity concentrations in urine: cumulative amount (A_{eu}) and excreted percentage recovered in urine over the dosing interval; and renal clearance (CL_R). The following parameters were calculated for each participant based on stool total radioactivity concentrations: cumulative amount (A_{ef}) and excreted percentage recovered in stool over the dosing interval. When possible, the mass balance was calculated by adding the total radioactivity recovered from urine, stool, and (if appropriate) vomitus.

2.5 Safety Assessments

Safety was evaluated by monitoring of reported AEs and by assessment of physical examination findings, vital signs, clinical laboratory parameters, and 12-lead ECGs at pre-specified time points (Online Resource: Appendices 2 and 3). All AEs were recorded from the time when participants signed the informed consent until the defined follow-up period (7 ± 2 days after discharge) and were classified

using version 18.1 of the Medical Dictionary for Regulatory Activities (MedDRA). Treatment-emergent AEs were defined as AEs that started or increased in severity on administration of or after the first dose of study drug.

2.6 Data Analysis

Statistical analyses were performed using SAS software Version 9.4 (SAS Institute, Inc., Cary, NC, USA). Pharmacokinetic analysis was performed using Phoenix WinNonlin Version 5.2.1 or higher (Pharsight Corporation, Mountain View, CA, USA). The sample size chosen for this study was based on other pharmacokinetic studies of a similar nature and not on power calculations. The safety analysis set consisted of all participants who had taken at least one dose of the study drug. The pharmacokinetic analysis set consisted of all participants in the safety analysis with primary pharmacokinetic data that were considered sufficient and interpretable; if all values were below the level of quantification, mean concentrations were reported as zero, and no descriptive statistics were reported. Unless otherwise specified, continuous variables were summarized using the following descriptive statistics: number of participants; mean; median; standard deviation (SD); minimum; and maximum. Categorical and count variables were summarized by the number and percentage of participants in each category.

3 Results

3.1 Participant Disposition and Demographics

All of the enrolled participants ($n = 8$) received a single oral dose of $[^{14}C]$-volixibat 50 mg, were included in the safety and pharmacokinetic analyses sets, completed the study, and were discharged from the clinical research center on day 7. At baseline, the participant characteristics (mean $\pm$ SD) were: age 32.1 ± 11.03 years; body weight 67.95 ± 8.380 kg; height 171.1 ± 9.78 cm; and body mass index 23.29 ± 3.170 kg/m^2. The majority of participants (62.5%) were non-white and none of the participants received concomitant medications during the study.

3.2 Pharmacokinetic Assessments

Very low concentrations of volixibat (range 0–0.179 ng/mL) were observed in plasma for up to 8 h following administration of a single, oral dose of $[^{14}C]$-volixibat 50 mg (Fig. 1). No radioactivity was observed in plasma or whole blood following administration of $[^{14}C]$-volixibat at any of the sampling time points. One participant

had a minimal, yet detectable concentration of volixibat in the urine following administration of $[^{14}C]$-volixibat (1.62 ng/mL, 5 days post-dose). The cumulative mass and percentage of volixibat recovered in the urine from this participant during the study period were 1762.56 ng and 0.0035%, respectively. Overall, individual radioactivity concentrations detected in urine were in the range 0.0910–8.64 ng equivalent/mL. The mean (SD) cumulative mass and percentage of total radioactivity recovered in urine during the study were 4423.51 (3290.684) ng and 0.01 (0.007)%, respectively. Owing to the lack of available concentration data, pharmacokinetic parameters could not be calculated for volixibat in plasma, whole blood, or urine.

The overall proportion (mean $\pm$ SD) of volixibat recovered in feces was $92.3 \pm 5.25\%$ (range 83.9–98.1%). Most of the administered radioactivity ($69.2 \pm 33.1\%$) was recovered in the first 24 h following $[^{14}C]$-volixibat administration and $87.6 \pm 6.06\%$ had been recovered by 72 h after dosing. Unchanged volixibat was the only quantifiable radioactive component detected in feces, representing 95.59% of the total radioactivity of the radiochromatogram and accounting for 88.0% of the administered dose. Accordingly, no metabolite analyses of $[^{14}C]$-volixibat in blood and urine samples were performed. No vomitus was collected within the 4 h following administration of $[^{14}C]$-volixibat.

