

Pharmacokinetics, Excretion, and Mass Balance of [¹⁴C]-Batefenterol Following a Single Microtracer Intravenous Dose (Concomitant to an Inhaled Dose) or Oral Dose of Batefenterol in Healthy Men

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Abstract

Inhaled batefenterol is an investigational bifunctional molecule for the treatment of chronic obstructive pulmonary disease. The excretion balance and pharmacokinetics of batefenterol using [¹⁴C]-radiolabeled drug administered orally and as intravenous (IV) infusion were assessed. In this 2-period, open-label study, 6 healthy male subjects received a single IV microtracer 1-hour infusion of 4 μg [¹⁴C]-batefenterol concomitant with inhaled nonradiolabeled batefenterol (1200 μg) followed by oral [¹⁴C]-batefenterol (200 μg) in period 2 after a 14-day washout. The primary end points included: the area under the concentration-time curve from time zero to last time of quantifiable concentration (AUC_{0-t}); maximum observed concentration (C_{max}); and time of occurrence of maximum observed concentration. Following IV administration, the geometric mean AUC_{0-t} of [¹⁴C]-batefenterol was 121.9 pgEq • h/mL; maximum observed concentration and time of occurrence of maximum observed concentration were 92.7 pgEq/mL and 0.8 hours, respectively; absolute oral bioavailability was 0.012%. The mean AUC_{0-t} ratio indicated that [¹⁴C]-batefenterol accounted for 85% of total circulating radioactivity in the plasma initially and declined rapidly following IV administration, but only ~0.2% of total circulating radioactivity following oral administration. Cumulative mean recovery of total radioactive [¹⁴C]-batefenterol in urine and feces was 6.31% and 77.6%, respectively. Overall, batefenterol exhibited low systemic bioavailability after inhaled and oral administration, and high fecal excretion and low urinary excretion following IV and oral administration.

Keywords

batefenterol, chiral inversion, pharmacokinetics, microtracer

Chronic obstructive pulmonary disease (COPD) is a common, often progressive, condition characterized by persistent respiratory symptoms and airflow limitation.^{1,2} Inhaled bronchodilators, such as long-acting muscarinic antagonists, long-acting β₂-adrenergic agonists, and inhaled corticosteroids are the mainstay of pharmacotherapy in COPD.^{1,2} The combination of a long-acting muscarinic antagonist with a long-acting β₂-adrenergic agonist results in greater bronchodilation in the airways than either component alone.²⁻⁴

Batefenterol (GSK961081⁵), a novel bifunctional molecule combining muscarinic acetylcholine receptor antagonism (M2 and M3) and β₂-adrenoreceptor agonism,⁶ is in development for the treatment of COPD.⁷ This bifunctional molecule has advantages over the use of 2 separate compounds. For example, the technical and clinical development pathway is simpler for a single compound than for a coformulation of

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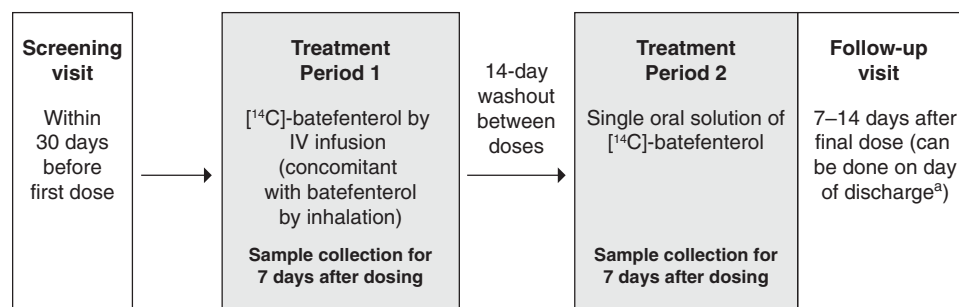


Figure 1. Study design. IV indicates intravenous

^aThe follow-up period was extended if radioactivity excretion was > 1% upon discharge.

2 separate compounds.⁸ Furthermore, due to the existence of a single pharmacokinetic (PK) profile for both pharmacologic activities, there is a potential to maximize the synergy between the 2 mechanisms.

Although the PK profile of batefenterol following inhalation has been examined in previous clinical studies,^{7,9,10} the absorption, elimination routes, and metabolic pathways of batefenterol in humans remain poorly characterized. Microtracer approaches combined with accelerator mass spectrometry (AMS) have been used in quantitative absorption, metabolism, and excretion studies; this novel approach enables detection of significantly lower doses of [¹⁴C]-labeled compounds compared with traditional analytical methods.¹¹⁻¹³ Further, *in vivo* studies in humans using radiolabeled compounds, primarily ¹⁴C, provide quantitative analyses of overall routes of excretion of drug-related material, PK of total drug-derived radioactivity in circulation relative to parent compound, as well as quantitation and characterization of metabolites in excreta and circulation.^{11,14}

In order to gather a large amount of information from a small number of subjects, this study had a novel design involving the use of an intravenous (IV) microtracer to define absolute bioavailability in combination with a human absorption, distribution, metabolism, and excretion study. The aim of this study was to assess the plasma PK and excretion balance of batefenterol in healthy human volunteers using ¹⁴C-radiolabeled batefenterol administered by IV and oral routes as surrogates for administration by inhalation.

