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ORIGINAL RESEARCH ARTICLE

Pharmacokinetics, Biotransformation, and Excretion of [14C]Etelcalcetide (AMG 416) Following a Single Microtracer Intravenous Dose in Patients with Chronic Kidney Disease on Hemodialysis

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Abstract Etelcalcetide (AMG 416) is a novel synthetic peptide calcium-sensing receptor activator in clinical development as an intravenous calcimimetic for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease (CKD) on hemodialysis. Etelcalcetide is composed of seven D-aminoacids with an L-cysteine linked to a D-cysteine by a disulfide bond. A single intravenous dose of [14C]etelcalcetide (10 mg; 26.3 kBq; 710 nCi) was administered to patients with CKD on hemodialysis to elucidate the pharmacokinetics, biotransformation, and excretion of etelcalcetide in this setting. Blood, dialysate, urine, and feces were collected to characterize the pharmacokinetics, biotransformation product profiles, mass balance, and formation of anti-etelcalcetide antibodies. Accelerator mass spectrometry was necessary to measure the microtracer quantities of C-14 excreted in the large volumes of dialysate and other biomatrices. An estimated 67 % of the [14C]etelcalcetide dose was recovered in dialysate, urine, and feces 176 days after dose administration. Etelcalcetide was primarily cleared by hemodialysis, with approximately 60 % of the administered dose eliminated in dialysate. Minor excretion was observed in urine and feces. Biotransformation resulted from disulfide exchange with endogenous thiols, and preserved the

etelcalcetide D-amino acid backbone. Drug-related radioactivity circulated primarily as serum albumin peptide conjugate (SAPC). Following removal of plasma etelcalcetide by hemodialysis, re-equilibration occurred between SAPC and L-cysteine present in blood to partially restore the etelcalcetide plasma concentrations between dialysis sessions. No unanticipated safety signals or anti-etelcalcetide or anti-SAPC antibodies were detected.

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Key Points

Hemodialysis was the predominant clearance and elimination pathway of etelcalcetide following a single dose of [14C]etelcalcetide in chronic kidney disease patients on hemodialysis.

Biotransformation resulted from reversible disulfide exchange with endogenous thiols, and the etelcalcetide D-amino acid backbone was preserved.

The majority of circulating etelcalcetide-related biotransformed moieties existed as serum albumin peptide conjugate (SAPC).

After removal of plasma etelcalcetide by dialysis, re-equilibration between SAPC and L-cysteine present in blood partially restored predialysis concentrations of etelcalcetide.

1 Introduction

Calcimimetics are a class of drugs that activate the parathyroid calcium-sensing receptor (CaSR) to inhibit parathyroid hormone (PTH) secretion [1, 2]. Dysregulation of CaSR signaling plays an important role in the
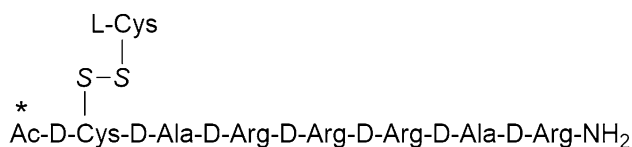
△ Adis

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          L-Cys
            |
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 Ac-D-Cys-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH₂
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Fig. 1 Structure of etelcalcetide. The drug substance was formulated as a single 10 mg dose of etelcalcetide (with 26.3 kBq of [^{14}C]etelcalcetide; 10 µg) in 2 mL liquid solution for bolus intravenous administration at the end of hemodialysis. The formulation was a sterile, preservative-free, aqueous solution containing 0.85 % sodium chloride and 10 mM succinic acid. *Ala, Arg, Cys* denote the alanine, arginine, and cysteine amino acids, *Ac* denotes the acetyl group on the N-terminus of the D-cysteine, and *NH₂* denotes amidation on the C-terminus of the D-arginine. The *asterisk* denotes the position of the C-14 radiolabel placed on the carbonyl carbon of the acetyl moiety

development of secondary hyperparathyroidism (SHPT), a frequent comorbidity for patients with chronic kidney disease (CKD) that is characterized by elevated PTH levels, mineral disturbances, and increased risk of complications and death [1].

Etelcalcetide (AMG 416) (Fig. 1) is a novel intravenous calcimimetic in clinical development for SHPT treatment. Etelcalcetide is a 1048 Da synthetic peptide comprising a linear chain of seven D-amino acids (referred to as the 'D-amino acid backbone') and an L-cysteine linked to the D-cysteine by a disulfide bond. Formation of a covalent disulfide bond between the D-cysteine of etelcalcetide and cysteine 482 of the CaSR results in allosteric activation of the receptor, enhanced signal transduction, and inhibition of PTH secretion [3]. In two phase III studies in patients with SHPT on hemodialysis, approximately 75 % of those administered etelcalcetide achieved a >30 % PTH reduction from baseline versus 8–10 % of those receiving placebo [4].

Pharmacokinetic profiles of etelcalcetide and [^{14}C]etelcalcetide-derived radioactivity (referred to as 'total C-14') in rat plasma showed a multiphase decline with a long terminal half-life ($t_{1/2}$) [5]. In rats with intact kidneys, total C-14 following a single intravenous dose was predominantly excreted in urine (77–84 % of administered dose; approximately 90 % of recovered dose). In bilaterally nephrectomized rats, recovery of total C-14 was low and elimination by nonrenal pathways was slow. Etelcalcetide biotransformation occurred in whole blood primarily by disulfide exchange with endogenous thiol-containing molecules; the D-amino acid backbone was unaltered. The predominant biotransformation product present in plasma was a covalently bound serum albumin peptide conjugate (SAPC) [5].

In clinical studies, the importance of renal clearance was indicated by a prolonged etelcalcetide $t_{1/2}$ in patients with CKD on hemodialysis (81.7–175 h) compared with healthy volunteers (18.4–20.0 h) [6–8]. These findings were consistent with the role of renal clearance determined in rats

[5]. Based on the nonclinical findings that biotransformation of etelcalcetide only occurs by reversible disulfide exchange [9], and because renal clearance is the primary mechanism of elimination in animals and humans with intact renal function [5, 7], dialytic clearance was expected to be the main elimination pathway in patients with limited or no kidney function undergoing hemodialysis. In previous clinical studies [8, 10, 11], the duration of pharmacokinetic data collections was limited and the effect of hemodialysis on the pharmacokinetics of etelcalcetide was unknown.

This study was conducted to elucidate the pharmacokinetics, biotransformation, and excretion of etelcalcetide in patients with CKD on hemodialysis. A therapeutic dose was administered that contained a microtracer quantity of [^{14}C]etelcalcetide in order to limit patient exposure to C-14 and to facilitate study conduct in a dialysis clinic. The microtracer approach necessitated using accelerator mass spectrometry (AMS) to achieve the sensitivity required for quantification of the low concentrations of radioactivity excreted in the large volumes of dialysate and other biomatrices over the long study duration.

