



Determination of Etelcalcetide Biotransformation and Hemodialysis Kinetics to Guide the Timing of Its Dosing

Katheryne Z. Edson¹, Benjamin M. Wu², Abhinaya Iyer¹, William Goodman³, Gary L. Skiles¹ and Raju Subramanian¹

¹Pharmacokinetics and Drug Metabolism, Amgen Inc., Thousand Oaks, California, USA; ²Clinical Pharmacology Modeling and Simulations, Amgen Inc., Thousand Oaks, California, USA; and ³Global Development, Amgen Inc., Thousand Oaks, California, USA

Introduction: Etelcalcetide, a novel calcimimetic agonist of the calcium-sensing receptor for treatment of secondary hyperparathyroidism in chronic kidney disease patients on hemodialysis, is a D-amino acid linear heptapeptide with a D-cysteine that is linked to an L-cysteine by a disulfide bond. In addition to binding to the calcium-sensing receptor, etelcalcetide is biotransformed by disulfide exchange in whole blood to predominantly form a covalent serum albumin peptide conjugate (SAPC). Key factors anticipated to affect the pharmacokinetics and disposition of etelcalcetide in chronic kidney disease patients on hemodialysis are the drug's intrinsic dialytic properties and biotransformation kinetics.

Methods: These factors were investigated using *in vitro* methods, and the findings were modeled to derive corresponding kinetic rate constants.

Results: Biotransformation was reversible after incubation of etelcalcetide or SAPC in human whole blood. The rate of SAPC formation from etelcalcetide was 18-fold faster than the reverse process. Clearance of etelcalcetide by hemodialysis was rapid in the absence of blood and when hemodialysis was initiated immediately after addition of etelcalcetide to blood. Preincubation of etelcalcetide in blood for 3 hours before hemodialysis resulted in formation of SAPC and decreased its clearance due to the slow rate of etelcalcetide formation from SAPC. Etelcalcetide hemodialysis clearance was >16-fold faster than its biotransformation.

Discussion: These results indicate that etelcalcetide should be administered after hemodialysis to avoid elimination of a significant fraction of the dose.

KI Reports (2016) 1, 24–33; <http://dx.doi.org/10.1016/j.ekir.2016.04.002>

KEYWORDS: calcimimetic; calcium-sensing receptor; disulfide bond; etelcalcetide; hemodialysis clearance; peptide agonist

© 2016 Amgen Inc. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Etelcalcetide (AMG 416, [Figure 1](#)) is a novel 8-amino acid peptide calcimimetic intended for treatment of secondary hyperparathyroidism in patients with chronic kidney disease (CKD) receiving hemodialysis treatment.^{1–4} Secondary hyperparathyroidism is a disorder in which the impairment of calcium, phosphate, and vitamin D homeostasis leads to excessive parathyroid hormone levels.⁵ Etelcalcetide covalently binds to and activates the calcium-sensing receptor of chief cells, thereby reducing parathyroid hormone secretion from the parathyroid gland.^{4,6} In clinical

studies, etelcalcetide resulted in rapid, sustained, dose-dependent reductions in serum parathyroid hormone.^{1,2}

Etelcalcetide is administered i.v. into the venous line of the hemodialysis blood circuit at the end of a dialysis session. After dose administration, etelcalcetide forms multiple products ([Figure 1](#)). Etelcalcetide is biotransformed in whole blood by disulfide exchange of the L-cysteine with other endogenous plasma thiols.⁷ A dynamic equilibrium between etelcalcetide and its biotransformation products is expected because of the reversible nature of the disulfide exchange reactions.⁸ The major biotransformation product formed in blood is a serum albumin peptide conjugate (SAPC) with a high molecular weight (67,365 Da) that is formed by covalent conjugation via the D-cysteine of etelcalcetide to the cysteine at amino acid 34 of human serum

Correspondence: Raju Subramanian, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, USA. E-mail: raju@amgen.com

Received 22 March 2016; revised 3 April 2016; accepted 8 April 2016; published online 21 April 2016

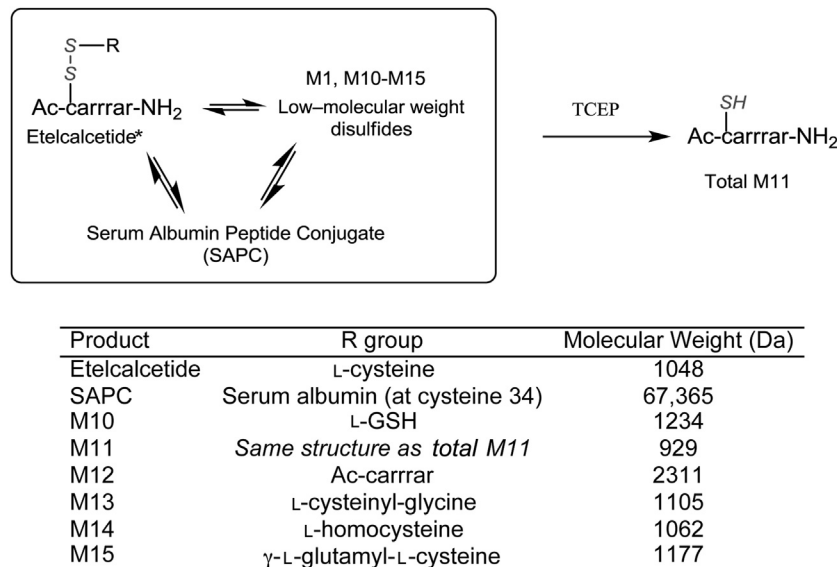


Figure 1. Biotransformation of etelcalcetide in whole blood. Products observed in plasma profiling are shown below; tris(2-carboxyethyl) phosphine (TCEP) reduction of plasma results in formation of total M11. The backbone remains intact in all biotransformation products. R-group moieties for etelcalcetide and biotransformation products are shown in the table. *Lower-case letters in “carrar” represent D-amino acids.

albumin.⁷ Minor biotransformation products of low molecular weight (<2000 Da) are formed with other plasma thiols such as L-glutathione and L-cysteinyl-glycine.⁷

Etelcalcetide is primarily eliminated by excretion in urine in healthy humans and rats with normal kidney function.^{2,7} In CKD patients on maintenance hemodialysis who have little or no residual kidney function, etelcalcetide is expected to be eliminated by hemodialysis. Modern high-flux clinical hemodialyzers remove small molecules such as urea (60 Da) and creatinine (113 Da) along with relatively small proteins (also called middle molecules) such as β_2 -microglobulin (11,900 Da), whereas larger proteins, such as serum albumin (~66,500 Da), are retained.^{9,10} Accordingly, dialysis is expected to result in the removal of the low-molecular weight biotransformation products of etelcalcetide, whereas SAPC would be retained because of its high molecular weight.

