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Characterization of Clinical Absorption, Distribution, Metabolism, and Excretion and Pharmacokinetics of Velsecorat Using an Intravenous Microtracer Combined with an Inhaled Dose in Healthy Subjects[S]

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ABSTRACT

This open-label, single-period study describes the human absorption, distribution, metabolism, excretion, and pharmacokinetics of velsecorat (AZD7594). Healthy subjects received inhaled velsecorat (non-radiolabeled; 720 μg) followed by intravenous infusion of carbon 14 (^{14}C)-velsecorat (30 μg). Plasma, urine, and feces were collected up to 168 hours post-dose. Objectives included identification and quantification of velsecorat and its metabolites (i.e., drug-related material) in plasma and excreta, and determining the elimination pathways of velsecorat by measuring the rate and route of excretion, plasma half-life ($t_{1/2}$), clearance, volume of distribution and mean recovery of radioactivity. On average, 76.0% of administered ^{14}C dose was recovered by the end of the sampling period (urine = 24.4%; feces = 51.6%), with no unchanged compound recovered in excreta, suggesting that biliary excretion is the main elimination route. Compared with intravenous ^{14}C-velsecorat, inhaled velsecorat had a longer $t_{1/2}$ (27 versus 2 hours), confirming that plasma elimination is absorption-rate-limited from the lungs. Following intravenous administration, $t_{1/2}$ of ^{14}C-drug-related material was longer than for unchanged velsecorat, and 20% of the ^{14}C plasma content was related to unchanged velsecorat. The geometric mean plasma clearance of velsecorat was high (70.7 l/h) and the geometric mean volume of distribution at steady state was 113 l. Velsecorat was substantially metabolized via O-dealkylation of the indazole ether followed by sulfate conjugation, forming the M1 metabolite, the major metabolite in plasma. There were 15 minor metabolites. Velsecorat was well tolerated, and these results support the progression of velsecorat to phase 3 studies.

SIGNIFICANCE STATEMENT

This study describes the human pharmacokinetics and metabolism of velsecorat, a selective glucocorticoid receptor modulator, evaluated via co-administration of a radiolabeled intravenous microtracer dose and a non-radiolabeled inhaled dose. This study provides a comprehensive assessment of the disposition of velsecorat in humans. It also highlights a number of complexities associated with determining human absorption, distribution, metabolism, and excretion for velsecorat, related to the inhaled route, the high metabolic clearance, sequential metabolite formation and the low intravenous dose.

Introduction

Velsecorat, formerly known as AZD7594 (3-(5-((1R,2S)-2-(2,2-difluoropropanamido)-1-(2,3-dihydrobenzo-[b][1,4]dioxin-6-yl)propoxy)-1H-indazol-1-yl)-N-((R)-tetrahydrofuran-3-yl) benzamide), is a novel inhaled, potent non-steroidal glucocorticoid receptor modulator in development for the treatment of asthma (Hemmerling et al., 2017; Brown et al., 2019; Prothon et al., 2019).

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ABBREVIATIONS: AMS, accelerator mass spectrometry; $AUC_{0\text{-}12h}$, area under the curve from 0 to 12 hours; $AUC_{0\text{-}t}$, area under the curve from 0 hours to last measurable concentration; ^{12}C, carbon-12; ^{14}C, carbon-14; C_{max}, maximum plasma concentration; DRM, drug-related material; ESI, electrospray ionization; ^{3}H, hydrogen-3 (tritium); hADME, human absorption, distribution, metabolism and excretion; ICH, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use; kBq, kilobecquerel; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLoQ, lower limit of quantification; PK, pharmacokinetics; pmol velsecorat Eq/l, picomoles of velsecorat equivalents (radioactivity) per l; $t_{1/2}$, terminal phase half-life; t_{max}, the time from dosing at which the C_{max} was apparent; t_x, time at point x; UPLC, ultra-performance liquid chromatography; V_{ss}, volume of distribution at steady state.

The characterization of human absorption, distribution, metabolism and excretion (hADME) and pharmacokinetics (PK) plays an important role during drug development and the approval process (Coppola et al., 2019). Data from hADME studies are critical in the understanding of rates and routes of excretion, metabolic pathways, and the identification, quantification, and exposure to metabolites, as well as in designing appropriate clinical drug–drug interaction studies (Coppola et al., 2019). These data can also be used to evaluate the presence of metabolites in humans that would also need to be confirmed present in animals used in preclinical safety testing (Coppola et al., 2019). hADME studies are most commonly performed using drug labeled with a radioactive isotope, most often hydrogen-3 (tritium; ^{3}H) or carbon-14 (^{14}C), and by the intended clinical route. After administration, the recovery of radioactivity is determined in excreta, and the metabolites and quantitative radio-chromatographic metabolite profiles are recorded in plasma and excreta (Coppola et al., 2019). However, for compounds intended to be administered via inhalation, there are both technical and ethical complications associated with administering a radioisotope (Beaumont et al., 2014). These complications include difficulties in manufacturing the radiolabeled material particle size to match that of the non-radiolabeled material and how to accurately quantify the inhaled and exhaled doses. This can be challenging due to the complexity of quantifying the remaining radioactivity in the inhalation device and potential contamination of radioactivity in the administration room. For example, administering radiolabeled drugs (^{3}H-mometasome furoate) via dry powder inhaler has previously resulted in highly variable mean total cumulative recovered radioactivity (range 63–99% after 168 hours) (Affrime et al., 2000). In addition, the long-term toxicological effect of inhaled ^{14}C isotopes in the lungs is not known. Therefore, hADME studies for inhaled compounds often use administration of oral and/or intravenous (IV) dosing as a surrogate.

The use of highly sensitive accelerator mass spectrometry (AMS) in hADME studies allows the administration of much lower quantities of ^{14}C (0.1–1 μCi [3.7–37 kBq]) compared with radioactivity measurements following standard hADME study designs (typical doses of 20–100 μCi [740–3700 kBq]) (Spracklin et al., 2020). High sensitivity is achieved because AMS measures ^{14}C by MS and not radioactivity (Lozac'h et al., 2018).

In this study, we used a microtracer design, which involves an intravenous ^{14}C-velsecorat dose administered concomitantly with a therapeutically relevant non-radiolabeled inhaled velsecorat dose using a commercial formulation and device (Ambery et al., 2018; Harrell et al., 2019). Intravenous ^{14}C-velsecorat acts as a surrogate for the inhaled velsecorat reaching the systemic circulation through the lung.

The objectives of this study were to determine the rates and routes of velsecorat excretion, evaluate the completeness of dose recovery in excreta, and identify and quantify velsecorat and its metabolites (i.e., drug-related material; DRM) in plasma, urine, and feces.

