

Pharmacokinetics and ADME Characterization of Intravenous and Oral [¹⁴C]-Linerixibat in Healthy Male Volunteers[§]

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ABSTRACT

Linerixibat, an oral small-molecule ileal bile acid transporter inhibitor under development for cholestatic pruritus in primary biliary cholangitis, was designed for minimal absorption from the intestine (site of pharmacological action). This study characterized the pharmacokinetics, absorption, metabolism, and excretion of [¹⁴C]-linerixibat in humans after an intravenous microtracer concomitant with unlabeled oral tablets and [¹⁴C]-linerixibat oral solution. Linerixibat exhibited absorption-limited flip-flop kinetics: longer oral versus intravenous half-life (6–7 hours vs. 0.8 hours). The short intravenous half-life was consistent with high systemic clearance (61.9 l/h) and low volume of distribution (16.3 l). In vitro studies predicted rapid hepatic clearance via cytochrome P450 3A4 metabolism, which predicted human hepatic clearance within 1.5-fold. However, linerixibat was minimally metabolized in humans after intravenous administration: ~80% elimination via biliary/fecal excretion (>90%–97% as unchanged parent) and ~20% renal elimination by glomerular filtration (>97% as unchanged parent). Absolute oral bioavailability of linerixibat was exceedingly low (0.05%), primarily because of a very low fraction absorbed (0.167%; fraction escaping first-pass gut metabolism (fg) ~100%), with high hepatic

extraction ratio (77.0%) acting as a secondary barrier to systemic exposure. Oral linerixibat was almost entirely excreted (>99% recovered radioactivity) in feces as unchanged and unabsorbed linerixibat. Consistent with the low oral fraction absorbed and ~20% renal recovery of intravenous [¹⁴C]-linerixibat, urinary elimination of orally administered radioactivity was negligible (<0.04% of dose). Linerixibat unequivocally exhibited minimal gastrointestinal absorption and oral systemic exposure. Linerixibat represents a unique example of high CYP3A4 clearance in vitro but nearly complete excretion as unchanged parent drug via the biliary/fecal route.

SIGNIFICANCE STATEMENT

This study conclusively established minimal absorption and systemic exposure to orally administered linerixibat in humans. The small amount of linerixibat absorbed was eliminated efficiently as unchanged parent drug via the biliary/fecal route. The hepatic clearance mechanism was mispredicted to be mediated via cytochrome P450 3A4 metabolism in vitro rather than biliary excretion of unchanged linerixibat in vivo.

Introduction

Primary biliary cholangitis (PBC) is a rare, chronic autoimmune liver disease caused by immune-mediated destruction of the intrahepatic bile ducts. This results in impaired bile acid flow and retention in the liver, leading to hepatic scarring, fibrosis, and ultimately cirrhosis and liver

failure (Boonstra et al., 2012; Gao et al., 2015). Cholestatic pruritus is a major symptom of PBC that significantly affects a patient's quality of life (Gotthardt et al., 2014; Hegade et al., 2015a; Jin and Khan, 2016; Hegade et al., 2019a; Lindor et al., 2019). The currently recommended treatments for pruritus can lead to symptomatic improvement in a subset of patients; however, a sizable population remains refractory and requires the utilization of invasive experimental treatments, such as nasobiliary drainage or even liver transplantation (Trivella and Levy, 2021). As such, an effective antipruritic drug therapy is an unmet clinical need in PBC (Trivedi et al., 2017).

Several putative pruritogens have been proposed in the treatment of PBC, including circulating bile acids (Hegade et al., 2015b), suggesting that the ileal bile acid transporter (IBAT), a solute carrier family transporter that mediates bile acid uptake from the gut lumen into enterocytes, may be a potential target for pruritus therapy (Hegade et al., 2015b; Al-Dury and Marschall, 2018). Inhibition of IBAT decreases bile acid reabsorption from the gastrointestinal (GI) tract, leading to decreased bile acid levels in the circulation and increased fecal bile acid excretion (Hegade et al., 2017, 2019b). Alongside pruritus, IBAT

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Information on GSK's data sharing commitments and requesting access can be found at: <https://www.clinicalstudydatarequest.com>.

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inhibition could benefit diabetic dyslipidemia by decreasing bile acid reabsorption and forcing the liver to increase de novo bile acid synthesis from cholesterol (Beysen et al., 2012). More recently, it has also shown utility in the treatment of chronic idiopathic constipation (Khanna and Camilleri, 2021) by increasing bile acid concentrations entering the colon, thereby stimulating colonic secretion and motility (Bampton et al., 2002).

Linerixibat (GSK2330672) is an oral small-molecule IBAT inhibitor, which has been shown to be effective in reducing PBC-associated pruritus and serum bile acids in two phase 2 studies (Hegade et al., 2017; Levy et al., 2020). Linerixibat was intentionally designed for minimal absorption from the GI tract (Wu et al., 2013). The rationale for this approach was to restrict drug exposure to the pharmacologic site of action while minimizing systemic exposure that may cause adverse events and drug-drug interactions (DDIs).

Preclinical studies demonstrated oral bioavailability of <1% in mice, rats, and dogs (Wu et al., 2013). However, in rodents this was caused by the low fraction absorbed from the GI tract, whereas in dogs the fraction absorbed was higher, which was consistent with relatively leakier canine intestines (Lennernäs, 1997), and high hepatic extraction served as a secondary barrier to systemic exposure of linerixibat. Although clinical trials have demonstrated minimal systemic linerixibat exposure in humans (Hegade et al., 2017; Ino et al., 2019), oral pharmacokinetics (PK) alone cannot prove that minimal GI absorption is the underlying cause. Understanding the basis of minimal oral systemic exposure is key in the context of linerixibat's drug development. Studying both intravenous and oral linerixibat PK in humans enables quantification of the contributions of the fraction absorbed from the GI tract and first-pass hepatic extraction to the minimal systemic exposure. Thus, the objectives of this study were to assess the PK, absorption, metabolism, distribution, and excretion of [¹⁴C]-linerixibat in humans after both intravenous and oral administration.

Materials and Methods

Materials. The [¹⁴C]-linerixibat (GSK2330672D) intravenous and oral solutions were obtained from Hammersmith Medicines Research Ltd (London, UK). Linerixibat (GSK2330672B) oral tablets were obtained from Wuxi Apptec Co., Ltd (Shanghai, China). Reference standards were provided by GlaxoSmithKline: GSK2330672B (free acid/free base, batch C13090301-QF17602, chemical purity 99.5%), [¹³C]-radiolabeled GSK2330672C (free acid/free base, batch R18284/139/2, radiochemical purity 97.8%), and [¹⁴C]-radiolabeled GSK2330672D (free acid/free base, batch DN27404-056A1, radiochemical purity 97.6%). All other solvents and reagents were of laboratory grade and purchased from commercial suppliers.

Study Design. This was a single-group, single-center, open-label, non-randomized, two-period, single-sequence [¹⁴C]-linerixibat mass balance study (ClinicalTrials.gov identifier NCT03992014) conducted at Hammersmith Medicines Research Centre (London, UK) between July 8, 2019 and August 26, 2019. The study adhered to the Declaration of Helsinki and was approved by the Health and Social Care Research Ethics Committee (National Health Service, UK). Written informed consent was obtained from participants prior to any study-specific procedures. All analyses were performed according to a predefined protocol.

The study included a screening visit and two treatment periods separated by approximately 7 days (≥ 13 days for oral doses) (Fig. 1). During the treatment periods, participants resided in the unit from the morning of day -1 (the day

before dosing) until all procedures were completed at 168 hours postdose (day 8). Participants were followed up 1–2 weeks after the last assessment in treatment period 2.