Subjects and Methods

Study Design

This was a 2-period, single-sequence crossover, non-randomized, open-label study in healthy male subjects conducted at a single site (GSK study number 201003; ClinicalTrials.gov registration number NCT02663089; Hammersmith Medicines Research, London, UK). The study was performed in accordance with the Declaration of Helsinki.¹⁵ Ethics Committee approval was ob-

tained from South Central-Oxford A Research Ethics Committee, Oxford A REC, Whitefriars, Level 3, Block B, Lewin's Mead, Bristol, UK. Written informed consent was obtained from all subjects. Each subject participated in the study for up to 11 weeks, having a screening visit (within 30 days prior to the first dose), 2 treatment periods, and a follow-up visit (Figure 1).

The absorption, distribution, metabolism, and excretion properties of inhaled batefenterol were determined by administering radiolabeled IV and oral doses and comparing the resulting data with the PK parameters following nonradiolabeled inhaled administration of batefenterol. The PK properties of the radiolabeled oral dose therefore reflect those of the swallowed portion of the inhaled dose, while the PK properties of the radiolabeled IV dose reflect those of the systemically absorbed portion of the inhaled dose.

In period 1, subjects received a single IV microtracer as a 1-hour infusion of [¹⁴C]-batefenterol (6.2 kBq; ~168 nCi) at a dose of 4 μg, after an overnight fast. Two minutes after the start of the IV infusion, subjects concomitantly received an inhaled 1200-μg nonradiolabeled dose of batefenterol (4 inhalations of batefenterol 300 μg) via Ellipta Dry Powder Inhaler (owned by or licensed to the GSK group of companies). Samples of blood, duodenal bile (single-time-point collection only), urine, and feces were collected up to 168 hours (7 days) after dosing.

After a 14-day washout period, subjects entered period 2, in which they received 200 μg [¹⁴C]-batefenterol (311 kBq; ~8.4 μCi) as an oral solution following an overnight fast. Following dosing, blood, urine, and fecal samples were collected for a minimum of 168 hours (up to day 8) and up to 336 hours (day 15), depending on the amount of radioactivity excreted by each subject.

Study Population

The study recruited healthy men between 30 and 55 years of age, a body weight of ≥50 kg, and a body mass index of 19 to 31 kg/m² (inclusive). Participating subjects were required not to have been involved in a

study involving a ^{14}C -labeled drug within the 12 months prior to enrollment. Details of all eligibility criteria are provided in the Supporting Information.

Sample Collections and Analysis

Blood samples for plasma total radioactivity, [^{14}C]-batefenterol, and nonradiolabeled batefenterol were collected before the dose (0), and 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 48, 72, 96, and 168 hours after the dose and shipped to the Department of Bioanalysis, GSK Research and Development, Ware, UK, for analysis (see Supporting Information for details of all analytical methods). Plasma total radioactivity was assessed by direct AMS, which had a lower limit of quantification (LLQ) of 0.924 pg batefenterol Equivalents (Eq)/mL (period 1) and 0.946 pgEq/mL (period 2). The slight variation in LLQ between the 2 periods was due to the differences in the background endogenous carbon radioactivity, which was calculated separately for each period. Plasma [^{14}C]-batefenterol was determined using a validated analytical method based on protein precipitation, followed by high-performance liquid chromatography (HPLC) + AMS. The LLQ of this method was 0.25 pg/mL using a 500- μL aliquot of plasma. Plasma batefenterol concentration was measured using a validated analytical method based on protein precipitation, followed by analysis by HPLC–tandem mass spectrometry (HPLC-MS/MS) (achiral assay). The LLQ was 25 pg/mL using a 50- μL aliquot of plasma. The potential for chiral inversion of the batefenterol S- to R-enantiomer was investigated by chiral derivatization and HPLC-MS/MS analysis, involving the comparison of pooled sample chromatograms and spiked control human plasma samples, which enabled chiral inversion to be detected to approximately a 10% level of the observed batefenterol C_{max} calculated from the period 1 profiles. Further details of the analytical methodologies are provided in the Supporting Information.

Urine and fecal samples were shipped and analyzed at the Department of Metabolism, Covance Laboratories Limited, Harrogate, North Yorkshire, UK, and/or Xceleron Inc. (now Pharmaron; Germantown, Maryland). Liquid scintillation counting (LSC) was performed daily on 24-hour urine collections and 24-hour fecal homogenates after day 6 to measure total radioactivity excreted in urine and feces, respectively. LSC was also used to screen the plasma samples prior to subsequent analysis by AMS. The LSC method uses an external standard procedure for quantification. The mean LLQ for total radioactivity by LSC was 0.253 ngEq/mL and by AMS was 0.197 pgEq/mL for urine measurements. For fecal sample analyses, the mean LLQ for total radioactivity by LSC was 2.38 ngEq/g and by AMS

was 2.59 pgEq/g. Further details of the LSC and AMS methodologies are provided in the Supporting Information.