2 Materials and Methods

2.1 Radiolabeled Drug and Formulation

[^{14}C]etelcalcetide drug substance (Fig. 1; hydrochloride salt; specific activity 70.9 nCi/mg of etelcalcetide freebase; high-performance liquid chromatography [LC] purity 99.5 %) was synthesized at Amgen Inc. (Thousand Oaks, CA, USA). The C-14 label was placed on the carbonyl carbon of the *N*-acetyl moiety in the D-amino acid backbone and the stability of the radiolabel at this position was demonstrated in nonclinical studies [5]. The drug substance was formulated as a single 10 mg dose of etelcalcetide with 26.3 kBq (710 nCi; approximately 10 µg) of [^{14}C]etelcalcetide in 2 mL liquid solution for bolus intravenous administration at the end of hemodialysis. The formulation was a sterile, preservative-free, aqueous solution containing 0.85 % sodium chloride, and 10 mM succinic acid, in a single-use 5 mL type 1 glass vial. The drug product vial for each patient was prepared at the clinical site by combining the drug substance in the liquid formulation and contained 4 mL of clear, colorless solution with AMG 416 concentration of 5 mg/mL at a final pH of 3.3.

2.2 Study Design and Objectives

This phase I, open-label study (Fig. 2) was conducted at a single center in the US (DaVita Clinical Research, Minneapolis, MN, USA) in compliance with the International Conference on Harmonisation Tripartite Guideline on
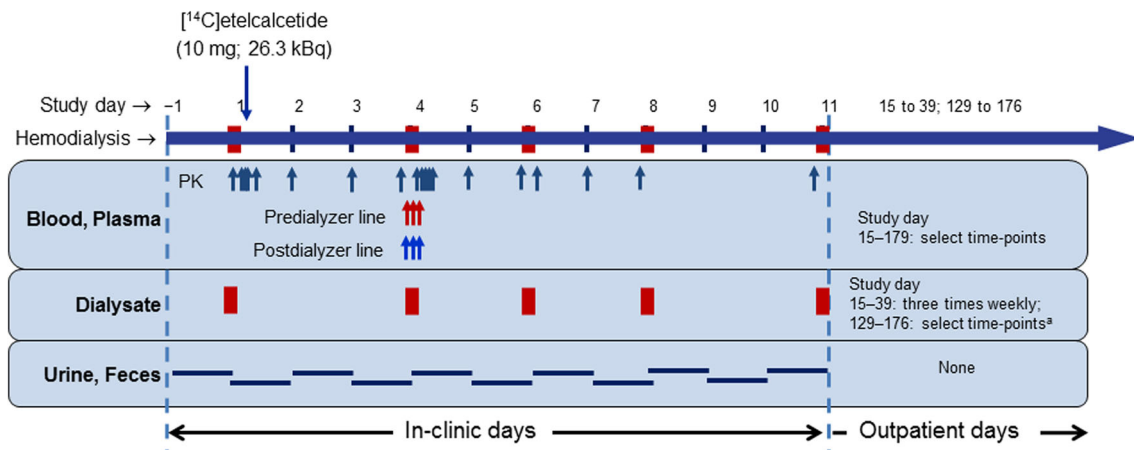
Fig. 2 Study scheme. Pharmacokinetic sampling was performed using the patient's nonhemodialysis arm at predose, 0.17, 1, 4, 8, 24, 48, 68, 72, 72.17, 73, 76, 80, 96, 116, 120, 144, 168, 192, 216, 236, 240, 332, 500, 668, and 908 h postdose. Predialyzer (arterial) and postdialyzer (venous) blood samples were withdrawn from the respective hemodialysis lines during dialysis on study day 4 at approximately 10 min, 2 h (midpoint), and the end of dialysis (range 3–4 h). Pharmacokinetic sampling was performed using the patient's nonhemodialysis arm after completion of the hemodialysis session on study day 4 at 0.17, 1, 4, and 8 h. Additional blood samples were collected for the extended pharmacokinetic collection period during study days 129–176. Serum for anti-etelcalcetide antibody analysis was collected on study days −1 and 39. Total dialysate was collected for each patient during the in-clinic period (study days 1–11) and on outpatient study days 14–39; a proportion of each patient's total dialysate was combined, and the pooled dialysate stored at −70 °C for C-14 analysis and biotransformation product profiling. Predose and postdose urine were collected if discharged on study days 1–11 in 24 h intervals, pooled, and stored at −70 °C for C-14 analysis and biotransformation product profiling. Predose and postdose feces were collected on study days 1–11, frozen at −70 °C, shipped to Covance Laboratories (Madison, WI, USA), combined by patient at 24 h intervals, and homogenized in ethanol/water (1:1 v/v); a portion was lyophilized and stored at room temperature for C-14 analysis, and a portion was stored at −70 °C for biotransformation product profiling. [a]Additional extended pharmacokinetic collection period (study days 129–176)

Good Clinical Practice. Patients provided written informed consent.

The specific objectives of the study were (i) to determine the rate, extent, and routes of radioactivity excretion of [14C]etelcalcetide in feces, dialysate, and urine over time; and (ii) to measure radioactivity concentrations in whole blood and plasma over time. Secondary objectives were to (i) measure etelcalcetide concentrations in plasma for comparison with total C-14 concentrations; (ii) evaluate the safety and tolerability of a single intravenous dose (10 mg) of etelcalcetide; and (iii) assess the effect of hemodialysis on the pharmacokinetics of etelcalcetide and total C-14.

2.2.1 Patient Selection

Six patients aged ≥18 years with stage 5 CKD on hemodialysis were enrolled in this study. Each patient had a stable dialysis prescription that was not anticipated to change significantly during the course of the study; a body mass index between 18 and 38 kg/m^2; corrected calcium >8.3 mg/dL; and PTH between 300 and 1200 pg/mL. Patients were excluded if they had received etelcalcetide or cinacalcet (a small molecule calcimimetic [12]) within 30 days. Patients were also excluded if any [14C]-labeled drug substance within 1 year to ensure that no residual drug-related C-14 is present in the samples from these subjects, which may confound the results obtained through AMS analysis.

2.2.2 Study Treatment

Patients were resident in the clinical study site for 12 days (i.e. study day −1 until completion of procedures on study day 11) and underwent hemodialysis three times per week for the duration of the study (Fig. 2). The hemodialysis time was fixed as per the patient prescription and its duration ranged from 3 to 4 h across the study subjects. All patients used the same dialyzer model (Optiflux® F180NR; Fresenius Medical Care, Waltham, MA, USA). At the end of hemodialysis on study day 1, patients received a single intravenous bolus dose of [14C]etelcalcetide into the arm opposite the arm used for hemodialysis, followed by a 5 mL saline flush.

2.3 Sample Collection

Blood, dialysate (entire amounts from each hemodialysis session), urine, feces from each discharge, and vomit from each emesis were collected (Fig. 2) and processed as described in the electronic supplementary methods. Blood samples were also collected from predialyzer and

postdialyzer (arterial and venous) lines during the hemodialysis session on study day 4 and after completion of hemodialysis. After the in-clinic period, patients returned to the site on an outpatient basis in accordance with their routine hemodialysis sessions up to study day 39. Four patients returned for two extended collection periods, each comprising three consecutive hemodialysis sessions (collection period 1: study days 129–148; collection period 2: approximately 1 month after collection period 1, corresponding to study days 157–176). Blood and dialysate were collected during each outpatient day visit. Blood samples were collected into potassium-EDTA (Fig. 2) for C-14 analysis, etelcalcetide and total M11 (TM11; described in Sect. 2.4) bioanalysis, and biotransformation product profiling. Blood, dialysate, urine, and feces were collected, processed, and stored for additional analyses as described (Fig. 2). Dialyzer cartridges were collected after each hemodialysis session for each patient. Serum for anti-etelcalcetide antibody analysis was collected on study days −1 and 39.

