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Research paper

An Affinity Capture Elution (ACE) assay for detection of anti-drug antibody to monoclonal antibody therapeutics in the presence of high levels of drug

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Abstract

Monoclonal antibody therapeutics typically have relatively long half-lives and can be dosed at high levels. Although formation of anti-drug antibodies (ADA) is relatively rare, detection of these antibodies can be very difficult in the presence of high circulating levels of drug. Typically these ADA are detected by bridging ELISAs which can be very sensitive to even low levels of drug. We describe an ELISA method based on affinity capture of ADA on solid-phase drug followed by removal of excess free drug, release and transfer of bound ADA and subsequent detection using biotinylated drug. The assay is both sensitive and highly tolerant to free drug with detection of 500 ng/ml of ADA readily achieved in the presence of 500 μg/ml of drug.

Keywords: Antibody assay; Acid dissociation; Immunogenicity; Therapeutic proteins; ELISA; Antigen interference

1. Introduction

Monoclonal antibody therapeutics are finding increased usage for treatment of a wide variety of clinical indications with over 18 approved products. The incidence of anti-drug antibodies (ADA) varies widely from almost 100% for some murine monoclonals to virtually undetectable for some humanized antibodies (Bourdage et al., 2005). These antibodies can have significant clinical consequence. Bendtzen et al. reported detection of anti-infliximab antibodies that apparently caused loss of bioavailability, infusion reactions and treatment failure (Bendtzen et al., 2006). Anti-rituximab antibodies caused reduced B-cell depletion and reduced blood levels of rituximab (Looney et al., 2004). There have been cases where neutralizing antibodies to endogenous proteins have caused life-threatening situations (Casadevall et al., 2002; Basser et al., 2002).

Humanized monoclonal antibodies typically have half-lives of 10–20 days and can often be administered at relatively large doses leading to blood levels of more than 500 μg/ml (Maloney et al., 1997). Preclinical studies typically use even higher doses to establish safety

Abbreviations: ELISA; Enzyme-Linked Immunosorbent Assay; ADA; anti-drug antibody; ACE; affinity capture elution; TMB; tetramethyl benzidine; OD; optical density; HRP; horseradish peroxidase.

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margins with necessary short washouts prior to evaluation. Bridging or double antigen format assays are often used to evaluate ADA for monoclonals due to their ability to detect multiple classes of antibody (Feldman et al., 2003: Pendley et al., 2003) . However, there is concern for the ability to detect low affinity antibody due to the requirement for monovalent binding to the solid phase and labeled drug during bridge formation. This monovalent binding also causes the assay to be very sensitive to free drug since blocking of only a single arm of the ADA molecule will prevent bridge formation. A double antigen format assay described for infliximab has drug interference at drug levels above 1.4 μg/ml (Baert et al., 2003). This presents a dilemma when developing preclinical and clinical protocol sampling points since antibodies may be undetectable while drug levels are high thus causing a requirement for washouts of several weeks to several months. These potentially long washout periods could in turn cause decreasing ADA titers, thus making detection of ADA difficult or impossible.

We have therefore developed a method that first dissociates ADA-free drug complexes with acid treatment followed by neutralization in the presence of solid-phase drug giving the ADA an opportunity to be affinity captured. After washing away excess free drug, ADA are eluted off with acid and subsequently bound to a fresh solid surface. Bound ADA are subsequently detected by addition of biotinylated drug followed by streptavidin-HRP and substrate. Results presented here indicate that this affinity capture elution (ACE) assay format is capable of detecting low ng/ml levels of ADA in the presence of a 1000-fold excess of free drug.

2. Materials and methods

2.1. Reagents

Wash buffer consists of 0.01 M Tris-buffered saline (TBS) (Fisher Scientific, Fair Lawn, NJ) with 0.05% Tween 20 (Sigma-Aldrich, St. Louis, MO) (TBST). Therapeutic monoclonal antibody (LY) was supplied by Eli Lilly and Co. (Indianapolis, IN). Affinity purified rabbit anti-human IgG (AffiniPure Rabbit Anti-Human IgG (H+L), was obtained from Jackson ImmunoResearch Laboratories, Inc, West Grove, PA. Poly HRP Streptavidin (SA-HRP) was obtained from Pierce (Pierce Biotechnology, Rockford, IL, N200).

2.2. Biotin-LY Conjugate (B-LY)

Therapeutic humanized monoclonal antibody (LY) was labeled using EZ-Link Sulfo-NHS-LC-Biotinylation Kits (Pierce, 21435) according to manufacturer's instructions. Briefly, LY was dialyzed overnight against phosphate buffered saline, combined with Sulfo-NHS-LC-Biotin in a 20:1 molar ratio and incubated for 1 hour at room temperature. The reaction mixture was dialyzed vs. PBS overnight. Protein content was analyzed using the BCA Protein Assay (Pierce, 23225) and was then diluted with an equal volume of glycerol and stored at −20 °C.

2.3. ACE assay

ELISA plates (Nunc Maxisorp 96 Microplates, Nunc, Rochester, NY) were coated with therapeutic humanized monoclonal antibody (LY) at a concentration of 5 μg/ml in BupH carbonate–bicarbonate buffer (Pierce) by adding 100 μl per well and incubating overnight at 4 °C. Samples were diluted 1:10 in TBS. Aliquots (100 μL) were acidified with 50 μL 300 mM acetic acid and incubated at room temperature for 5 min. LY coated plates were washed three times with TBST and 50 μL 1 M Tris, pH 9.5 was added. Acid-treated samples (100 μL) were added to the buffered, coated plates and allowed to incubate overnight at 4 °C. The following day, plates were washed three times with TBST followed by elution of bound ADA by addition of 65 μL 300 mM acetic acid for 5 minutes at room temperature. Fresh Nunc Maxisorp plates were then loaded with 50 μL of 1 M Tris pH 9.5 buffer. Fifty microliters of the acid eluate was transferred to the buffered Maxisorp plates followed by incubation for 1 h at room temperature to allow binding of eluted ADA to the wells. Plates were washed three times with TBST and blocked with Casein buffer for 1 hour at room temperature. After washing three times with TBST, 100 μL B-LY was added and incubated at room temperature for 1 h to allow binding to plate-bound ADA. Plates were washed three times with TBST and 100 μL SA-HRP was added and incubated at room temperature for 30 minutes at room temperature. Plates were washed three times with TBST and 100 μl of 3,3′,5,5′ tetramethylbenzidine (TMB) substrate (BioFX, Ewings Mills, MD) substrate was added and incubated for 30 min at room temperature. Color development was stopped by addition of 100 μl of 2 M phosphoric acid, and plates were read at 450 nm in a SpectraMax Plus plate reader.

2.4. Data analysis

Data was transferred to Excel 2003 where averages and standard deviations were calculated. The calculated numbers were transferred to Sigma Plot 8.0 for graphical