Published information about the pharmacokinetics and disposition of a small synthetic peptide, such as etelcalcetide, during hemodialysis is limited. Available evidence shows that the intrinsic dialytic properties of etelcalcetide and its biotransformation kinetics are likely to be the key determinants of etelcalcetide pharmacokinetics in CKD patients on hemodialysis. The objective of this study was to characterize these determinants using *in vitro* methods and model the resultant data to derive the kinetic rate constants of etelcalcetide dialysis and biotransformation. The findings were then useful to inform the timing of clinical dose administration.

MATERIALS AND METHODS

This section contains the key study methods. Full study methods including materials and bioanalysis are provided in the [Supplementary Methods](#) online.

Whole-Blood Incubations With Etelcalcetide and SAPC

Pooled whole blood from healthy volunteers ($n = 3$) or bovine blood was incubated in duplicate with either [¹⁴C]etelcalcetide (10 μ M, 0.3 μ Ci/ml), cold etelcalcetide (5 μ M), or SAPC (2.5 μ M) for up to 6 hours at 37 °C. Blood was centrifuged, and the resultant plasma was acidified with formic acid (1% vol/vol).

In Vitro Hemodialysis

The *in vitro* hemodialysis experimental setup is depicted in [Figure 2](#). *In vitro* hemodialysis experiments were performed under 3 experimental conditions using an Optiflux dialyzer.¹⁰ Peristaltic pump 1 moved the fluid (either dialysate or bovine blood; 0.5 liters) in reservoir A at 50 ml/min (Q_B) through the hemodialysis blood line tubing to the hollow fiber capillaries of the dialyzer. Peristaltic pump 2 moved dialysate (5 liters) from reservoir B at 250 ml/min (Q_D) to the dialyzer extracapillary space. The dialysate fluid in reservoir B was mixed with a magnetic stir bar, and the entire setup was maintained at ambient temperature (~22 °C).

Condition 1 assessed the intrinsic ability of etelcalcetide to cross the dialyzer membrane and determined whether it adhered to the dialyzer apparatus. No whole blood was used in this experiment.

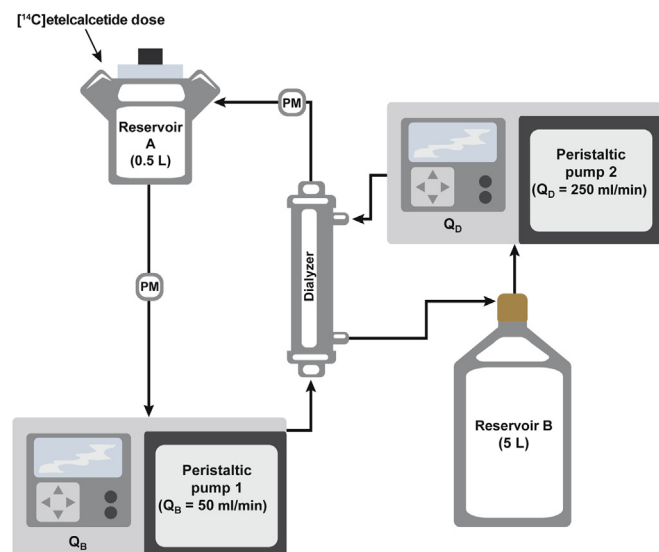


Figure 2. *In vitro* hemodialysis experimental setup. The dialyzer was operated in a closed-circuit loop. Reservoir A, also referred to as the “blood compartment,” contained 0.5 liters of either dialysate (for condition 1) or whole blood (for conditions 2 and 3), and the reservoir fluid was pumped by peristaltic pump 1 through the capillary bundle in the dialyzer at 50 ml/min (Q_B). Reservoir B, also referred to as the “dialysate compartment,” contained 5 liters of dialysate (in all experiments) that was pumped in a countercurrent direction by peristaltic pump 2 through the extracapillary space in the dialyzer at 250 ml/min (Q_D). PM, pressure monitor.

$[^{14}\text{C}]$ etelcalcetide and positive controls were added to reservoir A, and the pumps were started immediately. The spiked $[^{14}\text{C}]$ etelcalcetide dose was 1.1 μmol (3.15 μCi), resulting in a concentration of 2.1 μM (2.2 $\mu\text{g/ml}$). At each time point, aliquots were withdrawn from reservoirs A and B for radiometric analysis and measurement of etelcalcetide, urea, creatinine, and vitamin B₁₂. Samples for etelcalcetide measurement were acidified with formic acid (1% vol/vol). After the 2-hour hemodialysis procedure, the fluid in the dialyzer was drained, and then reservoir A was replaced with a bottle containing Solvable (PerkinElmer, Waltham, MA) (0.2 liters) to solubilize any etelcalcetide-related material nonspecifically bound to the dialyzer membrane. Solvable was circulated at 50 ml/min for 20 minutes, and samples were collected for radiometric analysis.

Condition 2 emulated starting hemodialysis immediately after etelcalcetide dosing in the clinic. Bovine whole blood (0.5 liters) was warmed to 37 °C. $[^{14}\text{C}]$ etelcalcetide was added and hemodialysis was started immediately. Sodium heparin (100 U) was spiked into the blood every 30 minutes. Positive controls were added to reservoir A. The spiked $[^{14}\text{C}]$ etelcalcetide dose was the same as condition 1. At each time point, aliquots were withdrawn from reservoirs A and B for radiometric analysis and

measurement of etelcalcetide, urea, creatinine, and vitamin B₁₂. A Solvable washout procedure was performed similar to condition 1.