Entero-Test (Entero-Test: HDC Corp., Mountain View, California)¹⁶ was used for sampling of duodenal bile to conduct qualitative assessment of drug metabolites during period 1 (IV dosing). The Entero-Test was inserted approximately 3.5 hours before the start of the IV infusion while subjects were in a fasted state, and removed approximately 2.5 hours after the end of the IV infusion. Approximately 0.5 hours after the end of the IV infusion (2 hours before Entero-Test string withdrawal) a food cue was used to stimulate gallbladder emptying.

The characterization and quantification of batefenterol metabolites in plasma, urine, feces, and duodenal bile was investigated by GSK.

Pharmacokinetic Assessments

The primary end points were the area under the concentration-time curve from time zero (predose) extrapolated to infinite time ($\text{AUC}_{0-\text{inf}}$) of total drug-related material, area under the concentration-time curve from time zero (predose) to last time of quantifiable concentration (AUC_{0-t}), maximum observed concentration (C_{max}), time of occurrence of C_{max} (t_{max}), and the terminal phase half-life ($t_{1/2}$) of total plasma radioactivity. Additional primary end points included volume and clearance of total radioactivity (following IV dose only) and urinary and fecal cumulative excretion as a percentage of the total radioactive dose over time.

Secondary end points were $\text{AUC}_{0-\text{inf}}$, AUC_{0-t} , C_{max} , t_{max} , $t_{1/2}$ for batefenterol and [^{14}C]-batefenterol following IV, inhaled, and oral doses; volume and clearance of batefenterol and [^{14}C]-batefenterol following IV dose; and absolute bioavailability (F; oral and inhaled).

Safety Assessments

Safety was assessed by monitoring adverse events (AEs), clinical laboratory parameters, electrocardiogram, and vital signs. AEs were recorded throughout the study treatment until the follow-up visit.

Statistical Analyses

Safety and study population data were reported using the all-subjects population (and PK data were reported using the PK population). The all-subjects population included all subjects who had received at least 1 dose of study medication. The PK population comprised those subjects from the all-subjects population for whom a PK sample was obtained and analyzed.

No formal sample size calculation was performed for this study. A sample size of 6 was deemed appropriate to investigate the primary objective.¹⁷ PK

Table 1. Demographic and Baseline Characteristics (All-Subjects Population)

Parameter	Total (N = 6)
Age, y, mean (SD)	37.0 (4.34)
Male, n (%)	6 (100)
BMI (kg/m ²), mean (SD)	22.87 (2.904)
Height (cm), mean (SD)	177.5 (7.58)
Weight (kg), mean (SD)	71.77 (7.186)
Not Hispanic or Latino, n (%)	6 (100)
Race, n (%)	
African American/African heritage	3 (50)
White—White/Caucasian/European heritage	2 (33)
Mixed race	1 (17)

BMI indicates body mass index; SD, standard deviation.

analysis was performed by Quanticate Ltd. (Hitchin, UK) under the direct auspices of Clinical Pharmacology Modelling & Simulation, GSK, and with support from Covance Laboratories Ltd. (Harrogate, UK) and Xceleron Inc. (Germantown, Maryland) for the excretion mass balance elements of the study and sample analysis by LSC and AMS, respectively. Plasma batenfenterol, [¹⁴C]-batenfenterol, and total radioactivity concentration-time data were analyzed by non-compartmental methods with WinNonlin version 6.4 (Certara USA, Inc., Princeton, New Jersey), using the actual sampling times recorded during the study. [¹⁴C]-batenfenterol data provided for the IV route of administration are corrected for presence of all parent drug (of note, the IV dose specific activity was such that it comprised ~50% nonlabeled batenfenterol, which was detectable by the achiral HPLC-MS/MS assay for parent drug).

Anonymized individual participant data and study documents can be requested for further research from www.clinicalstudydatarequest.com.

Results

Demographics and Baseline Characteristics

Of the 11 subjects screened, 6 were enrolled and all completed the study. The mean age (standard deviation) was 37.0 (4.34) years, and mean (standard deviation) body mass index was 22.87 (2.9) kg/m². Baseline demographics and characteristics are summarized in Table 1.

Pharmacokinetic Results

PK parameters and changes over time for total radioactivity following IV [¹⁴C]-batenfenterol + inhaled batenfenterol and oral [¹⁴C]-batenfenterol, and concentrations of batenfenterol in plasma following IV administration and inhalation are summarized in Table 2 and Figure 2. Overall, following IV dosing, both total radioactivity and [¹⁴C]-batenfenterol concentrations

in plasma peaked rapidly, reached a plateau during IV infusion (up to 1 hour) and declined rapidly after the infusion was stopped. Both IV [¹⁴C]-batenfenterol and inhaled batenfenterol had a short plasma terminal elimination ($t_{1/2}$: 3.2 hours and 3.4 hours, respectively). However, $t_{1/2}$ and parameters associated with $t_{1/2}$ should be interpreted with caution, as the period over which they were calculated was less than twice the resultant half-life.

[¹⁴C]-batenfenterol had a small (17.1 L) apparent volume of distribution at steady state following IV administration. The absolute bioavailability following a 1200- μ g inhaled dose in healthy subjects was 1.29% (95% confidence interval, 0.72, 2.31) based on AUC_{0-t} data (Table 3), but was probably underestimated due to the low plasma concentrations encountered.