2.4 Bioanalysis of Etelcalcetide and TM11

Plasma concentrations of etelcalcetide and TM11 were determined by LC–tandem mass spectrometry (MS/MS) as described in the electronic supplementary methods. TM11 is the sulfhydryl (reduced) form of the D-amino acid peptide backbone in etelcalcetide [5].

2.5 Accelerator Mass Spectrometry Analysis

The C-14 content in whole blood, plasma, dialysate, urine, and fecal samples was determined by AMS at Xceleron Inc. (Germantown, MD, USA) following a graphitization procedure as described in the electronic supplementary methods.

2.6 Biotransformation Product Profiling

Radioprofiles and biotransformation product identification were performed on pooled samples of plasma, urine, and dialysate with and without tris(2-carboxyethyl phosphine) (TCEP) reduction as described in the electronic supplementary methods. Structure assignments of etelcalcetide and its biotransformation products were based on comparisons to known structures from nonclinical studies [5], authentic standards, calculations, and interpretations from high-resolution MS and LC–MS/MS data.

2.7 Pharmacokinetic Analysis

All patients who received a single dose of etelcalcetide were included in the pharmacokinetic analysis set.

Noncompartmental analyses were performed on individual plasma etelcalcetide, plasma C-14, and plasma TM11 concentration–time data from samples taken from the patient's nonhemodialysis arm during the nonhemodialysis period using Phoenix WinNonlin v.6.3 software on Citrix (Pharsight®, St. Louis, MO, USA). Mean (standard deviation) concentration–time profiles were generated using Phoenix WinNonlin v.6.3 (Pharsight®), as were all descriptive statistics. Pharmacokinetic parameters (e.g. maximum observed drug concentration [C_{max}], time to reach C_{max} [t_{max}], area under the plasma concentration–time curve [AUC], hemodialysis clearance [CL_{HD}], and extraction ratio [E_{HD}]) were estimated as described in the electronic supplementary methods.

2.8 Estimation of Carbon-14 Excretion on Nonsampled Days

The total C-14 recovery in dialysate over the period between day 39 and the start of the extended collection period was estimated by interpolation. The total C-14 excretion rate on nonsampled days was estimated with the assumption that the dialysis excretion rate was a first-order process (electronic supplementary Fig. 1). A straight line ($Y = \mathrm{slope} \cdot X + \mathrm{intercept}$) regression was applied to the log-transformed excretion rate (Y) versus time (X) profile. Regression analysis was applied for the measured data from study day 27 to the last time point; these correspond to six time points from study days 27 to 39, and six time points in the two extended pharmacokinetic collection periods.

The cumulative total C-14 excretion in urine and feces on nonsampled days (study days 11–176) was estimated for each patient by extrapolation using the following equations:

$$CE_{U,SD11\,to\,SD176} = \left(\frac{CE_{U,SD1\,to\,SD10}}{CE_{D,SD4\,to\,SD11}}\right) \times CE_{D,SD14\,to\,SD176}$$

$$CE_{F,SD11\,to\,SD176} = \left(\frac{CE_{F,SD1\,to\,SD10}}{CE_{D,SD4\,to\,SD11}}\right) \times CE_{D,SD14\,to\,SD176}$$

where CE is the cumulative excretion in respective matrices (U = urine, F = feces, D = dialysate), and SD is study day.

2.9 Safety Analysis

All patients in the study were included in the safety analysis set. Adverse events were classified according to the Medical Dictionary for Regulatory Activities version 17.0 [13], and graded according to the Common Terminology Criteria for Adverse Events version 4.0 [14]. Adverse events were reported through study day 39 or the last visit of the extended pharmacokinetic collection period.

2.10 Anti-Etelcalcetide Antibody Analysis

A validated biosensor immunoassay was used to screen for and confirm the presence of binding anti-etelcalcetide and anti-SAPC antibodies (electronic supplementary methods).

3 Results

3.1 Pharmacokinetic Analyses

Demographics of the six subjects are provided in Table 1. Mean concentration–time profiles of total C-14 in blood and plasma, etelcalcetide, and TM11 (in plasma) after a single intravenous bolus administration of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) to patients with CKD on hemodialysis are shown in Figs. 3a, b and 4. Etelcalcetide concentrations in plasma decreased during hemodialysis and rebounded slowly after the end of hemodialysis (Fig. 3a). Total C-14 concentration in blood was lower than that in plasma over the time course (Fig. 3a). Mean blood to plasma radioactivity concentration ratio was 0.6, indicating limited distribution of radioactivity into blood cells. Pharmacokinetic parameters during the nonhemodialysis period for total C-14, etelcalcetide, and TM11 in plasma are shown in Table 2.

Estimates of pharmacokinetic parameters during hemodialysis are summarized in Table 3. Arterial and venous plasma etelcalcetide concentrations were determined and used to calculate E_{HD} and CL_{HD} for etelcalcetide (mean E_{HD} 0.385; CL_{HD} 7660 mL/h). Total C-14 in blood and plasma, etelcalcetide in plasma, and TM11 in plasma from the predialyzer arterial line were greater than the corresponding concentrations in the postdialyzer venous line (Fig. 4). Changes in the arterial and venous concentrations of blood and plasma total C-14 and plasma TM11 were too small to determine their E_{HD} and CL_{HD}.

Table 1 Patient demographics

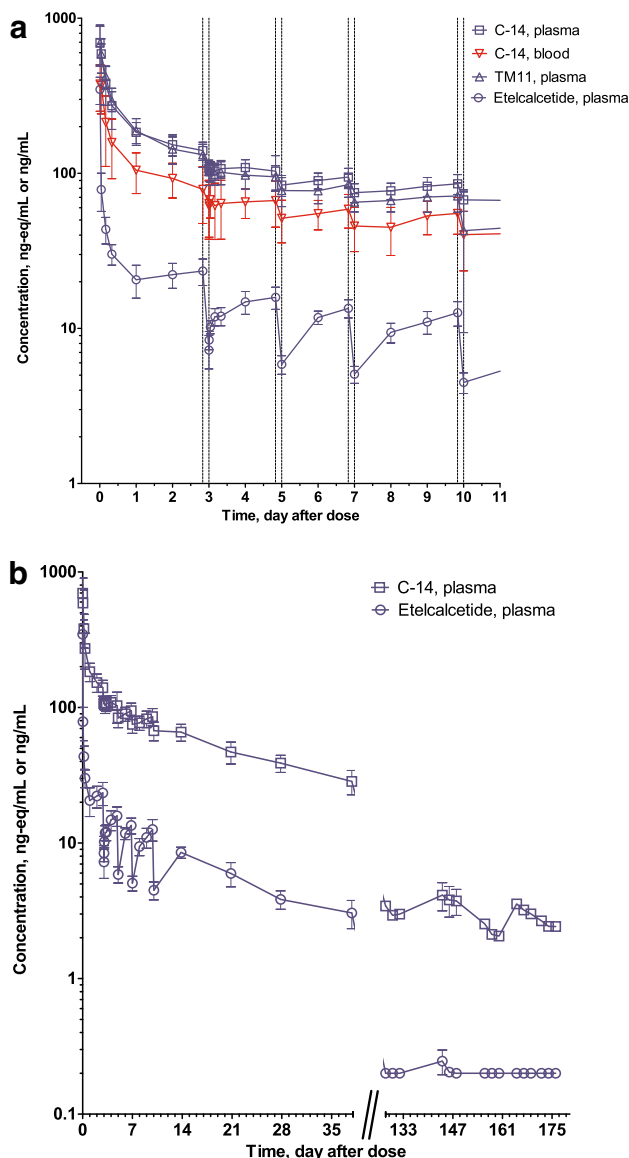| Demographic	[^{14}C]etelcalcetide [$N = 6$]
Sex, n (%)	
Male	3 (50.0)
Female	3 (50.0)
Median age, years (range)	62 (33–75)
Race, n (%)	
Black (or African American)	5 (83.3)
White	1 (16.7)
Median height (range), cm	171.9 (162–184)
Median weight (range), kg	91.3 (51.7–120.5)
Median body mass index (range), kg/m^2	32.3 (19.7–35.6)