3.3 Safety and Tolerability

AEs were reported in all eight participants (Table 1); all were mild in severity, none were serious, and none led to study discontinuation. Of the 18 AEs that were reported, 16 were classed as gastrointestinal disorders, of which 12 were diarrhea. Most of the diarrhea events ($N = 9$) were reported on day 1 and all lasted for less than 1 day. The remaining AEs were scratch (one event reported in one participant) and headache (one event reported in one participant). All but 1 of the 18 AEs (scratch) were considered to be treatment related.

No clinically meaningful changes from baseline were observed in mean hematology, serum biochemistry, coagulation, or urinalysis clinical laboratory values during the study (Table 2). One participant met potentially clinically important (PCI) criteria for urate concentration in serum [increase of >119 µmol/L from baseline and above the upper limit of normal (487.777 µmol/L)] on days 3 and 7; this individual had urate concentrations of 648.387 µmol/L at screening, 398.550 µmol/L at baseline (day 1), 523.468 µmol/L on day 3, and 588.902 µmol/L on day 7. These elevations in urate from baseline were not reported as AEs and were not considered by the study
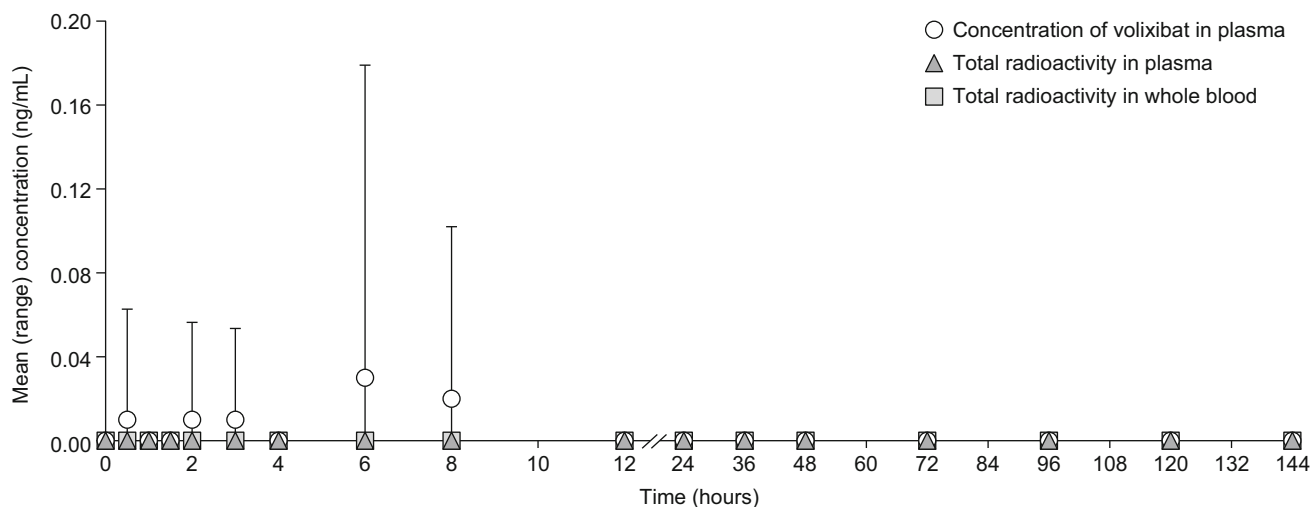
Fig. 1 Plasma and whole blood concentrations in participants following administration of volixibat. Median values in plasma and whole blood were zero at all time points. Data are from the pharmacokinetic analysis set

Table 1 Summary of treatment-emergent AEs in individuals ($n = 8$) exposed to [14C]-volixibat 50 mg

Event	N, n (%)
Any AE	18, 8 (100)
AEs related to the study drug	17, 8 (100)
AEs by system order class and preferred term	
Gastrointestinal disorders	16, 8 (100)
Diarrhea	12, 8 (100)
Abdominal distention	1, 1 (12.5)
Abdominal pain upper	1, 1 (12.5)
Change of bowel habit	1, 1 (12.5)
Flatulence	1, 1 (12.5)
Injury, poisoning, and procedural complications	1, 1 (12.5)
Scratch	1, 1 (12.5)
Nervous system disorders	1, 1 (12.5)
Headache	1, 1 (12.5)

Data are the number of events (N) and number of participants experiencing the event [n (%)] in the safety analysis set. Participants were counted once per category per treatment. AEs were classified by the preferred term using version 18.1 of the Medical Dictionary for Regulatory Activities. Participants were counted once per system organ class and once per preferred term

AE adverse event

investigator to be of clinical significance. None of the participants met the PCI criteria for hematology, coagulation, or urinalysis laboratory values (data not shown). No clinically meaningful changes from baseline in vital signs or ECG parameters were reported during the study (Table 3). None of the PCI abnormalities in vital signs or ECG parameters were considered by the study investigator to be of clinical significance or were reported as AEs (data not shown).