Materials and Methods

Velsecorat for inhalation was supplied by AstraZeneca (Mölndal, Sweden). Selcia, Ltd. (Essex, UK) provided the ^{14}C-velsecorat, which was diluted with unlabeled velsecorat by AstraZeneca to get the correct specific radioactivity, as part of the drug substance preparation, and manufactured into a solution for IV infusion by Quotient Sciences (Nottingham, UK). All other solvents and reagents were of analytical grade and were acquired from commercial suppliers.

Study Design

This was an open-label, single-center, single-period study in healthy subjects conducted at Quotient Sciences between June and July 2019 (NCT04002427). The study adhered to the Declaration of Helsinki and was approved by the Wales Research Ethics Committee 2, Cardiff; each subject gave their written informed consent prior to any study procedures.

Following a screening visit, in a single treatment period, subjects were administered non-radiolabeled velsecorat as an inhalation powder in a dry powder inhaler (SD3FL; AstraZeneca, Mölndal, Sweden) at a nominal dose of 792 µg (delivered dose 720 µg) (**Fig. 1**). The SD3FL inhaler is a multi-dose device-metered inhaler similar in form and function to the Genuair inhaler marketed in Europe as Eklira Genuair. Fifteen minutes after the inhaled dose, each subject received 30 μg of ^{14}C-velsecorat (containing approximately 180 nanocurie [6.7 kBq] ^{14}C) via intravenous infusion over 1 hour. ^{14}C-velsecorat was dissolved at 5 µg/ml so that 30 μg was administered in a volume of 6 ml. The specific radioactivity of ^{14}C in this formulation was 133 Bq/nmol.

During the study, each subject was exposed to an effective radioactive dose of 0.01 millisieverts, well within the safety limits defined by the World Health Organization category 1 and the International Commission on Radiologic Protection category 1 (<0.1 millisievert; minor risk) (International Commission on Radiologic Protection, 1991). The dose of radioactivity was classed as a microtracer dose, as it contains ≤37 kBq (1 μCi) of ^{14}C.

Study Population

Healthy men and non-pregnant, non-lactating women aged 18–55 years, who were non-smokers, with a body mass index of 18.5–35.0 kg/m^2 and without clinically relevant disease were eligible for the study. Participants were confined to the study center for the duration of the study. A full list of inclusion and exclusion criteria is provided in Supplemental Table 1.

Sample Collection and Processing

Blood samples were collected pre-dose and up to 168 hours post-dose (Supplemental Fig. 1). Analysis of plasma total ^{14}C content, plasma ^{14}C-velsecorat, quantitative profiles of metabolites and metabolite characterization was performed by TNO (Leiden, The Netherlands). PK analysis of non-radiolabeled velsecorat was performed by Covance Laboratories (Harrogate, UK).

Urine samples were quantitatively collected at 0–6, 6–12, and 12–24 hours post-dose on the first day and then in 24-hour collection intervals up to 168 hours (morning of day 8). Feces was quantitatively collected at 24-hour collection intervals from the dose administration (0 hours) up to 168 hours (morning of day 8) and homogenized by progesterone receptor A (Assen, The Netherlands). Fecal samples per subject per 24-hour interval were diluted in 1% carboxymethyl cellulose solution (1% carboxymethyl cellulose solution: feces, 4:1) and homogenized.

Mass Balance and Excretion. To determine total radioactivity excreted in urine and feces, for each 15-µl sample of urine or 30 mg of fecal homogenates (diluted or undiluted) were dried and placed in the elemental analyzer, which acted as an autosampler and combustion device for the AMS. Urine samples were analyzed once, and fecal homogenates were analyzed in triplicate. The lower limit of quantification (LLoQ) was 3.08 picomoles of velsecorat equivalents (radioactivity) per l (pmol of velsecorat Eq/l) for urine measurements and 19.8 pmol of velsecorat Eq/l in fecal samples.

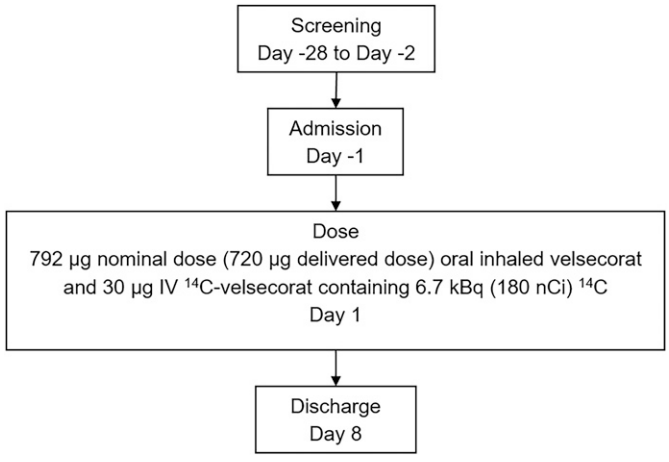

Fig. 1. Study design.

Pharmacokinetic Assessments. The level of non-radiolabeled velsecorat in plasma was determined using a validated analytical method based on protein precipitation, followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) by Covance Laboratories (Prothon et al., 2019). The LLoQ of velsecorat in plasma was 10 pmol/L. As the ^{14}C-velsecorat IV dose contained both ^{12}C-velsecorat (unlabeled velsecorat) and ^{14}C-velsecorat, the unlabeled velsecorat concentrations measured by LC-MS/MS were corrected prior to PK parameter estimation in SAS (v9.4) for the reporting of bioanalytical data to obtain unlabeled velsecorat pertaining to the inhaled dose only as follows:

Concentration of inhaled velsecorat at time x (t_x) = ^{12}C-velsecorat (inhaled and intravenous doses) as measured by LC-MS/MS at t_x – (^{12}C-velsecorat [IV dose at t_x]), where ^{12}C-velsecorat in the ^{14}C-labeled intravenous dose was calculated as follows at each time point:

Proportion of ^{12}C-velsecorat (intravenous dose) × ^{14}C-velsecorat at t_x, where proportion of ^{12}C-velsecorat (intravenous dose) = (1 – [specific activity of IV ^{14}C-velsecorat/maximum possible ^{14}C-velsecorat specific activity]).

Velsecorat plasma concentration refers to the inhaled dose only, and ^{14}C-velsecorat refers to the total concentration of velsecorat pertaining to the intravenous dose, i.e., the sum of the unlabeled velsecorat and ^{14}C-velsecorat as given by the composition of the formulation.

Plasma ^{14}C-velsecorat was determined using protein precipitation followed by ultra-performance liquid chromatography (UPLC) analysis with gradient elution. The compound peak was collected and subjected to AMS analysis. The dynamic range of the method qualified prior to the sample analysis was 1.00–128 megabecquerels/m (7.50–961 pmol velsecorat/l). Assay performance was assessed by measuring quality control samples on three individual occasions, and the carryover was determined to be 0.718%. A dilution factor of 10-fold was qualified in one series. The LLoQ for ^{14}C-velsecorat in plasma was 7.50 pmol of velsecorat/l upon extraction of 150 µl of plasma. Plasma total radioactivity was directly analyzed using the sensitive AMS method, which had an LLoQ of 4.88 picomoles of velsecorat equivalents (radioactivity) per l (pmol velsecorat Eq/l).