Condition 3 emulated starting hemodialysis after etelcalcetide had sufficient time to form biotransformation products (Figure 1) and establish equilibrium with blood components. After $[^{14}\text{C}]$ etelcalcetide addition, the blood was incubated for 3 hours at 37 °C before hemodialysis. Heparin (100 U) was added every hour. The spiked $[^{14}\text{C}]$ etelcalcetide dose was 0.11 μmol (3.15 μCi), resulting in a blood concentration of 0.21 μM (0.22 $\mu\text{g/ml}$). Heparin addition and blood and dialysate sampling were the same as in condition 2. A Solvable washout procedure was performed similar to condition 1.

Kinetic Modeling

Model 1 was developed using ADAPT 5 software¹¹ (University of Southern California, Los Angeles, CA) to simultaneously fit the concentration data obtained from the etelcalcetide and SAPC biotransformation kinetics experiments in human whole blood. Maximum likelihood with additive, proportional, or both additive and proportional error model¹² was implemented. The differential equations and details are in the [Supplementary Methods](#).

Model 2 was developed using Phoenix 64, version 1.4 (Certara, Princeton, NJ), and was an extension of model 1 to fit the etelcalcetide kinetic data from the closed-loop *in vitro* hemodialysis experiments. The differential equations and details are provided in the [Supplementary Methods](#).

RESULTS

In Vitro Biotransformation Profile

$[^{14}\text{C}]$ etelcalcetide biotransformation profile in human and bovine whole blood

Biotransformation of etelcalcetide was characterized previously *in vitro*.⁷ Etelcalcetide was biotransformed in human whole blood by disulfide exchange with endogenous plasma thiols to form the disulfide products identified previously (Figure 1).⁷ The time course of etelcalcetide disappearance and protein conjugate formation was similar over 6 hours for bovine and human blood ([Supplementary Figure S1A](#)). The ¹⁴C profiles at each time point were also similar, with etelcalcetide as the most abundant low-molecular weight (<2000 Da) component, M13 as the most abundant low-molecular weight biotransformation product, and protein conjugate as the most abundant overall component ([Supplementary Figure S2](#)). The protein conjugate in human plasma was previously determined to be SAPC, which is formed by disulfide exchange of the L-cysteine in etelcalcetide with the reduced cysteine at amino acid position 34 in serum

albumin.⁷ Cysteine 34 is preserved in bovine serum albumin, and the protein conjugate formed in bovine blood was thus inferred to be the bovine analogue of SAPC. Based on the aforementioned results, bovine blood was deemed an appropriate surrogate for human blood with respect to etelcalcetide biotransformation. Although [¹⁴C]etelcalcetide biotransformation was slower at 25 °C than at 37 °C (Supplementary Figure S1B), the same biotransformation products were formed at the lower temperature, and their rates of formation did not differ substantially.

In Vitro Biotransformation Kinetics

Etelcalcetide Biotransformation in Human Whole Blood

The biotransformation kinetics of etelcalcetide is shown in Figure 3a. Approximately 90% of etelcalcetide was biotransformed by 3 hours, and SAPC accounted for ~60% of etelcalcetide-related products, as measured by tryptic peptide LC-MS/MS (Supplementary Figure S3). M11, the sulfhydryl (reduced) form of the D-amino acid peptide backbone in etelcalcetide, was present in minor amounts in plasma after etelcalcetide incubation in whole blood. M11

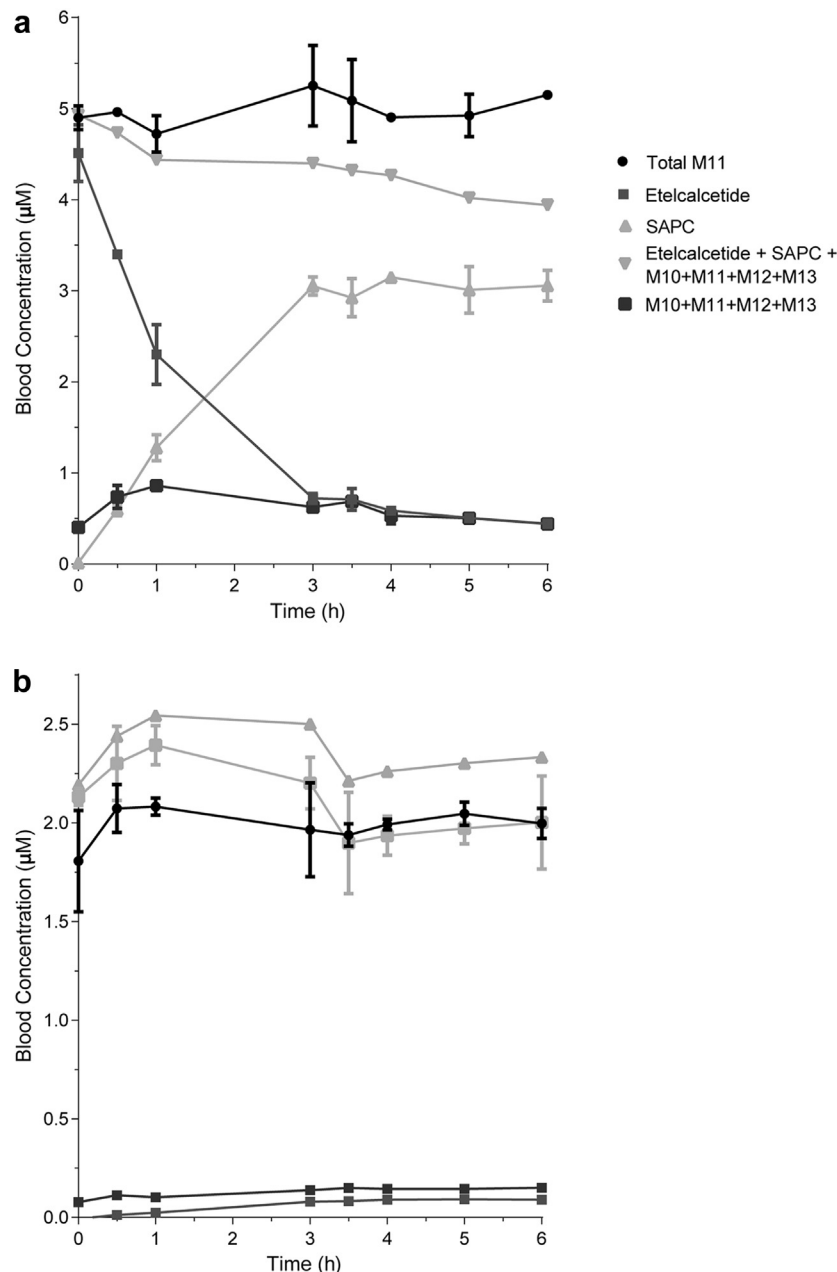


Figure 3. Formation of biotransformation products following etelcalcetide and serum albumin peptide conjugate (SAPC) incubations in human whole blood. (a) Following duplicate incubation of etelcalcetide (5 μM) in whole blood. (b) Following duplicate incubation of SAPC (2.5 μM) in whole blood. Biotransformation products M10, M11, M12, and M13 are combined in a single line. Etelcalcetide and biotransformation products were quantified in duplicate at each time point.