4 Discussion

This phase 1, open-label study, investigating the mass balance and ADME profile of a single, oral dose of [14C]-volixibat 50 mg in healthy men, found that volixibat concentrations in plasma were very low or undetectable for up to 8 h after administration. Consistent with a drug that has minimal absorption and the findings of another phase 1 study, the pharmacokinetic parameters for volixibat could not be calculated [42]. Volixibat is a benzothiepine-based structure (Fig. 2) [43, 44]. The presence of a negatively charged sulfonate moiety is thought to prevent strong interaction with the intestinal cell membrane, which may explain the minimal absorption of volixibat. Phase 1 studies of other ASBT inhibitors indicate that low bioavailability is a class characteristic [45, 46]. In these studies, the parent compound was either undetectable or only quantifiable at picomolar levels after repeat daily dosing for up to 2 weeks.

In the present study, no radioactivity was detected in plasma or whole blood, and a very low percentage of the administered volixibat was recovered from urine (detectable in the urine of only one participant). Based on the profiling and identification of radioactivity in feces, [14C]-volixibat was not metabolized after oral administration and was excreted unchanged from the parent compound almost exclusively in feces. Radiolabeling studies of other ASBT inhibitors have either not been conducted or have not been published; so, the ADME profiles of these compounds cannot be compared with that of volixibat.

The ADME profile of [14C]-volixibat observed in the present study is consistent with the findings of previous nonclinical studies (Shire, data on file). Systemic exposure to volixibat was found to be minimal following oral

Table 2 Quantitative clinical laboratory results in individuals ($n = 8$) exposed to [^{14}C]-volixibat 50 mg

Parameter	Baseline	Final on-treatment assessment	Change from baseline
Hematology			
Basophils, $\times 10^9$/L	0.050 (0.0535)	0.025 (0.0463)	−0.025 (0.0707)
Eosinophils, $\times 10^9$/L	0.163 (0.1302)	0.125 (0.0463)	−0.038 (0.0916)
Hematocrit, fraction of 1	0.4393 (0.01823)	0.4244 (0.02234)	−0.0149 (0.01271)
Hemoglobin, g/L	147.38 (7.596)	143.00 (8.246)	−4.38 (4.241)
Lymphocytes, $\times 10^9$/L	2.150 (0.9243)	1.725 (0.6798)	−0.425 (0.4921)
Monocytes, $\times 10^9$/L	0.500 (0.0756)	0.413 (0.0641)	−0.087 (0.0641)
Neutrophils, $\times 10^9$/L	3.250 (0.8264)	3.063 (0.8651)	−0.188 (0.7680)
Platelets, $\times 10^9$/L	223.0 (43.44)	213.1 (46.85)	−9.9 (16.82)
Erythrocytes, $\times 10^{12}$/L	4.891 (0.1999)	4.778 (0.2321)	−0.114 (0.1499)
Leukocytes, $\times 10^9$/L	6.11 (1.141)	5.36 (0.953)	−0.75 (1.117)
Biochemistry			
Albumin, g/L	46.4 (2.13)	46.4 (2.83)	0.0 (2.27)
Alkaline phosphatase, U/L	63.0 (20.84)	61.0 (24.04)	−2.0 (6.57)
Alanine aminotransferase, U/L	15.8 (7.87)	17.4 (7.76)	1.6 (4.10)
Aspartate aminotransferase, U/L	19.0 (3.70)	18.6 (2.92)	−0.4 (2.72)
Bicarbonate, mmol/L	28.4 (2.07)	27.9 (2.30)	−0.5 (1.77)
Bilirubin, μmol/L	10.26 (6.128)	8.55 (2.771)	−1.71 (4.639)
Blood urea nitrogen, mmol/L	5.44 (1.266)	6.46 (1.758)	1.03 (1.371)
Calcium, mmol/L	2.403 (0.0656)	2.430 (0.0487)	0.027 (0.0625)
Chloride, mmol/L	100.5 (0.93)	97.9 (1.36)	−2.6 (2.07)
Creatinine, μmol/L	90.60 (11.324)	92.84 (14.921)	2.24 (7.837)
γ-glutamyl transferase, U/L	21.9 (16.71)	23.3 (16.32)	1.4 (4.10)
Glucose, mmol/L	5.15 (0.389)	4.58 (0.260)	−0.58 (0.453)
Potassium, mmol/L	4.09 (0.210)	4.33 (0.231)	0.24 (0.160)
Phosphate, mmol/L	1.211 (0.1713)	1.235 (0.1726)	0.024 (0.1390)
Protein, g/L	73.4 (4.00)	71.9 (4.05)	−1.5 (3.89)
Sodium, mmol/L	140.5 (1.31)	139.1 (2.59)	−1.4 (2.97)
Urate, μmol/L	343.53 (53.371)	359.88 (104.153)	16.36 (75.695)
Coagulation			
Activated partial thromboplastin time, s	30.85 (3.678)	31.65 (3.799)	0.80 (1.900)
Prothrombin international normalized ratio	1.04 (0.074)	1.04 (0.092)	−0.00 (0.076)
Prothrombin time, s	13.19 (0.848)	13.08 (0.907)	−0.11 (0.790)
Urinalysis			
pH	5.63 (1.061)	5.50 (0.756)	−0.13 (1.458)
Specific gravity	1.0179 (0.00940)	1.0205 (0.00743)	0.0026 (0.01011)
Ketones			
Negative, n (%)	7 (87.5)	7 (87.5)	
Trace, n (%)	1 (12.5)	1 (12.5)	
Leukocyte esterase, negative, n (%)	8 (100)	8 (100)	
Nitrite, negative, n (%)	8 (100)	8 (100)	
Occult blood, negative, n (%)	8 (100)	8 (100)	
Urine bilirubin, negative, n (%)	8 (100)	8 (100)	
Urine glucose, negative, n (%)	8 (100)	8 (100)	
Urine protein, negative, n (%)	8 (100)	8 (100)	