UPLC. Analytical UPLC-photodiode array was performed on an Acquity H Class Plus system (Waters Corp., Milford, Massachusetts, USA) using a BEH C18 reserved-phase column (2.1 × 150 mm, 1.7 µm) and the corresponding guard column, UV detection at 318 nm and linear gradient (15–50% over 22.54 minute) of acetonitrile into MilliQ water (0.1% formic acid), with a flow rate of 0.35 ml/min.

Quantification and Characterization of Metabolites. A time proportional plasma pool was prepared from each individual (representing the area under the plasma concentration time curve from 0–12 hours [$AUC_{0\text{-}12h}$]) by combining volumes in proportion to the time interval between individual samples as previously described (Hamilton et al., 1981). One single-plasma $AUC_{0\text{-}12h}$ pool was prepared by mixing equal volumes of each subject's pool, representing approximately 50% of the total AUC of total radioactivity. A 24-hour plasma pool was also prepared by mixing equal volumes from each of the individual 24-hour samples to evaluate any additional metabolites appearing in plasma at a later timepoint.

Individual pooled urine samples were prepared for each subject up to 72 hours, corresponding to the time needed to recover 95% of radioactivity via this route. The samples were prepared using equal percentage per timepoint based on volume. A single urine pool was then prepared by mixing equal volumes of each individual pool. This pool contained DRM corresponding to 23% of the dose administered, which is approximately 95% of the total DRM excreted in urine.

For feces, individual pooled fecal homogenate samples were prepared for each individual up to 96 hours, corresponding to the time needed to recover 94% of radioactivity via this route. The samples were prepared using equal percentages per timepoint based on total weight of fecal homogenates. A single fecal homogenate pool was then prepared by mixing equal weights of each individual pool. The combined fecal pool contained 49% of the administered dose, which is approximately 94% of the total DRM excreted in the feces.

Investigation of velsecorat metabolism in the lungs was not performed; previous in vitro studies using lung microsomes did not indicate metabolism of velsecorat (unpublished data).

Metabolites were identified and quantified in plasma, urine, and feces using an Acquity H class Plus UPLC system (Waters Corp., Milford, Massachusetts, USA) coupled to a high-resolution MS (Q Exactive, ThermoFisher Scientific, Massachusetts, USA) and AMS (4110Bo-AMS, High Voltage Engineering Europa B.V., Amersfoort, The Netherlands), respectively. Prior to analysis, the Q-Exactive system was calibrated in electrospray ionization (ESI)-positive and -negative mode using the corresponding calibration mixtures recommended by the supplier (ThermoFisher Scientific) with acceptance criteria of 3 ppm. Each pool sample was injected three times; one to run in ESI-positive mode, the second in ESI-negative mode, and for the third, the flow was fully collected (split flow off, no fractionation) in one container to enable column recovery calculations by means of total radioactive analysis by AMS. The pre-dose pools were injected twice without fractionation. One was run in ESI-positive mode and the other in ESI-negative mode as background references. Plasma (700 µl) and fecal homogenates (200 mg) were extracted by protein precipitation, and urine pools were injected directly without further processing. Post-column, the eluent was split to generate online high-resolution MS and off-line AMS analyses.

Proposals of metabolite structures were based on data acquired from high-resolution MS and MS/MS analyses. Molecular weights of each metabolite were proposed based on the protonated adduct in the positive ionization MS mode and the deprotonated molecule in the negative ionization mode. High-resolution MS data were used to propose the molecular composition. The retention times for each metabolite compared between sample matrices agreed well both for LCMS and LC-AMS profiling. Velsecorat was identified by comparison of retention time and high resolution MS and MS/MS spectra with the synthesized standard. Tentative structures of metabolites were proposed based on their product ion spectra and a detailed analysis of fragmentation pathways of velsecorat in the positive ionization mode.

Safety. Safety and tolerability of velsecorat were assessed by physical examination and measurement of vital signs, 12-lead ECGs, and routine laboratory tests (including urinalysis) pre-dose, over the first 24 hours post-dose and prior to discharge. Adverse events were monitored throughout the study.

Statistical Analysis. A sample size of 6 was chosen to investigate the primary objective of the study (Penner et al., 2009). One subject was excluded from mass balance and metabolite analysis due to incomplete urine sampling; however, a sample size of 5 was considered sufficient to fulfill the objectives of the study. Plasma PK of inhaled velsecorat, ^{14}C-velsecorat and total radioactivity in plasma were analyzed at Quotient Sciences by non-compartmental analysis methods in Phoenix WinNonlin V8.0 (Certara; New Jersey, USA). The partial $AUC_{0\text{-}12h}$ for ^{14}C-velsecorat and total radioactivity was calculated post hoc.

Results

Demographic and Baseline Characteristics

Eleven volunteers were screened, with six (four women and two men) included; no discontinuation was reported, although one subject was excluded from mass balance and metabolite analysis due to incomplete urine sampling. The mean (standard deviation; S.D.) age was 37 (10) years with a mean (S.D.) body mass index of 29 (1.8) kg/m^2.

Mass Balance and Excretion. The mean recovery of total radioactivity over time is shown in **Fig. 2**. An average of 76.0% (range 72.1–78.8%) of the radioactivity administered was recovered by the end of the sampling period (168 hours), most of which was recovered within 72 hours. Approximately 24.4% was recovered in the urine and 51.6% in feces. Within the first 24 hours, approximately 14.0% and 6.9% of the total radioactivity was recovered in the urine and feces, respectively.

Pharmacokinetic Results. Following inhaled administration, plasma concentrations of velsecorat were observed in all subjects at the first post-inhalation dose sampling timepoint of 0.5 hours. The geometric mean maximum plasma concentration (C_{max}) was 509 pmol/l and the median time from dosing at which the C_{max} was apparent (t_{max}) occurred at 0.88 (range 0.50–1.32) hours post-dose. There was a steep decline in velsecorat concentrations immediately after C_{max}, followed by a slower decline thereafter. The geometric mean terminal phase half-life ($t_{1/2}$) was 27 hours (Fig. 3A; Table 1). Small fluctuations (secondary peaking) in plasma concentrations were observed over the first 48 hours post-dose. The geometric mean absolute bioavailability following inhalation of velsecorat was 66% (coefficient of variation 22.0%) based on AUC from time zero extrapolated to infinite time ($AUC_{0\text{-}\infty}$; Table 1).

