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Research paper

Clinical immunogenicity of the D-amino acid peptide therapeutic etelcalcetide: Method development challenges and anti-drug antibody clinical impact assessments

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ABSTRACT

The immunogenicity risk assessment and bioanalytical strategy for novel therapeutics should account for both unique biophysical properties and potential consequences of immunogenicity. When assessing the immunogenicity risk of etelcalcetide, a peptide agonist of the calcium-sensing receptor, we considered the potential that the D-amino acid 'backbone' and biotransformation of etelcalcetide could allow the drug to act as a hapten. As a consequence, we validated and implemented a surface plasmon resonance immunoassay platform with both etelcalcetide and etelcalcetide-'carrier' surfaces to detect anti-drug antibodies (ADA). No evidence of in-vitro neutralizing activity with surrogate controls was detected despite multiple immunization approaches and a sensitive cell-based activity assay. Therefore, a neutralizing assay was not implemented for clinical support. We conducted an integrated analysis of immunogenicity data pooled from two pivotal placebo-controlled trials to define the clinical impact of anti-etelcalcetide antibodies. While both pre-existing and developing anti-etelcalcetide antibodies were detected, we show here that they have no consequences for clinical exposure, efficacy, or safety of etelcalcetide.

1. Introduction

Immunogenicity as it applies to drug development typically refers to an adaptive immune response reactive with a therapeutic. Patients and physicians are concerned with immunogenicity because of the potential for anti-drug antibodies (ADA) to alter safety and efficacy. Regulatory agencies acknowledge the potential impact of ADA, and provide guidelines for both assay development (FDA, 2016) and immunogenicity assessment strategy (EMA, 2015; FDA, 2014).

Etelcalcetide (AMG 416) is a novel calcimimetic for the treatment of secondary hyperparathyroidism in chronic kidney disease (CKD) patients receiving hemodialysis. Etelcalcetide is a synthetic peptide with a molecular weight of 1048 Da and is composed of seven linear D-amino acids (referred to as the "D-amino acid backbone") with the N-terminal D-cysteine linked to an L-cysteine by a disulfide bond (Walter et al., 2013). The N-terminal D-cysteine and the C-terminal arginine in etelcalcetide are capped with acetyl and amide groups, respectively (Fig. 1A). The D-amino acid backbone in etelcalcetide covalently binds via a disulfide bond to cysteine 482 in the calcium sensing receptor (CaSR) of chief cells in the parathyroid gland and activates the CaSR, resulting in a reduction of PTH secretion (Alexander et al., 2015). Etelcalcetide is biotransformed in whole blood by disulfide exchange of the L-cysteine to form reversible conjugates with endogenous thiols present in plasma. The major human circulating biotransformation product is the serum albumin peptide conjugate (SAPC) (Subramanian et al., 2016a). SAPC is formed by covalent disulfide bond conjugation of the D-cysteine in the etelcalcetide D-amino acid backbone to the L-cysteine at amino acid 34 of serum albumin.

Given the novel structure of etelcalcetide and presence of biotransformed products (Park and Kitteringham, 1990), a conservative immunogenicity testing scheme was implemented when Amgen acquired this program. Surface plasmon resonance (SPR) is capable of detecting low affinity ADA responses and all immunoglobulin isotypes. We developed an SPR immunoassay that utilizes both the native therapeutic as well as SAPC. The ultimate goal of immunogenicity support is to provide meaningful and specific information about the incidence and consequences of ADA in a product's label (Shankar et al., 2015). To that end, we performed an antibody impact analysis on clinical data to

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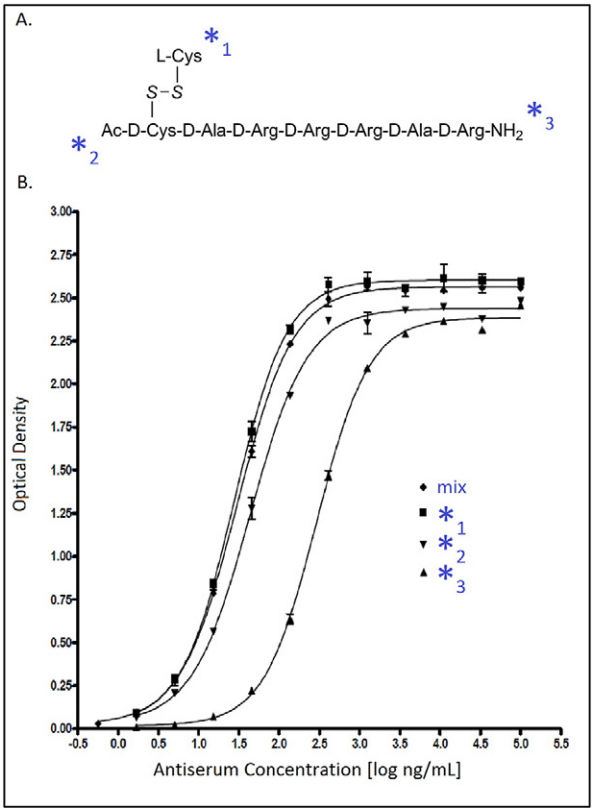

Fig. 1. Structure of etelcalcetide and immunoreactivity position effects. Structure of etelcalcetide is depicted in black (Panel A). Blue asterisks indicate positions labeled with biotin for the position effect immunoreactivity (Panel B). Shown in Panel B is immunoreactivity (determined by streptavidin-capture colorimetric ELISA) of drug-specific antiserum from a single immunized rabbit. The capture reagents are variants of etelcalcetide, biotin-labeled at the positions indicated in Panel A.

determine whether the presence of anti-etelcalcetide antibodies altered exposure, efficacy, or safety of etelcalcetide.