formed upon tris(2-carboxyethyl)phosphine reduction is referred to as “total M11” (Figure 1) because it represents all etelcalcetide-related material in plasma with an intact D-amino acid backbone. An authentic standard of M13 was not available; thus, the quantitation curve for M10 was used to estimate concentrations of M13 in plasma. The sum total of monitored products at 1 and 6 hours was ~90% and 77% of total M11, respectively (Figure 3a).

SAPC Biotransformation in Human Whole Blood

SAPC also underwent disulfide exchange in human whole blood to form etelcalcetide as the predominant product (~3%) and M10 to M13 as minor products (collectively 8%; Figure 3b). The conversion rate to thiol exchange products was significantly slower for SAPC compared with etelcalcetide. Approximately 12% of SAPC was biotransformed, and a plateau was reached by 3 hours.

In Vitro Hemodialysis

Condition 1: Hemodialysis of a Buffered Aqueous Solution (Dialysate) Immediately After Addition of Etelcalcetide

Recovery of total C-14, which represented the total [¹⁴C]etelcalcetide-derived radioactivity, was quantitative (114% of the spiked dose; Figure 4a). Etelcalcetide concentration in reservoir A (“blood compartment”) declined exponentially and appeared in reservoir B (“dialysate compartment”) at a similar rate (Figure 4b). Hemodialysis cleared approximately 70% of the dose in the first 15 minutes with almost all of it present as intact etelcalcetide in the dialysate compartment. A minor amount of the administered dose (3.3%) was recovered in the Solvable washout, which was likely due to small amounts of fluids remaining in the apparatus after it was drained. These results demonstrated that the dialyzer readily cleared etelcalcetide. Also, etelcalcetide did not bind to the dialyzer assembly.

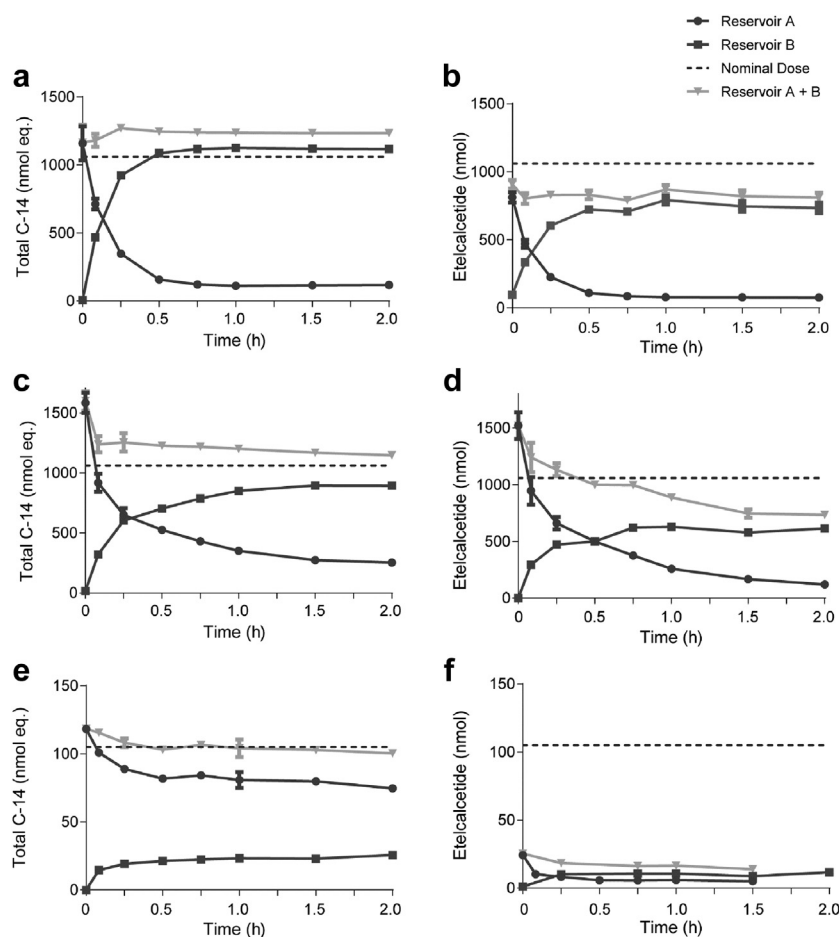


Figure 4. Hemodialysis clearance of etelcalcetide. (a and b) Total C-14 (a) and intact etelcalcetide (b) immediately after etelcalcetide was dosed into a buffered aqueous solution (dialysate) in reservoir A. (c and d) Total C-14 (c) and intact etelcalcetide (d) immediately after etelcalcetide was dosed into bovine blood in reservoir A. (e and f) Total C-14 (e) and intact etelcalcetide (f) after a 3-hour preincubation period at 37 °C after etelcalcetide was spiked into bovine blood in reservoir A. Etelcalcetide and total C-14 were quantified from triplicate aliquots from each time point. Total C-14, total [¹⁴C]etelcalcetide-derived radioactivity; eq., etelcalcetide equivalents.