The batenfenterol plasma concentrations following oral administration were below the LLQ of the achiral HPLC-MS/MS parent assay (25 pg/mL). The absolute bioavailability of [¹⁴C]-batenfenterol administered as a 200- μ g oral dose was 0.29% (95% confidence interval, 0.26%, 0.34%) based on imputed AUC_{0-t} data (details of the imputation are provided in the Supporting Information). However, the oral absolute bioavailability was 0.012% based on pooled [¹⁴C]-batenfenterol samples (using an HPLC+AMS assay with improved sensitivity, to provide a LLQ of 0.25 pg/mL) and AUC_{0-t}.

The mean AUC_{0-t} ratio indicated that [¹⁴C]-batenfenterol accounted for 85% of total circulating radioactivity in the plasma following IV administration (Table 2), although at later time points (>2 hours) in the concentration-time profile the majority of drug-related material in the systemic circulation was not due to parent drug. Only a single elimination phase was observed for batenfenterol following both IV and inhaled administrations to the limit of quantification.

A post hoc analysis comparing the ratio of total drug-related material in the systemic circulation to parent drug using combined data from periods 1 and 2 (IV and oral administrations) made it possible to calculate the fraction of the drug surviving metabolism (0.15), the hepatic extraction ratio (0.44), and the fraction of the drug absorbed via the gut following oral administration (0.14). Details of the calculation are provided in the Supporting Information.

Mass Balance and Excretion

The radioactivity recoveries (as a percentage of the administered radiolabeled dose) over time following IV [¹⁴C]-batenfenterol and oral [¹⁴C]-batenfenterol are summarized in Figures 3 and 4.

Following administration of an IV dose of [¹⁴C]-batenfenterol (concomitant with an inhaled non-radiolabeled dose), 83.9% (3.73) of the original dose of radioactivity was recovered by 168 hours. The