Data are mean (standard deviation) unless otherwise stated. Data are from the safety analysis set

Table 3 Changes from baseline in vital signs and electrocardiogram parameters in individuals ($n = 8$) exposed to [^{14}C]-volixibat 50 mg

Parameter	Baseline	Final on-treatment assessment	Change from baseline
Pulse, beats/min	65.6 (20.57)	57.6 (7.44)	−8.0 (22.06)
Systolic blood pressure, mmHg	112.5 (12.90)	109.3 (4.68)	−3.3 (10.66)
Diastolic blood pressure, mmHg	69.9 (11.70)	66.0 (4.81)	−3.9 (9.49)
Temperature, °C	36.56 (0.160)	36.75 (0.227)	0.19 (0.181)
Respiration rate, breaths/min	15.8 (2.31)	15.3 (2.12)	−0.5 (3.16)
Heart rate, beats/min	62.3 (13.81)	58.8 (7.32)	−3.5 (14.81)
PR interval, ms	157.1 (23.70)	159.1 (16.78)	2.0 (12.97)
QRS interval, ms	102.9 (10.55)	101.6 (7.80)	−1.3 (4.13)
QT interval, ms	408.3 (35.37)	411.8 (16.99)	3.5 (35.86)
RR interval, ms	990.4 (195.84)	1023.8 (119.35)	33.4 (212.03)
QTcB interval, ms	412.90 (16.390)	408.09 (14.003)	−4.81 (11.981)
QTcF interval, ms	410.91 (14.574)	409.17 (9.857)	−1.75 (9.404)