All participants received a [¹⁴C]-linerixibat intravenous microtracer (100 μ g; 3-hour infusion) concomitant with 90-mg linerixibat oral tablets during treatment period 1 and a 90-mg [¹⁴C]-linerixibat oral solution during treatment period 2; all participants received their doses on site. The 90-mg oral dose of linerixibat was determined from a previous phase 2a study (Hegade et al., 2017). The [¹⁴C]-linerixibat intravenous microtracer dosing regimen was extrapolated by allometry from preclinical in vivo data (see Supplemental Data) and in vitro-to-in vivo extrapolation (IVIVE) using data from in vitro analyses of human liver microsomes (presented in this paper). The relatively long (3-hour) intravenous infusion of the relatively high microtracer dose (100 μ g) was selected to ensure adequate characterization of the intravenous concentration-time curve considering projected rapid human elimination of intravenous dose (projected half-life 0.8 hours, see Supplemental Data) as well as to reduce the maximal concentration expected at the end of intravenous infusion to ensure it does not exceed established safety at the low concentrations achieved after oral linerixibat administration in previous human studies (Hegade et al., 2017).

For both treatment periods, participants underwent an overnight fast of at least 8 hours. A blood sample was collected at 0 hours, which was immediately followed by either a 90-mg oral dose of linerixibat administered as two 45-mg tablets along with a 3-hour intravenous infusion of 100 μ g of [¹⁴C]-linerixibat (treatment period 1) or a 90-mg [¹⁴C]-linerixibat dose administered as an oral solution (treatment period 2). Participants continued fasting for 2 hours after dosing. The last blood sample was collected on day 8 of treatment period 1 and on day 7 (completing on day 8) of treatment period 2. Urine and fecal samples were collected every 24 hours for a total of 168 hours after oral dosing for both treatment periods. Duodenal bile was collected on day 1 of treatment period 1. For both treatment periods, the last vital sign assessment, electrocardiogram, and brief physical examination were completed on day 8.

Each participant received 9.25 kBq (250 nCi) in treatment period 1 and approximately 4.96 MBq (134.1 μ Ci) in treatment period 2. The total amount of radioactivity administered to each participant in the study was 4.97 MBq (134.3 μ Ci). It was estimated that the combined total effective dose for the two treatment periods would be <1 millisievert. On this basis, the maximum administered activity complied with recommendation of 1 millisievert maximum for category IIa projects by International Commission on Radiologic Protection (ICRP, 1992).

Study Population. Healthy male participants between 30 and 55 years of age with body weight ≥ 50 kg, body mass index 19–31 kg/m², and a history of regular bowel movements were eligible for the study. The additional eligibility criteria were nonsmokers or smokers who had not regularly smoked for 6 months prior to screening, no history of drug abuse, and those not exposed to significant radiation in the 3 years prior to the study. Standard participant criteria for a human radiolabeled disposition study were used (see Supplemental Methods for full eligibility criteria).

Sample Collection and Processing. Blood samples were collected at preselected time points up to day 7 after dosing and transferred into dipotassium EDTA tubes. The maximum amount of blood collected from each participant over the duration of the study did not exceed 600 ml. Plasma was separated by centrifugation. Bile samples were collected via an Entero-test noninvasive string device (Neo-Medical Inc., Sparks, NV) in treatment period 1 only. The Entero-test device was swallowed 3.5 hours before the oral dose or start of the intravenous infusion to allow peristaltic transit to the duodenum. A food cue was used to stimulate gall bladder emptying at 2 hours after the start of intravenous infusion, and the string was withdrawn 1 hour later. All samples were stored frozen prior to shipment for analysis.

Mass Balance and Excretion. Total radioactivity excreted in urine and fecal samples was determined by using liquid scintillation counting (LSC) and accelerator mass spectrometry (AMS) for treatment period 1 (Pharmaron ABS, Inc., Germantown, MD) and by LSC for treatment period 2 (Covance Laboratories

ABBREVIATIONS: AMS, accelerator mass spectrometry; AUC, area under the plasma concentration-time curve; $Cl_{h,iv,blood}$, hepatic blood clearance; CYP, cytochrome P450; DDI, drug-drug interaction; E_h , hepatic extraction ratio; F, absolute oral bioavailability; fa, fraction absorbed; fg, fraction escaping first-pass gut metabolism; F_h , fraction escaping first-pass hepatic extraction; GI, gastrointestinal; IBAT, ileal bile acid transporter; IVIVE, in vitro-to-in vivo extrapolation; LC, liquid chromatography; LC-MS/MS, LC with tandem mass spectrometry; LLQ, lower limit of quantification; LSC, liquid scintillation counting; OATP, organic anion-transporting protein; PBC, primary biliary cholangitis; PK, pharmacokinetics; QC, quality control.

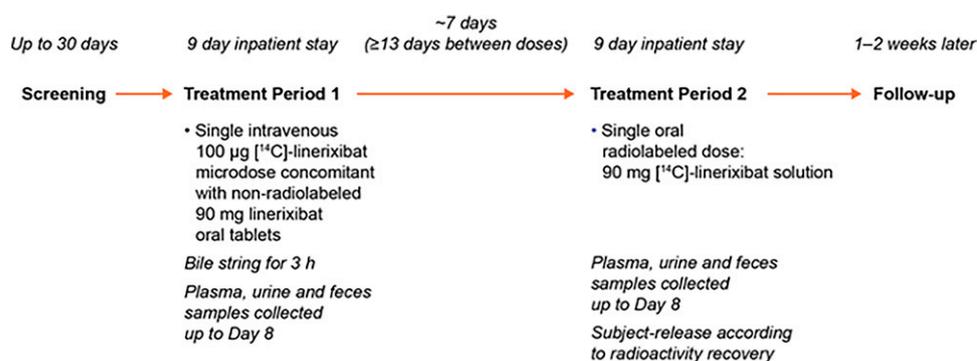


Fig. 1. Study schematic.

Limited, Harrogate, UK). For LSC, counting efficiency and quench correction were achieved automatically by an external standard ratio method. The lower limit of quantification (LLQ) for LSC was assigned as twice the mean background disintegration rate.

AMS instrument standards were analyzed alongside the samples for graphitization. For measurement of total radioactivity by AMS, urine and fecal samples underwent combustion (oxidation) and graphitization (reduction), and radioactivity was determined using a single stage accelerator mass spectrometer-250 (NEC, Middleton, WI). For treatment period 1, the LLQ value using AMS for urine samples was 2.97 pg linerixibat Eq/ml and for fecal samples was 17.2 pg Eq/ml. For the assessment of radioactivity in feces, one participant was a recovery outlier and was not included in the mean calculations.

Pharmacokinetic Assessments. Plasma concentrations of [¹⁴C]-linerixibat (parent drug) were determined by liquid chromatography (LC) and AMS (LC+AMS), and plasma concentrations of [¹²C]-linerixibat were determined by LC with tandem mass spectrometry; the details of these assays are presented in Supplemental Methods.

Quality-control (QC) samples were analyzed with each batch of study samples against separately prepared calibration standards. For measurement of samples containing linerixibat using LC-MS/MS, four concentrations were used. For measurement of samples containing [¹⁴C]-linerixibat using LC+AMS, three concentrations were used. To pass acceptance, no more than one-third of the QC results were to deviate from the nominal concentration by >15% (LC-MS/MS) or >20% (LC+AMS), and ≥50% of the results from each QC concentration were required to be within 15% (LC-MS/MS) or 20% (LC+AMS) of nominal. The applicable analytical runs met all predefined run acceptance criteria.

The total radioactivity of plasma from treatment period 1 was determined with AMS and from treatment period 2 with LSC and AMS. For plasma total radioactivity measured by AMS, the mean LLQ was 16.1 pg Eq/ml for treatment period 1 and 25.2 pg Eq/ml for treatment period 2. Further details of bioanalytical methodologies are provided in the Supplemental Methods.

Quantification and Characterization of Metabolites. To create pooled plasma samples at 1–3 hours after treatment period 1, individual participant plasma samples were vortexed, and equal volumes of 1-, 2-, and 3-hour samples were pooled across participants. Using an area under the plasma concentration-time curve (AUC) approach (Hamilton et al., 1981), individual participant plasma samples were vortexed and pooled across all participants for the preparation of plasma AUC_{0–12h} samples after treatment period 2.

