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Antibody drug quantitation in coexistence with anti-drug antibodies on nSMOL bioanalysis

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ABSTRACT

Therapeutic monoclonal antibodies (mAbs) are developed for treatment of diverse cancers and autoimmune diseases. For expansion of mAbs approval against unapproved diseases and pharmaceutical development, pharmacokinetics study is very important. Bioanalysis provides one of the most essential index against pharmacokinetics information. So far, we developed useful method for bioanalysis of mAbs in plasma or serum, nSMOL: nano-surface and molecular-orientation limited proteolysis. This method can provide accurate and reproducible value of mAbs content in plasma. Quantification of mAbs using ELISA is strongly influenced by endogenous ligand or anti-drug antibodies. In this report, we exhibited the role of nSMOL proteolysis coupled to LC-MS/MS analysis against quantification of mAbs bound to some binding molecules. The ligands against mAbs do not affect quantification of mAbs concentration in plasma using nSMOL proteolysis. On the other hands, some anti-drug antibodies (ADA), such as idiotypic antibodies, inhibit quantification of mAbs using nSMOL proteolysis. Acid dissociation has some efficacy in accurate value of quantitation of ADA binding mAbs using nSMOL proteolysis coupled to LC-MS/MS analysis. Accordingly, we consider that nSMOL method will contribute to understanding of mAb PK data and therapeutic reference combining with ADA measurements.

Introduction

Large amounts of monoclonal antibodies are continually encountered in circulation, the host may mount significant anti-drug antibodies (ADAs) response. Recently, humanized antibodies have increased to produce because of preventing the immunogenicity, but humanization could not reduce enough immunogenicity [1]. ADAs might be expected to diminish the effectiveness of mAbs as a therapeutic drug, and perhaps have other metabolic consequences. For example, Bevacizumab has reported to be able to induce antibodies (human anti-Bevacizumab or anti-drug antibodies) in patients receiving Bevacizumab [2]. Ranibizumab, a monoclonal antibody fragment targeting VEGF, also induced anti-drug antibodies in 17% of treated patients [3]. Furthermore, ADAs against Nivolumab were determined in samples collected during Nivolumab treatment [4]. However, this is highly dependent on the sensitivity of the assay. On April 22, 2016, guidance on immunogenicity testing of therapeutic proteins was released from FDA [5], so that evaluation of immunogenicity will be essential for development of mAb drugs [6,7]. The detection of ADAs is performed using a bridging electrochemiluminescence immunoassay, which provides a semi-quantitative assessment of immunogenicity for ADA. Immunogenicity of mAbs is needed for approval.

Immunogenicity interfered with the accuracy assessment by enzyme-linked immunosorbent assay (ELISA) method. Thus, ADAs may interfere with the quantification of mAbs levels in PK/PD analysis. Assay tolerance to such interference may depend on assay platform and reagents. Various approaches have been developed to improve ADAs tolerance in mAbs analysis but limited success was observed. Then, we provide the information about quantification of ADAs binding mAbs using nSMOL proteolysis coupled to LC-MS/MS bioanalysis.

As observed with other antibodies, the pharmacokinetics of Bevacizumab is well described. Overall, in all clinical trials, Bevacizumab disposition was characterized by a low clearance, a limited volume of the central compartment, and a long elimination half-life. This enables target therapeutic Bevacizumab plasma levels to be maintained with a range of administration schedules (such as once every 2 or 3 weeks). Moreover, Nugue G et al. suggested that serum Bevacizumab concentration is useful clinical pharmacodynamics marker [8]. Hence, credible methodologies for measurements of mAb in plasma are crucial for the assessment of exposure–response

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Table 1
The list of ADAs.

ADA name	Immunogen	Format	Affinity (Kd, nM)
HCA168	Trastuzumab	Human Fab	0.02 nM
HCA176	Trastuzumab	Human IgG1	0.4 nM
HCA177	Trastuzumab	Human IgG1	0.02 nM
MAB11130	F (ab)2 fragment of Trastuzumab	Mouse IgG1	NA
HCA185	Bevacizumab	Human IgG1	0.4 nM
HCA182	Bevacizumab	Human Fab	0.4 nM
MAB11128	F (ab)2 fragment of Bevacizumab	Mouse IgG1	NA
8G6G3D8	Nivolumab	Mouse IgG	NA

relationships in support of efficacy and safety evaluations, and dose selection for chemotherapeutic clinicians.

For the quantification of therapeutic mAbs in biological specimens, classical ligand binding assays such as ELISA is the most widely used technique. However, in some cases, an immunological-based assay is not the most appropriate method for quantifying mAbs. For example, mAbs can be analogs of endogenous IgGs with a minor change to their amino acid sequence, the standard ELISA unable to differentiate between endogenous and exogenous variants. In these cases, mass spectrometry-based methodologies are available for quantify the mAbs, which offer superior selectivity over an immunoassay, and significantly shorten method development times.