Fig. 3 Mean (SD) concentration versus nominal time profiles of total C-14 in blood and plasma, etelcalcetide in plasma, and TM11 in plasma following a single intravenous bolus administration of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) to patients with CKD on hemodialysis (during nonhemodialysis periods). **a** Predose to 10 days postdose (study days 1–11). **b** Predose to 175 days postdose (study days 1–39 and 129–176). Each pair of *vertical dashed lines* represents a hemodialysis period. *C-14* [^{14}C]etelcalcetide-derived radioactivity, *CKD* chronic kidney disease, *SD* standard deviation, *TM11* total M11

Etelcalcetide concentrations in plasma were lower than the corresponding concentration of total C-14 in plasma, indicating the presence of circulating biotransformation products (Fig. 3a, b). TM11 concentrations in plasma were similar to the concentrations of total C-14 in plasma. A regression analysis of the pooled data in which TM11 and total C-14 concentrations were available for a given time point across all patients demonstrated a goodness of fit (R^2)

Fig. 4 Mean (SD) concentration versus nominal time profiles of total C-14 in plasma, and etelcalcetide in plasma during the first hemodialysis period postdose on study day 4 following a single intravenous bolus administration of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) to patients with CKD on hemodialysis. *Vertical dashed lines* represent the hemodialysis period. *C-14* [^{14}C]etelcalcetide-derived radioactivity, *CKD* chronic kidney disease, *HD* hemodialysis, *SD* standard deviation
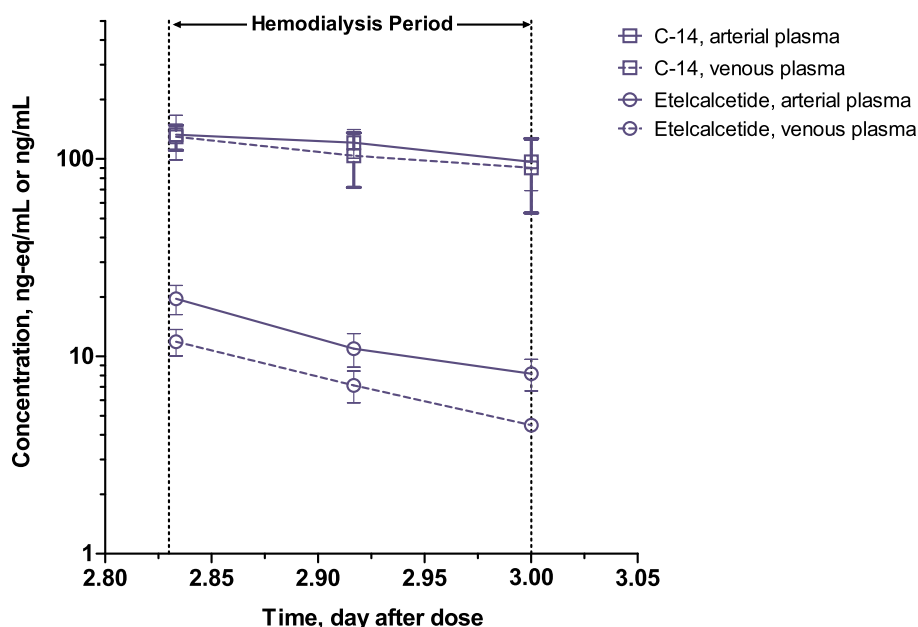

Table 2 Summary of pharmacokinetic parameters during the nonhemodialysis period for analytes total C-14, etelcalcetide, and total M11 in plasma after a single intravenous bolus administration of [^{14}C] etelcalcetide (10 mg; 26.3 kBq) in patients with CKD on hemodialysis

	Total C-14			Etelcalcetide			Total M11		
	n	Mean (SD)	CV%	n	Mean (SD)	CV%	n	Mean (SD)	CV%
t_{max}, minute[a]	6	10 (10–12)	NR	6	10 (10–11)	NR	6	10 (10–60)	NR
C_{max}[b]	6	697 (207)	29.7	6	347 (69.5)	20.0	6	705 (172)	24.4
t_{last}, day[a]	6	164 (37.6–174)	NR	6	37.8 (37.6–145)	NR	6	13.8 (9.8–20.7)	NR
C_{last}[a,b]	6	3.01 (2.06–24.7)	NR	6	2.50 (0.210–2.80)	NR	6	57.9 (50.8–62.7)	NR
$t_{1/2,z}$, day	4	35.9 (2.99)	8.3	2	NR (NR)	NR	0	NR (NR)	NR
AUC_{3d}[b]	6	585 (98.8)	16.9	6	81.6 (8.4)	10.3	6	590 (101)	17.1
AUC_{10d}[b]	6	1220 (172)	14.1	6	160 (16.9)	10.6	6	1150 (190)	16.4
Ratio to ^{14}C									
$\quad AUC_{3d}$	NR	NR (NR)	NR	6	0.142 (0.0219)	15.4	6	1.01 (0.124)	12.2
$\quad AUC_{10d}$	NR	NR (NR)	NR	6	0.132 (0.0151)	11.5	6	0.947 (0.0973)	10.3

Values were rounded to three significant figures, except for CV% and t_{max}, which are reported to one decimal place and two significant figures, respectively

AUC area under the plasma concentration–time curve, AUC_{3d} AUC from time zero to 3 days (68 h) postdose, AUC_{10d} AUC from time zero to 10 days (240 h) postdose, *CKD* chronic kidney disease, C_{last} last observed plasma concentration, C_{max} maximum observed drug concentration, *CV* coefficient of variation, *NR* not reported, *SD* standard deviation, t_{last} time C_{last} was observed, t_{max} time to reach C_{max}, *total C-14* [^{14}C]etelcalcetide-derived radioactivity, $t_{1/2,z}$ apparent terminal half-life

[a] Median (range) values are provided for t_{max}, t_{last}, and C_{last}

[b] The total C-14 parameter values are reported in ng-eq/mL (C_{max} and C_{last}) and day·ng-eq/mL (AUC_{3d} and AUC_{10d}), whereas plasma etelcalcetide parameter values are reported in ng/mL (C_{max} and C_{last}) and day·ng/mL (AUC_{3d} and AUC_{10d})

value of 0.97. Plasma AUC ratios between TM11 and total C-14 were also close to unity (Table 2), indicating that all [^{14}C]-labeled components were quantitatively converted to TM11 by chemical reduction; therefore, the D-amino acid backbone in etelcalcetide was predominantly intact in the C-14 products present in plasma.