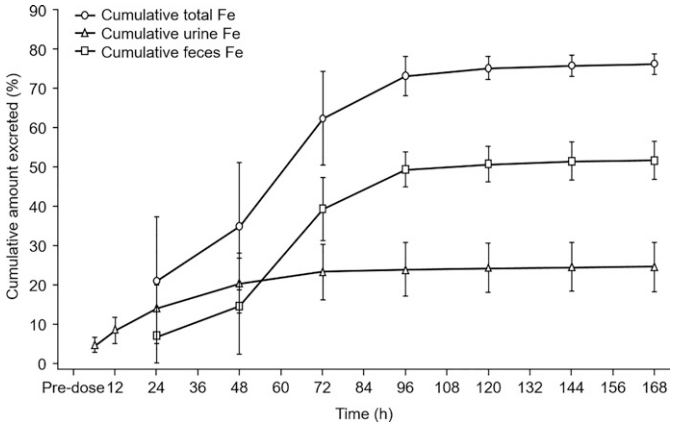

Fig. 2. Cumulative arithmetic mean (±S.D.) of radioactivity recovery over time following intravenous administration of carbon 14-velsecorat. N = 5.

Following the start of the 1-hour intravenous infusion, plasma concentrations of ^{14}C-velsecorat and total radioactivity were observed in all subjects at the first sampling timepoint after the start of the intravenous infusion (Supplemental Fig. 1). Median t_{max} occurred at 1.00 (range 0.75–1.17) hour, i.e., at the end of the intravenous infusion, for ^{14}C-velsecorat and 1.00 (range 0.75–1.05) hour for total radioactivity (total ^{14}C). There was a rapid biphasic decline in ^{14}C-velsecorat concentrations, and concentrations were measurable up to 8–12 hours, with a mean terminal $t_{1/2}$ of 2 hours (Fig. 3A; Table 1). For total ^{14}C, a slower triphasic decline was observed, with quantifiable concentrations lasting 48–98 hours in most subjects (168 hours in one subject) post-dose, and a mean terminal $t_{1/2}$ of 18 hours (Fig. 3B; Table 1). The geometric mean C_{max} of ^{14}C-velsecorat and total radioactivity was 543 pmol/L and 565 pmol velsecorat Eq/L, respectively, showing that at this early timepoint all DRM was related to unchanged velsecorat.

The geometric mean clearance of ^{14}C-velsecorat from plasma was 70.7 l/h, and the geometric mean volume of distribution at steady state (V_{ss}) was 113 l.

Based on the AUC ratio from time zero to the last quantifiable ^{14}C-velsecorat concentration and total ^{14}C concentration (AUC_{0-t}), the ^{14}C-velsecorat accounted for 19.0% of circulating plasma total ^{14}C (i.e., ^{14}C-DRM). Approximately 50% of the total ^{14}C AUC was recovered within 0–12 hours.

Metabolism. Analysis of a plasma pool covering the first 12 hours post-dose revealed that most DRM was related to unchanged velsecorat (40%) and metabolite M1 (22%), with remaining radioactivity distributed over approximately 15 minor metabolites (Fig. 4A; Table 2). Metabolite M1 was identified as a metabolite formed by O-dealkylation of the indazole ether (M2), followed by sulfate conjugation (Fig. 5); we did not investigate which sulfotransferase mediated the formation of the M1 metabolite as part of this study. At 24 hours, the major component in plasma was metabolite M1, accounting for 68% of the radioactivity, with only minor amounts of unchanged velsecorat (5%) (Fig. 4B; Table 2).

In the urine pool, the major part of the detected ^{14}C was attributed to metabolite M1 (85% by metabolite peak integration of the chromatogram), representing 20% of the administered dose. The second most abundant metabolite was M22, identified as the glucuronide conjugate of M2, representing 2% of the administered dose (Table 3). No unchanged velsecorat was detected in the urine pool. The reconstructed AMS chromatogram of the urine pool is shown in Fig. 6A.

The metabolite profile of the fecal homogenates was more complex, including partially resolved or co-eluting metabolite fractions. The main metabolite in feces was metabolite M2, accounting for 30% of the total

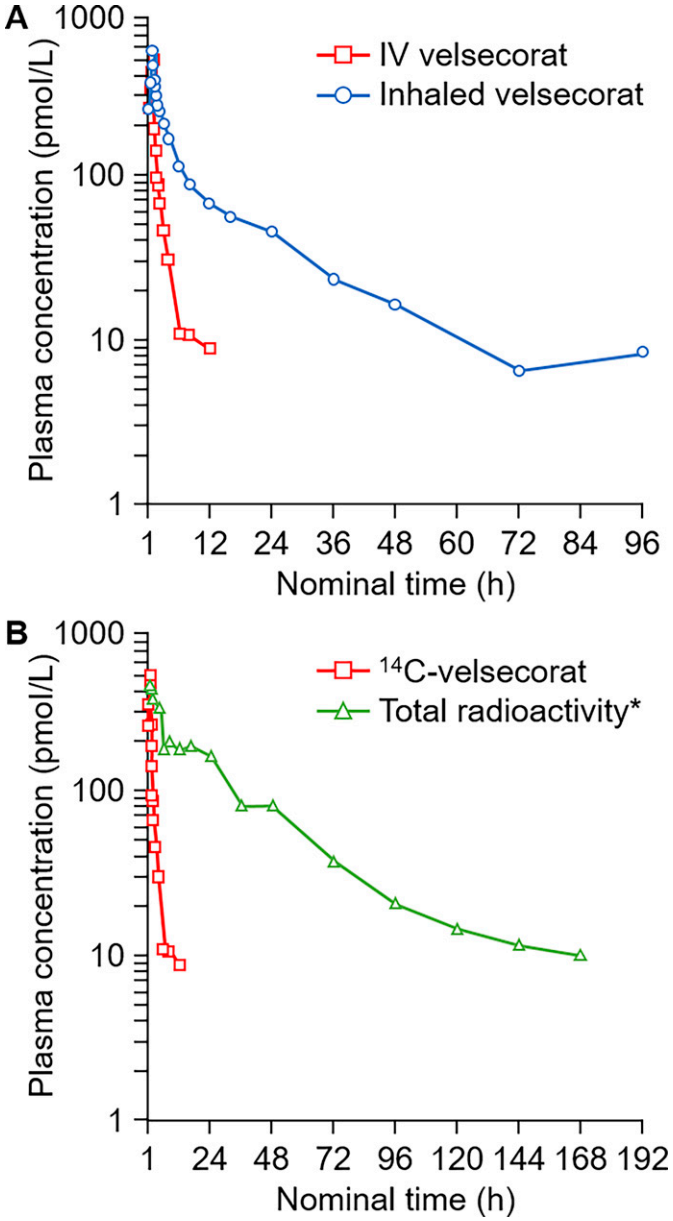

Fig. 3. Geometric mean plasma concentrations profiles following inhalation of 720 µg velsecorat and intravenous infusion of 30 µg carbon 14 (^{14}C-velsecorat) in healthy subjects: A) unchanged velsecorat pertaining to intravenous dose and inhaled dose; B) total radioactivity (^{14}C; unchanged velsecorat and metabolites [drug-related material]; pmol velsecorat Eq/l) and ^{14}C-velsecorat following intravenous administration. *Unit is pmol velsecorat Eq/l.

