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Research paper

Detection of drug specific circulating immune complexes from in vivo cynomolgus monkey serum samples ☆

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ABSTRACT

Background: Administration of a biotherapeutic can result in the formation of anti-drug antibodies (ADAs). The resulting ADA can potentially form immune complexes (ICs) with the drug leading to altered pharmacokinetic (PK) profiles and/or adverse events. Furthermore the presence of such complexes may interfere with accurate PK assessment, and/or detection of ADA in immunogenicity assays. Here, we present two assays to detect the presence of drug–ADA immune complexes in cynomolgus monkeys.

Results: Serum samples were analyzed for IC formation in vivo. 8/8 tested animals were positive for drug specific IC. Depending on the time point tested 4/8 or 7/8 animals tested positive for ADA during drug dosing. All 8 animals were confirmed positive for ADA during the washout phase, indicating drug interference in the bridging assay. Relative amount of IC over time was determined and its correlation with PK and ADA was then assessed. Multivariate data analysis demonstrates good correlation between signals obtained from the anti-drug and FcγRIIIa based capture assays, although due to its biological characteristic FcγRIIIa based assay captured only a subset of drug specific IC. In one animal IC remained in circulation even when the drug levels decreased below detection limit.

Conclusion: Results from this study indicate the presence of IC during administration of an immunogenic biotherapeutic. Potential application of these assays includes detection of ADA in an IC during high drug levels. The results on the kinetics of IC formation during ADA response can complement the understanding of PK and ADA profiles. Moreover, the presence of IC indicates possible ADA interference in standard PK assays and potential underestimation of total drug exposure in toxicology studies. In addition this study also highlights the need to understand downstream in vivo consequences of drug–ADA IC as no animals under investigation developed adverse events.

1. Introduction

Biotherapeutics are revolutionizing the treatment of many diseases due to multiple advantages of this class of molecules. One of the limitations with this modality of treatment is their ability to trigger an immune response. This entails the innate as well as both the cellular and humoral arms of the immune system that can result in the formation of anti-drug antibodies (ADAs) leading to altered pharmacokinetic profiles, loss of efficacy (Chirmule et al., 2012; Vugmeyster et al., 2012) and in extreme cases hypersensitivity reactions (Brennan et al., 2010).

Abbreviations: ADA, anti-drug antibody; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BSA, bovine serum albumin; BUN, blood urea nitrogen; CP, cut point; CREA, creatinine; FcγR, Fc-gamma receptor; HQC, high quality control sample; HPLC, high pressure liquid chromatography; HRP, horseradish peroxidase; IC, immune complex; LBA, ligand binding assay; LQC, low quality control sample; mAB, monoclonal antibodies; MALS, multi-angle light scattering; MRD, minimum required dilution; MSD, Meso Scale Discovery; NQC, negative quality control sample; PK, pharmacokinetic; PBS, phosphate based saline; QC, quality control; SEC, size exclusion chromatography; UV, ultra violet.

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K_2HPO_4, 150 mM NaCl (pH 6.8 with HCl), with 0.02% Sodium Azide buffer. Three online detectors were utilized: Agilent diode array UV/vis spectrophotometer, Wyatt Technologies mini-Dawn three angle laser light scattering detector and Wyatt Optilab DSP interferometric refractometer. Data were collected and analyzed using Astra (Wyatt) and Chemstation (Agilent) software.

2.5. Bridging assay

Study samples and positive control QCs were diluted 20 fold (MRD20) in 1% BSA in PBS with 0.05% Tween 20 (1% PTB) containing experimentally optimized concentrations of ruthenium labeled drug and biotin labeled drug for 1 h. Following bridge formation, samples were incubated for 30 min on a pre-blocked MSD streptavidin coated Gold 96 well plate and read using 4x MSD Read Buffer T with Surfactant on a MSD Sector Imager 2400 Model 1250 (Meso Scale Discovery, Gaithersburg, MD). Unless otherwise stated all incubation steps were carried out shaking at 400 rpm in temperature controlled incubator at 24 °C and all washes were repeated 4 times.

2.6. Immune complex assays

Taking advantage of the unique structure of our biotherapeutic molecule and the absence of any cross reactive epitopes with an IgG molecule we proceeded with the classical approach of capturing the drug and detecting the IgG bound to the drug as a means of detecting immune complex. In order to elicit the presence of any complexes believed to be large enough to have potential in vivo FcγR mediated effects we chose to develop a second assay format utilizing FcγRIIIa capture that would capture a subset of drug specific immune complexes capable of binding to the low affinity FcγRIIIa.