3.2 Excretion

The estimated overall mean recovery, the mean sum of measured recovery on sampled days and estimated recovery on nonsampled days (Sect. 2.8), of radioactivity was 67.2 % after 175 days (Table 4). Hemodialysis was the

Table 3 Summary of pharmacokinetic parameter values during hemodialysis for etelcalcetide in arterial and venous plasma after a single intravenous bolus administration of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) in patients with CKD during the first hemodialysis period postdose on study day 4

	Analyte etelcalcetide [$N = 6$]	
	Mean (SD)	CV%
$AUC_{A,HD}$, ng·day/mL	1.79 (0.467)	26.1
$AUC_{V,HD}$, ng·day/mL	1.09 (0.264)	24.2
E_{HD}	0.385 (0.0679)	17.6
Q_B, mL/min	533 (51.6)	9.7
HCT	37.3 (5.33)	14.3
Q_P, mL/min	333 (31.3)	9.4
CL_{HD}, mL/h	7660 (1380)	18.0

Values were rounded to three significant figures, except for CV%, which is reported to one decimal place

$AUC_{A,HD}$ area under the arterial plasma concentration–time curve obtained during the hemodialysis session on study day 4, $AUC_{V,HD}$ area under the venous plasma concentration–time curve obtained during the hemodialysis session on study day 4, *CKD* chronic kidney disease, CL_{HD} hemodialysis clearance, *CV* coefficient of variation, E_{HD} hemodialysis extraction ratio, *HCT* hematocrit; Q_B blood flow rate through the dialyzer during hemodialysis, Q_P plasma flow rate through the dialyzer during hemodialysis, *SD* standard deviation

Table 4 Cumulative excretion of radioactivity following administration of a single intravenous dose of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) in patients with CKD on hemodialysis

	Estimated cumulative recovery from study days 1–176, mean (SD) % Administered C-14 dose[a]
Dialysate	
Measured	35.29 (4.17)
Estimated	24.26 (2.71)
Urine	
Measured	0.75 (1.13)
Estimated	2.40 (3.79)
Feces	
Measured	1.17 (0.13)
Estimated	3.34 (0.57)
Total	67.19 (1.37)

CKD chronic kidney disease, *SD* standard deviation

[a] Mean and SD values computed with values from four patients who completed the extended pharmacokinetic collection period

predominant elimination pathway (Table 4; Fig. 5); an estimated mean of 59.6 % of the administered dose (88.7 % of recovered dose) was eliminated in dialysate. The mean total C-14 elimination $t_{1/2}$ was similar for dialysate (40.2 days) and plasma (35.9 days). A small proportion of the dose was excreted in urine (3.2 % of administered dose) and feces (4.5 % of administered dose). Radioactivity was below the lower limit of quantification in the sole vomit sample. Etelcalcetide and its biotransformation products did not bind to an appreciable extent to the dialyzer membrane and cartridge. Only trace levels of radioactivity (0.005 % of administered dose) were recovered in extracts from the dialyzer used in the first dialysis session; thus, dialyzers were not further analyzed.

3.3 Biotransformation

Radiochromatograms of pooled plasma, urine, and dialysate samples are shown in Fig. 6 and electronic supplementary Fig. 2, while radiochromatograms of TCEP-treated plasma, urine, and dialysate samples are shown in electronic supplementary Fig. 3. MS and MS/MS fragmentation data for etelcalcetide and its biotransformation products are presented in Table 5 and were used to develop the etelcalcetide biotransformation scheme shown in Fig. 7.

In plasma, nearly all of the C-14 chromatogram peaks (Fig. 6a; Table 6) were identifiable in the LC-high-resolution mass spectrometry (HRMS) profile (Table 5). Etelcalcetide biotransformation resulted predominantly from disulfide exchange. In plasma from the first interdialytic period after dosing (AUC_{68hr}), intact etelcalcetide accounted for approximately 17 % of the total C-14 AUC_{3d} after dose administration and before the first postdose dialysis session. Covalent protein conjugates, presumed to be SAPC based on nonclinical characterization [5], were the most abundant biotransformation product in plasma (73 % of the total radioactivity in the pooled plasma) (Table 6). SAPC formation in plasma was confirmed by LC-HRMS analysis of the Lys-C digest of SAPC. Other biotransformation products were each <4 % of the total C-14 AUC_{68hr}. In plasma from the second interdialytic period ($AUC_{72–116h}$) (Fig. 6b), intact etelcalcetide accounted for approximately 13 % of the total C-14 AUC; covalent protein conjugates were the most abundant biotransformation product in plasma (83 % of the total radioactivity); other biotransformation products were each <2 % of the total C-14 $AUC_{72–116h}$. No desacetylated biotransformation product or the corresponding reduced form, both leading to loss of the radiolabel, was observed in plasma or TCEP-reduced plasma. TCEP reduction of the plasma resulted in formation of, predominantly, a single TM11 peak (84 %) (Table 7).

In dialysate (Fig. 6c), the biotransformation product profile consisted of all components observed in plasma, except the protein conjugates. TCEP reduction of the dialysate resulted in a major TM11 peak (77.8 %) and a minor amount of reduced M5 (1.1 %) (Table 7). Several

Fig. 5 Cumulative recovery of radioactivity following administration of a single intravenous dose of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) to patients with CKD on hemodialysis ($n = 6$). Excretion in **a** dialysate; **b** urine; and **c** feces. *CKD* chronic kidney disease, *PK* pharmacokinetic
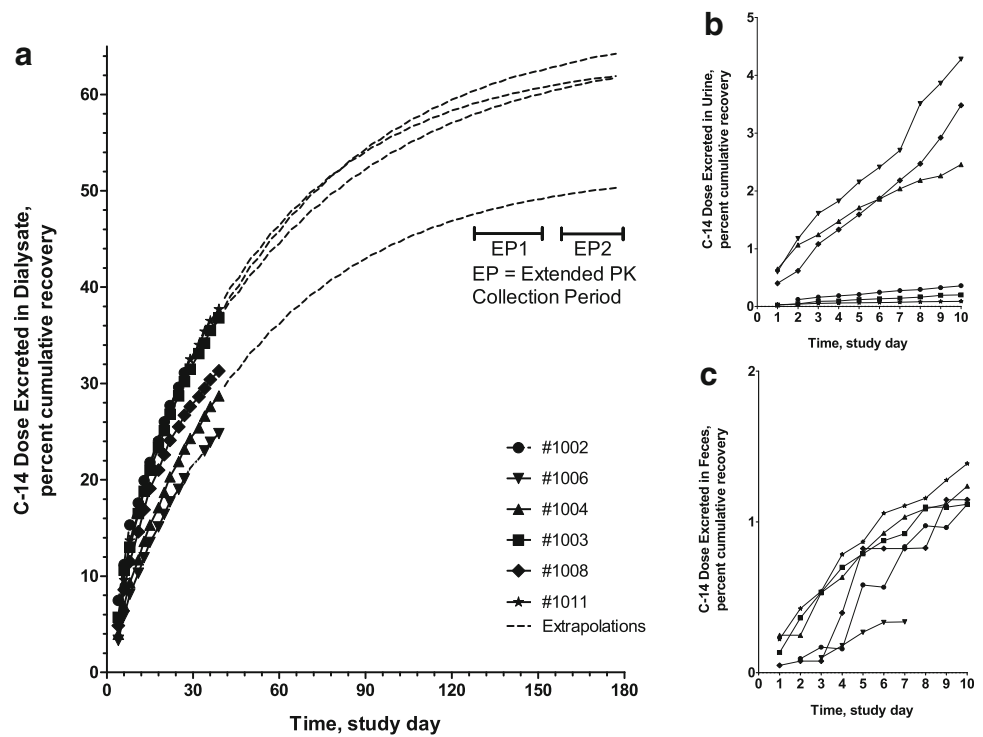

