Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 249–254

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Evaluation of a generic immunoassay with drug tolerance to detect immune complexes in serum samples from cynomolgus monkeys after administration of human antibodies

Kay Stubenrauch*, Uwe Wessels, Ulrich Essig, Rudolf Vogel, Julia Schleypen

Pharma Research Penzberg, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg, Germany

A R T I C L E I N F O

Article history:
Received 18 September 2009
Received in revised form
22 December 2009
Accepted 23 December 2009
Available online 4 January 2010

Keywords:
Anti-drug antibody
Immune complex
Drug interference
Cynomolgus monkey
Immunogenicity

A B S T R A C T

Current state of the art bridging ELISA technologies for detection of anti-drug antibodies (ADAs) against therapeutic antibodies bear the risk of false-negative results due to interference by circulating drug. Methods to remove the drug in the sample or sample pre-treatment techniques such as acid dissociation of the immune complexes are limited, laborious and may destroy ADAs resulting again in false-negative results. The immune complex ELISA described in this publication provides a simple solution. It is designed to analyze samples from cynomolgus monkeys dosed with human antibodies; it can be used for all human antibodies since it is independent of the specific antibody and its target. The generic applicability of the ADA assay is enabled by the use of (1) a murine anti-human Fc monoclonal antibody (MAb) as capture reagent; (2) a murine anti-cynomolgus monkey IgG MAb as detection reagent; and (3) an ADA positive control conjugate consisting of cynomolgus IgG complexed with human IgG. In its basic version, the generic ADA ELISA specifically detects only immune complexes formed in vivo. Validation of the ADA assay revealed a lower limit of quantitation of 15.6 ng/mL in serum samples. Intra-assay and inter-assay precision was characterized by a coefficient of variation of less than 10% and accuracy was within 8%. Matrix effects were low as evidenced by a mean recovery of 95%. In vitro pre-incubation of the serum samples with drug makes also the free ADA in the sample amenable to measurement by the immune complex ELISA as demonstrated by analysis of ADAs from two cynomolgus monkey studies with two different antibodies. The generic and versatile nature of this ADA assay favors its use in pilot pharmacokinetic and safety studies in cynomolgus monkeys during candidate selection of antibodies. The assay can help to explain unexpected drug clearance profiles, loss of efficacy or safety events caused by immune complexes and guide further development.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Monoclonal antibodies have become an increasingly important class of drugs in a variety of diseases and represent the majority of protein candidates currently in clinical development [1]. It is accepted standard practice and expected under current regulatory guidance that potential antibody formation to therapeutic biologics is being evaluated during non-clinical safety studies to describe and assess its impact on the toxicity profile [2]. Assay methodologies currently available to study the immune response against therapeutic biologics include enzyme linked immunoassays (ELISA), surface plasmon resonance (SPR), chemiluminescence and flow cytometry assays for binding antibodies and cell-based assays to assess the neutralizing potential of anti-drug antibodies (ADAs) [3]. In non-clinical studies, ADAs can affect drug exposure, thereby complicating the interpretation of the toxicity, pharmacokinetic and pharmacodynamic data [4]. In addition, immune complexes of drug and ADAs may cause toxic effects. Non-clinical safety and efficacy studies very often are conducted in cynomolgus monkeys, due to insufficient target cross-reactivity of human-specific antibodies with rodent or non-rodent laboratory animals, such as mouse, rat, rabbit and dog.

Despite the emergence of novel technologies to measure ADAs such as SPR, microtiter plate-based ELISAs are still the most widely used format for testing ADAs due to their high-throughput efficiency, relative simplicity and high sensitivity [5]. ADA ELISAs are most often designed in a bridge format which provides high selectivity and pan-species ADA detection capability, making it feasible to implement a single assay format for both non-clinical and clinical studies. In the double-antigen bridging ELISAs, the drug is immobilized on the surface of microtiter plates allowing specific binding of ADAs in the sample which in turn are recognized by the drug

* Corresponding author. Tel.: +49 8856 60 7869; fax: +49 8856 60 79 7869.
E-mail address: kay-gunnar.stubenrauch@roche.com (K. Stubenrauch).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.12.029

labelled with a detection system. An apparent disadvantage of the bridging ELISA using drug for capture and detection of ADAs is the possible interference of free drug present in the sample. Free drug may compete with solid phase drug for capture of the ADA and lead to a false-negative result [6]. In addition, bridging ELISAs are incapable to detect drug–ADA complexes. Considerable efforts are underway to dissociate the drug–ADA complex to detect ADA despite the presence of bound drug or to enable detection of the complex [5].

The present work describes an ELISA to measure ADAs in serum samples from cynomolgus monkeys exposed to human antibodies despite drug interference and to measure drug–ADA immune complexes. The ELISA uses a murine monoclonal antibody directed against the constant part (Fc) of human immunoglobulin (Ig) to capture the antibody (drug) of the drug–ADA complex, and a murine monoclonal antibody specifically directed against cynomolgus monkey IgG to detect cynomolgus monkey ADA. The antibody specific for cynomolgus monkey IgG was successfully generated in our lab despite the high sequence homology of cynomolgus immunoglobulins and human immunoglobulins of 85–98% [7].

This assay principle, thereby, provides a versatile generic format for measurement of ADAs in serum of cynomolgus monkey independent of the drug and the drug target. In its basic version, the generic ADA ELISA specifically measures immune complexes formed in vivo and, by definition, does not detect unbound ADAs. By introducing a simple pre-incubation step in which the serum sample is spiked with drug to form immune complexes, the generic ADA ELISA can be used to measure total ADA levels.