^{14}C, and representing 15% of the administered dose. Up to 13 additional metabolites were identified in the 5 major metabolite fractions of the profile, altogether representing approximately 56% of the total ^{14}C in the chromatogram (Table 3). The reconstructed AMS chromatogram of the feces pool is shown in Fig. 6B.

Nearly all metabolites with assigned proposed structures were formed via one of three major routes: O-dealkylation of the indazole ether (M2); ring opening of the 1,4-dioxane ring (M46); and ring opening of the oxolane ring (M47). A proposed metabolic scheme for velsecorat is shown in Fig. 5.

Safety

No deaths or serious adverse events were reported during the study, and no subjects withdrew from the study or permanently discontinued

TABLE 1

Summary of PK parameters following 720 μg inhaled velsecorat and 30 μg of intravenous carbon 14-velsecorat

Parameter	Inhaled Velsecorat (720 μg)N = 6	^{14}C-Velsecorat (30 μg)N = 6	Total RadioactivityN = 6
t_{max}, hours[†], median (range)	0.88 (0.50–1.32)	1.00 (0.75–1.17)	1.00 (0.75–1.05)
C_{max}, pmol/l	509 (34.1)	543 (52.9)	565 (45.2)[‡]
AUC_{0-t}, pmol.hours/l	10,600 (40.7)	671 (31.6)	3,380 (15.0)
AUC, pmol.hours/l	11,100 (38.3)	700 (30.9)	3,630 (13.0)
AUC_{0-12h}*, pmol.hours/l	NC	681	1880
$t_{1/2}$, hours	26.9 (26.7)	1.99 (20.2)	18.4 (14.6)
CL/F | CL, l/hour	107 (38.3)	70.7 (30.7)	13.6 (13.0)
V_z/F | V_z, l	4,140 (52.6)	203 (23.2)	362 (20.5)
V_{ss}, L	—	113 (30.5)	276 (27.9)
MRT AUC_{0-t}, hours	—	1.20 (17.2)	14.9 (23.2)
MRT AUC, hours	—	1.59 (18.7)	20.3 (21.1)
F AUC_{0-t}, %	65.90 (22.7)	—	—
F AUC, %	66.31 (21.6)	—	—

All values are geometric mean (CV%) unless otherwise specified.
AUC, area under the curve; AUC_{0-t}, the area under the concentration-time curve from dosing to the last measurable concentration; CL, total body clearance of drug from plasma (intravenous dose only); CL/F, apparent total body clearance of drug from plasma (inhaled dose only); C_{max}, the maximum observed plasma concentration; CV%, percentage of coefficient of variation; F, absolute bioavailability; MRT, mean residence time of the unchanged drug in the systemic circulation (IV dose only); NC, not calculated; PK, pharmacokinetics; $t_{1/2}$, terminal phase half-life; t_{max}, the time from dosing at which the C_{max} was apparent; V_{ss}, volume of distribution at steady state (intravenous dose only); V_z, volume of distribution (intravenous dose only); V_z/F, apparent volume of distribution (inhaled dose only)
*The values were calculated post hoc.
[†]Time is recorded from the start of the 1 h intravenous infusion
[‡]Units expressed as pmol velsecorat Eq/l

study treatment due to an adverse event. Two mild adverse events, assessed as unrelated to treatment, were reported during the study: one dysmenorrhea and one vessel puncture site pain. There were no clinically significant changes in clinical laboratory tests, vital signs, ECGs, physical examinations, or urinalysis.

Discussion

In this study investigating the metabolism and disposition of velsecorat in healthy adults, most of the administered ^{14}C-dose was recovered in urine and feces within 72 hours. The geometric mean clearance of velsecorat was high, and velsecorat was substantially metabolized, with all DRM excreted as metabolites after IV administration. Velsecorat was well tolerated, with two mild adverse events reported, both assessed as unrelated to treatment.

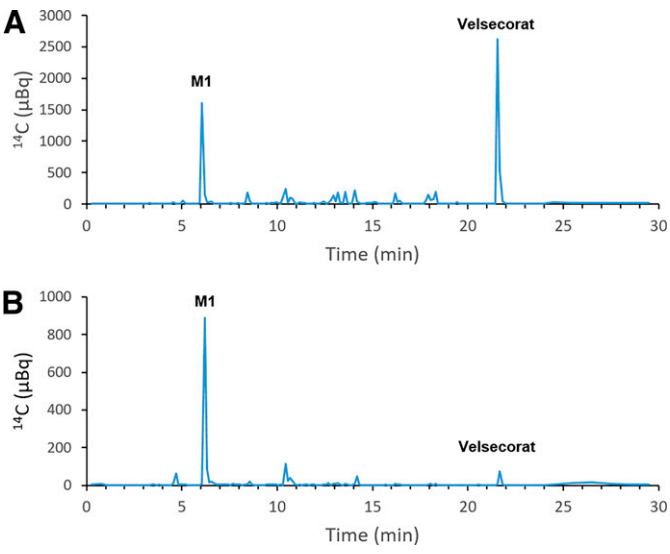

Fig. 4. Reconstructed accelerator mass spectrometry chromatogram of pooled human plasma extracts following intravenous administration of carbon 14-velsecorat: A) area under the curve from 0 to 12 hours pool; B) 24-hour pool. N = 5.

An average of 76% of the intravenously administered ^{14}C-dose was recovered, slightly below the target recovery of >80% (Roffey et al., 2007; Coppola et al., 2019). The average excretion of the administered dose was <1% in the penultimate and last 24-hour collection intervals (120–144 and 144–168 hours, respectively). This indicates that using <1% DRM recovery per 24-hour collection interval as a release criterion would not have changed the result, and achieving an 80% recovery would require extending the collection time for several more days. Low recovery could be due to inaccuracy in dose preparation and/or delivery or loss of the radiolabel on the molecule in expired air or to endogenous metabolic routes or tissue binding. In a velsecorat preclinical rat ADME study, there was no indication of radioactivity in expired air in the first 24 hours post-dose, nor in the carcass at study termination. Mass balance recovery was 80–90% following sampling for 168 hours. Furthermore, recovery is generally low when the circulating half-life of total radioactivity is >50 hours and the majority of DRM is recovered in feces (Roffey et al., 2007), which is consistent with our finding that 52% of the administered ^{14}C dose was recovered in feces. In the present study, 24% of the dose was recovered in urine as DRM 168 hours post-administration, with no unchanged velsecorat detected, which is consistent with previous findings that urinary excretion of unchanged velsecorat was negligible (Prothon et al., 2019).