Data are mean (standard deviation). Data are from the safety analysis set

QTcB interval QT interval corrected using Bazett's formula, QTcF interval QT interval corrected using Fridericia's formula
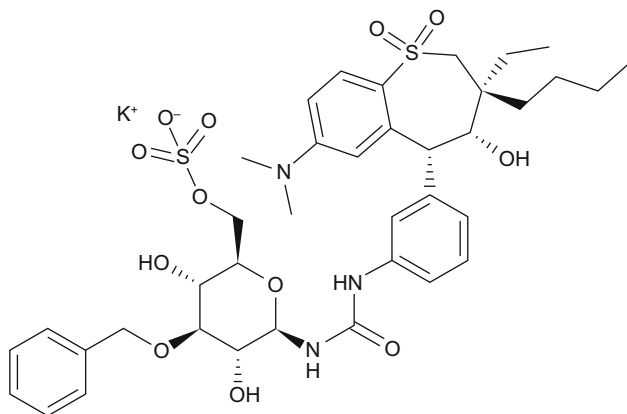
Fig. 2 Structure of volixibat[a]. [a]Chemical name: potassium((2R,3R,4S,5R,6R)-4-benzyloxy-6-(3-[3-((3S,4R,5R)-3-butyl-7-dimethylamino-3-ethyl-4-hydroxy-1,1-dioxo-2,3,4,5-tetrahydro-1H-benzo[b] thiepin-5-yl)-phenyl]-ureido)-3,5-dihydroxy-tetrahydropyran-2-ylmethyl)sulfate ethanolate, hydrate

administration of pharmacologically relevant doses in rats (20 mg/kg) and dogs (10 mg/kg). Plasma levels of volixibat were generally below the lower limit of quantitation (1 ng/mL) and the drug was recovered almost entirely in feces, with less than 1% of the administered dose excreted in urine. Quantitative whole-body autoradiography investigating the distribution of volixibat following administration of a single oral dose of [^{14}C]-volixibat 20 mg/kg to rats found that radioactivity was confined to the lumen and mucosa of the gastrointestinal tract, with elimination complete within 48 h. In vitro studies have demonstrated the slow metabolism of volixibat, mainly via hydroxylation and demethylation. This finding supports the lack of detection of metabolites of volixibat in the present study.

The safety profile of volixibat in the present study was consistent with that observed in previous phase 1 studies of volixibat administered once daily at doses of 0.5–80 mg for up to 28 days in healthy adults and patients with type 2 diabetes mellitus [34, 38], as well as in overweight and obese adults [42]. Overall, treatment with a single oral dose of [^{14}C]-volixibat 50 mg was generally well tolerated and no safety concerns were evident. The most frequently reported AEs were gastrointestinal disorders, predominantly diarrhea. A preponderance of these AEs can be expected given that volixibat increases BA concentrations in the colon, as indicated by increases in total fecal BA excretion [34, 38, 42]. In the colon, elevations in BA stimulate colonic motility [47] and secretion of water [48, 49], thereby leading to reduced colonic transit time and watery stool [50]. Long-term studies of ASBT inhibitors will establish whether these gastrointestinal AEs are treatment limiting or, conversely, decrease in frequency with treatment duration and have little effect on adherence.

As with any small phase 1 study in selected healthy adults, the generalizability of the results to a 'real-world' population with disease may be limited. Volixibat is in development for the treatment of NASH, a disease of the liver with multiple comorbidities affecting individuals likely to be receiving other pharmacotherapies. Any of these factors could affect the behavior of the drug compared with that in a healthy population in a trial setting. However, the current study was designed to minimize the potential for confounding effects from other drugs and substances to enable evaluation of the ADME, safety, and tolerability of volixibat. The effects of volixibat in patients with NASH and associated comorbidities, including patients with type 2 diabetes mellitus and obesity, will be

evaluated by other studies in the ongoing clinical trial program. In the present study, the absence of a placebo arm precludes comparisons of safety and tolerability. In addition, physicians and patients were aware of the treatment, which could have influenced the reporting of AEs and physicians' judgments about the relationship between AEs and treatment. The volixibat trial program includes randomized, double-blind studies designed to address these limitations and to minimize potential sources of bias.

5 Conclusions

Volixibat was engineered to have low systemic availability and limit its action to the intestinal lumen. In this study, radiolabeled volixibat could not be detected in plasma or whole blood, and AEs were almost exclusively restricted to gastrointestinal disorders. The minimal systemic absorption of volixibat confirmed in the present study suggests that volixibat may not have clinically significant effects on the metabolism of other drugs. A drug to treat patients with NASH that has a low potential for drug–drug interactions would be beneficial because this patient population is often receiving numerous pharmacotherapies for associated comorbidities. However, the potential for drug–drug interactions in the gut or those arising from inhibition of ASBT needs to be explored further in preclinical and clinical studies. A 48-week, double-blind, placebo-controlled, phase 2 trial (ClinicalTrials.gov identifier: NCT02787304) has commenced to evaluate the efficacy, safety, and tolerability of volixibat in adults with NASH.

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