Individual participant urine (0–24 hours) and homogenized fecal (0–120 hours) samples from treatment period 1 were pooled based on a ratio of the total weight of the sample excreted at each time point and were made to represent ≥95% of the total excreted radioactivity across all participants (Penner et al., 2009). For treatment period 2, representative individual participant fecal samples were created according to excretion balance data; weighed aliquots of selected samples of feces (from time intervals containing >2% of the administered dose) were combined proportionally to total sample weight to give a single pooled representative sample per participant. The pooled fecal samples were extracted once with acetonitrile containing 0.2% formic acid and once with methanol containing 0.2% formic acid. Combined extracts were dried down and reconstituted with methanol containing 10% DMSO. Samples were diluted with 0.1% formic acid in water prior to LC-MS/MS analysis with quantitation by microtiter plates scintillation counting from collected fractions. The total radioactivity of pooled

treatment period 2 (oral) feces samples was expressed as a percentage of the total administered radioactive dose. Calculations were based on the percentage of administered dose and the pooled weight ratio data of the individual samples.

Crossparticipant pools of all sample types [plasma AUC_{0–12h}, urine (0–24 hours) and fecal (0–120 hours, treatment period 1) samples] were prepared after each individual participant pool was created by taking a constant proportion the participant pool. Predose plasma, urine, and fecal samples were created. These pools were used to obtain background values to subtract from the postdose pools for analysis by AMS. Prior to analysis by LC+AMS, plasma samples were extracted with acetonitrile and then methanol containing 0.2% formic acid, reconstituted in 10% DMSO in methanol, and diluted with 0.1% formic acid in water; fecal samples were extracted with acetonitrile containing 0.1% formic acid and then with methanol containing 0.2% formic acid (3×) and diluted with 0.1% formic acid in water, and urine was centrifuged. All the samples analyzed by AMS were spiked with [¹²C]-linerixibat reference standard.

Duodenal bile was first extracted with acetonitrile from the bile-string samples, and this was followed by a second extraction with water. Aliquots of the bile extracts were mixed with scintillation fluid and measured separately by LSC; vials were individually counted for 15 minutes. After analysis and review of each sample, certain extracts were combined prior to further analysis by LC+AMS.

Radiochromatograms of plasma (treatment periods 1 and 2) and urine, homogenized feces, and bile string (treatment period 1) were generated by collecting LC fractions, which was followed by graphitization and analysis of fractions by AMS. Column recovery was calculated based on total amount of radioactivity (¹⁴C) injected and the amount recovered. Metabolites were quantified in plasma, urine, fecal (treatment period 1), and bile-string extracts using AMS. Further details are provided in the Supplemental Methods.

Metabolite identification for the treatment period 2 feces was conducted using high-resolution mass spectrometry, wherein the chromatographic retention time, accurate mass, and multi stage mass spectrometry fragmentation pattern were used for structure elucidation of metabolites. Metabolites and linerixibat assignments in the AMS radiochromatograms were made by retention time matching with either a corresponding linerixibat reference standard UV peak, metabolites from treatment period 2 feces, or metabolite peaks from prior metabolite studies (unpublished data).

Pharmacokinetics and Statistical Analysis. No formal sample size calculation was performed for this study. As the objective was to gain a better understanding of the PK, excretory routes, and metabolic profile of linerixibat, inclusion of 4–6 participants was deemed sufficient (Penner et al., 2009).

Plasma linerixibat, [¹⁴C]-linerixibat, and total radioactivity concentration-time data were analyzed by noncompartmental methods with PhoenixWinNonlin Version 6.3 (Certara USA, Inc., Princeton, NJ) using the actual sampling times for derivation of PK parameters. Linerixibat plasma kinetics in terms of C_{max}, time to C_{max}, AUC_{0–t}, AUC_{0–24} and AUC_{0–inf}, terminal phase rate constant, apparent terminal phase half-life, clearance, volume of distribution at steady state, absolute bioavailability, fraction of drug escaping first-pass hepatic clearance, hepatic extraction ratio, and fraction absorbed were calculated from plasma concentration-time data.

The hepatic plasma clearance was calculated as the difference between linerixibat total plasma clearance and renal clearance. Hepatic blood clearance (Cl_{h,iv,blood}) was determined by adjusting the hepatic plasma clearance by the linerixibat blood-

to-plasma ratio of 0.678 determined in vitro (unpublished data). The hepatic extraction ratio (E_h) was then determined by dividing $Cl_{h,iv,blood}$ by hepatic blood flow utilizing the literature reference value of 1660 ml/min (Edginton et al., 2006). The fraction of linerixibat that escapes first-pass liver extraction (F_h) was determined as one minus the E_h . The fraction of linerixibat absorbed (f_a), including the fraction escaping first-pass gut metabolism ($f_g \sim 100\%$), was determined by dividing the absolute oral bioavailability (F) by the F_h .

Results

A total of six participants were enrolled in this study, and all participants completed the study. Mean age was 41.2 (S.D. 8.1; range 33–53) years, and mean body mass index was 24.0 (S.D. 1.1; range 22.9–26.0) kg/m^2 .

Linerixibat and total drug-related radioactivity in the systemic circulation rapidly attained steady state during the 3-hour intravenous infusion and declined rapidly at the end of the infusion with detectable concentrations at up to 6 hours (Fig. 2). The short elimination half-life (0.828 hours) after intravenous administration was consistent with the high systemic clearance (61.8 l/h) and low volume of distribution (16.3 l) of linerixibat (Table 1). The exposure ratio of plasma linerixibat to plasma total radioactivity as well as the metabolite profiling showed that linerixibat was the predominant contributor to total radioactivity in the circulation (86% of total radioactivity in plasma), demonstrating linerixibat was minimally metabolized. Elimination of intravenous radioactivity was 80% fecal and 20% renal, predominantly as unchanged parent drug (90%–97% of fecal, bile-string, and urinary radioactivity) (Fig. 3A; Tables 1 and 2).

Standard in vitro studies identified oxidative metabolism in human liver microsomes as the most likely route of linerixibat clearance, which was mediated by cytochrome P450 (CYP) 3A4 but not the other major CYP enzymes (unpublished data). Scaled unbound intrinsic clearance of linerixibat in human liver microsomes was high (241 l/h) based on an in vitro incubation half-life of 39.4 minutes at 1 mg/ml microsomal protein concentration [microsome fraction unbound = 0.299; 39.7 mg microsomal protein/g of liver and 24.5 g of liver/kg body weight (Barter et al., 2007, 2008), assuming 70 kg human body weight, as described previously (Obach et al., 1997, 1999)]. This microsomal intrinsic

clearance was extrapolated to human using IVIVE based on the well stirred liver model, and the human clearance was predicted to be 31 l/h [plasma fraction unbound = 0.245; blood-to-plasma ratio = 0.678; human hepatic blood flow = 96.6 l/h (Yang et al., 2007)]. This prediction was within 1.5-fold of the high hepatic clearance observed in this study (80% of human systemic elimination of intravenous dose).

The geometric mean half-life of linerixibat was approximately an order of magnitude longer after oral versus intravenous administration (6–7 hours vs. 0.8 hours). Based on the observed shorter half-life after intravenous administration compared with oral administration, linerixibat displays absorption-limited, flip-flop kinetics; therefore, the terminal slope of the oral concentration-time profile (Table 1, λ_z) reflects the oral absorption rate constant, which was estimated to be 0.1 $hour^{-1}$. Linerixibat exhibited minimal oral absorption (absolute oral bioavailability of 0.0517%) primarily due to the very low fraction absorbed (0.167%; $f_g \sim 100\%$, see Table 3 results below). High hepatic extraction (77.0%) acted as a secondary barrier to systemic exposure, accounting for the approximately 4-fold lower oral bioavailability versus fraction absorbed. Time of C_{max} was highly variable after oral administration of both tablet and solution formulations with the large range overlapping across the two (Table 1).