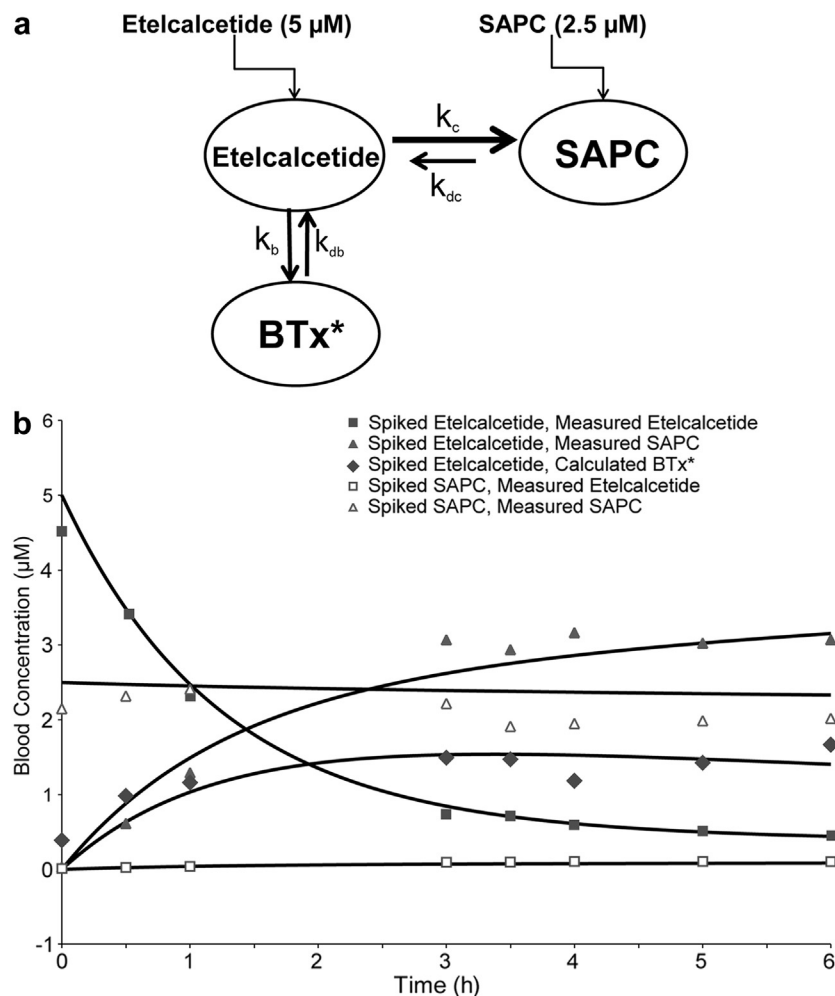


Figure 5. Modeling of the kinetic profiles obtained following etelcalcetide and serum albumin peptide conjugate (SAPC) incubations in human whole blood. (a) Model 1. (b) Observed versus model-predicted concentration-time curves. The differential equations and initial conditions are described in Materials and Methods. BTx^* represents the collective concentration of all etelcalcetide biotransformed products including M10 to M13 but excluding SAPC. k_b and k_{db} are rate constants for the disulfide exchange between etelcalcetide and BTx^* . k_c and k_{dc} are the rate constants for disulfide exchange between etelcalcetide and SAPC. Pooled whole blood from healthy volunteers was incubated in duplicate with etelcalcetide or SAPC. Model 1 was fitted using measured concentrations of etelcalcetide and SAPC and the calculated concentration of BTx^* in whole blood, based on mean values of duplicate samples.

Condition 2: Hemodialysis of Bovine Whole Blood Immediately After Addition of [^{14}C]etelcalcetide

Figure 4c and d show the kinetic changes of total C-14 and the intact etelcalcetide during hemodialysis performed under condition 2. The spiked total C-14 in bovine whole blood was dialyzed rapidly out of the blood compartment and appeared in the dialysate compartment at a similar rate (Figure 4c). As expected, intact etelcalcetide decreased over the 2-hour hemodialysis period (Figure 4d). Based on the biotransformation kinetics described above, the decrease of etelcalcetide in this experiment was attributed to exchange of L-cysteine with thiols in blood. The entire spiked ^{14}C dose (108%) was recovered in the blood and dialysate after 2 hours, indicating that etelcalcetide-related products did not bind significantly to the dialyzer assembly. In the first 15 minutes of hemodialysis, 59% and 57% of the spiked radioactivity and intact etelcalcetide, respectively, were dialyzed.

The drug in the blood compartment at time 0 appeared to be greater than the calculated [^{14}C]etelcalcetide dose. This was likely due to an experimental limitation wherein [^{14}C]etelcalcetide spiked in whole blood could not be mixed completely before the first sample was obtained from reservoir A at time 0. The hemodialysis experiment was performed at ambient room temperature because it was not feasible to maintain the dialysis setup at a physiological temperature (37 °C) for the duration of the 2-hour experiment; however, the kinetics of etelcalcetide biotransformation is similar at both temperatures (Supplementary Figure S1) because it proceeds via a chemical disulfide exchange reaction.¹³

A minor amount of ^{14}C (0.2% of the spiked dose) was recovered during the washout period. Similar to condition 1, this demonstrated that etelcalcetide did not bind to the dialyzer assembly when blood was present.

Condition 3: Hemodialysis of Bovine Whole Blood After a 3-Hour Preincubation at 37 °C With [¹⁴C]etelcalcetide

Figure 4e and f show the kinetic changes in total C-14 and intact etelcalcetide during hemodialysis following a 3-hour preincubation of etelcalcetide in bovine whole blood at 37 °C. As previously demonstrated (Figure 1),⁷ etelcalcetide underwent disulfide exchange with endogenous thiols in blood. Low-molecular weight disulfide exchange products (1000–2300 Da) were readily dialyzed. However, the predominant product of disulfide exchange was SAPC (67 kDa), which had a significantly higher molecular weight than the cutoff of the dialyzer membrane. Consequently, the proportion of the total C-14 eliminated from the blood compartment with a 3-hour preincubation period (23% in 15 minutes) was attenuated compared with no preincubation in condition 2 (59% in 15 minutes). Additionally, the proportion of the dose eliminated in 2 hours only reached 24% with preincubation versus 84% eliminated without preincubation.

Dialysis of Positive Controls: Urea, Creatinine, and Vitamin B₁₂

Positive controls (urea, creatinine, and vitamin B₁₂) were cleared from the blood compartment at similar rates in conditions 1 and 2 (Supplementary Figure S4). Estimated urea and creatinine clearance in condition 1 was 50 and 47 ml/min, respectively, which was the expected clearance for this dialyzer at the operated flow rate. This suggests that the *in vitro* hemodialysis setup approximated clinical dialyzer clearance for these solutes. Vitamin B₁₂ was cleared from the blood compartment at a rate similar to that of creatinine and urea, as 44% and 8% of the dose remained in bovine blood at 15 and 120 minutes, respectively.