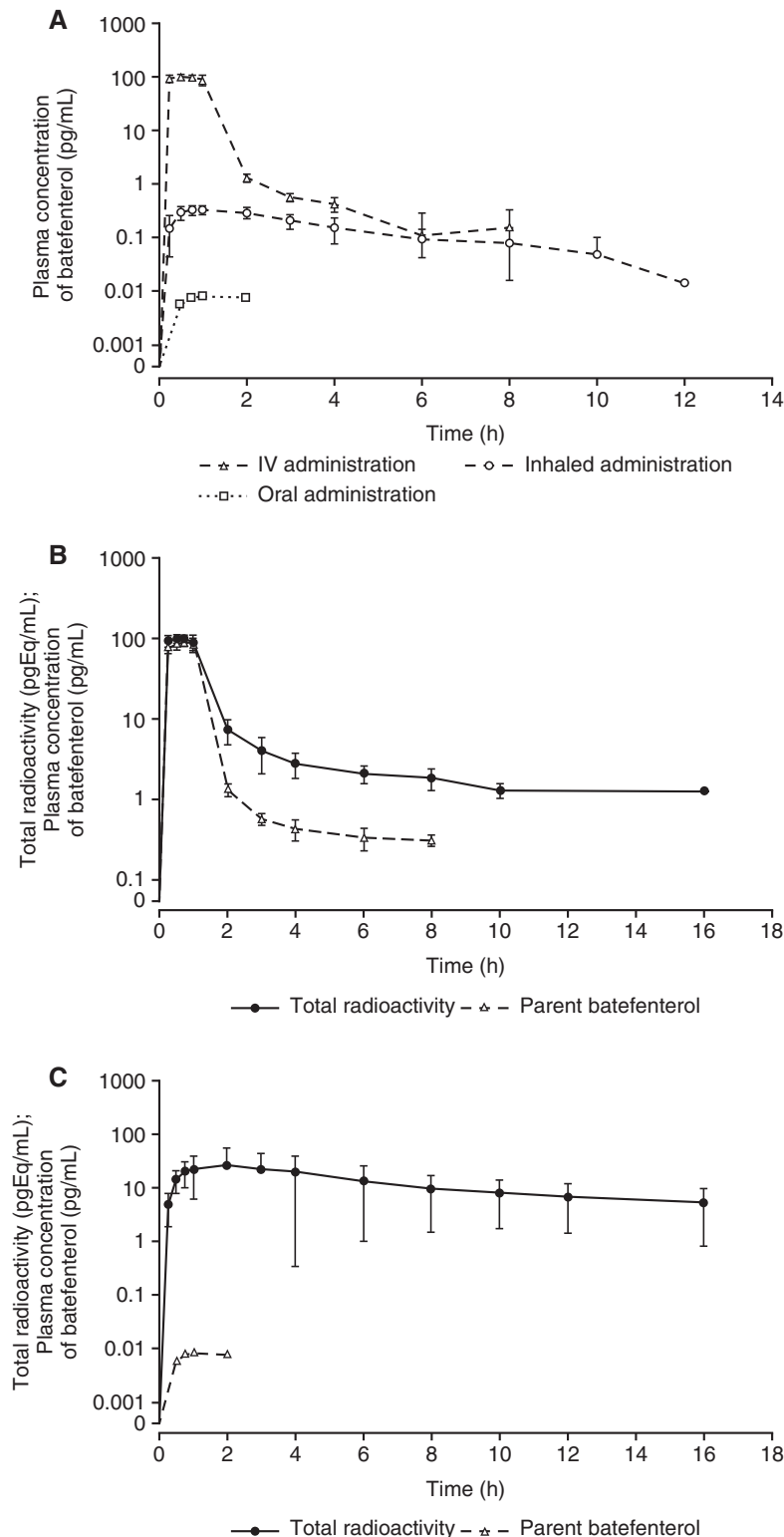


Figure 2. Arithmetic mean (SD) concentrations of (A) plasma batefenterol following IV administration (data provided by LC+AMS assay) with concomitant inhaled administration (data by LC/MS assay), and oral administration (data by LC+AMS assay of pooled samples) (PK population; oral and inhaled doses normalized to 4- μ g IV dose); (B) total radioactivity (data by AMS) and batefenterol in plasma following IV administration (data by LC+AMS); and (C) total radioactivity (data by AMS) and batefenterol in plasma following oral administration (data by LC+AMS of pooled samples). AMS indicates accelerator mass spectrometry; IV, intravenous; LC, liquid chromatography; MS mass spectrometry; PK, pharmacokinetic; PO, oral administration; SD, standard deviation.

Table 2. Summary of PK Parameters Following 4- μg IV [^{14}C]-Batefenterol + 1200- μg Inhaled Batefenterol and 200- μg Oral [^{14}C]-Batefenterol (All-Subjects Population)

Parameter (Unit)	Route of Administration	N	n	Arithmetic Mean (SD)	Geometric Mean	95% CI	CV%
Total radioactivity following 4 μg IV [^{14}C]-batefenterol (+1200 μg inhaled batefenterol) and 200 μg oral [^{14}C]-batefenterol ^a							
AUC _{0-inf} (pgEq • h/mL)	IV	6	5	144.4 (31.3)	141.5	(106.5, 187.9)	23.2
	Oral	6	3	517.9 (623.3)	313.1	(16.2, 6068.6)	177.6
AUC _{0-t} (pgEq • h/mL)	IV	6	6	124.1 (25.4)	121.9	(98.3, 151.2)	20.7
	Oral	6	6	387.8 (381.8)	260.3	(93.2, 727.1)	126.8
C _{max} (pgEq/mL)	IV	6	6	93.4 (12.5)	92.7	(80.2, 107.1)	13.8
	Oral	6	6	35.5 (27.8)	28.1	(13.1, 60.3)	83.5
t _{1/2} (h) ^b	IV	6	5	8.2 (3.5)	7.66	(4.5, 13.0)	44.7
	Oral	6	3	32.1 (33.5)	22.5	(1.9, 268.2)	130.4
t _{last} (h) ^c	IV	6	6	—	10.0	(8.1, 16.0)	NA
	Oral	6	6	—	96.0	(24.0, 168.0)	NA
t _{max} (h) ^c	IV	6	6	—	0.8	(0.5, 1.0)	NA
	Oral	6	6	—	1.0	(0.8, 2.0)	NA
IV [^{14}C]-batefenterol							
AUC _{0-inf} (pg • h/mL)	IV	6	6	106.3 (15.4)	105.5	(91.5, 121.7)	13.6
AUC _{0-t} (pg • h/mL)	IV	6	6	104.6 (14.7)	103.8	(90.4, 119.2)	13.2
AUC _{0-inf} ratio	IV	6	5	0.7 (0.2)	0.74	(0.57, 0.95)	21.0
AUC _{0-t} ratio	IV	6	6	0.9 (0.2)	0.85	(0.71, 1.03)	18.0
CL (L/h)	IV	6	6	38.2 (4.9)	37.9	(32.9, 43.7)	13.6
C _{max} (pg/mL)	IV	6	6	104.0 (13.4)	103.3	(90.6, 117.8)	12.5
t _{1/2} (h) ^b	IV	6	6	3.9 (2.4)	3.2	(1.5, 6.8)	80.7
V _{ss} (L)	IV	6	6	18.1 (6.5)	17.1	(11.7, 25.1)	37.9
V _z (L)	IV	6	6	203.1 (104.6)	177.2	(94.3, 333.1)	66.0
t _{last} (h) ^c	IV	6	6	—	7.0	(4.0, 8.1)	NA
t _{max} (h) ^c	IV	6	6	—	0.6	(0.4, 1.0)	NA
Inhaled batefenterol							
AUC _{0-inf} (h*pg/mL)	Inhaled	6	5	573.5 (259.6)	516.0	(260.4, 1022.6)	59.5
AUC _{0-t} (h*pg/mL)	Inhaled	6	6	440.5 (176.2)	402.2	(236.1, 685.1)	54.2
CL/F (L/h)	Inhaled	6	5	2657.1 (1686.2)	2325.4	(1173.5, 4608.0)	59.5
C _{max} (pg/mL)	Inhaled	6	6	107.3 (23.4)	105.2	(83.8, 132.0)	21.9
t _{1/2} (h) ^b	Inhaled	6	5	4.0 (2.2)	3.4	(1.6, 7.7)	71.6
t _{last} ^c (h)	Inhaled	6	6	—	9.0	(3.0, 12.0)	NA
t _{max} ^c (h)	Inhaled	6	6	—	0.8	(0.5, 2.0)	NA