As expected for a minimally absorbed drug, the oral [^{14}C]-linerixibat dose was almost entirely excreted (>99% of the recovered radioactivity) in feces as unchanged and unabsorbed linerixibat. Three very minor oxidative metabolites, M8, M9, and M10, were detected, but they accounted for negligible radioactivity after correcting for coeluting radiochemical impurity E and degradant K (Table 3), thus supporting f_g being approximately 100%. Consistent with low oral fraction absorbed and approximately 80%/20% fecal/renal excretion of intravenous [^{14}C]-linerixibat, urinary elimination of orally administered radioactivity was negligible (<0.04% of dose).

After administration of oral [^{14}C]-linerixibat solution, the exposure to total radioactivity was far higher and longer than to parent drug (Fig. 4; individual participant data in Supplemental Figure S1). The geometric mean C_{max} and exposure ratios of plasma linerixibat to plasma total radioactivity showed that linerixibat represented only 2%–6% of the total radioactivity in plasma. This low fraction of parent linerixibat

Fig. 2. Parent [^{14}C]-linerixibat and total drug-related radioactivity concentration-time profiles after 100 μg i.v. microdose of [^{14}C]-linerixibat ($n = 6$). The intravenous microdose of [^{14}C]-linerixibat (infused over 3 hours) was administered concomitantly with a nonradiolabeled 90-mg tablet oral dose.

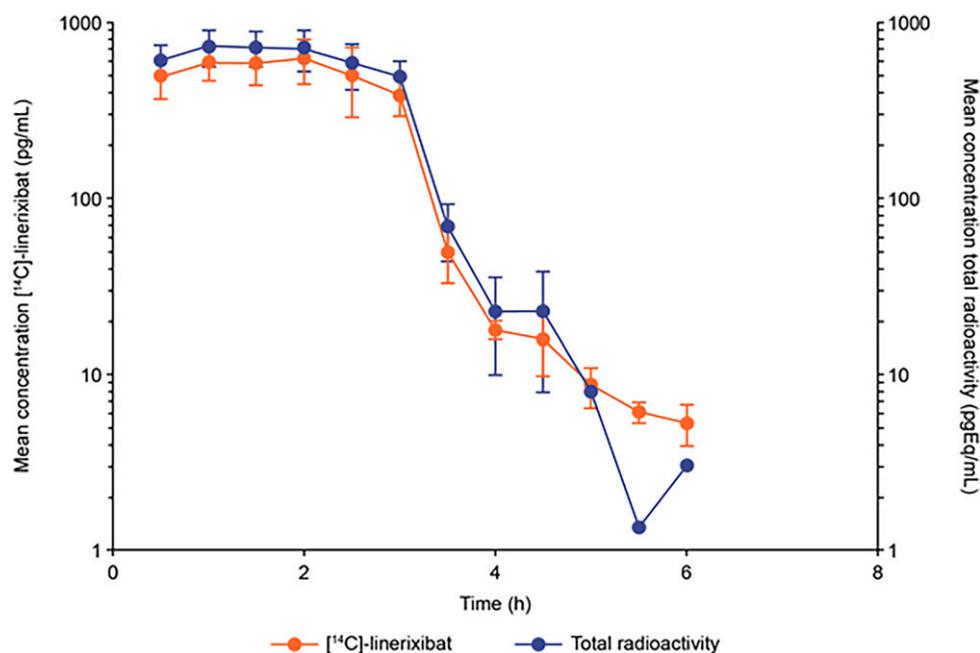


TABLE 1
Summary of pharmacokinetic parameters for linerixibat (n = 6, unless stated otherwise)

PK Parameter (Units)	Summary Statistics	90-mg Linerixibat Oral Tablets	100- μ g [14 C]-Linerixibat Intravenous Infusion	90-mg [14 C]-Linerixibat Oral Solution
C_{max} (pg/ml)	Geometric mean (CVb%)	120 (108)	638 (27.3)	158 (270)
	95% CI	(47.8, 302)	(481, 845)	(34.3, 726)
t_{max} (h)	Median	2.25	1.49	7.50
	Range	(0.500, 5.50)	(0.983, 2.50)	(2.00, 48.1)
AUC_{0-t} (h•pg/ml)	Geometric mean (CVb%)	749 (125)	1560 (26.8)	1040 (79.4)
	95% CI	(270, 2070)	(1190, 2060)	(501, 2170)
AUC_{0-inf} (h•pg/ml)	n	3	6	3
	Geometric mean (CVb%)	1550 (25.8)	1570 (26.7)	1630 (95.3)
λ_z (h $^{-1}$)	95% CI	(826, 2920)	(1190, 2070)	(222, 12000)
	Geometric mean (CVb%)	0.102 (124)	0.836 (18.26)	0.110 (64.3)
$t_{1/2}$ (h)	95% CI	(0.009, 1.13)	(0.692, 1.01)	(0.026, 0.479)
	n	3	6	3
V_{ss} (L)	Geometric mean (CVb%)	6.76 (124)	0.828 (18.3)	6.25 (64.3)
	95% CI	(0.614, 74.4)	(0.685, 1.00)	(1.45, 27.0)
$Cl_{iv,plasma}$ (ml/min)	Geometric mean (CVb%)	NA	16.3 (35.7)	NA
	95% CI	NA	(11.4, 23.5)	NA
$Cl_{R,iv,plasma}$ (ml/min)	Geometric mean (CVb%)	NA	1030 (27.3)	NA
	95% CI	NA	(779, 1370)	NA
$Cl_{h,iv,plasma}$ (ml/min)	Geometric mean (CVb%)	NA	163 (37.2)	NA
	95% CI	NA	(112, 238)	NA
$Cl_{h,iv,blood}$ (ml/min)	Geometric mean (CVb%)	NA	867 (26.4)	NA
	95% CI	NA	(660, 1140)	NA
E_h	Geometric mean (CVb%)	NA	1280 (26.4)	NA
	95% CI	NA	(974, 1680)	NA
F_h	Geometric mean (CVb%)	NA	0.770 (26.4)	NA
	95% CI	NA	(0.586, 1.01)	NA
fa^a	Geometric mean (CVb%)	NA	0.244 (68.4)	NA
	95% CI	NA	(0.113, 0.527)	NA
F	Geometric mean (CVb%)		0.00167 (73.7)	NA
	95% CI		(0.000739, 0.00380)	NA
F	Geometric mean (CVb%)		0.000517 (120)	NA
	95% CI		(0.000192, 0.00140)	NA

AUC_{0-t} , area under the conc.-time curve from time 0 (predose) to last time of quantifiable conc. within a participant across all treatments; AUC_{0-inf} , area under the conc.-time curve from time 0 (predose) extrapolated to infinite time; λ_z , elimination rate constant; CI, confidence interval; $Cl_{h,iv,plasma}$, hepatic clearance from plasma; $Cl_{iv,plasma}$, clearance; $Cl_{R,iv,plasma}$, renal clearance; CVb, between participant variability; F, absolute oral bioavailability for linerixibat; n, number of participants with available data to estimate the PK parameter; NA, not assessed; $t_{1/2}$, half-life; t_{max} , time of occurrence of C_{max} ; V_{ss} , volume of distribution.

^a fa includes the fraction escaping first-pass gut metabolism ($fg \sim 100\%$)

accounting for oral plasma total radioactivity concentrations was conceptually inconsistent with parent linerixibat accounting for the majority of radioactivity administered via intravenous infusion. It also differs from previous profiling of human plasma obtained after 14 days of oral 90-mg twice-daily nonradiolabeled linerixibat dosing, wherein only the parent drug but no linerixibat-related metabolites were observed in circulation (Nunez et al., 2016). The low fraction of parent linerixibat accounting for plasma total radioactivity after oral dosing in treatment period 2 is likely an artifact of 2.4% radiochemical impurities for a drug with <0.2% fraction absorbed. Furthermore, these radiochemical impurities had a 70-fold-higher specific activity than isotopically diluted [14 C]-linerixibat in the oral dose formulation and were profiled using AMS, which provides a measure of total radiocarbon (see supplement for discussion of results presented in Fig. 5).