2.7. Anti-drug capture assay

A monoclonal mouse anti-drug antibody was generated in house and was labeled with biotin using EZ-Link NHS-LC-Biotin according to the manufacturer's recommendations (Thermo Scientific Rockford, IL) for use as a capture reagent. Streptavidin 96-well black plate (Greiner Bio-one, Monroe, NC) was coated with this monoclonal anti-drug capture antibody at 1.2 μg/mL for 1 h. The plates were then blocked with 5% BSA in PBS. LowCross buffer (Candor Bioscience GmbH) was used as a dilution buffer for samples and detection antibody to decrease non-specific binding of serum IgG. According to the manufacturer's literature (Candor Bioscience GmbH) LowCross buffer contains a proprietary formulation capable of reducing interference, non-specific binding and matrix effect. It is possible that the formulation may contain non-physiological concentrations of salts or higher levels of detergents which might have the potential to alter the size or composition of immune complexes. LowCross buffer was used in assay format B with anti-IgG + IgM detection due to its ability to decrease assay background and increase assay sensitivity to detect immune complexes irrespective of their size as compared to other buffers tested. Although it is possible that the equilibrium of IC size was potentially affected by LowCross buffer, we were still successful in detecting immune complexes using additional assays. As an example, LowCross buffer was not used in assay where presumably larger immune complexes bind to FcγRIII (format C).

Samples and QCs were diluted 50 fold (MRD50) in LowCross buffer and were incubated on the plate for 30 min and washed 4 times with PBS containing 0.05% Tween 20. Detection antibody was used at 20 ng/mL and was purchased from Jackson ImmunoResearch (affinity purified HRP labeled anti-human IgG + IgM (H + L), cat # 309-035-107). Cross reactivity of this reagent to cyno IgG was determined experimentally (data not shown). Following 1 h incubation with the detection reagent and washes as described earlier the HRP activity was detected using luminol based SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific Rockford, IL). All washes were repeated total of 4 times using ELx405 Select CW plate washer (Biotek, Winooski, VT). Luminescence signal was recorded using SpectraMax M5e (Molecular Devices Sunnyvale, CA).

2.8. FcγRIIIa capture assay

Streptavidin 96-well black plate (Greiner Bio-one, Monroe, NC) was coated with an anti-polyhistidine monoclonal antibody labeled with biotin at 1 μg/mL (R&D Systems, cat # BAM050) for 1 h and blocked with 5% PTB. This step was followed by a wash and capture of recombinant cynomolgus monkey FcγRIIIa bearing 6HIS residues (Sino Biological, Inc. cat # 90013-C08H) at 1 μg/mL for 1 h. QCs and study samples were diluted 50 fold in 1% PTB followed by an overnight capture. Detection of captured drug specific complex was conducted using 400 ng/mL of monoclonal mouse anti-drug antibody custom labeled with HRP (Innova Biosciences Lightning-Link HRP Conjugation Kit, cat # 701-0000). All washes were conducted 5 times. HRP activity was detected using luminol based SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Scientific Rockford, IL). Luminescence signal was recorded using SpectraMax M5e (Molecular Devices Sunnyvale, CA).

2.9. Assay cut point determination

Cut point was determined using methods and calculations as previously described (Shankar et al., 2008) using normal serum from at least 30 cynomolgus monkeys. Cut point factor corresponding to 5% false positive rate was used for all three assays. The same serum lots were used in all three assays.

2.10. PK sandwich ELISA

Measurement of drug level in study samples was conducted using standard colorimetric sandwich assay format. A commercially obtained biotinylated anti-drug goat polyclonal antibody at 1 μg/mL was used to coat the Greiner streptavidin plate for 90 min. Standards, analytical QCs and study samples were diluted 10 fold (MRD10) in PTB and incubated on the plate for 90 min. Captured analyte was detected with custom made purified rabbit anti-drug antibody at 1 μg/mL. The detection reagent bound to a distinct portion of the drug away from the capture reagent binding site. Secondary detection was conducted using donkey anti-rabbit IgG HRP (Jackson Immuno Research, cat # 711-035-152) at 1/50,000 dilution. Each step was followed by 5 washes. TMB peroxidase substrate was added to detect HRP enzymatic activity. Following color development the reaction