AUC_{0-inf} indicates area under the concentration-time curve from time zero (predose) extrapolated to infinite time; AUC_{0-inf} ratio, the ratio of the area under the concentration-time curve from time zero (predose) extrapolated to infinite time for [^{14}C]-batefenterol IV/total radioactivity; AUC_{0-t}, area under the concentration-time curve from time zero (predose) to last time of quantifiable concentration; AUC_{0-t} ratio, the ratio of the area under the concentration-time curve from time zero (predose) to last time of quantifiable concentration for [^{14}C]-batefenterol IV/total radioactivity; CI, confidence interval; CL, clearance; C_{max}, maximum observed concentration; CV%, coefficient of variation; IV, intravenous; NA, not applicable; t_{1/2}, terminal phase half-life; t_{last}, time to reach last observed plasma concentration; V_z, volume of distribution using the area method; V_{ss}, apparent volume of distribution at steady state.

^aAll batefenterol plasma concentrations following oral [^{14}C]-batefenterol were nonquantifiable (NQ); [^{14}C]-batefenterol AUC_{0-t} from pooled samples was 0.614 h*pgEq/mL.

^bt_{1/2} should be interpreted with caution because the period over which it was calculated was less than twice the resultant half-life.

^cData presented as median (range).

cumulative mean recovery of total [^{14}C]-batefenterol radioactivity in urine and feces was 6.31% (2.23) and 77.6% (3.45), respectively. Meanwhile, following oral dosing of [^{14}C]-batefenterol, the mean (standard deviation) cumulative total recovery at 168 hours was 84.22% (1.48); 0.47% (0.50) in urine and 83.88% (1.66) in feces following exclusion of 1 subject, who exhibited abnormally low mass balance recovery following oral

[^{14}C]-batefenterol dosing compared with the other subjects. No dosing or sampling issues were deemed to contribute to this anomalous value.

Metabolism

Characterization and quantification of metabolites formed in humans indicated that elimination of batefenterol following IV and oral administration

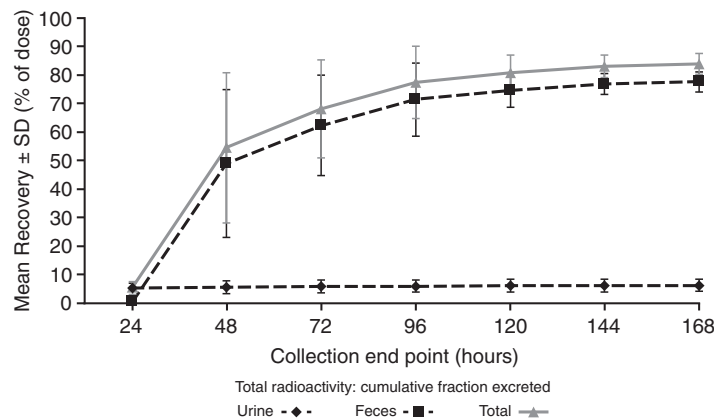
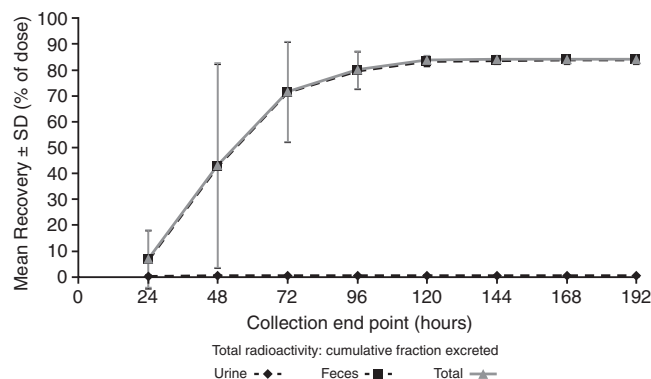
Table 3. Absolute Bioavailability of Inhaled Batefenterol (1200 μg , Dosed Concomitantly With 4- μg IV [^{14}C]-Batefenterol) and Oral [^{14}C]-Batefenterol (200 μg) (All-Subjects Population)

Test Route of Administration	PK Parameter	Geometric Mean AUC (dose)		Absolute Bioavailability	
		Test route	IV	Geometric Mean (%) (CVb%)	95% CI
Inhaled	$F_{0-\text{inf}}$	516 (1200 μg)	105 (4 μg)	1.62 (67.8)	(0.75, 3.47)
	$F_{0-\text{t}}$	402 (1200 μg)	104 (4 μg)	1.29 (60.1)	(0.72, 2.31)
Oral	$F_{0-\text{inf}}^{\text{a}}$	15.27 (200 μg)	105 (4 μg)	0.29 (13.6)	(0.25, 0.33)
	$F_{0-\text{t}}^{\text{a}}$	15.27 (200 μg)	104 (4 μg)	0.29 (13.2)	(0.26, 0.34)
	$F_{0-\text{t}}^{\text{b}}$	0.614 (200 μg)	104 (4 μg)	0.012	—

AUC indicates area under the curve concentration; CI, confidence interval; CVb%, coefficient of variation; $F_{0-\text{inf}}$, absolute bioavailability from time zero (predose) extrapolated to infinite time; $F_{0-\text{t}}$, absolute bioavailability from time zero (predose) to last time of quantifiable concentration within a subject across all treatments; IV, intravenous; LLQ, lower limit of quantification; PK, pharmacokinetic.