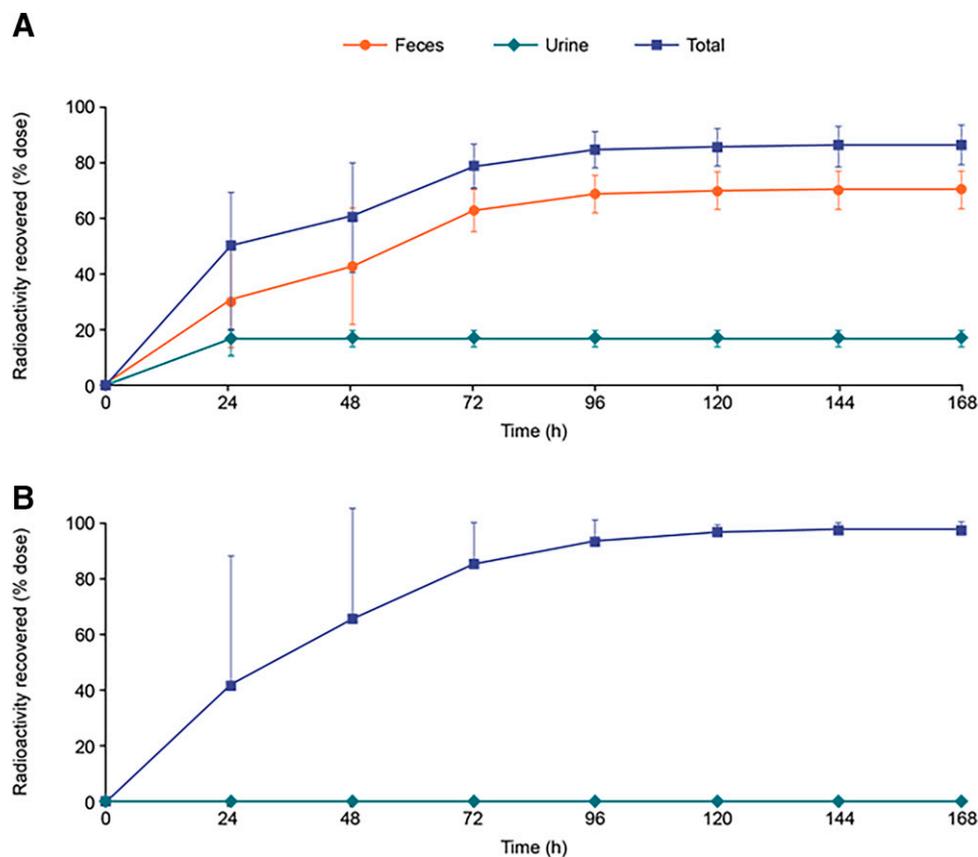
Discussion

Characterization of the human PK and metabolism of [14 C]-linerixibat after both intravenous and oral administration is important in the context of a drug that has minimal systemic exposure and in which the GI lumen is the site of pharmacology. Only by assessing both modes of administration can we quantify the contribution of fraction absorbed from the GI tract and of first-pass hepatic extraction to the minimal systemic exposure. The current study established that linerixibat exhibits minimal systemic exposure due to low intestinal absorption. Furthermore, intestinal absorption was slow, resulting in flip-flop kinetics that

were limited by absorption rate, as evidenced by a longer oral versus intravenous half-life (6–7 hours vs. 0.8 hours). Technically, fraction absorbed is $fa \times fg$ and, typically, direct calculation of fg would have been possible based on oral and intravenous metabolite load data given that oral metabolite load = intravenous metabolite load + metabolites generated on first pass through the GI and the liver (Harrell et al., 2019). Unfortunately, the oral metabolite load calculation for linerixibat is not feasible since the major contributors to oral radioactivity exposure were radiochemical impurities and their metabolites. However, minimal first-pass gut-wall metabolism ($fg \sim 100\%$) is substantiated by negligible recovery of metabolites in excreta after oral administration of [14 C]-linerixibat (Table 3). Finally, there is no evidence that linerixibat accesses enterocytes and their metabolic enzymes because of exceedingly low passive permeability, and although linerixibat is taken up into hepatocytes by OATP 1B, it is not transported by intestinal OATP2B1 (unpublished data).

These results have a direct impact on late-stage clinical development of linerixibat. In vitro screening of both perpetrator and victim DDI risks flagged linerixibat as both a substrate and inhibitor of OATP1B1 ($IC_{50} = 2.69 \mu M$), OATP1B3 ($IC_{50} = 0.265 \mu M$) and CYP3A4 ($K_I = 1.3 \text{ mM}$, $k_{inact} = 9.6 \text{ hour}^{-1}$, $f_{u,inc} = 0.3$) (unpublished data). Based on PK results from this study and current regulatory guidelines, linerixibat's potential as a perpetrator or victim of DDIs via CYP3A4 and OATP1B does not meet the criteria for clinical evaluation (US FDA, 2020a).

Fig. 3. Fecal, urinary, and total recovery of radioactivity after administration of (A) an intravenous [^{14}C]-linerixibat microtracer over 3 hours ($n = 6$, mean \pm S.D.) and (B) a 90-mg [^{14}C]-linerixibat oral solution ($n = 5^a$, mean \pm S.D.). $n = 5$ for all time points, as one participant's recovery was only 20.8% of the dose and considered anomalous compared with the 97% recovery in the other five participants (outlier by Q test; no fecal samples on study days 2 and 6 when the other five participants produced daily fecal samples; prune juice given from day 6 to elicit bowel movements, resulting in feces with no detectable radioactivity from day 7 onward).



Although linerixibat is an OATP1B1 and OATP1B3 substrate *in vitro*, the present study clearly established minimal intestinal absorption as the basis of minimal systemic exposure of linerixibat, with the liver acting as a secondary barrier to systemic exposure with a hepatic extraction of 77.0%. Assuming complete inhibition of hepatic OATP1B, oral bioavailability would increase at the most from 0.05% to the fraction absorbed of <0.2%. Consequently, systemic exposure would increase at most only by approximately 4-fold, which would be well within systemic safety margins. Because of linerixibat exhibiting flip-flop kinetics limited by absorption rate, potential inhibition of hepatic clearance would not increase exposure after oral dosing because absorption is approximately 10-fold-slower than elimination. As such, only the hepatic first-pass effect is relevant to victim DDI potential.

Based on the results of the present study, further clinical studies to assess the effect of renal impairment are not scientifically justified. The systemic exposure of linerixibat after oral administration is not expected to be impacted by renal impairment or by a reduction in hepatic clearance due to circulation of elevated levels of uremic toxins due to its

minimal oral absorption, negligible dose recovery in urine after oral administration, and flip-flop kinetics.

Although linerixibat is a CYP3A4 substrate *in vitro*, inhibition of metabolic clearance by a coadministered CYP3A4 inhibitor is not expected to impact linerixibat disposition or systemic PK. This is because orally administered linerixibat was eliminated almost entirely as unabsorbed and unchanged parent drug in feces. About 1 in 2000 orally administered linerixibat molecules that do enter the systemic circulation is eliminated as unchanged drug in bile/feces (approximately 80%) and urine (approximately 20%).

Intrinsic clearance of linerixibat in human liver microsomes was high (241 l/h) with metabolism exclusively via CYP3A4 and no other major CYP isoforms (unpublished observations). IVIVE based on the well stirred liver model predicted a human hepatic clearance of 31 l/h, which was within 1.5-fold of the observed human hepatic clearance (80% of systemic clearance). Therefore, it was surprising that human clearance occurred primarily by direct excretion of parent drug with negligible metabolism. Even after a thorough literature review, it appears that linerixibat is the only reported example of this phenomenon, which may be related to the unusual physicochemical properties needed to achieve minimal absorption (Wu et al., 2013). An interesting future direction may be to determine whether a more complex *in vitro* hepatocyte system that re-establishes bile canaliculi while maintaining CYP metabolic activity is capable of describing *in vivo* hepatobiliary disposition of linerixibat.