Fig. 6 HPLC radiochromatogram of **a** AUC pooled plasma (0–68 h); **b** AUC pooled plasma (approximately 72–116 h); **c** pooled dialysate from the first hemodialysis session following a single intravenous dose of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) to patients with CKD on hemodialysis. *AUC* area under the plasma concentration–time curve, *CKD* chronic kidney disease, *dpm* disintegrations per minute, *HPLC* high-performance liquid chromatography, *SAPC* serum albumin peptide conjugate
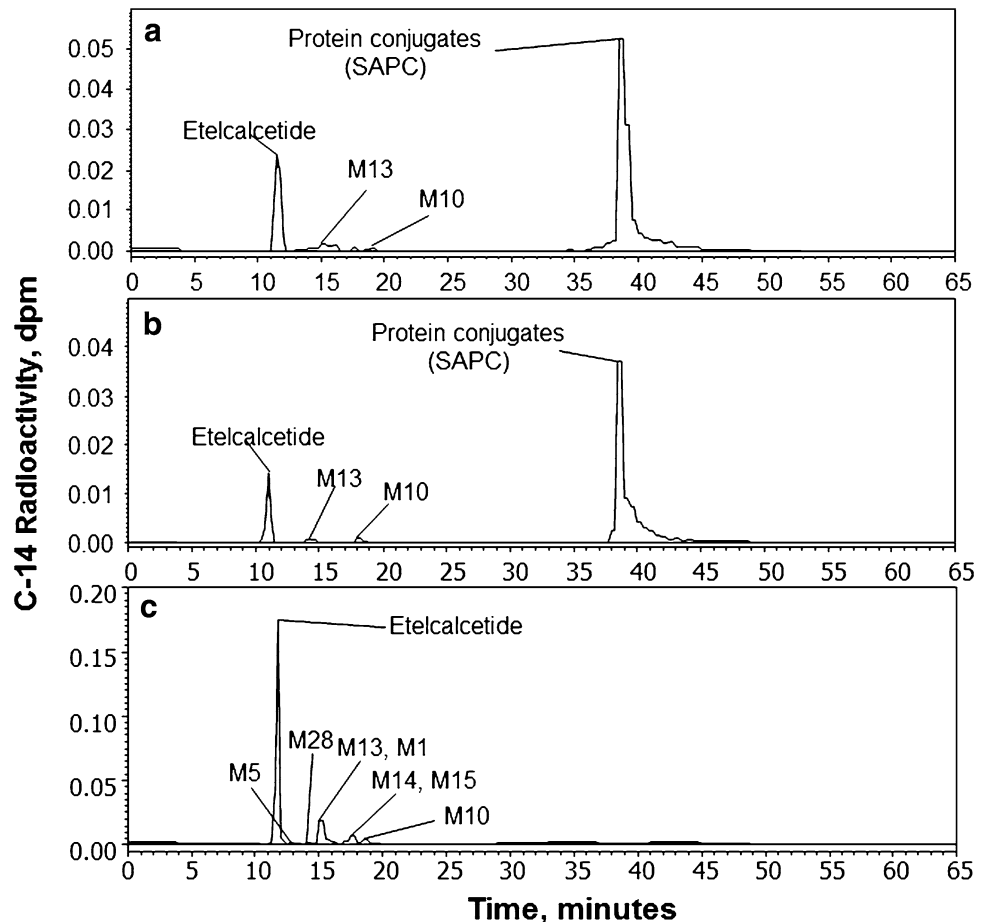
Table 5 Summary of representative LC-HRMS data for etelcalcetide and its biotransformation products

Component	RT (min)	Calculated m/z $(2+)$[e]	Observed m/z $(2+)$[e]	Difference (ppm)	Product ion data
Etelcalcetide[a]	12.40	524.7718	524.7707	−2.1	448, 464, 481
SAPC[b]	35.14	672.7604	672.7707	−15.3	299, 448, 714, 742, 770, 795
M1[a]	16.62	505.2482	505.2482	0	448, 464
M2a[a]	26.28	NA	617.2879	NA	448, 465, 481
M2b[a]	27.74	NA	617.2866	NA	448, 465, 481
M3[a]	28.02	638.8091	638.8088	−0.5	448, 465, 481
M4[a]	29.69	574.7798	574.7802	0.7	448, 464, 481, 524
M5[a]	13.58	525.2638	525.2630	−1.5	448, 464, 481
M7[a]	18.71	698.8303	698.8295	−1.1	448, 464, 481, 617
M9[a]	22.85	NA	560.7634	NA	448, 464, 481
M10[c]	20.45	617.8038	617.8073	5.7	448, 465, 481
M11[d]	20.41	465.2698	465.2716	3.9	448
M13[a]	16.29	553.2826	553.2825	−0.2	448, 464
M14[a]	18.18	531.7796	531.7789	−1.3	448, 465
M15[c]	19.47	589.2931	589.2968	6.3	448, 464, 481, 524
M28[a]	14.64	489.2621	489.2620	−0.2	NA
M29[a]	27.59	545.7771	545.7767	−0.7	448, 464, 481

NA not available, *LC* liquid chromatography, *HRMS* high-resolution mass spectrometry, *m/z* mass-to-charge ratio, *ppm* parts per million, *RT* retention time, *SAPC* serum albumin peptide conjugate, *TCEP* tris(2-carboxyethyl phosphine)

[a] From human urine data

[b] From Lys-C digest of SAPC

[c] From human dialysate data

[d] From TCEP-treated human urine data

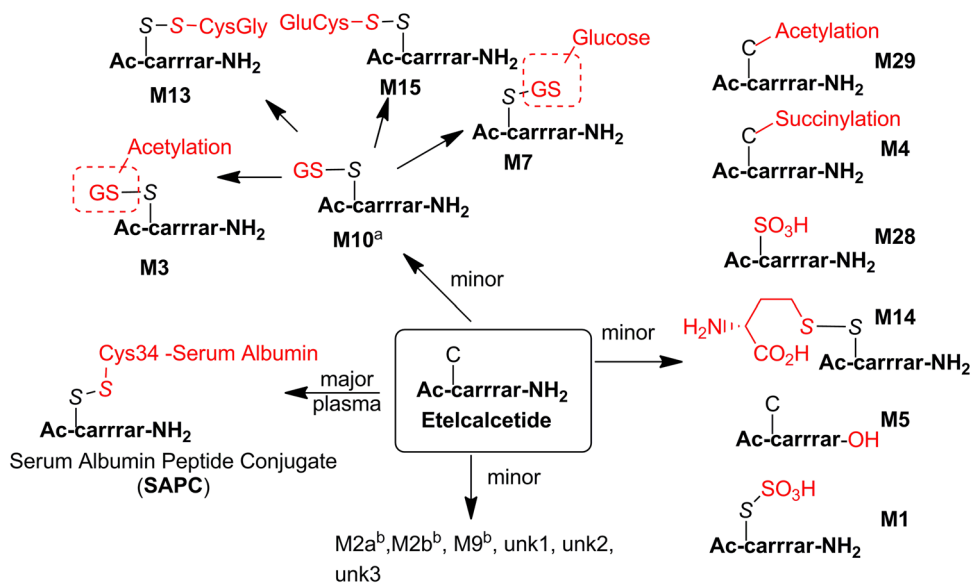[e] Charge state of the precursor molecular ion except the m/z for SAPC peptide was from a 5+ charge state