Modeling of Biotransformation and Hemodialysis Kinetics

Biotransformation Kinetics Model

Time course data from etelcalcetide and SAPC incubations in human whole blood were simultaneously fit with model 1 (Figure 5a) to derive rate constants for disulfide exchange. Model prediction versus observed data is displayed in Figure 5b, and rate parameter estimates are listed in Table 1. Model 1 described the observed data well, with coefficients of variance <33% for each rate constant. The rate of disulfide exchange from etelcalcetide to form SAPC was 18-fold faster than the reverse process (k_c vs. k_{dc}). The rate of disulfide exchange from etelcalcetide to BTx* (i.e., collective concentration of all etelcalcetide biotransformed products including M10–M13 but excluding

Table 1. Rate constant estimates from the model fitting of the biotransformation kinetics and hemodialysis clearance data sets

Parameter	Estimate	SE (% CV)
Model 1: Etelcalcetide biotransformation kinetics		
k_c (min ⁻¹)	0.007	6.65
k_{dc} (min ⁻¹)	0.00038	8.37
k_b (min ⁻¹)	0.00525	16.7
k_{db} (min ⁻¹)	0.0025	32.5
Etelcalcetide proportional error	0.185	18.7
SAPC additive error (μM)	0.0175	70.4
SAPC proportional error	0.133	19.8
BTx* additive error (μM)	0.241	25.0
Model 2: Etelcalcetide hemodialysis kinetics		
Condition 1		
k_{AB} (min ⁻¹)	0.118	0.35
k_{BA} (min ⁻¹)	0.00932	0.52
Etelcalcetide _{ResA} proportional error	0.119	0.035
Etelcalcetide _{ResB} proportional error	33.8	0.52
Conditions 2 and 3		
k_{AB} (min ⁻¹)	0.0718	22.2
k_{BA} (min ⁻¹)	0.0364	46.8
Etelcalcetide _{ResA} proportional error	0.446	10.1
Etelcalcetide _{ResB} proportional error	0.123	18.8

Model 1 was fitted using measured concentrations of etelcalcetide and SAPC and the calculated concentration of BTx* in whole blood, based on mean values of duplicate samples. Model 2 was fitted using measured amounts of etelcalcetide in reservoirs A and B, based on mean values of triplicate samples.

CV, coefficient of variation; SAPC, serum albumin peptide conjugate. k_c and k_{dc} are the conjugation and deconjugation rate constants of etelcalcetide and SAPC. k_b and k_{db} are the conjugation and deconjugation rate constants of etelcalcetide and BTx*. BTx* represents the collective concentration of all etelcalcetide biotransformed products excluding SAPC (see Materials and Methods for details). k_{AB} and k_{BA} are forward and reverse rate constants of etelcalcetide hemodialysis clearance between reservoirs A and B. Etelcalcetide_{ResA} and etelcalcetide_{ResB} are etelcalcetide amounts in reservoirs A and B.

SAPC) was 2.1-fold faster than the reverse process (k_b vs. k_{db}).

In Vitro Hemodialysis Model

The concentration-time data from *in vitro* hemodialysis experiments under conditions 1, 2, and 3 were fitted simultaneously with model 2 (Figure 6a) using 1 set of rate constants (Table 1). Model 2 described the observed data well (Figure 6b and c), with coefficients of variance <50%. In the absence of blood (condition 1), the rate constant for etelcalcetide decline in reservoir A (k_{AB}) was 0.118 min⁻¹, corresponding to hemodialysis clearance of 59 ml/min. This value was close to the theoretical maximum of 62.5 ml/min obtainable under the flow rates used in the experiment.¹⁴ The clearance value was also higher than the flow rate from reservoir A, likely due to the convection gradient in the hemodialysis setup. In the presence of blood (conditions 2 and 3), k_{AB} for etelcalcetide decline in reservoir A was 0.0718 min⁻¹, which corresponded to a hemodialysis clearance of 36 ml/min (~70% of blood flow rate).

DISCUSSION

Multiple factors are likely to affect the pharmacokinetics and disposition of etelcalcetide in a clinical hemodialysis