Biliary excretion was studied in bile duct-cannulated male Han Wistar rats and male Beagle dogs after oral administration of [^{14}C]-linerixibat (unpublished data). The original conclusion of these studies was that fecal excretion was the major elimination pathway for oral [^{14}C]-linerixibat (rat = 93.9% and dog = 82.0% of dosed radioactivity), whereas

TABLE 2

[^{14}C]-Linerixibat-related radioactivity after intravenous infusion of radiolabeled microdose

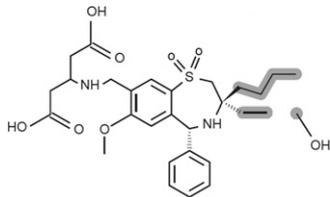
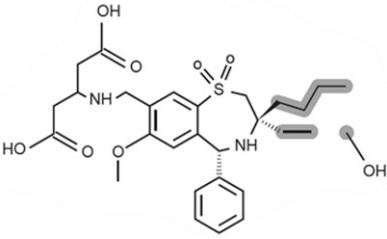
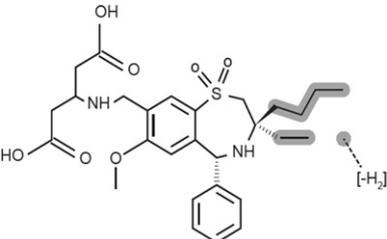
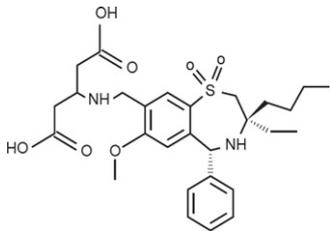
Component	Nominal Retention Time	% Radioactivity			
		Plasma	Urine	Bile	Feces
	<i>min</i>				
Impurity E	35.2	0.68	1.46	1.13	NA
Linerixibat	36.4	85.64	97.28	96.22	90.57
P3 ^a	42.0	1.24	NA	NA	NA
P4 ^a	47.4	1.78	NA	NA	NA

NA, not assessed.

^aIdentity unknown.

TABLE 3

Mean quantification of the radioactive components in fecal extracts after a single oral dose of [¹⁴C]-linerixibat at 90 mg (134.1 μCi) using offline analysis^a

Metabolite ID	Metabolite Structure	Radioactivity in Feces, Mean (%) of Administered Dose ^c	Radioactivity in Spiked Feces, %
M8 ^b	 Oxidation	1.08 (1.03)	ND
K ^b	Unassigned		1.05
M9	 Oxidation	0.14 (0.14)	ND
M10 ^b	 Oxidation	1.32 (1.27)	ND
E ^b	Dehydrogenation (two isomers)		1.58
Linerixibat (parent)		93.9 (90.5)	97.4
	Total quantified	96.5 (93.0)	100
	Dose in sample analyzed, %	93.2	NA
	Dose in total sample, %	96.4	NA

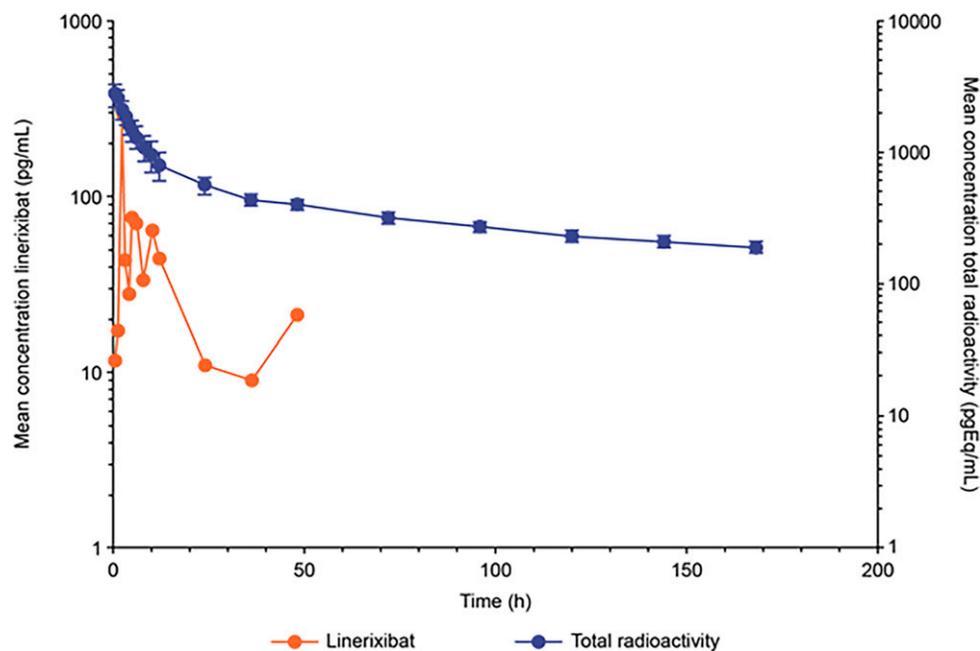
NA, not assessed; ND, not determined.

^an = 5; a single participant was a recovery outlier and was not included in the mean calculations.^bBased on further calculations, it was determined that the majority of the radioactivity of these peaks were from degradant K and impurity E.

biliary and urinary excretion represented minor elimination routes (rat bile = 3.0% and urine = 0.1%; dog bile = 6.7% and urine = 2.7% of dosed radioactivity). The outcome of the present clinical study resulted in re-examination of preclinical oral [¹⁴C]-linerixibat mass balance studies, assuming radioactivity recovered in bile and urine represents absorbed radioactivity. Upon re-examination of these preclinical results in terms of elimination of absorbed radioactivity, it becomes evident that biliary excretion of unchanged linerixibat is also the major route of elimination in rats and dogs. Biliary/urinary recovery of absorbed radioactivity in rats and dogs was 97%/3% and 71%/29%, respectively, which is consistent with ~80%/20% linerixibat-related material excretion in humans. Rat and dog biliary radioactivity consisted primarily of

parent linerixibat 73% and 75%, respectively; mono-oxygenated metabolites ≤6.5% and 19.5%, respectively; and radiochemical impurity-related radioactivity ≥13% and 3.4%, respectively. Mechanistically, the first step in linerixibat biliary excretion is mediated by OATP1B1 and OATP1B3 hepatic uptake (unpublished data); subsequent canalicular secretion is not mediated by P-glycoprotein or breast cancer resistance protein (unpublished data), leaving multidrug resistance-associated protein 2 as the most likely canalicular transporter (Patel et al., 2016) but one that is not studied during drug development because of lack of clinical evidence supporting involvement of multidrug resistance-associated protein 2 in clinical DDIs (US FDA, 2020a).

Fig. 4. Parent linerixibat and total drug-related radioactivity concentration-time profiles after 90-mg oral solution of [^{14}C]-linerixibat ($n = 6$). Individual participant data shown in Supplemental Figure S1.