The longer estimated plasma terminal $t_{1/2}$ of inhaled velsecorat (27 hours) than that of ^{14}C-velsecorat following the IV dose (2 hours) confirms that the elimination of velsecorat is absorption-rate-limited from the lungs, which is consistent with previous data (Chen et al., 2017). This slow absorption, governed by a slow dissolution rate due to low solubility, results in a long pulmonary residence time and supports once-daily administration.

TABLE 2

Quantification of the major components in plasma extracts following intravenous administration of carbon 14-velsecorat

	AUC_{0-12h} Pool	24-hour Pool
	% Sample Radioactivity	
Velsecorat	39.7	5.3
M1	22.1	67.6

AUC, area under the curve; IV, intravenous.
N = 5

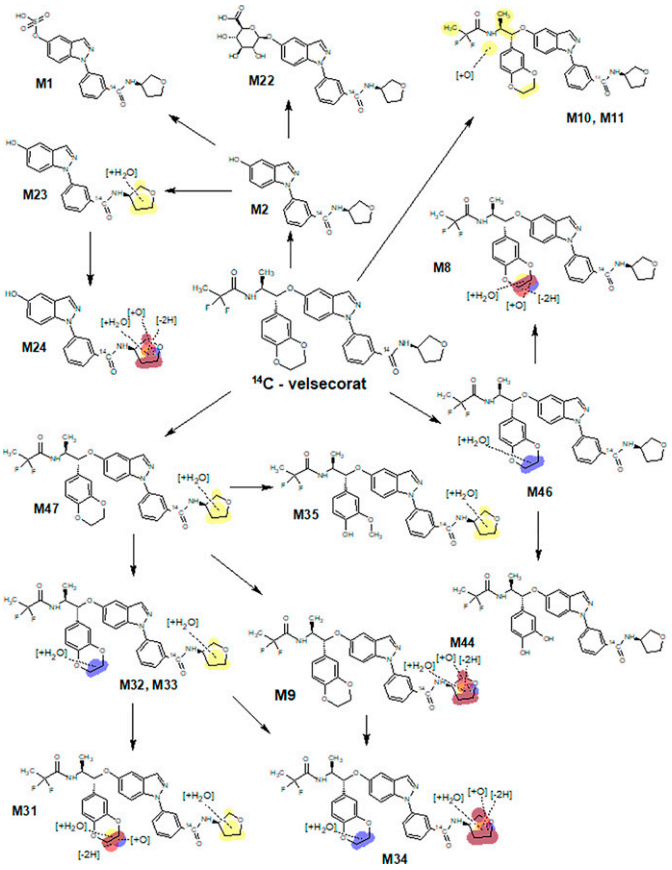

Fig. 5. Proposed metabolic scheme for velsecorat in humans. Potential sites of metabolism, identified by tandem mass spectrometry fragmentation analysis, are highlighted in the metabolite structures by colored Markush.

Following IV administration, the geometric mean V_{ss} was higher than total body water, indicating substantial distribution of velsecorat into tissue. The geometric mean clearance of velsecorat from plasma was high and consistent with a high extraction ratio. Exposure to ^{14}C-velsecorat accounted for approximately 20% of circulating DRM based on $AUC_{0\text{-}t}$ ratio. However, the percentage of unchanged velsecorat may be a slight underestimation in relation to DRM. ^{14}C-velsecorat was generally quantifiable up to 8–12 hours post-dose following the intravenous dose, resulting in a large proportion of the plasma curve under LLoQ, as shown by the 24-hour pool, where 5% of the DRM was attributed to unchanged velsecorat. This may also have resulted in an overestimation of the absolute bioavailability (66%), which was substantially higher than that reported in a previous velsecorat clinical study (46%) (Sadiq et al., 2017). The oral bioavailability of inhaled velsecorat was previously reported as negligible (<1% at 1200 μg); thus, absolute bioavailability for velsecorat following the inhaled dose represents the fraction of the dose absorbed via the lung in this study (Prothon et al., 2019).

To quantify and characterize the metabolites in plasma, two pools were prepared from $AUC_{0\text{-}12h}$, representing approximately 50% of radioactivity and a single timepoint 24-hour sample. This was because the radioactivity spread over time meant that producing an AUC pool representing the recommended 80% of the total AUC of DRM (Penner et al., 2009; Coppola et al., 2019) would result in extensive dilution, limiting detection and quantification. Instead, the 24-hour pool was prepared and analyzed to evaluate any additional metabolites appearing in plasma at a later timepoint. Analysis of the plasma metabolite profile in the AUC pool of the first 12 hours post-dose revealed that ∼40% of ^{14}C content was related to unchanged velsecorat, and that M1 was the major metabolite, corresponding to >22% of the radioactivity. This supports our results showing that ^{14}C-velsecorat comprised approximately 20% of circulating DRM. The remaining radioactivity was spread over ≥15 metabolites of ∼3% abundance each. In the 24-hour pool, the majority of DRM was attributed to M1 (68%). These findings suggest that the total contribution of metabolite M1 to the total AUC of DRM is >10%, i.e., above the threshold where additional animal studies might be warranted, according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation_en.pdf; https://database.ich.org/sites/default/files/M3_R2_Q%26As_R2_Q%26As_0.pdf). However, the ICH guidelines acknowledge that for a daily administered dose <10

TABLE 3

Quantification and identification of components in human urine and feces following intravenous administration of carbon 14-velsecorat

		% Matrix Content (% of dose)	
Compound	Structure Modification	Urine	Feces
Velsecorat	Unchanged compound		
M2	O-dealkylation of the indazole ether		30 (14.7)
M1	Sulfate conjugate of M2	84.9 (19.6)	4.4 (2.2)
M22	Glucuronide conjugate of M2	8.1 (1.9)	
M44 a	Ring deethylation of 1,4-benzodioxane		25.4 (12.5)
M46 a	Ring opening of 1,4-benzodioxane (diol)		
M8 a	M46 + oxidation of 1 alcohol to acid		
M23 a	M2 + hydrolysis of oxolane ring		12.5 (6.2)
M24 a	M23 + oxidation of 1 alcohol to acid		
M32 a or M33 a	Hydrolysis of both the oxolane and 1,4-benzodioxane ring		11.8 (5.8)
M31 a	M32 + oxidation of 1 alcohol to acid in the opened 1,4-benzodioxane ring		
M34 a	M32 + oxidation of 1 alcohol to acid in the hydrolyzed oxolane ring		
M47 a	Hydrolysis of the oxolane ring		3.2 (1.6)
M9 a	M47 + oxidation of 1 alcohol to acid		
M10 a	Monooxygenation of velsecorat		
M11 a	Monooxygenation of velsecorat		
M35 a	M47 + decarbonylation of the 1,4-benzodioxane ring		2.8 (1.4)