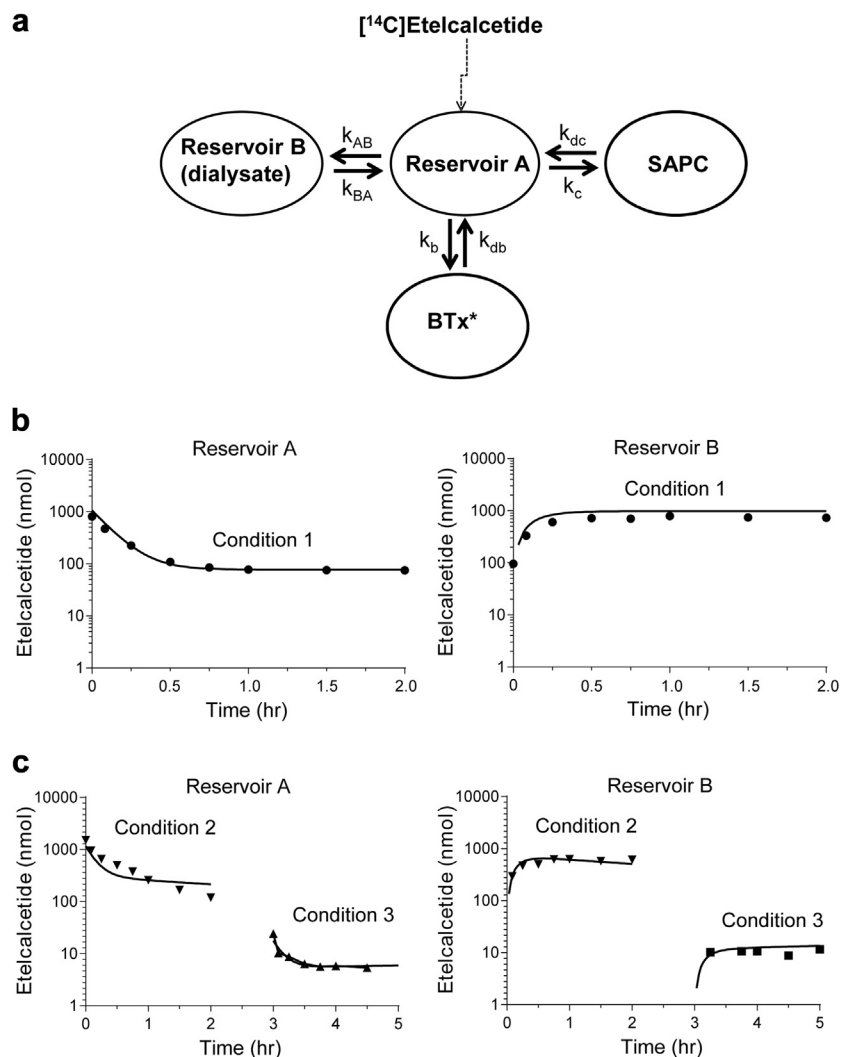


Figure 6. Simultaneous modeling of the hemodialysis clearance profiles obtained following etelcalcetide incubation in bovine whole blood. (a) Model 2. (b) Observed versus model-predicted concentration-time curves in condition 1. (c) Observed versus model-predicted concentration-time curves in conditions 2 and 3. Conjugation and deconjugation rates (k_c , k_{dc} , k_b , k_{db}) were fixed to the values obtained from model 1 fitting of the *in vitro* biotransformation kinetics data. k_{AB} and k_{BA} are forward and reverse rate constants of etelcalcetide hemodialysis clearance between reservoirs A and B. BTx* represents the collective concentration of all etelcalcetide biotransformed products including M10 to M13 but excluding serum albumin peptide conjugate (SAPC). Model 2 was fitted using measured amounts of etelcalcetide in reservoirs A and B, based on mean values of triplicate samples.

setting. These include the drug's intrinsic dialytic properties, biotransformation kinetics, non-covalent plasma protein binding and blood-to-plasma partition ratio, permeability, and the extent and rate of its distribution into and out of peripheral tissue compartments. The non-covalent plasma protein binding, the blood-to-plasma ratio, and permeability have been previously reported.⁷ The rate of etelcalcetide distribution into peripheral tissue compartments has not been determined; however, that was unlikely to affect this investigation because of the drug's very low permeability.

In this study, the dialytic properties and biotransformation kinetics of etelcalcetide were investigated by *in vitro* methods. The results of this investigation were used to provide insights into the disposition of etelcalcetide that would be difficult to determine in a

clinical setting. Etelcalcetide and its biotransformation products did not bind to the polysulfone dialyzer, which showed that the *in vitro* apparatus was free of that potential confounding artifact. In the absence of blood, as expected for a molecule of etelcalcetide's molecular weight, the dialyzer cleared etelcalcetide at a rate that was near the theoretical maximum and was similar to the clearance rates of the control solutes urea, creatinine, and vitamin B₁₂.¹⁴

Etelcalcetide and its biotransformation products exist in a dynamic equilibrium state. Consequently, as etelcalcetide and its low-molecular weight biotransformation products are removed by hemodialysis, they are reformed from SAPC by re-equilibration. The overall removal of etelcalcetide is therefore dependent on the proportion of the drug present during dialysis in dialyzable and non-

dialyzable forms and the rates at which these forms interconvert. A rate of conversion from SAPC to etelcalcetide that is fast relative to etelcalcetide dialytic clearance would result in rapid removal of total etelcalcetide. A rate of conversion that is slow relative to dialytic clearance would result in rapid removal of low-molecular weight forms of the drug, and then additional removal would occur at a rate determined by the rate of etelcalcetide formation from SAPC. SAPC does not cross the dialyzer membrane, so the proportion of total etelcalcetide present in the form of SAPC can be eliminated during dialysis only as rapidly as the conversion from SAPC to etelcalcetide.

In this study, the etelcalcetide hemodialysis clearance rate was shown to be >16-fold faster than its conversion to SAPC, and the rate of conversion of SAPC to etelcalcetide was far slower (~200-fold) than the dialytic rate constant. Consequently, administration of etelcalcetide during or shortly before dialysis would result in its rapid removal in the dialysate. When etelcalcetide was preincubated in blood before hemodialysis for a period of time sufficient to reach equilibrium with SAPC (preincubation for 3 hours was tested in the experiments), the dialysis of all etelcalcetide-related components as measured by total radioactivity was slow. This finding is consistent with rapid formation of SAPC and a slow rate of the reverse process. The conversion of etelcalcetide to SAPC was 18-fold faster than conversion of SAPC to etelcalcetide, which is consistent with the finding that SAPC was the predominant etelcalcetide-related species in blood.⁷ This is also consistent with the >7-day plasma half-life of etelcalcetide in CKD patients on hemodialysis.^{3,15} As previously established⁷ and reaffirmed, SAPC was the predominant biotransformation product. Other low-molecular weight products were minor compared with SAPC and are therefore not likely to contribute significantly to the overall disposition or elimination of etelcalcetide during hemodialysis.

For drugs requiring i.v. dosing to hemodialysis patients, it is most convenient to administer the dose during hemodialysis (commonly into the venous line of the dialyzer). For example, erythropoietin (molecular weight ~34 kDa) can be dosed at any time during a hemodialysis session because it has minimal passage across a dialyzer membrane. In this *in vitro* investigation, it was demonstrated that introduction of etelcalcetide into the blood immediately before hemodialysis resulted in rapid elimination. The elimination rate constant of 0.0718 min^{-1} translated to a half-life of approximately 10 minutes. In 1 hour of dialysis (~6 half-lives), 98.5% of the drug would be cleared by

dialysis, because only a small amount of SAPC can be formed under these conditions.

A drug's non-covalent binding to plasma proteins and its blood-to-plasma partition ratio affect hemodialysis clearance.¹⁶ In bovine blood, etelcalcetide was only 40% bound non-covalently to plasma proteins (data not shown). This is consistent with the *in vitro* finding that the elimination rate constant in blood was 60% of that in the absence of blood. Non-covalent binding of etelcalcetide to plasma proteins in human blood was also low (~50%).⁷ The etelcalcetide blood-to-plasma ratio was close to the hematocrit value (~0.5) and hence is not expected to appreciably affect how much etelcalcetide in plasma is cleared during hemodialysis.

