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Research paper

Development of a panel of highly sensitive, equivalent assays for detection of antibody responses to velaglucerase alfa or imiglucerase enzyme replacement therapy in patients with Gaucher disease

Márcia Séllos-Moura, Samad Barzegar, Luying Pan, Philip Shi, Susan Oommen, Judith Durant, Juan A. Ruiz *

Department of Bioanalytical and Biomarker Development, Shire Human Genetic Therapies, 300 Shire Way, Lexington, MA 02421, United States

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ABSTRACT

Anti-drug antibodies are elicited by virtually all therapeutic proteins, and standardized assays are required for clinical monitoring of patients as well as for comparing antibody response to different therapeutic proteins in clinical trials. Velaglucerase alfa and imiglucerase are enzyme replacement therapies for the long-term treatment of type 1 Gaucher disease, a lysosomal storage disease resulting from an inherited deficiency of the enzyme glucocerebrosidase. We used state-of-the-art tools to develop a panel of assays for detection and characterization of antibody responses to velaglucerase alfa and imiglucerase. Highly-sensitive, direct bridging electrochemiluminescence screening assays were developed using samples from treatment-naïve individuals with type 1 Gaucher disease to set cut points. A mouse anti-glucocerebrosidase monoclonal antibody used as a calibrator was shown to have similar affinity and binding kinetics for anti-velaglucerase alfa and anti-imiglucerase antibodies. A quantitative radioimmunoprecipitation assay for IgG antibodies was developed to eliminate false-positives from the highly sensitive screening assay. Using 59 samples from treatment-naïve individuals with type 1 Gaucher disease, the confirmatory cut points were calculated to be 1.42 ng/mL for anti-velaglucerase alfa antibodies and 3.23 ng/mL for anti-imiglucerase antibodies. Isotype-specific indirect electrochemiluminescence assays were developed for IgE, IgA, and IgM subclasses. The IgE subclass assay was shown to be more sensitive than the confirmatory assay using sheep anti-glucocerebrosidase polyclonal antibody cross-linked with fragments specific to human IgE, with cut points for anti-velaglucerase alfa or anti-imiglucerase antibodies determined to be 0.53 and 0.55 ng/mL, respectively. An assay that detects inhibition *in vitro* of velaglucerase alfa and imiglucerase hydrolysis of a synthetic substrate in the presence of antibodies was developed to test for neutralizing antibodies. Using 52 individual healthy human donor samples and 35 samples from treatment-naïve individuals with type 1 Gaucher disease, cut points for the velaglucerase alfa and imiglucerase neutralizing antibody assays were determined to be 20%, such that a sample with greater than 20% inhibition of enzyme activity in the presence of antibodies was considered positive for neutralizing antibodies. In conclusion, highly sensitive and equivalent methods were developed and validated to directly compare antibody response to velaglucerase alfa and imiglucerase treatments in patients with Gaucher disease, and may contribute to future internationally standardized assays for antibody detection in patients with Gaucher disease.

Abbreviations: ADA, anti-drug antibody; BSA, bovine serum albumin; CPM, counts per minute; DPBS, Dulbecco's Phosphate Buffered Saline solution; ECL, electrochemiluminescence; EMA, European Medicines Agency; FDA, Food and Drug Administration; HBS-EP, HEPES-buffered saline with EDTA and surfactant P20; Ig, immunoglobulin; LC-SPDP, long spacer arm cross-linker succinimidyl 6-[3′-2-pyridyldithio-propionamido] hexanoate; LOD, limit of detection; LOQ, limit of quantification; NAb, neutralizing antibodies; RSD, relative standard deviation; RU, resonance units; SD, standard deviation.

* Corresponding author. Tel.: +1 781 482 0749; fax: +1 781 482 2965.
E-mail address: jruiz@shire.com (J.A. Ruiz).

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1. Introduction

Anti-drug antibodies occur with virtually all therapeutic proteins, although the incidence varies considerably, ranging from less than 10% of patients to nearly 100% (Schellekens, 2004). Factors pre-disposing to antibody development are a complex interaction between the therapeutic proteins, the formulation, dose, rate of administration, excipients, and patient-specific factors (Patten and Schellekens, 2003), making it very difficult to predict which individuals will develop antibodies to therapeutic proteins.

Type 1 Gaucher disease was one of the first enzyme deficiencies to be treated with a replacement enzyme. Gaucher disease is a lysosomal storage disorder resulting from deficiency of glucocerebrosidase; the lack of this enzyme leads to accumulation of glucosylceramide within macrophages, which in turn leads to spleen, liver, bone, and hematologic abnormalities (Goker-Alpan, 2010). Replacement of the deficient enzyme by infusion of purified or recombinant human enzyme is associated with improvement in symptoms (Barton et al., 1991; Grabowski et al., 1995). Initially, replacement enzyme was purified from human placental tissue (Ceredase®, Genzyme Corporation, Cambridge, MA), but was limited by supply, and subsequently a recombinant protein produced in transformed Chinese hamster ovary cells, imiglucerase (Cerezyme®, Genzyme Corporation, Cambridge, MA), was made more widely available. Typically, patients receive infusions every 2 weeks, usually in the long-term if not for the remainder of their lives. Imiglucerase has been used in this way in several thousand patients to date, with a consistent rate of elicitation of antibodies, at approximately 15% (Starzyk et al., 2007).