Co-eluting peaks, percentages are presented as sum.
IV, intravenous
N = 5

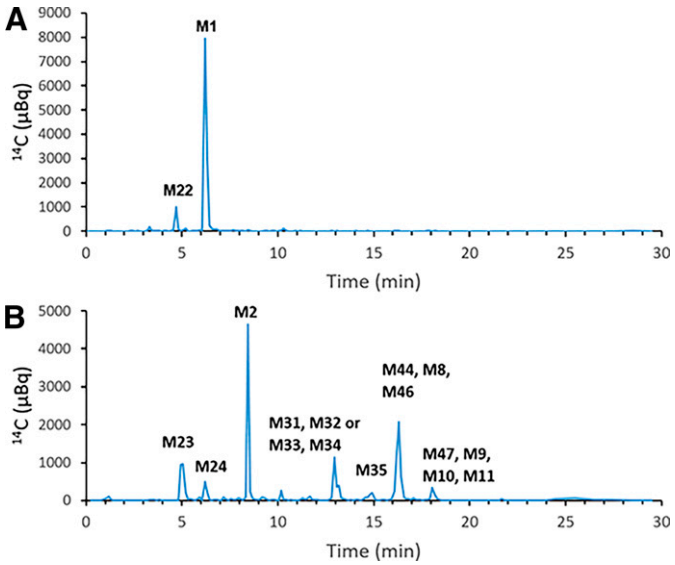

Fig. 6. Reconstructed accelerator mass spectrometry chromatogram of A) pooled human urine (0–72 hours) and B) pooled human fecal homogenate extracts (0–96 hours) following intravenous administration of carbon 14-velsecorat. N = 5.

mg, fractions of DRM >10% might be considered appropriate and that a conjugated metabolite, such as M1, is of less regulatory concern (https://database.ich.org/sites/default/files/M3_R2_Q%26As_R2_Q%26As_0.pdf).

Nearly all metabolites with assigned structure proposals involved at least one of the following three sites of metabolism: the indazole ether, the 1,4-dioxane ring and the oxolane ring. Both rings were prone to mono-oxygenation of an α-carbon, followed by ring opening forming an aldehyde and reduction to an alcohol (M46 and M47). Alternatively, the aldehyde was oxidized to carboxylic acid (M8, M9, M24, M31, and M34). The vast majority of velsecorat metabolism involved O-dealkylation of the indazole ether (M2). Metabolites formed via O-dealkylation of the indazole ether, i.e., M1, M2, M22, M23, and M24, accounted for >40% of the given dose recovered in excreta, indicating that the metabolic pathway resulting in metabolite M2 has a significant contribution to clearance. Attempts to characterize metabolites formed from the left-hand-side of velsecorat after oxidative dealkylation to form M1 were unsuccessful, possibly since the given dose was low and the left side of the molecule was unlabeled. Furthermore, the left-hand-side metabolites after cleavage might be devoid of MS response, or this fragment was extensively metabolized to minor metabolites appearing below the detection limit. Further characterization of left-hand-side metabolites of velsecorat are planned.

In this study, the IV dose (^{14}C-velsecorat) was used concomitantly with an inhaled therapeutically relevant dose as a surrogate for the inhaled dose, as in previous studies (Ambery et al., 2018; Harrell et al., 2019). Due to the negligible oral bioavailability of velsecorat (Chen et al., 2017; Prothon et al., 2019), it was expected that IV dosing would result in a similar metabolite profile as post-inhalation. Furthermore, following oral dosing of ^{14}C-velsecorat in rats, very low tissue distribution of radioactivity (i.e., DRM) has been observed, as well as lower urinary excretion of radioactivity versus IV administration, indicating low absorption of DRM from the gut (unpublished data). Based on in vitro studies, CYP3A4 is the major metabolizing enzyme of velsecorat and also the main enzyme contributing to the formation of metabolite M2, which we showed to be a major route of metabolism of velsecorat in humans (>40%) (unpublished data). Thus, as CYP3A4 is one of the major enzymes present in the gut, it is unlikely that oral dosing would have resulted in any additional metabolites compared with the IV route.

The use of a radiolabeled intravenous microtracer dose allowed the determination of the mass balance, excretion routes and levels of circulating metabolites in humans, with exposure to radiation similar to natural background levels (Young et al., 2014; Vuong et al., 2015; Lozac'h et al., 2018). Furthermore, the implementation of UPLC to achieve good chromatographic separation, followed by fraction collection and AMS analysis, resulted in high-resolution ^{14}C profiles in four human pools (two plasma, one urine, and one fecal homogenate). Accurate analysis with LC-MS/MS enabled the identification of all major and a number of minor metabolites in the three matrices.

Limitations included a chemical dose level of ^{14}C-velsecorat (30 μg) that was too low to provide structural information about components of the metabolic profile, whereas addition of the higher inhaled unlabeled dose (720 μg) facilitated the metabolite identification. However, all the quantitative information, including abundance of metabolites, was provided based on the intravenous dose.

As data from a single intravenous dose indicated a major metabolite of velsecorat in plasma, there is a potential need for further data to fully evaluate the quantitative exposure to this major metabolite in plasma at steady state following inhalation. For example, new repeat dose toxicity studies in animals could be used to assess and ensure adequate exposure in safety testing, as well as to investigate the potential activity of the major metabolite, M1, and any drug-drug interactions with CYP3A4 inhibitors.

In conclusion, velsecorat was substantially metabolized via three major routes: O-dealkylation of the indazole ether (M2), followed by sulfate conjugation (M1); ring opening of the 1,4-dioxane ring (M46); and ring opening of the oxolane ring (M47). On average, 76% of administered ^{14}C-velsecorat dose was recovered, with biliary excretion as the main elimination route and no unchanged compound recovered in excreta. Velsecorat had a high clearance, volume of distribution, and bioavailability, and confirmed absorption-rate limited elimination following inhalation. Velsecorat was well tolerated by the healthy subjects. These results support the progression of velsecorat to phase 3 studies.

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Authorship Contributions

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Conducted experiments: Pelay-Gimeno, de Ligt, Verheij.

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Wrote or contributed to the writing of the manuscript: Holmberg, Weidolf, Necander, Bold, Sidhu, Pelay-Gimeno, de Ligt, Verheij, Jauhiainen, Psallidas, Wählby Hamrén, Prothon.

