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REVIEW

Current Status of Anti-Drug Antibody Analysis Using Immunocapture-Liquid Chromatography/Mass Spectrometry

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ABSTRACT

During drug development, anti-drug antibodies (ADA) is closely monitored for immunogenicity assessment. Ligand binding assay is currently the most widely used platform for ADA detection. Very recently, immunocapture-LC/MS was developed for ADA analysis including isotyping and semi-quantitation. This mini-review summarizes literatures pertaining to immunocapture-LC/MS ADA analysis with focus on methodology, applications and regulatory aspects. Current thinking and considerations as well as outlook are also presented.

KEYWORDS: anti-drug antibody (ADA), ADA isotyping, immunocapture, LBA, LC/MS.

INTRODUCTION

Therapeutic proteins and peptides have potential to cause immunogenicity by provoking an immune response in the body of a human and other animal [1,2], resulting in anti-drug antibodies (ADA) that can pose problems for both patient safety and product efficacy. Clinical consequences can range from relatively mild to serious adverse events [3-5], such as anaphylaxis, cytokine release syndrome, and cross-reactive neutralization of endogenous proteins mediating critical functions. ADA can affect drug efficacy, biodistribution and drug clearance, and complicate the interpretations of toxicity, pharmacokinetic (PK) and pharmacodynamic (PD) data [6-8]. Characterization and analysis of ADA is thus a vital element of immunogenicity assessment during drug development. Enzyme-linked immunosorbent assays (ELISA) and electrochemiluminescence (ECL) immunoassays [9], are the most widely used platform for ADA detection. The ADA detection involves a tiered approach of screening, confirmation and titration. In addition, neutralizing ability, isotyping, domain-mapping, relative binding affinity, cross-reactivity with endogenous proteins, or complement activating ability of ADA may be conducted, which are driven by product-specific, indication-specific, or risk assessment-based objectives [10-12].

However, the traditional ADA LBA could be subject to interference from circulating high-concentration therapeutic or soluble targets, and special sample treatments such as acid dissociation may be required to overcome the drug interference [13-

15]. Very recently, immunocapture-LC/MS has been developed as a supplemental tool for ADA analysis. This mini-review summarizes the literatures pertaining to immunocapture-LC/MS ADA analysis with focus on methodology, applications and regulatory aspects. Current thinking and considerations based on literature and outlook are also discussed

Indirect ADA detection by LC/MS

The first LC/MS-based attempt for assessing immunogenicity was to indirectly detect ADA in the presence of excess protein therapeutic in support of clinical programs addressing the safety and tolerability of human growth hormone analogues [16]. To overcome drug interference, excess therapeutic drug was spiked to completely saturate available ADA binding sites. Drug-ADA complexes were then isolated using Protein-G magnetic beads, followed by elution and digestion. Resultant peptide from the target therapeutic protein instead of ADA was quantified by LC coupled with matrix-assisted laser desorption MS and the results were correlated to the binding capacity of total ADA. Due to indirect ADA detection, however, this approach did not permit ADA isotyping.

The indirect method was also evaluated for detecting ADA for PEGylated biotherapeutics, where flexible PEG chain interfered with ADA capture using biotinylated drug [17]. In another application, LC/MS was used to evaluate neutralizing Ab (NAb) assay by simultaneously quantitating residual mAb drug, endogenous IgG and a NAb-positive control in bead extraction and acid dissociation (BEAD) eluates [18]. The low levels of the residual drug and human IgG in the BEAD eluates indicate that the BEAD efficiently removed high-concentration drug and serum components from the serum samples. Meanwhile, the NAb-positive control recovery (42%) in the BEAD provided an acceptable detection limit for the cell-based assay. Although the LC/MS method was not for measuring ADAs in the study samples, the capability of LC/MS for detecting multiple immunoglobulins (IGs) was clearly demonstrated.

Human ADA detection by LC/MS

Very recently, Chen et al. developed an immunocapture-LC/MS assay for simultaneous ADA isotyping and semi-quantitation in human plasma [19,20]. The assay was designed for analysis of pre-existing ADA (PEA) from biotherapeutics that did not contain human Ig Fc and had not been tested with post-dose clinical samples. The hybrid LC/MS ADA assay workflow included immunocapture of ADA and/or ADA-drug complexes on magnetic beads, separation from plasma, elution, digestion and LC/MS analysis. ADA and/or ADA-drug complex were first captured via immunoaffinity using either biotinylated drug or a biotinylated mouse mAb targeting the drug. With biotinylated drug as the capture reagent, drug interference could be an issue in the presence of high-level drug. On the other hand, immunocapture using the anti-drug mAb eliminated potential drug interference, providing that the capture Ab was not competing with ADA for binding to the drug which was demonstrated by complete recovery of drug with the mAb in the presence of low, medium and high titer ADA samples. With either capture approach, the captured ADA and/or drug-ADA complexes were immobilized on streptavidin magnetic beads, and separated from plasma by a magnet. After washing residual plasma components off the beads, ADA was released from the beads under acid conditions and subjected to overnight trypsin digestion followed by LC/MS analysis. Due to lack of a human ADA positive control (PC), commercial human IG of different isotypes and subclasses were used as surrogates to generate calibration curves for semi-quantitation of ADA. Unlike ADA, however, the surrogate IGs should not bind to the immunocapture reagents and were therefore spiked into the magnetic beads eluent of blank human plasma after the immunocapture steps. The assay for each ADA isotype fallen within the range of 0.1–10 µg/mL.

