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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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Immunogenicity against biotherapeutics is mounted against a foreign portion of the protein (Jefferis and Lefranc, 2009; Harding et al., 2010), although immunogenicity towards recombinant human proteins may be a result of breaking self-tolerance (Eckardt and Casadevall, 2003; Schellekens and Casadevall, 2004; Torosantucci et al., 2014). Irrespective of the cause of immunogenicity an immunogenic response leads to the induction of humoral responses in the form of anti-drug antibodies. ADA found in circulation binds to the biotherapeutic resulting in the formation of immune complexes. Formation of immune complexes is the natural response of the immune system designed to neutralize and remove foreign molecules from circulation. Most downstream adverse effects of ADA generally require the formation of an IC intermediate with the drug which in turn mediates Fc mediated clearance, reduced PK, reduced efficacy, immune complex disorders, or altered potency. In extreme cases, formation of ADA may lead to severe adverse events like pure red-cell-aplasia triggered by anti-epoetin alfa antibodies cross reacting with the patient's own erythropoietin (Eckardt and Casadevall, 2003).

Current state of the art methods in bioanalysis utilizes a bridging assay where a ternary complex or "bridge" is formed between the capture reagent (e.g., biotin-labeled drug), ADA, and the detection reagent (e.g., ruthenium-labeled drug). This format has the ability to detect multivalent molecules such as IgG or IgM antibodies. However, this assay as well as most other assay formats suffer from drug interference, where biotherapeutic drug found in circulation during immunogenicity sampling prevents detection of ADA in the same sample (Hart et al., 2011). This interference is in fact caused by biotherapeutic drug being bound to ADA and several approaches e.g. acid dissociation of the sample, have been developed to minimize this interference (Butterfield et al., 2010).

The issue of drug interference has consequences for establishing clinical rate of immunogenicity which is a regulatory requirement. For example, the reported immunogenicity of adalimumab is 5%, however, true immunogenicity rate is difficult to determine and is dependent on the assay format used (van Schouwenburg et al., 2010). In fact, current data supports that a substantial number of patients develop adalimumab ADA (van Schouwenburg et al., 2013a) of which 99% are neutralizing and lead to loss of efficacy (van Schouwenburg et al., 2013b).

Moreover, anti-adalimumab antibodies were recently reported to be found in small complexes with adalimumab of sizes consistent with IgG dimer using density gradient centrifugation (van Schouwenburg et al., 2013b). Although immune complexes were present in circulation there was no high rate of hypersensitivity reactions reported clinically. According to adalimumab prescribing information hypersensitivity reaction including anaphylaxis was rare, with allergic reactions occurring in about 1% of patients. These results suggest that although formed, not all types of immune complexes will result in anaphylaxis or immune complex disease.

Therefore, when a humoral immune response is generated against a therapeutic protein and whenever the therapeutic molecule is present in circulation, the ADA and the therapeutic protein will be present both separately as well as bound together as an immune complex. In this investigation, in order to detect immunogenicity, we used the hypothesis that immune complexes are present in circulation during an immunogenic response. To investigate this hypothesis we have selected a biotherapeutic molecule lacking an Fc portion in order to take bioanalytical advantage of the Fc portion on the ADA for detection of immune complexes made of drug and ADA. In this study we clearly demonstrated that immunogenicity can be detected not only through the detection of ADA but also through the detection of drug specific immune complexes that are likely to be present in circulation at any time point at which both ADA and biotherapeutic are present, but not necessarily detectable in circulation. Furthermore, the immune complexes formed in circulation had the ability to bind to Fcγ receptors, however, the presence of biotherapeutic drug–ADA IC did not lead to adverse events in non-human primates.

2. Materials and methods

2.1. Biotherapeutic drug

Biologic molecule used in this investigation was an engineered 31 kDa monovalent recombinant human protein. Unlike most biotherapeutics this protein was not engineered on an immunoglobulin backbone and as a result lacked Fc. The preclinical lot of this material was manufactured using a bacterial expression system with standard molecular biology techniques.

2.2. Anti-drug antibody (ADA)

ADA used as a positive control in bridging assay and as ADA component of drug specific immune complexes were generated by hyper-immunizing cynomolgus monkeys with the biotherapeutic drug using standard protocols available with the vendor. Sera were collected at multiple time points and were pooled together. Polyclonal ADA was purified using Protein G sepharose affinity chromatography and was followed by affinity purification on a column with immobilized biotherapeutic drug. Affinity purified ADA was eluted using 0.1 M Sodium Citrate buffer at pH 3.0, then buffer exchanged into PBS and stored at −80 °C.

2.3. Formation of immune complexes

Biotherapeutic drug–ADA immune complexes were formed in PBS buffer by combining both components of the immune complex at twice the final concentration and allowing the binding to proceed for 1 h in a temperature controlled incubator at 24 °C, followed by an equilibration step overnight at 4 °C. Preformed immune complexes were spiked into pooled cynomolgus monkey serum (Bioreclamation) for analysis of immune complexes in matrix and analyzed at indicated quality control (QC) concentration levels. Concentrations of immune complex QC used in IC assays were always based on the final concentration of IgG (ADA) in the immune complexes.

2.4. SEC–HPLC–MALS

Immune complexes formed in vitro were characterized using Agilent 1100 series with HPLC Shodex Protein KW 803 column (8 mm × 300 mm). Samples were injected using the Agilent auto sampler and run at 0.5 mL/min with 200 mM