^aOral $F_{0-\text{inf}}$ and oral $F_{0-\text{t}}$ calculations were based on imputed AUC values as all plasma batefenterol data were nonquantifiable. A value of 15.27 $\text{pg} \cdot \text{h/mL}$ was used, based on an imputed concentration time series of predose = 0 ng/L ; 0.25 h = 50 ng/L (assay LLQ); and 0.5 h = 25 ng/L (half assay LLQ).

^bOral $F_{0-\text{t}}$ calculation based on pooled [^{14}C]-batefenterol $\text{AUC}_{0-\text{t}}$, 0.614 $\text{pgEq} \cdot \text{h/mL}$.

**Figure 3.** Arithmetic mean (\pm SD) total radioactivity recovery over time following IV administration of [^{14}C]-batefenterol (PK population). IV indicates intravenous; PK, pharmacokinetic; SD, standard deviation.**Figure 4.** Arithmetic mean (\pm SD) radioactivity recovery over time following oral administration of [^{14}C]-batefenterol (PK population). Data presented are those for which the subject, who exhibited abnormally low mass balance recovery following oral [^{14}C]-batefenterol dosing compared with the other subjects was excluded. PK indicates pharmacokinetic; SD, standard deviation.

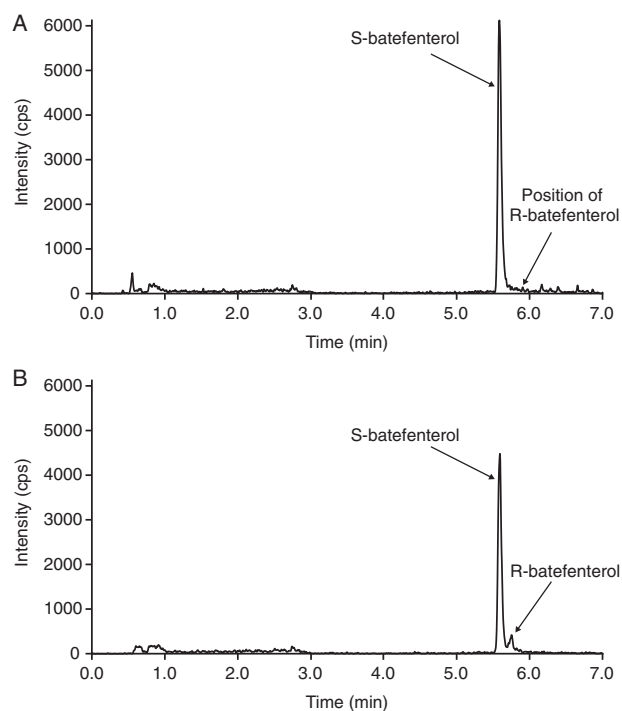


Figure 5. (A) Representative LC-MS/MS chromatogram of pooled human plasma samples from a single subject following administration of batefenterol (1200 µg) by inhalation; (B) LC-MS/MS chromatogram of a human plasma sample spiked with batefenterol (150 pg/mL) and R-batefenterol (at a 10% ratio; 15 pg/mL). cps indicates counts per second; LC-MS/MS, liquid chromatography–tandem mass spectrometry.

was mainly as unchanged batefenterol with metabolism by amide hydrolysis, carbamate hydrolysis, N-dealkylation, or glucuronidation, excreted via biliary, fecal, and urinary routes. Following IV infusion, unchanged batefenterol was observed to be a major circulating component at early time points, with metabolites formed by hydrolysis and glucuronidation contributing to a greater extent later in the sampling time course (confirming the divergent PK data for concentrations of batefenterol versus total radioactivity as shown in Figure 2B). However, following oral administration the major circulating components were metabolites formed by hydrolysis, oxidative deamination, and glucuronidation, whereas unchanged batefenterol was only a minor component (confirming the PK data shown in Figure 2C).

Chirality

A representative HPLC-MS/MS chromatogram of single-subject pooled human plasma following inhalation of batefenterol (1200 µg) is shown in Figure 5A. An HPLC-MS/MS chromatogram of batefenterol at 150 pg/mL (approximately representative of C_{max}) and the batefenterol R-enantiomer at a 10% ratio

Table 4. Summary of All AEs (All-Subjects Population)

	[¹⁴ C]- Batefenterol IV + Batefenterol IH (n = 6)	[¹⁴ C]- Batefenterol Oral (n = 6)	Total ^a (N = 6)
Subjects with any AE(s), n (%)	4 (67)	3 (50)	4 (67)
Cough	3 (50)	0	3 (50)
Headache	1 (17)	1 (17)	2 (33)
Nasal congestion	1 (17)	0	1 (17)
Dizziness	0	1 (17)	1 (17)
Eye irritation	0	1 (17)	1 (17)
Catheter site pain	1 (17)	0	1 (17)
Oral herpes	1 (17)	0	1 (17)

AE indicates adverse event; IH, inhaled; IV, intravenous.

^aTotal number of subjects experiencing the event.