While a polycloncal ADA response can differ in amino acid sequence in their variable regions, the amino acid sequences of the heavy chain constant regions are highly con-

served for each isotype/subclass. The LC/MS ADA analysis was based on the detection of surrogate peptides from the heavy chain constant regions, which were unique, yet universal for each ADA isotype/subclass. As such, LC/MS detection of the unique peptides allowed simultaneous ADA isotyping and semi-quantitation. These unique peptides consisted of 6–12 amino acids. A micro-flow LC coupled to a triple quadrupole mass spectrometer was used. Chromatographic separation was performed on an Acquity UPLC Peptide BEH C18 column (1 mm x 50 mm, 1.7 µm, 300 Å), and the total runtime was 7.5 min per sample. The mass spectrometer was operated in positive multiple-reaction-mode (MRM) for detecting the unique peptides of all human ADA isotypes and subclasses.

Proof-of-concept of this methodology was demonstrated by detecting PEA for a biotherapeutic protein in blank human plasma. All of the PEA positive samples determined by ECL were found to be also positive by the immunocapture-LC/MS assay. LC/MS isotyping indicated that the ADA was attributed predominately to IgG1. The ADA levels were further determined semi-quantitatively using the calibration curves. Only 2 out of the 11 PEA positive samples had relative levels, at 0.660 and 0.680 µg/mL IgG.

Drug interference has been evaluated for PEA detection using an immunocapture-LC/MS ADA assay in human sera from 50 healthy donors [17]. In the presence of 120 µg/mL of drug (mAb), no apparent impact on ADA detection was observed.

With immunocapture-LC/MS assay using biotinylated drug as capture reagent, only one free ADA binding arm is needed for the biotinylated drug to capture ADA, whereas both ADA binding arms need to be free for a drug bridge assay to work [21]. Therefore, it was stated that the LC/MS ADA assay is expected to have better drug tolerance than drug bridging assay [21]. However, no side-to-side comparison between the two assays has been reported.

Monkey ADA detection by LC/MS

The first case-study using immunocapture-LC/MS ADA assay was recently reported by Roos et al. [22]. The assay was developed for detecting cynomolgus monkey ADA in support of a GLP toxicology study with weekly intravenous dosing of a biotherapeutic protein for 6 weeks. Drug levels in the ADA samples were from 0.229 to 1530 µg/mL far exceeding the drug tolerance limit of an initial ECL assay, and thus prevented ADA detection by the ECL assay. To overcome drug interference, an immunocapture-LC/MS assay and a new ECL assay with Protein G sample pretreatment were developed in parallel. For the LC/MS assay, a biotinylated mAb was employed to capture drug and drug-ADA complexes. Monkey ADA (IgG1-4) was monitored using a unique peptide VVSVLTVTHQDWLNGK. The LC/MS runtime was 3.5 min. Due to lack of a monkey ADA PC, ADA samples from a different monkey study using the same drug were used for the assay qualification. The assay precision and accuracy were determined to be within 30%. The immunocapture-LC/MS assay was able to detect ADA in the week 3 or later time point samples, and the results were in good agreement with the new ECL assay.

In another application, Staelens reported simultaneous detection, semi-quantification and isotyping (IgG, IgM and IgE) of ADA in monkey serum by immunocaptue-LC/MS [23]. Biotinylated drug coated on magnetic beads was used to capture the monkey ADA. Immunocapture recovery was estimated to be 47.8% using a monoclonal rabbit IgG. The LC/MS system consisted of C18 chromatography (ACE chromatography, 250 mm x 2.1 mm, 2.5 µm) and API5500 QTrap MS. Unique peptides for MS detection were DTLMISR (IgG1-4), GTVNLTWSR (IgE) and GQPLSPEK (IgM). The quantification ranged from 0.5 to 10 µg/mL using commercial reference monkey Ig materials. Samples from a 4-week toxicology study were analyzed using both the immunocapture-LC/MS and a LBA. A good correlation (r = 0.8656) was obtained between the relative amounts of ADA from the LC/MS assay and the ECL optical signals.