2. Materials and methods

2.1. Etelcalcetide SPR immunoassay

Etelcalcetide and SAPC were covalently immobilized onto separate flow cell surfaces of a carboxymethyl-dextran coated sensor chip (Carboxymethyl-5 (CM5); GE Biacore cat # BR-1000-12) by amine coupling chemistry. Assay controls and serum samples were diluted in sample buffer (50 mM citric acid, 0.5%carboxymethyl dextran (CMD), 0.005% tween-20, 3 mM EDTA, 200 mM NaCl, pH 5.3) and individually injected across both surfaces utilizing separate injections. To confirm that human sample binding was attributable to antibodies, a solution of goat anti-human IgA + IgG + IgM (Jackson cat # 109-005-064) secondary antibody was injected for confirmation after completion of each sample injection. The immobilized surface was then regenerated using 50 mM HCl before injection of the next sample. Subjects were considered positive for the presence of anti-etelcalcetide antibodies if both screening and confirmatory cut-points were exceeded for either etelcalcetide or SAPC assay surfaces.

2.2. SAPC conjugation chemistry and characterization

Human serum albumin (HSA; CSL Behring) was diluted to 5 mg/mL. Treatment with dithiothreitol (DTT) resulted in HSA with reduced cys-34 by liquid chromatography-mass spectrometry (LCMS). The HSA solution was passed through desalting columns and eluted with pH 2.5 citrate buffer. A pyridyl sulfide-activated D-amino acid backbone of etelcalcetide was added as a solid and the mixture was held overnight at ambient room temperature without agitation. This mixture was passed through G-50 spin columns and eluted with 50 mM pH 2.4 citrate buffer and further purified via size exclusion chromatography (SEC). A high resolution mass spectrometric analysis of the synthetically prepared SAPC (Subramanian et al., 2016b) showed a predominant monoconjugate of the etelcalcetide D-amino acid backbone with HSA. Amino acid sequence analysis of the peptide digest determined the conjugation with the D-cysteine in etelcalcetide D-amino acid backbone had occurred exclusively at cysteine 34 of HSA.

2.3. Animal immunization schemes for surrogate positive controls

Surrogate positive control antibodies were generated through hyperimmunization of rabbits (N = 4 per campaign), as described in Section 3.2. In each campaign, adjuvanted priming subcutaneous injections used 100–200 μg of covalently conjugated etelcalcetide. Boosts utilized approximately 100 μg. Four injections total were administered throughout a 70 day protocol. In cases where the poly(lactic-*co*-glycolic acid) (PLGA)-complexed etelcalcetide was included, approximately 10% of each injection volume was comprised of the PLGA complex.

2.4. In-vitro neutralizing antibody assay development

Phosphorylation of ERK1/2, a signaling event that occurs downstream of the CaSR (McNeil et al., 1998), was selected as the endpoint for the neutralizing antibody assay. CaSR transfected HEK293T cells were maintained in RPMI 1640 supplemented with 10% FBS and 2 mM L-glutamine. On assay day −1, cells were washed in PBS and staged in the absence of serum for 18–24 h. Prior to stimulation with etelcalcetide, cells were washed and placed in assay buffer which was optimized for etelcalcetide activity. Assay buffer was composed of 10 mM HEPES (pH 7.75), 130 mM NaCl, 4.2 mM KCl, 0.5 mM $MgCl_2$, 0.8 mM $CaCl_2$, 5 mM glucose. Test samples were diluted to 15% serum (3× intermediate) in assay buffer, combined with etelcalcetide diluted in assay buffer, and incubated at room temperature in a 96 well plate to allow for potential antibody binding. After 30 min, 200,000 HEK293T-CaSR cells were added and the plate was incubated for 3 h at 37 °C. While phosphorylation assays typically utilize much shorter incubation times, a 3 h incubation allowed for significant reduction in the concentration of etelcalcetide while still maintaining a sufficient assay window. At the end of the incubation period, cells were lysed on ice and an MSD whole cell lysate kit was utilized to measure phosphorylation of ERK1/2 (MSD cat # K151DWD). Briefly, the plate-bound capture antibody binds to phosphorylated ERK1/2 and after washing, a ruthenylated total ERK1/2 secondary antibody was used to generate electrochemiluminescent signal.

2.5. Clinical studies

The design and outcomes of these studies have been described elsewhere (Block et al., 2017). Briefly, two parallel, phase 3, randomized, placebo-controlled 26-week treatment trials which were identical in design were conducted in 1023 patients receiving hemodialysis with moderate to severe secondary hyperparathyroidism (Amgen study nos. 20120229 and 20120230; NCT00345839 and NCT01788046, respectively). The trials were approved by institutional review boards at participating study sites and registered at ClinicalTrials.gov. All patients, regardless of treatment assignment, received standard of care with phosphate binders and calcitriol or active vitamin D analogs, as prescribed by the individual Investigator. In both studies, serum specimens for immunogenicity analysis were collected on day 1 (predose), week 12, week 27, and at safety follow-up.