(15 pg/mL) in spiked control human plasma is shown in Figure 5B. No evidence of chiral inversion of the batefenterol S- to R-enantiomer was observed.

Safety

Four (67%) subjects experienced 1 or more AEs, with cough (n = 3; 50%) and headache (n = 2; 33%) the most frequent (Table 4). There were no deaths, serious AEs, or AEs leading to discontinuation of the study drug or withdrawal from the study. There were no clinically significant changes in any clinical laboratory parameter, electrocardiogram, or vital signs in the study population.

Discussion

This study examined the PK and excretion balance of batefenterol in humans using [¹⁴C]-batefenterol in healthy male volunteers. The study drug was administered by radiolabeled IV and oral routes as surrogates for inhaled administration, with the PK properties of the radiolabeled IV dose reflecting those of the systemically absorbed portion of the inhaled dose, and the PK properties of the radiolabeled oral dose reflecting those of the swallowed portion of the inhaled dose.

Consistent with previous experience with the drug,⁷ batefenterol was well tolerated in the 6 subjects recruited, with no serious AEs or AEs leading to discontinuation or study withdrawal. Batefenterol had a low inhaled bioavailability (1.29%) and very low bioavailability (~0.01%) following oral administration, with drug-related material excreted predominantly in the feces following both IV and oral administrations. It was apparent that the bioavailability of batefenterol

following inhaled administration might have been underestimated due to the low plasma concentrations and assay limitations. While a low inhaled bioavailability was anticipated for batenfenterol from preclinical studies, the value obtained in this study is lower than for most other molecules in the same class. This is likely a reflection of the physicochemical properties and low permeability (or first-pass metabolism following oral administration as evidenced by the low calculated value of the fraction of the drug surviving metabolism) of batenfenterol.¹⁸ The low inhaled bioavailability does not reflect the actual drug dose delivered to the airways via the Ellipta device; rather, it reflects the systemically absorbed portion of the inhaled dose, which is desired to be small, potentially indicating a prolonged availability of batenfenterol in the airways and an extended duration of action.

A moderate plasma clearance of batenfenterol was observed following IV dosing, and the low volume of distribution suggests low tissue distribution. Overall, the low systemic exposure suggests a favorable tolerability profile. This, combined with an inferred prolonged lung availability, suggests the potential for a high therapeutic index for batenfenterol.

Radiolabeled [¹⁴C]-batenfenterol drug-related material was excreted primarily in the feces, with only a small amount recovered from the urine following both IV and oral doses. These data show that elimination of batenfenterol was likely predominantly via hepatic clearance, or at least via first-pass metabolism and biliary elimination. This likely impact of first-pass metabolism is supported by the observation that the total radioactivity in the systemic circulation was much higher than parent drug following oral administration. Plasma elimination half-life following a 4- μ g IV dose derived from [¹⁴C]- batenfenterol was lower compared with that derived from total radioactivity, indicating that 1 or more metabolites may have contributed to longer residence time and hence the longer estimated $t_{1/2}$. The mass balance (Figures 3 and 4) was within acceptable limits for studies of this type in humans.¹⁹

In common with similar studies of this nature, this study is limited by the small number of subjects ($n = 6$). However, the sample size was deemed appropriate for the current investigation.¹⁷ Of note, the terminal phase was not readily defined, as the concentrations of batenfenterol in plasma were close to the limit of quantification. In addition, $t_{1/2}$ estimates should be treated with caution because the period over which they were calculated was less than twice the resultant half-life. When estimating the volume of distribution and clearance following [¹⁴C]-batenfenterol IV with concomitant inhaled nonradiolabeled batenfenterol, the nonradiolabeled batenfenterol data were derived by sub-

tracting the nonradiolabeled portion (~50%) of the IV systemic data from the achiral HPLC-MS/MS concentration data. This approach was necessary due to very low bioavailability (~1.29%) following inhaled batenfenterol, which meant that the systemic concentrations of batenfenterol (nonradiolabeled portion) after the IV dose were detected to a significant degree using achiral HPLC-MS/MS, even though the IV dose was 300-fold lower than the inhaled dose. Strengths of the study design include the concomitant administration of IV and inhaled doses, which facilitated the comparison of PK parameters from the 2 routes of administration without concerns over the nonlinearity or nonequivalent clearance effects. Nonlinearity is a common concern for microdose administrations, and crossover study designs can suffer from nonequivalent clearance effects to assess absolute bioavailability. The study design presented here negates or at least minimizes both of these potential issues.^{20,21} Furthermore, adequate mass balance was achieved in this study, allowing the successful determination of the excretion of batenfenterol.

In summary, batenfenterol exhibited low systemic bioavailability following administration via inhaled and oral routes, with low tissue distribution, moderate plasma clearance, and low systemic absorption via the lung. Batenfenterol-related material was primarily excreted in the feces with low urinary excretion, indicating that hepatic clearance, or at least first-pass metabolism, and biliary elimination are the likely major routes of elimination, which was confirmed by quantification and characterization of the metabolites present in the circulation, as well as in excreta.

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Declaration of Conflicting Interests

FvdB and DR have no conflicts of interest. CA, GY, TF, ALL, AP, AH, and PD-Y are employees of GSK and hold stocks or shares in the company.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.