Fig. 7 Proposed biotransformation scheme for [^{14}C]etelcalcetide in humans. *Lower* and *upper case single-letter* amino acid abbreviations refer to D- and L-amino acids, respectively. *G* L-glutathione, minor each <5 % of C-14 dose (urine) or C-14 chromatogram (plasma), *m/z* mass-to-charge ratio. [a]The structure was confirmed by an authentic standard. [b]The structure has an intact peptide backbone but the exact modification could not be determined. The observed doubly charged m/z of ^{12}C-precursor for M2a and M2b and M9 were 617.2879, 617.2866, and 560.7634, respectively. The mass shifts for these products were 185.0322, 185.0296, and 71.9832 Da, respectively. These values were not available for unknowns 1, 2, and 3

Table 6 Summary of biotransformation product profile in plasma, dialysate, and urine following intravenous administration of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) in patients with CKD on hemodialysis

Name	Proposed Structure	% C-14 Chromatogram[c]		
		Plasma[d]	Dialysate[e]	Urine[f]
Etelcalcetide	L-cysteine disulfide	17.4	48.0	22.3
M1[a]	Thiosulfate disulfide	ND	10.3	5.5
M13[a]	L-cysteinyl-L-glycine disulfide	1.4		
M2a	m/z 617.28[b]	ND	ND	4.1
M2b[a]	m/z 617.28[b]	ND	ND	2.5
M29[a]	Acetylated etelcalcetide	ND	[g]	
M3	Acetylated M10	ND	ND	1.5
M4	Succinylated etelcalcetide	ND	ND	2.3
M5	Des-amido etelcalcetide	ND	0.4	1.8
M7[a]	Glucose conjugate of M10	ND	ND	4.7
M14[a]	L-homocysteine disulfide	ND	4.0	
M15[a]	γ-glutamic acid-L-cysteinyl disulfide	ND		ND
M9	m/z 560.76[b]	ND	[g]	3.4
M10	L-glutathione disulfide	0.5	2.1	ND
M28	Thiosulfate	ND	0.8	1.1
Protein conjugates	SAPC[g]	72.9	ND	ND
Unk1	NA	ND	ND	6.9
Unk2	NA	ND	ND	1.6
Unk3	NA	ND	ND	28.6

CKD chronic kidney disease, *m/z* mass-to-charge ratio, *NA* not applicable, *ND* not detected, *SAPC* serum albumin peptide conjugate, *Unk1, Unk2, Unk3* unknown peaks

[a] Biotransformation products were co-eluted

[b] The precursor ion was detected by mass spectrometry; D-amino acid back bone was intact, but the structure could not be determined

[c] % C-14 chromatogram = peak area of etelcalcetide or its biotransformation product divided by the total peak area of all etelcalcetide-related components

[d] AUC$_{68h}$ pool (see sample pooling in electronic supplementary methods). Each unidentified component was <4 % in C-14 chromatogram

[e] Day 4 pool (see sample pooling in electronic supplementary methods). Each unidentified component was <7 % in C-14 chromatogram

[f] Day 1–10 pool (see sample pooling in electronic supplementary methods). Each unidentified component was <3 % in C-14 chromatogram

[g] Peak characterized by liquid chromatography/high-resolution mass spectrometry analysis. SAPC was inferred to be the predominant component in the protein conjugates based on in vitro characterization

Table 7 Summary of biotransformation product profile in TCEP-treated plasma, dialysate, and urine following intravenous administration of [^{14}C]etelcalcetide (10 mg; 26.3 kBq) in patients with CKD on hemodialysis

Name	Proposed structure	% C-14 chromatogram[a]		
		Plasma[b]	Dialysate[b]	Urine[b]
TM11	Sulfhydryl (reduced form) of etelcalcetide backbone	83.8	77.8	67.4
M5-DesCys	Des-amido M11	1.4	1.1	3.2

CKD chronic kidney disease, *TCEP* tris(2-carboxyethyl phosphine)

[a] % C-14 chromatogram = peak area of the etelcalcetide or its biotransformation product divided by the total peak area of all drug-related components

[b] Each unidentified component was <5 % in C-14 chromatogram

unidentified peaks were observed in the pooled day 4 dialysate before and after TCEP, each individually <7 and <5 %, respectively. No desacetylated biotransformation product or the corresponding reduced form was observed in dialysate or TCEP-reduced dialysate. In urine, the most abundant component was intact etelcalcetide (approximately 25 % of the ^{14}C-chromatogram). In addition to etelcalcetide, 12 known biotransformation products were detected in urine (electronic supplementary Fig. 2). Peaks from unidentified components were each <1 % of the administered dose. TCEP reduction of the urine resulted in formation of, predominantly, a single TM11 peak (67.4 %) (Table 7). Several unidentified peaks were observed in the TCEP-reduced urine, each individually <5 % of the ^{14}C-chromatogram. No desacetylated biotransformation product or its reduced form was observed in urine or TCEP-reduced urine. Feces were not profiled for biotransformation products because of the low amount of fecally excreted radioactivity. Cumulative excretion ratios in urine and feces on nonsampled days were estimated for each patient (electronic supplementary Table 1).

3.4 Safety

Treatment-emergent adverse events occurred in five patients and included constipation (n = 2), contact dermatitis, flatulence, nausea, pneumonia, upper respiratory tract infection, and vomiting (each n = 1); events were mild in intensity, with the exception of one serious adverse event of severe pneumonia. Of these, only constipation and flatulence were considered treatment-emergent adverse events.

3.5 Anti-Etelcalcetide Antibodies

No antibodies against either etelcalcetide or SAPC were detected in six predose and six postdose (study day 39) samples.

4 Discussion

Etelcalcetide is a novel synthetic peptide composed almost entirely of D-amino acids. Minimal literature is available on the human disposition of peptides predominantly composed of D-amino acids, since there is no approved drug in this chemical class. The objective of this study was to characterize the pharmacokinetics, biotransformation, and excretion following a single microtracer intravenous dose of [^{14}C]etelcalcetide to patients with CKD on hemodialysis. Higher quantities of C-14 more typical for human drug disposition studies were considered but not pursued because of the difficulty in controlling radioactivity contamination if hospitalizations were required during the study. One patient required hospitalization, and microtracer labeling obviated the need for contamination controls. The microtracer approach required the use of AMS for C-14 measurement in biofluids and dialysate. Remarkable detection limits were enabled by AMS, with C-14 concentrations of approximately 0.25 mBq (approximately 6.7 fCi/g) of ^{14}C (approximately 0.06 ng equivalents of etelcalcetide/g) quantified in an approximately 0.35 g dialysate sample prepared from approximately 150 kg of dialysate collected on study day 176.