On the other hand, liquid chromatography-mass spectrometry (LC-MS) has become one of the most widely used methods in pharmaceutical laboratories. Recently, tandem LC-MS (LC-MS/MS) have been applied to mAbs as an alternative to ELISA for the bioanalysis of preclinical samples [9]. Although LC-MS/MS provides high sensitivity and high specificity for quantifying target analytes in complicated biological matrices, the associated severe matrix effects result in large quantification errors. To address this issue, we proposed a novel strategy for decreasing contaminant from various biological matrix using nSMOL proteolysis [10]. The nSMOL method is designed as solid-solid proteolysis for Fab-selective limited proteolysis. This developed proteolysis has made it possible not only to minimize sample complexity, but also to maintain the sequence specificity of peptides in CDRs. In this article, we investigate whether ADAs affect the interference of quantification values of mAbs using nSMOL strategy.

Materials and methods

Reagents and materials

Trypsin-immoblized glycidyl methacrylate (GMA)-coated nano-ferrite particle FG bead with surface activation by NHS group was purchased from Tamagawa Seiki (Nagano, Japan). Toyopearl AF-rProtein A HC-650F resin was from Tosoh (Tokyo, Japan). nSMOL Antibody BA Kit was from Shimadzu (Kyoto, Japan). Trastuzumab and Bevacizumab was obtained from Chugai Pharmaceutical (Tokyo, Japan). Nivolumab was obtained from Ono Pharmaceutical (Osaka, Japan). Individual male and female human EDTA-2K treated-plasma was purchased from Kohjin Bio (Saitama, Japan). Modified trypsin was purchased from Sigma-Aldrich (St. Louis, MO). n-Octyl-β-D-thioglucopyranoside (OTG) was purchased from Dojindo Laboratories (Kumamoto, Japan). P14R, internal standard synthetic peptide, was purchased from Sigma-Aldrich. Ultrafree-MC GV centrifugal 0.22 μm filter was purchased from Merck Millipore (Billerica, MA). Recombinant human ErbB2 Fc chimera and recombinant human His-tagged PD-1 were purchased from R&D systems (Minneapolis, MN). Recombinant human vascular endothelial growth factor (VEGF-A_{165}) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Anti-Trastuzumab antibodies (HCA169, HCA176, and HCA177) and anti-Bevacizumab antibodies (HCA182 and HCA185) were purchased from Bio-Rad (Raleigh, NC). MAB11128 and MAB11130 were purchased from Abnova (Taipei, Taiwan). Anti-Nivolumab was purchased from Genscript (Piscataway, NJ) (Table 1). Other reagents, buffers, and solvents were purchased from Sigma-Aldrich and Wako Pure Chemical Industries.

nSMOL proteolysis

In this study, the concentration of mAbs in plasma samples was quantified using nSMOL proteolysis coupled to LC-MS/MS analysis. nSMOL method was described in our previous report [10] and we briefly explain in this report. An aliquot of mAb-spiked human plasma was diluted 10-fold in PBS containing 0.1% OTG. The Ig fraction in plasma was collected with Protein A resin (50% slurry) with gentle vortexing at 25 °C for 15 min. Non-specific absorption on Protein A resin was washed twice with 300 μl of PBS containing 0.1% OTG and then with 300 μl of PBS, and then washed resin suspended in 75 μl of 25 mM Tris-HCl (pH8.0) containing 10 fmol/μl P14R. nSMOL proteolysis was carried out using 10 μg modified-trypsin immobilized FG-beads with gentle vortexing at 50 °C for 5 h in saturated vapor atmosphere. After nSMOL proteolysis, reaction was quenched by adding 10% formic acid at a final concentration of 0.5%. The peptide solution was recovered only by centrifugation (10,000 × g for 1 min) with to remove Protein A resin and trypsin immobilized FG-beads. These analytes were transferred into low protein binding polypropylene vials, and then performed LC-MS analysis without other solid-phase purification, evaporative concentration, and reconstitution. nSMOL method has been already validated prior to analysis by using Trastuzumab, Bevacizumab, or Nivolumab-spiked plasma [11–13].

Binding of ADAs or ligands to mAbs

The ADA- or ligand-treated mAbs were incubated for 30 min at room temperature with gently vortexing under indicated conditions. After incubation, 10 μl of human plasma added to each samples and quantified mAbs concentrations using nSMOL proteolysis coupled with LC-MS/MS analysis.

Table 2
MRM transition of signature candidate peptides from Trastuzumab, Bevacizumab, and Nivolumab.

Selected peptide	Transition mass (m/z)	Q1 [V]	Collision [V]	Q3 [V]	Role
Trastuzumab	542.8 → 404.7	−20	−18	−30	Quantitation
IYPTNGYTR	542.8 → 808.4	−20	−18	−28	Structure
	542.8 → 610.3	−20	−25	−22	Structure
Bevacizumab	542.3 → 797.4	−38	−18	−30	Quantitation
FTFSLDTSK	542.3 → 898.5	−38	−20	−34	Structure
	542.3 → 650.3	−38	−19	−34	Structure
Nivolumab	550.8 → 661.5	−24	−18	−24	Quantitation
ASGITFSNSGMHWVR	550.8 → 746.4	−26	−16	−28	Structure
	550.8 → 785.4	−26	−25	−30	Structure