In 2010, another replacement glucocerebroside, velaglucerase alfa (VPRIV®, Shire Human Genetic Therapies, Cambridge, MA) was approved by the Food and Drug Administration (FDA), European Medicines Agency (EMA), and other regulatory agencies for use in patients with type 1 Gaucher disease. Velaglucerase alfa is produced by gene-activation of human glucocerebrosidase in a human fibroblast cell line, and contains the native human enzyme sequence. On X-ray crystallography, velaglucerase alfa is similar to imiglucerase; however, imiglucerase differs at amino acid position 495 where histidine substitutes for the natural arginine, and the glycosylation patterns indicate markedly different mannose structures (Brumshtein et al., 2010). Given the complex nature of antibody elicitation, whether these factors influence the rate of antibody formation is unknown.

With alternative enzyme replacement therapies for type 1 Gaucher disease available, physicians considering treatment options will require high-quality data on the development of antibodies in patients treated with imiglucerase or velaglucerase alfa. We therefore developed and validated a panel of highly sensitive and equivalent assays for the detection and characterization of anti-velaglucerase alfa and anti-imiglucerase antibodies.

2. Materials and methods

2.1. Overview

Identical methods were developed to evaluate patient sera for anti-velaglucerase alfa and anti-imiglucerase antibodies. The bridge electrochemiluminescent (ECL) immunoassay, in which the drug is alternatively labeled with capture or detection functional groups, detected all immunoglobulin subclasses and was considered the antibody screening assay. The radioimmunoprecipitation (RIP) assay was confirmatory for the presence of IgG antibodies, and the Ig subclass electrochemiluminescent immunoassays were confirmatory assays for the presence of IgA, IgM, and IgE antibodies. A diagram of the testing flowchart is shown in Fig. 1. The antibody screening assays and IgG assays were calibrated and quantitative, using human antibody-positive controls. The IgA, IgM, and IgE assays were semi-quantitative and utilized synthetic positive controls, since naturally occurring IgA, IgM, or IgE antibodies against velaglucerase alfa or imiglucerase were not available. To further test whether antibodies neutralized enzyme activity *in vitro*, assays were also developed to measure inhibition *in vitro* of velaglucerase alfa and imiglucerase hydrolysis of the substrate 4-nitrophenyl-β-D-glucopyranoside.

2.2. Materials

The ECL assays were read on a SECTOR™ Imager 2400 (Meso Scale Discovery, Gaithersburg, MD) using Meso Scale Discovery Workbench® Software. Streptavidin-coated high bind MA2400 96-microwell plates were also purchased from Meso Scale Discovery, as were the Sulfo-TAG™ NHS-Ester Kit for ruthenium-complex labeling and the read buffer S (4×) for ECL assay. Flat-bottomed Nunc MaxiSorp ELISA plates were purchased from Nalge Nunc International (Rochester, NY).

EZ-Link® Sulfo-NHS-LC-Biotinylation Kits and BCA™ Protein Assay Kits were acquired from Pierce (Pierce Protein Research Products from Thermo Fisher Scientific, Rockford, IL). Protein G Sepharose 4 Fast Flow columns and ECL Blocker B were acquired from GE Healthcare (Piscataway, NJ). Dulbecco's Phosphate Buffered Saline solution (DPBS) was obtained from Invitrogen (Carlsbad, CA). Protease-free bovine serum albumin (BSA) was obtained from American Bioanalytical (Natick, MA).

Purified sheep anti-glucocerebrosidase polyclonal antibody and mouse anti-glucocerebrosidase monoclonal antibody were both prepared by Shire Human Genetic Therapies. The sheep anti-glucocerebrosidase polyclonal antibody (G140) was raised in sheep hyperimmunized with velaglucerase alfa and cross-reactive with imiglucerase, and purified using Protein G columns (hence was an IgG antibody) and screened by ELISA. The mouse anti-glucocerebrosidase monoclonal antibody (clone number TK9E4-D1-F2-002 "9E4") was raised against velaglucerase alfa in *BALB/c* mice and was cross-reactive to imiglucerase; as with the polyclonal antibody, it was purified using Protein G columns and screened by ELISA. The goat anti-mouse IgG, Fc antibody used for the kinetic study of assay reagents was purchased from MP Biomedical/Cappel (Solon, OH). Pooled and individual normal human sera and cynomolgus monkey serum were obtained from Bioreclamation (Hicksville, NY). Gaucher disease serum positive for imiglucerase antibody was obtained from a patient screened for entry into a Shire Human Genetic Therapies clinical study who was subsequently excluded because baseline serum samples revealed a pre-existing high titer antibody to imiglucerase that cross-reacted with velaglucerase alfa. Goat-anti-human antibody (IgA, IgM, or IgE specific) was obtained from Jackson Immuno Research (IgA) and Chemicon