Other methods have been reported to reduce drug interference by sample pre-treatment techniques such as acid dissociation of drug–ADA complexes, or drug removal [8]. However, drug removal might also lead to removal of the drug–ADA complex. Acid dissociation to destroy the immune complexes is limited for high affinity ADAs, in addition ADA may be irreversibly denatured by acid pretreatment resulting in false-negative samples [3].

The generic ADA assay format described here is a novel and versatile method to generically detect immune complexes of human antibodies with ADAs from cynomolgus monkeys, or to analyze total ADA levels in the presence of high levels of circulating drug.

2. Experimental

2.1. Chemicals and reagents

The monoclonal antibodies used in the experiments were recombinant fully human monoclonal IgG antibodies directed against the human insulin-like growth factor-1 receptor (anti-IGF-1R) and against a human cellular surface target (MAb 2), respectively. The antibodies were produced at Roche Diagnostics GmbH, Penzberg, Germany.

The murine IgG1 monoclonal antibody R10Z8E9 was produced by Roche Diagnostics GmbH, Penzberg, Germany. This antibody is directed against a conformation epitope on the CH2 domain of all four subclasses of human Fc gamma [9]. To prepare biotinylated anti-human Fcγ-pan R10Z8E9, 10 mg/mL of the purified MAb were incubated at pH 8.5 in a molar ratio of 1:5 with D-biotinoyl-aminocaproic acid-N-hydroxysuccinimide ester dissolved in DMSO. The reaction was stopped after 60 min by addition of L-lysine and the surplus of the labeling reagent was removed by dialysis against 50 mM potassium phosphate buffer, with 150 mM NaCl, pH 7.5.

The murine monoclonal antibody against cynomolgus monkey IgG was generated from mice immunized with purified cynomolgus monkey IgG. Antibodies from hybridoma supernatants were purified by protein A chromatography. This anti-cynomolgus monkey IgG MAb (anti-cyno IgG) was digoxigenylated by incubating 2 mg/mL of the purified MAb at pH 8.1 in a molar ratio of 1:5 with digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester dissolved in DMSO. The reaction was stopped after 60 min by addition of L-lysine and the surplus of the labeling reagent was removed by dialysis against 50 mM potassium phosphate buffer, with 150 mM NaCl, pH 7.5.

Serum samples from chimpanzee, rhesus macaque, marmoset monkey and baboon were obtained by standard methods from individual zoo animals. Serum samples of 10 different individual cynomolgus monkeys were provided by Covance, Muenster, Germany. Batches of pooled cynomolgus monkey sera for use as matrix in the ELISA for calibration standards and quality control samples as well as for dilution of analyte samples were from Covance, Muenster, Germany, and from Bioreclamation Inc., Hicksville, NY, USA. Serum from dog, rat, CD-1 and NMRI mice was obtained from commercial sources (Charles River, Wilmington, MA, USA). The polyclonal antibody against human Fcγ (PAb anti-human Fcγ) was purchased from Dianova GmbH, Hamburg, Germany. The polyclonal anti-digoxigenin-horse radish peroxidase (HRP) conjugate (Fab fragments) was from Roche Diagnostics GmbH, Mannheim, Germany (cat. no. 11633716-001). The following reagents were obtained from Roche Diagnostics GmbH, Mannheim, Germany: 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) substrate (cat. no. 11684302-001) and the wash buffer for the ELISA: phosphate-buffered saline (PBS; 50 mM K_3PO_4; 150 mM NaCl; pH 7.5)/0.05% polysorbate 20 (Tween 20) (cat. no. 11332465-001). Ready to use LowCross buffer (order no. 100500) was obtained from Candor Bioscience GmbH, Weissensberg, Germany, and was used as dilution and assay buffer in the ELISA. All chemicals were of analytical grade.

2.2. Biacore assay for determination of specificity of binding

The specificity of the murine anti-cynomolgus monkey IgG MAb was evaluated by surface plasmon resonance (SPR) technology with the Biacore® 2000 instrument (Biacore, Uppsala, Sweden) using a CM5 sensor chip (BR-1000-14; Biacore, Uppsala, Sweden). Coating of an antibody to this chip was achieved by standard amine coupling. Unless otherwise stated, all incubations were performed in HBS buffer (HEPES, NaCl, pH 7.4) at 25 °C. A saturating amount of anti-cyno IgG and PAb anti-human Fcγ, respectively, was immobilized by amine coupling on different flow cells of the same CM5-chip. All animal and human sera were diluted in HBS buffer containing 1 mg/mL CM-dextran at a final concentration of 1%. Binding was analyzed by injection of the 1 in 100 diluted sera and incubation for 60 s. Dissociation was measured by washing the chip surface with HBS buffer for 180 s. Using BIAevaluation software V4.1 from Biacore, Sweden, the dissociation constant values (=K_{Diss}) were calculated with a 1:1 Langmuir fitting model. For all animal sera this calculation was based on the assumption that the IgG level was 15 mg/mL. The signal values 80 s after start of the injection of the test antibody have been chosen for the comparison of the amount of bound IgG (resonance signal units (RU) in Table 1).

2.3. Preparation of calibration standards and quality control samples

Conjugates of cynomolgus monkey IgG (Cyno IgG) with human IgG (H-IgG) were prepared for use as calibration standards (CS) and quality control (QC) samples. N-succinimidyl-3-acetylthiopropionate (SATP) was added to Cyno IgG purified from cynomolgus serum by ion exchange chromatography and gel filtration in a molar ratio of 1:5 (IgG:SATP). The mixture was incubated for 60 min at 25 °C at pH 7.1. The reaction was stopped by adding L-lysine and the surplus of SATP was removed by dialysis. In par-

Download English Version:

https://daneshyari.com/en/article/1221921

Download Persian Version:

https://daneshyari.com/article/1221921

Daneshyari.com