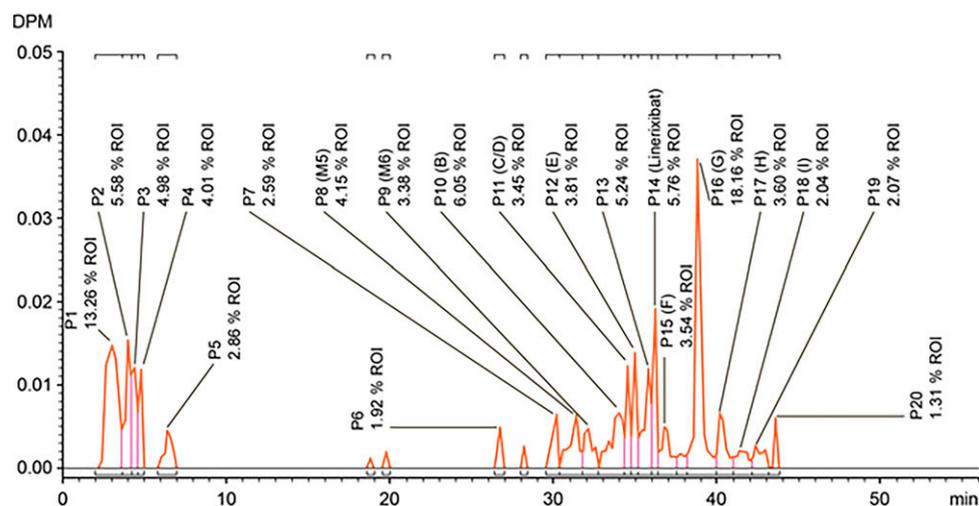


Typically, in the development of small-molecule drugs, the primary purpose of a radiolabeled study is the characterization of human in vivo metabolism to ensure that preclinical rodent and nonrodent toxicology species provide adequate coverage of the major human metabolites (US FDA, 2020b). After intravenous administration, unchanged linerixibat was the predominant form in human circulation and excreta (Fig. 2; Table 2). This was consistent with nonradiolabeled metabolite profiling of human plasma obtained after 90-mg oral linerixibat dosed twice-daily for 2 weeks (Nunez et al., 2016). However, the plasma radiochromatogram (derived by AMS) after oral administration was complex (Fig. 5). As described in Results, the observed complexity can be attributed to 2.4% of radiochemical impurities, with an estimated 70-fold-higher specific activity than the isotopically diluted parent drug, which exhibits low oral absorption (<0.2%). Despite this complex finding, the overall metabolite profile supports the absence of major human metabolites. AMS radiochromatogram of crossparticipant plasma pool extract

($\text{AUC}_{0-12 \text{ hour}}$) after oral administration of 90-mg oral solution of [^{14}C]-linerixibat is described in detail in the Supplemental Results and depicted in Fig. 5.

In conclusion, the present two-period clinical study of [^{14}C]-linerixibat intravenous and oral PK and disposition established linerixibat as a minimally absorbed drug that after oral administration is almost entirely recovered as unabsorbed and unchanged parent drug in feces. This study supports that, after oral administration, linerixibat is effectively restricted to the GI lumen, the site of pharmacology for IBAT inhibition. Additionally, this study demonstrated flip-flop oral absorption-rate-limited systemic PK. These results have considerable impact on late-stage clinical pharmacology studies as discussed above. Finally, linerixibat presents a fascinating novel case study in which human clearance by CYP3A4 metabolism was quantitatively predicted to be high, but in vivo metabolism was found to be minimal, with clearance predominantly by direct biliary/fecal excretion of the unchanged parent drug.

Fig. 5. AMS radiochromatogram of cross-participant plasma pool extract ($\text{AUC}_{0-12 \text{ h}}$) after oral administration of 90-mg oral solution of [^{14}C]-linerixibat. DPM, disintegrations per minute; ROI, region of interest.



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Wrote or contributed to the writing of the manuscript: Zamek-Gliszczynski, Kenworthy, Bershas, Sanghvi, Pereira, Mudunuru, Crossman, Pirhalla, Thorpe, Dennison, McLaughlin, Allinder, Swift, O'Connor-Semmes, Young.

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**Pharmacokinetics and ADME characterization of intravenous and oral [¹⁴C]-linerixibat
in healthy male volunteers**

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Supplemental Methods:*Selection of study population**Key inclusion criteria*

- Healthy male participants between 30 and 55 years of age (both inclusive), with body weight ≥ 50 kg, body mass index within the range 19.0–31 kg/m² (inclusive); and capable of giving written informed consent.
- Non-smoker or ex-smoker who had not regularly smoked for 6 months prior to the screening.
- History of regular bowel movements (averaging one or more bowel movements per day).
- A participant was eligible to participate if he agreed to use contraception with female partners of childbearing potential.

Key exclusion criteria

- Participants with a current or history of liver disease, cholecystectomy, known hepatic or biliary abnormalities (exception of Gilbert's syndrome or asymptomatic gallstones).
- Lymphoma, leukemia or any malignancy within the past 5 years except for basal cell or squamous epithelial carcinomas of the skin that was resected with no evidence of metastatic disease for 3 years.
- Any current medical condition (eg, psychiatric disorder, senility, dementia or other condition), clinical or laboratory abnormality or examination finding that the investigator considers would put the participant at unacceptable risk.
- Regular use or history of drug abuse or use of tobacco- or nicotine-containing products within 6 months prior to the study.

- Regular alcohol consumption within 6 months prior to the study defined as an average weekly intake of >21 units. One unit is equivalent to 8 g of alcohol: a glass (~240 mL) of beer, 1 small glass (~100 mL) of wine or 1 (~25 mL) measure of spirits.
- Past or intended use of over-the-counter or prescription medication, including analgesics (eg, paracetamol), herbal medications or grapefruit and Seville orange juices within 14 days prior to the first dose of study intervention until completion of the follow-up visit.
- Administration of any other ileal bile acid transporter inhibitor in the 3 months prior to screening.
- Had enrolled in a clinical trial and had received an investigational product within 3 months before the first dose in the current study. Had participated in a clinical trial involving administration of ¹⁴C -labeled compound(s) within the last 12 months.
- Had exposure to more than 4 new chemical entities within 12 months before the first dose in the current study.
- Received a total body radiation dose of greater than 10.0 mSv (upper limit of International Commission on Radiological Protection category II) or exposure to significant radiation (eg, serial X-ray or computed tomography scans, barium meal, etc.) in the 3 years before this study.
- Alanine transaminase or bilirubin >1.5x upper limit of normal. Presence of hepatitis B surface antigen at screening or positive hepatitis C antibody test result at screening or within 3 months before the first dose of study intervention. Positive human immunodeficiency virus antibody test.
- Screening estimated glomerular filtration rate <45 mL/min/1.73m² based on the Modification of Diet in Renal Disease study equation (Levey et al., 2006).

- Had positive pre-study drug/alcohol screen and urinary cotinine levels indicative of smoking.
- QT duration corrected for heart rate by Fridericia's formula (QTcF) >450 msec on electrocardiogram performed at Screening.
- Had a supine blood pressure that was persistently higher than 140/90 mmHg taken in triplicate, a supine mean heart rate outside the range of 40–100 beats per minute at screening or prior to the first dose.
- Has had an occupation that required monitoring for radiation exposure, nuclear medicine procedures or excessive X-rays within the past 12 months.
- Loss of more than 400 mL blood during the 3 months before screening.
- Unwillingness or inability to follow the procedures outlined in the protocol, including the use of the string bile collection device.
- History of sensitivity to linerixibat, its components or a history of drug allergy or any other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicated the participation.

Summary of the prediction of linerixibat human intravenous (IV) pharmacokinetics (PK) by allometry and static in vitro-to-in vivo extrapolation (IVIVE)

Various approaches were used to predict human clearance: multiple species simple allometry (3 species and 2 species) with floating exponent, fraction unbound intercept correlated method, two species rat-dog allometry with a forced exponent, as well as IVIVE extrapolation of clearance from human liver microsomes (mixed-sex pool of 150 donors). Simple multi-species allometry was used to predict the volume of distribution (V_{ss}), as well as terminal volume (V_z) needed for prediction of human terminal half-life.

Linerixibat was predicted to exhibit high clearance in humans, on average 1051 mL/min (83% of hepatic blood flow) [range 538–1357 mL/min (43–108% of hepatic blood flow)], low V_{ss} of 48.00 L, and a short terminal half-life of 0.811 h (based on V_z of 73.81 L and terminal elimination rate constant (K_e) of 0.855 1/h).