In summary, the biotransformation process of etelcalcetide and the influence of hemodialysis on etelcalcetide elimination are complex. The *in vitro* incubation and hemodialysis models used here provided a practical way to characterize etelcalcetide properties that would otherwise be difficult to study in clinical settings. Based on the rate constant comparisons of biotransformation and hemodialysis *in vitro*, etelcalcetide would be significantly eliminated if administered during hemodialysis. The findings from this study support dosing etelcalcetide immediately after hemodialysis in CKD patients with secondary hyperparathyroidism, as tested in clinical studies.^{1,3}

DISCLOSURE

All authors are or were employed by and are shareholders of Amgen, which is developing etelcalcetide for the treatment of secondary hyperparathyroidism in chronic kidney disease patients on maintenance hemodialysis. Jonathan Latham of PharmaScribe LLC received financial support from Amgen to assist with preparation and submission of the manuscript.

ACKNOWLEDGMENTS

Amgen provided financial support for the conduct of the research and preparation of the article. We thank Sean Zhu for the biotransformation profile methods and Joel Esmay for measuring plasma protein binding; Professor Larry Cowgill at University of Davis for previous exploratory analysis and consultation; Justin Murray for stable label standards; Rob Milburn for SAPC synthesis; and Nathan Teuscher at Certara and Theresa Yurasczek for facilitating the modeling in Phoenix. Holly Tomlin (Amgen Inc.) and Jonathan Latham (on behalf of Amgen Inc.) supported the preparation and submission of the manuscript. Divya Ganesan and Ninad Ranade (CACTUS on behalf of Amgen Inc.) provided journal formatting and editing support.

SUPPLEMENTARY MATERIAL

Supplemental figures show the etelcalcetide biotransformation time-course in human (at 25 °C and 37 °C) and bovine (at 37 °C) whole blood (Figure S1); bovine and human plasma 14C profiles (Figure S2); sample preparation and mass spectral properties of SAPC (Figure S3); and hemodialysis clearance of positive controls (urea, creatinine, and vitamin B12) in experiments 1 and 2 (Figure S4).

Supplementary material is linked to the online version of the paper at www.kireports.org.

REFERENCES

- Bell G, Huang S, Martin KJ, et al. A randomized, double-blind, phase 2 study evaluating the safety and efficacy of AMG 416 for the treatment of secondary hyperparathyroidism in hemodialysis patients. *Curr Med Res Opin.* 2015;31:943–952.
- Martin KJ, Bell G, Pickthorn K, et al. Velcalcetide (AMG 416), a novel peptide agonist of the calcium-sensing receptor, reduces serum parathyroid hormone and FGF23 levels in healthy male subjects. *Nephrol Dial Transplant.* 2014;29:385–392.
- Martin KJ, Pickthorn K, Huang S, et al. AMG 416 (velcalcetide) is a novel peptide for the treatment of secondary hyperparathyroidism in a single-dose study in hemodialysis patients. *Kidney Int.* 2014;85:191–197.
- Walter S, Baruch A, Dong J, et al. Pharmacology of AMG 416 (Velcalcetide), a novel peptide agonist of the calcium-sensing receptor, for the treatment of secondary hyperparathyroidism in hemodialysis patients. *J Pharmacol Exp Ther.* 2013;346:229–240.
- Cunningham J, Locatelli F, Rodriguez M. Secondary hyperparathyroidism: pathogenesis, disease progression, and therapeutic options. *Clin J Am Soc Nephrol.* 2011;6:913–921.
- Alexander ST, Hunter T, Walter S, et al. Critical cysteine residues in both the calcium-sensing receptor and the allosteric activator AMG 416 underlie the mechanism of action. *Mol Pharmacol.* 2015;88:853–865.
- Subramanian R, Zhu X, Kerr S, et al. Nonclinical pharmacokinetics disposition, and drug-drug interaction potential of a novel D-amino acid peptide agonist of the calcium sensing receptor AMG 416 (etelcalcetide) [e-pub ahead of print]. *Drug Metab Dispos.* 19 February 2016. pii: dmd.115.068007.
- Singh R, Whitesides GM. Thiol-disulfide interchange. In: Patai S, Rappoport Z, eds. *Sulphur-Containing Functional Groups*. Chichester, UK: John Wiley & Sons Inc.; 1993:634–647.
- Maduell F, Navarro V, Cruz MC, et al. Osteocalcin and myoglobin removal in on-line hemodiafiltration versus low- and high-flux hemodialysis. *Am J Kidney Dis.* 2002;40:582–589.
- Fresenius Optiflux® Dialyzers. Fresenius Medical Care North America, Waltham, MA; 2011. Available at: http://fmcna-dialyzers.com/dialyzers-site/pdf/Optiflux_Product_Brochure_Section/101_046-01_Optiflux_High_Flux.pdf. Accessed December 14, 2015.
- D'Argenio DZ, Schumitzky A, Wang X. *ADAPT 5 User's Guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software*. Los Angeles, CA: Biomedical Simulations Resource; 2009.
- Werner M, Brooks SH, Knott LB. Additive, multiplicative, and mixed analytical errors. *Clin Chem.* 1978;24:1895–1898.
- Fernandes PA, Ramos MJ. Theoretical insights into the mechanism for thiol/disulfide exchange. *Chemistry.* 2004;10:257–266.
- Depner TA. Hemodialysis adequacy: basic essentials and practical points for the nephrologist in training. *Hemodial Int.* 2005;9:241–254.
- Chen P, Melhem M, Xiao J, et al. Population pharmacokinetics analysis of AMG 416, an allosteric activator of the calcium-sensing receptor, in subjects with secondary hyperparathyroidism receiving hemodialysis. *J Clin Pharmacol.* 2015;55:620–628.
- Tsuruoka S, Kitoh Y, Kawaguchi A, et al. Clearance of imidapril, an angiotensin-converting enzyme inhibitor, during hemodialysis in hypertensive renal failure patients: comparison with quinapril and enalapril. *J Clin Pharmacol.* 2007;47:259–263.