Pharmacokinetics and ADME characterization of intravenous and oral [^{14}C]-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/m^2 (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 ^{14}C -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/1.73m^2 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 (Ke) 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 Aquity 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^{o}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),

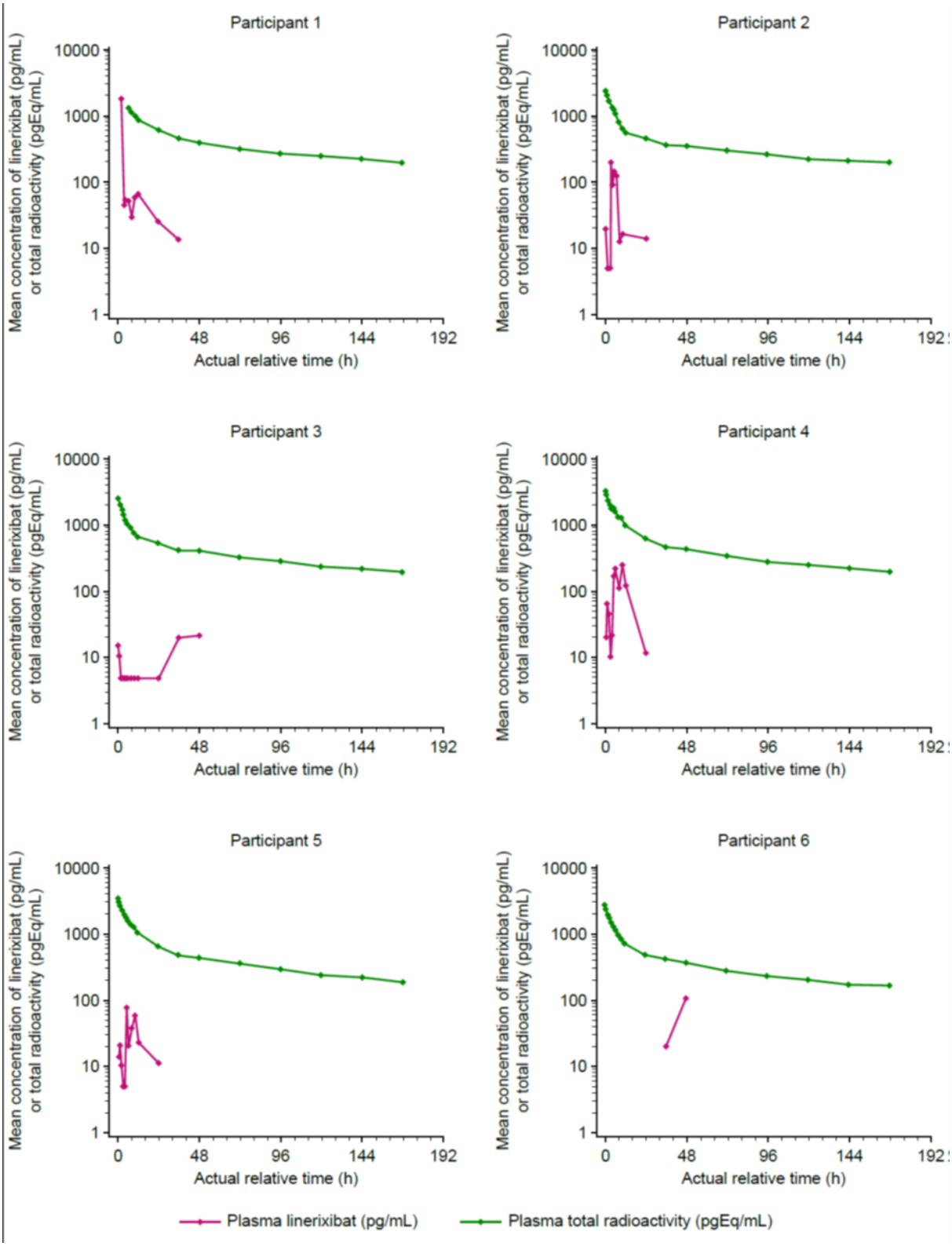
were placed in sample tubes containing pre-baked copper oxide powder, prior to combustion at 900°C for 1 h. The CO_2 thus formed was cryogenically transferred into borosilicate tubes, which were heated to 550°C for 5 h to complete the graphitization process. The cobalt/graphite was carefully tipped onto 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 [^{14}C]-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 [^{14}C]-linerixibat profiling. The major circulating component was chromatographically consistent with moiety G observed in preclinical [^{14}C]-linerixibat studies (data not shown). Moiety G has only been detected after oral administration of [^{14}C]-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 [^{14}C]-

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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