High resolution MS has also been also used to detect ADA in monkey plasma [24]. In this case, high levels of circulating drug (up to 1.0 mg/mL) prevented ADA detection by a

traditional ECL assay, a LC/MS assay was developed for simultaneous detection of ADA (IgG1), therapeutic mAb and target receptor. MS analysis clearly indicated the presence of the drug and the target cell fragments. However, the ADA detection was inconclusive, most likely due to incomplete removal of therapeutic mAb that could contribute to the MRM channel of the monkey ADA IgG1 peptide TTPPVLDSDGSYFLYSK.

Current thinking and considerations

Since the immunocapture-LC/MS method for direct ADA detection was first reported at the 2015 Workshop on Recent Issues in Bioanalysis (WRIB) [19], it has generated high interest among pharmaceutical industry and regulators to understand the advantages and challenges of using this novel platform versus the traditional LBA for ADA [25]. Based on an unofficial survey [26], more than a dozen of major pharmaceutical companies are currently evaluating LC/MS as a supplemental tool for immunogenicity assessment. However, many questions and issues are yet to be addressed in order for this technology to be widely used. Panel discussions on LC/MS ADA analysis have been featured in the last four (2015–2018) WRIB and published in annual white papers [25-28]. The panel consisted of several representatives form industry as well as regulatory agencies. The white papers present current thinking and considerations of the panel participants, which are summarized in this section. In my personal opinion, those thinking and considerations are worthy of further discussions as the LC/MS ADA technology is still in its infancy and a lot of questions are yet to be addressed.

The opinion of the 2017 WRIB panel concluded that LC/MS assay may offer some unique capabilities, including high specificity, reduced drug interference [21], reduced false positives, and ability of ADA isotyping [25]. However, more data and case studies are needed to support these statements. Immunocapture-LC/MS could be an alternative platform when there are unique challenges that cannot easily be overcome by LBA, such as novel or unusual matrices (e.g., mucosal secretions), when LBA assays show poor selectivity (e.g., low drug tolerability), or as a confirmatory tool of ADA positive sample sets. Due to its high specificity and ability of measuring relative amounts of ADA, a single LC/MS ADA assay may be used for screening, confirmation and titration . Currently, ADA isotyping is primarily driven by clinical safety signals, and is conducted case by case. When needed, specificity, multiplexing capability and capacity of LC/MS assay would greatly benefit ADA isotyping and characterization.

While cut-point has been used in LC/MS assays, in principal single-to-noise ratio (S/N) could be also used [25]. In LC/MS analysis, an S/N ratio of 3 is usually regarded as the limit of detection while S/N ratio of 10 as the limit of quantitation. However, the S/N approach could potentially underestimate ADA positive incidents, and further investigations are needed to validate this approach. As for ADA data reporting, LBA in most cases uses ADA titers whereas LCMS uses ADA relative concentrations, and both indicate the magnitude of immune response. Sponsors are encouraged to contact regulators to discuss the appropriate approach for reporting ADA responses.

Probably the most challenging issues for immunocapture-LC/MS ADA are assay sensitivity, validation and quality control (QC). At current state, immunocapture-LC/MS ADA assay sensitivity is lower than LBA, mainly due to interference from endogenous non-specific binding to magnetic beads. Further cleanup to remove endogenous IGs is a must in order to improve the LC/MS assay sensitivity. An ADA PC should be used for assay development, validation and QCs, estimating immunocapture recovery and establishing assay range [25]. To this end, a humanized PC (or the PC containing human Fc) can be used because the signature peptides are located in the highly conserved Fc region. However, using peptides that are unique to the PC and do not occur in ADA could also be useful to enable the use of non-humanized PCs.

No LC/MS ADA data have yet been submitted to regulatory agencies. The WRIB panel representatives from the US Food and Drug Administration stated that they would like

to have sponsors to present to regulators assay validation and comparison with LBA to get some confidence in the correlation [25]. They see potential use in a regulated environment, but would like to open a dialog to get specifics on their applications, in particular regarding assay validation [25,26]. To this end, the 2017 WRIB panel suggested that a hybrid approach using both LBA and LC/MS could be considered: LBA for ADA screening/confirmatory and immunocapture-LC/MS for characterization on the confirmed positive samples. This would allow comparison of the two assays and permit to evaluate the correlation [25].

Outlook

Immunocapture-LC/MS represents a novel platform for ADA detection and may have the potential to serve as a supplemental tool for immunogenicity assessment. It offers some unique capabilities, such as high specificity and ability for simultaneous semi-quantitation and ADA isotyping. Recognizing that it is still in its infancy, some technical details and practical considerations are yet to be addressed. Further development should focus on:

1. improve immunocapture-LC/MS assay sensitivity by reducing endogenous non-specific binding to magnetic beads;
2. implement an appropriate ADA PC for assay development, validation and QC;
3. assess immunocapture recovery for generating more quantitative measurements; and
4. demonstrate reasonable correlation with LBA. As this technology has generated high interest in the industry and regulatory agencies, more case studies are anticipated to be reported in the very near future.

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