Nonclinical investigations indicated that etelcalcetide and its biotransformation products exist in a dynamic equilibrium in blood [9]. Etelcalcetide undergoes biotransformation to form conjugates by disulfide exchange with endogenous thiols present in blood [5, 9]. These products were previously observed in vitro (human and rat) and in vivo (rat) [5]. In rats, a majority of etelcalcetide-related moieties in circulation existed in the form of covalent protein conjugates inferred to be SAPC. SAPC was characterized in plasma following in vitro incubations of etelcalcetide in whole blood; it was demonstrated to be a disulfide conjugation of the etelcalcetide D-amino acid backbone to the L-cysteine 34 of serum albumin [5].

In vitro kinetic studies showed that under the conditions tested, the rate of etelcalcetide CL_{HD} was >16-fold faster than its rate of conversion to SAPC, and the rate of conversion of SAPC to etelcalcetide was approximately 200-fold slower than the dialytic rate constant [9]. Re-equilibration to form etelcalcetide from SAPC occurred at a slow rate compared with etelcalcetide elimination by dialysis [9]. Formation of etelcalcetide from SAPC is possible because of abundant quantities of L-cysteine present in plasma [13].

Based on the nonclinical findings, we hypothesized that etelcalcetide biotransformation in humans would predominately result from disulfide exchange [5]. Moreover, with a dialyzer molecular weight cutoff of 10 kDa, we hypothesized that in patients with CKD on hemodialysis the drug-related species that would be eliminated during dialysis would be parent etelcalcetide and low-molecular-weight biotransformation products. Little change in total C-14 was expected during dialysis due to restricted dialysis of SAPC and the slow rate at which SAPC converts back to etelcalcetide or to any other low-molecular-weight products. Finally, we anticipated that upon cessation of dialysis, re-equilibration would occur and partially restore plasma etelcalcetide concentrations. To test these hypotheses, drug-derived products in plasma and dialysate were characterized and the pharmacokinetics of total C-14 and etelcalcetide in blood and plasma were determined. In addition, the dialyzer input and output blood was sampled during the first dialysis session, and intensive blood sampling was conducted after dialysis cessation.

The shape of the pharmacokinetic profiles of etelcalcetide and total C-14 were similar. The concentration–time courses of plasma etelcalcetide and SAPC (inferred from the total C-14 pharmacokinetic profile) outside the hemodialysis periods paralleled each other. In general, the SAPC concentration was approximately fivefold higher than the etelcalcetide concentration. The etelcalcetide to SAPC ratio decreased during the hemodialysis session when etelcalcetide and other low-molecular-weight biotransformation products were cleared. After dialysis, a partial restoration of circulating etelcalcetide concentrations in plasma occurred (Fig. 6b). These observations were consistent with the hypothesis that most of the radiolabeled, etelcalcetide-related species maintain a state of dynamic equilibrium with etelcalcetide, and that re-equilibration occurs following dialytic removal of etelcalcetide. This was further corroborated by direct determination of etelcalcetide and total C-14 clearance by dialysis, as measured by their respective concentrations entering and exiting the dialyzer during hemodialysis (Fig. 4). Only a small proportion (approximately 5 %) of the total C-14 that entered the dialyzer was removed. The nonclinical characterization of SAPC [5], the partial characterization of

SAPC in these patients with CKD, and the near quantitative conversion of total C-14 to TM11 in plasma, are all consistent with a presumption that most of the total C-14 present in blood was in the form of SAPC. Accordingly, little removal of total C-14 occurred because SAPC has a molecular weight of approximately 67 kDa, which is well above the dialyzer cutoff and thus prevented its removal. In contrast to limited removal of total C-14, approximately 50 % of the etelcalcetide that entered the dialyzer was removed, which was consistent with the proportion of unbound etelcalcetide in plasma. Etelcalcetide noncovalent binding to plasma proteins (fraction unbound) in patients with CKD was 0.59 [5]. These findings and the equilibrium that exists are consistent with the high volume of distribution for etelcalcetide reported in a previous population pharmacokinetic analysis [10], which can be attributed in part to reversible disulfide exchange of the etelcalcetide D-amino acid backbone between all available endogenous thiols and the L-cysteine readily available in plasma.

Recovery of total C-14 was estimated to be 67 % at study termination (day 176). The recovery is an estimate because the dose excreted in dialysate had to be partially interpolated. In the later part of the study, patients were released from the clinic and received some hemodialyses in settings where radioactivity was not quantified. The majority of the radioactivity was directly quantified. The predominant clearance pathway of total C-14 was hemodialysis, with the balance recovered in urine and feces. The parent drug was the predominant species present in dialysate and urine. Approximately 60 % of the [14C]etelcalcetide dose (approximately 89 % of total C-14 eliminated) was removed by dialysis as the parent drug. The radiolabeled species in feces was not determined because of the relatively small quantities of total C-14 present. The lack of higher recovery is likely due to the long C-14 elimination half-life in patients with stage 5 CKD. Extension of the study to increase recovery of the C-14 dose was not feasible. The recovery of thiol-containing drugs in disposition studies is generally low [15], particularly in patients undergoing hemodialysis for thiol-containing drugs normally cleared in the urine. For example, recovery of captopril, an angiotensin-converting enzyme inhibitor, was inversely proportional to the stage of renal impairment [16]. Compliance with sample collection in this study was high; only two dialyses were missed in one patient due to hospitalization. Loss of C-14 as carbon dioxide was unlikely because there was no evidence of acetyl moiety loss in any etelcalcetide biotransformation products, and <0.2 % of an [acetyl-14C]etelcalcetide dose in rats was recovered in expired air [5].

Previous in vitro assessments demonstrated that etelcalcetide was unlikely to be metabolized by cytochromes P450 (CYP450) or peptidases, or subject to

excretion by transporters [5]. The biotransformation products detected in this study confirmed etelcalcetide was not metabolized by CYP450 or peptidases, little or no direct excretion of the drug occurred, and biotransformation occurred by disulfide exchange.

No unanticipated safety events were detected in this study. Antibody formation has the potential to alter the exposure and safety profile of therapeutics with a molecular weight <10 kDa [17, 18]. No anti-etelcalcetide antibodies were detected in serum using an assay designed to detect etelcalcetide or SAPC-reactive antibodies.

5 Conclusions

The current study provided insight into the complex pharmacokinetics of etelcalcetide that was not available from previous clinical studies. The majority of radioactivity (67 %) that was eliminated from a single dose of [^{14}C]-labeled etelcalcetide 176 days after administration was removed by hemodialysis. Biotransformation was by disulfide exchange with endogenous thiols, and no alteration of the D-amino acid backbone was detected. Most of the circulating etelcalcetide-related biotransformed moieties existed as SAPC. After removal of plasma etelcalcetide by hemodialysis, re-equilibration between SAPC and L-cysteine in blood resulted in partial restoration of etelcalcetide plasma concentrations between hemodialysis sessions.

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