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Characterization of clinical ADME and pharmacokinetics of velsecorat using an intravenous microtracer combined with an inhaled dose in healthy subjects

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Drug Metabolism and Disposition

Supplemental data

Table S1. Main inclusion and exclusion criteria for eligibility to enter the study

Inclusion criteria	Exclusion criteria
Provision of signed and dated, written informed consent prior to any study specific procedures	History of any clinically significant disease or disorder which, in the opinion of the investigator, may have either put the subject at risk because of participation in the study, or influenced the results of the subject's ability to participate in the study
Healthy male or non-pregnant, non-lactating female subjects aged 18–55 years with suitable veins for cannulation or repeated venepuncture	History or presence of clinically significant gastrointestinal, hepatic or renal disease, or any other condition known to interfere with absorption, distribution, metabolism or excretion of drugs
Body mass index of 18.5–35.0 kg/m^2, and weighing ≥50 kg and ≤100 kg, as measured at screening	Any clinically significant illness, medical/surgical procedure, or trauma within 4 weeks of the first administration of IMP
Had regular bowel movements (i.e., average stool production of ≥1 and ≤3 stools per day)	Subjects with Gilbert's syndrome or subjects with a history of cholecystectomy or gallstones
Willing and able to communicate and participate in the whole study	Subjects who had a pregnant partner
Agreed to adhere to the contraception requirements	Any confirmed clinically significant abnormalities in clinical chemistry, hematology or urinalysis as judged by the investigator
Demonstrated the ability to use the study inhalation device properly	Any confirmed clinically significant abnormal findings in vital signs or 12-lead ECG as judged by the investigator

	Any positive result at screening for serum HBsAg, HCV Ab or HIV
	Had received any other new chemical entity (defined as a compound which has not been approved for marketing) within 3 months of the first administration of IMP in this study. The period of exclusion began 3 months after the final dose. Note: subjects consented and screened, but not randomized in this study or a previous Phase I study were not excluded
	Plasma donation within 1 month of screening or any blood donation/loss of >500 mL of blood during the 3 months prior to screening
	History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the investigator or history of hypersensitivity to drugs with a similar chemical structure or class to velsecorat or the formulation excipients including lactose. Hay fever was allowed unless it was active
	Current smokers and those who had smoked within the last 12 months. A breath carbon monoxide reading of >10 ppm at screening and admission
	Current users of e-cigarettes and nicotine replacement products and those who had used these products within the last 12 months
	Females of childbearing potential who were pregnant or lactating (all female subjects must have had a negative urine pregnancy test at screening and admission). A woman was considered of childbearing potential unless she was permanently sterile (hysterectomy, bilateral salpingectomy and bilateral oophorectomy) or was post-menopausal (had no menses for 12 months without an alternative medical cause and a serum LH concentration >8 IU/L and an FSH concentration ≥40 IU/L)

	Confirmed positive screen for drugs of abuse at screening or admission to the clinical unit or positive screen for alcohol at screening or admission to the clinical unit
	Herbal preparations/medications were not allowed throughout the study. These herbal medications included, but were not limited to, St. John's wort, kava, ephedra (ma huang), gingko biloba, dehydroepiandrosterone, yohimbe, saw palmetto and ginseng. Subjects were to stop using these herbal medications 14 days prior to administration of velsecorat
	Use of any prescribed or non-prescribed medication including antacids, H2 antagonists, proton pump inhibitors, analgesics (other than paracetamol/acetaminophen up to 4 g/day, hormone replacement therapy and hormonal contraception), vitamins and minerals during the 14 days prior to administration of velsecorat or longer if the medication has a longer half-life. Exceptions may have applied on a case by case basis, if considered not to interfere with the objectives of the study, as agreed by the PI and sponsor’s medical monitor
	Known or suspected history of alcohol or drug abuse within the past 2 years or regular alcohol consumption in males >21 units per week and females >14 units per week (1 unit = ½ pint beer, or a 25 mL shot of 40% spirit, 1.5 to 2 Units = 125 mL glass of wine, depending on type)
	Subjects who were study site employees, or immediate family members of a study site or sponsor employee
	Subjects who had previously been enrolled in this study
	Radiation exposure, including that from the present study, excluding background radiation but including diagnostic x-rays and other medical exposures, exceeding 5 mSv in the last 12 months or 10 mSv in the last 5 years. No occupationally exposed worker, as defined in the Ionizing

	Radiation Regulations 2017, was permitted to participate in the study
	Subjects who had been enrolled in an absorption, distribution, metabolism or excretion study in the last 12 months
	Judgment by the investigator that the subject should not participate in the study if they had any ongoing or recent (i.e., during the screening period) minor medical complaints that may have interfered with the interpretation of the study data or were considered unlikely to comply with the study procedures, restrictions and requirements
	History of infantile bronchiolitis, a history of asthma, adverse reaction or allergy to the inhaled medication or any excipients
	Upper respiratory tract infection (excluding otitis media) within 14 days of the first study day, or lower respiratory tract infection within the last 3 months
	Subjects who are unable to demonstrate their ability to fulfil the physical demands of the study
	Failure to satisfy the investigator of fitness to participate for any other reason

ECG, electrocardiogram; FSH, follicle-stimulating hormone; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C virus antibody; HIV human immunodeficiency virus; IMP, investigational medicinal product; LH, luteinizing hormone; mSv millisievert

Figure S1. Study design: Sampling schedule for collecting blood (plasma) samples

Study day	1																		2	3	4	5		6	7	8
Time relative to start of inhaled‡ dosing (hours)	**Pre- dose**	**0**	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.38**	**1.5**	**1.75**	**2**	**2.25**	**2.5**	**3.25**	**4.25**	**6.25**	**8.25**	**12.25**	**16.25**	**24.25**	**36.25**	**48.25**	**72.25**	**96.25**	**120.25**	**144.25**	**168.25***
Time relative to start of intravenous‡ dosing (hours)	**Pre- dose**	**-0.25**	**0**	**0.25**	**0.5**	**0.75**	**1**	**1.13**	**1.25**	**1.5**	**1.75**	**2**	**2.5**	**3**	**4**	**6**	**8**	**12**	**16**	**24**	**36**	**48**	**72**	**96**	**120**	**144**	**168***
Plasma samples for ^{14}C-velsecorat and total radioactivity†	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Plasma samples for inhaled velsecorat†	X			X	X	X	X					X			X	X	X	X	X	X	X	X	X	X	X	X	X
Whole blood samples for total radioactivity	X				X		X					X			X	X	X	X		X	X	X	X	X	X	X	X
Plasma samples for metabolite profiling and identification	X				X		X					X			X	X	X	X		X	X	X	X	X	X	X	X

*Discharge

‡The inhaled velsecorat dose was administered approximately 15 minutes before the start of the 14C-velsecorat IV infusion. The IV infusion was administered over 1 h

†After 24 h, the IV and inhaled samples coincided