PK analysis

For plasma [^{14}C]-linerixibat, the lower limit of quantification (LLQ) was 2.65 pg/mL using a 500 μL aliquot of ethylenediaminetetraacetic acid (EDTA) plasma; the higher limit of quantification (HLQ) was 329 pg/mL. Plasma concentrations of linerixibat (parent drug) were determined by LC-tandem mass spectrometry (LC-MS/MS; Covance, Madison, WI, USA).

For plasma concentrations of [^{12}C]-linerixibat, the LLQ was 10 pg/mL using a 100 μL aliquot of EDTA plasma; the HLQ was 10,000 pg/mL.

For plasma [^{12}C]-linerixibat, an aliquot of 100 μL of plasma was extracted by solid phase extraction and typically 15 μL was injected onto the LC-MS/MS system. The LC system utilized an Acquity UPLC HSS T3 1.8 μm , 2.1 x 50 mm column (Waters, Elstree, UK), mobile phases of 10 mM Ammonium Bicarbonate and a 1:50:50 mixture of 10 mM Ammonium Bicarbonate: Acetonitrile: Methanol acetonitrile at a flow rate of 0.6 mL/min with a column temperature of 35°C. Under gradient conditions the typical retention time of linerixibat was 0.9 min.

Bioanalytical methods

Preparation of samples for accelerator mass spectrometry (AMS)

Analysis by AMS requires conversion of samples via a two-step process of combustion (oxidation) to carbon dioxide (CO_2) and then graphitization (reduction). The process used was as follows: aliquots of each sample and, if appropriate, carbon carrier (sodium benzoate),

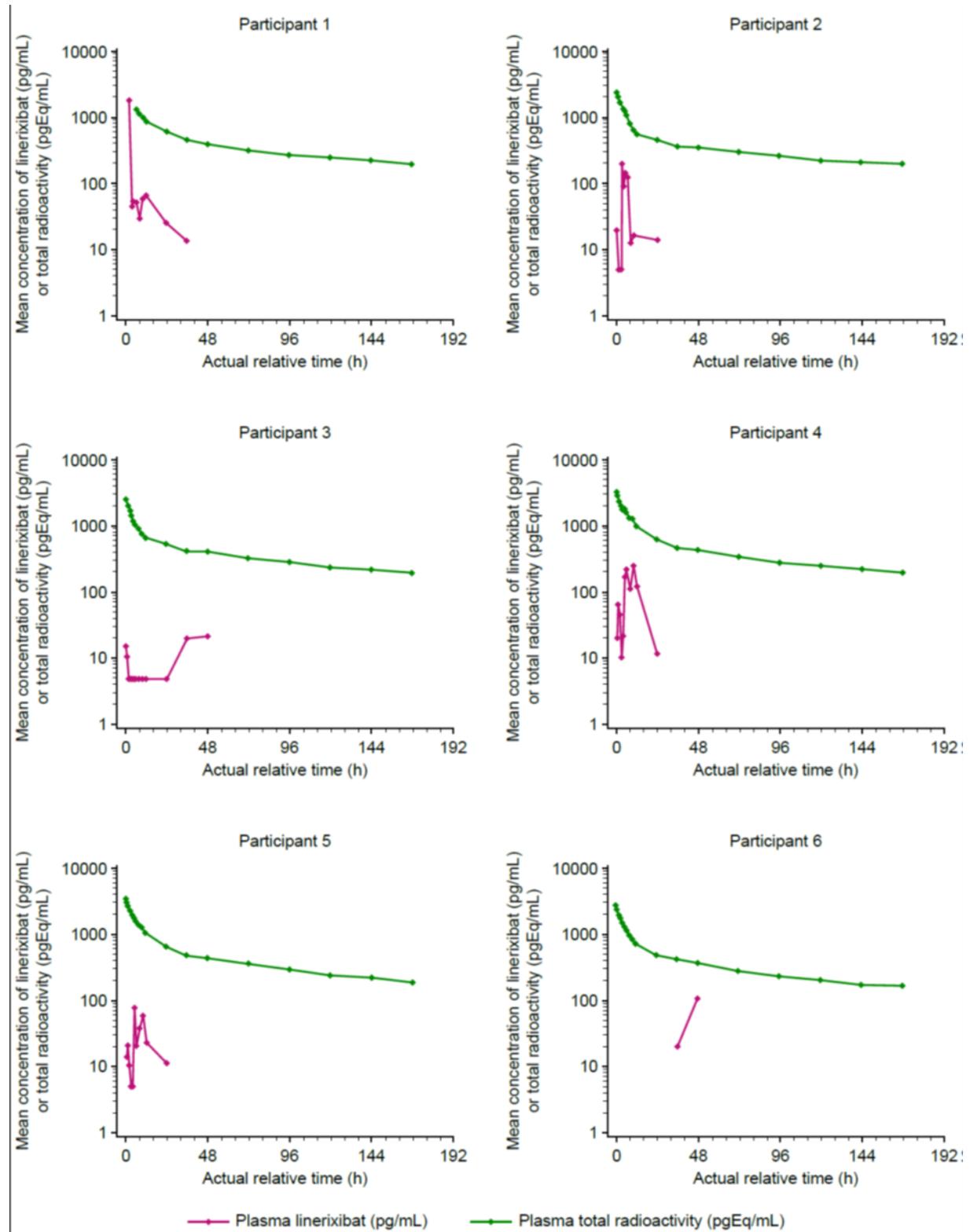
were placed in sample tubes containing pre-baked copper oxide powder, prior to combustion at 900°C for 1 h. The CO₂ thus formed was cryogenically transferred into borosilicate tubes, which were heated to 550°C for 5 h to complete the graphitization process. The cobalt/graphite was carefully tipped onto an aluminum cathode and compressed into place at 100–200 psi in a Parr Pellet Press before analysis by AMS. The data from the AMS and the carbon content of the control sample were combined to provide radiocarbon levels for each sample.

Supplemental Results

Although high relative to linerixibat, circulating total radioactivity following oral administration was low, so plasma samples were profiled using a highly sensitive AMS methodology. AMS provides a measure of only radiocarbon, and the radiochemical impurities (~2.4%) had a 70-fold higher specific activity than isotopically diluted [¹⁴C]-linerixibat in the oral dose formulation. Following administration of the radiolabeled oral solution, minor components in the human plasma profiles (1–6% of plasma radioactivity) included: linerixibat and radio-specific impurity E at comparable levels; oxidative metabolites M5 and M6; three peaks corresponding to radiochemical impurities and/or their metabolites; and nine minor peaks (**Figure 5**). The minor peaks would not have been detected by the less sensitive instrumentation that was used in nonclinical [¹⁴C]-linerixibat profiling. The major circulating component was chromatographically consistent with moiety G observed in preclinical [¹⁴C]-linerixibat studies (data not shown). Moiety G has only been detected after oral administration of [¹⁴C]-linerixibat, but not following chronic non-radiolabeled linerixibat administration to humans (Nunez et al., 2016) or following much higher chronic oral doses of non-radiolabeled linerixibat to rats and dogs (unpublished observations). Moiety G eluted after parent linerixibat, suggesting a more lipophilic molecule; structurally, linerixibat is not expected to form more lipophilic metabolites. As such, moiety G is believed to be a radiochemical impurity or metabolite thereof, but plasma drug-related material was consistently insufficient for structural identification. Moiety G (18% of human oral plasma radioactivity) was observed in both radiolabeled dog plasma (5% of oral plasma radioactivity) and rat liver (7% of radioactivity); mouse and rat plasma radioactivity were insufficient for profiling (data not shown). A fraction of the human oral plasma radioactivity (13%) was not retained on column and eluted in the solvent front, consistent with an injection column breakthrough artifact. Although the oral plasma [¹⁴C]-

linerixibat profile is complex and confounded by radiochemical dose impurities, it overall supports the absence of major human-specific linerixibat metabolites.

Supplementary Figure S1: Participant-level data for parent linerixibat and total drug-related radioactivity concentration-time profiles following 90-mg oral solution of [14 C]-linerixibat



References

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