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

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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.

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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

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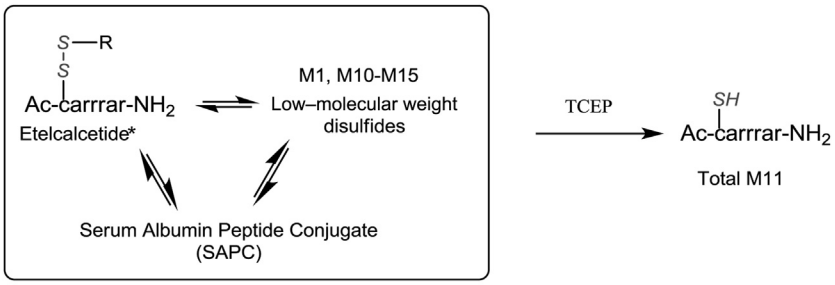

Product	R group	Molecular Weight (Da)
Etelcalcetide	L-cysteine	1048
SAPC	Serum albumin (at cysteine 34)	67,365
M10	L-GSH	1234
M11	*Same structure as total M11*	929
M12	Ac-carrrar	2311
M13	L-cysteinyl-glycine	1105
M14	L-homocysteine	1062
M15	γ-L-glutamyl-L-cysteine	1177

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 "carrrar" 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 [^{14}C]etelcalcetide (10 μM, 0.3 μCi/ml), cold etelcalcetide (5 μM), or SAPC (2.5 μM) 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.