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Note

Fully validated LCMS bioanalysis of Bevacizumab in human plasma using nano-surface and molecular-orientation limited (nSMOL) proteolysis

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ABSTRACT

The chemistry of nano-surface and molecular-orientation limited (nSMOL) proteolysis is the Fab-selective limited proteolysis by making use the difference of protease nanoparticle diameter (200 nm) and antibody resin pore diameter (100 nm). In this report, we have demonstrated that the full validation for Bevacizumab bioanalysis in human plasma using nSMOL. The immunoglobulin fraction was collected by Protein A resin from plasma, then nSMOL reaction was performed using the FG nanoparticle-immobilized trypsin under the nondenaturing physiological condition at 50 °C for 6 h. After removal of resin and nanoparticles, the signature peptide of Bevacizumab complementarity-determining region (CDR) and internal standard $P_{14}R$ were simultaneously quantified by LCMS multiple reaction monitoring (MRM). This nSMOL method quantification of Bevacizumab showed sensitivity of 0.146 μg/ml and linearity of 0.146–300 μg/ml. The intra- and inter-assay precision of lower limit of quantification (LLOQ), low quality control (LQC), middle quality control (MQC), and high quality control (HQC) was 7.94–15.2% and 14.6%, 7.15–13.5% and 11.7%, 2.63–6.47% and 5.83%, and 3.09–4.35% and 4.45%, respectively. These results indicate that nSMOL is also significant method for Bevacizumab bioanalysis in human plasma.

1. Introduction

Bevacizumab, a novel humanized monoclonal antibody (mAb) against vascular endothelial growth factor A (VEGF-A), is currently approved by the US Food and Drug Administration (FDA) on the market based on its effectiveness in metastatic cancer [1]. Public data exhibits that the global sales value of Bevacizumab in 2012 exceeded $6.3 billion, ranking the fourth among the 10 best-selling mAb drugs. Bevacizumab was approved for the treatment of colorectal, lung, breast, glioblastoma, kidney, and ovarian cancer. The main mechanism by which Bevacizumab exerts anti-tumor activity is by preventing VEGF-A from binding with its receptors, thus resulting in inhibition of the VEGF pathway, which is a crucial driver for tumor growth, angiogenesis, invasion, and metastasis [2].

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 [3]. Hence, credible methodologies for measurements of mAb in plasma are crucial for the assessment of exposure–response 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 enzyme-linked immunosorbent assay (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 or nucleotide sequence, the standard ELISA unable to differentiate between endogenous and exogenous variants. In these cases, mass spectrometry-based methodologies is available for quantify the mAbs, which offer superior selectivity over an immunoassay, and significantly quicker method development times.

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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 [4]. 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 [5]. 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 CDR. In this article, we describe the development of a bioanalytical assay using nSMOL proteolysis coupled with LC-MS/MS for the bioanalysis of Bevacizumab.

2. Experimental section

The detail experimental procedures are described in supplemental materials.

3. Results

3.1. Confirmation of Bevacizumab signature peptides by LCMS-IT-TOF MS and ClustalW analysis

Identification of tryptic Bevacizumab peptides by LCMS-IT-TOF MS and Mascot analysis showed that 6 tryptic peptides were identified using nSMOL proteolysis, and 3 peptides were from CDR containing peptides (data not shown). Fig. S3 showed the peptide configuration of Bevacizumab in 3D structure by nSMOL method. It was revealed that the nSMOL proteolytic sites were mainly associated with the flexible, unfolding region on CDR. Furthermore, Bevacizumab specific peptides were selected with some criteria for accurate quantitation like our presented report of Trastuzumab bioanalysis [6]. Finally, we have selected 3 candidate signature peptides (FTFSLDTSK, STAYLQMNSLR, and VLIYFTSSLHSGVPSR) of the CDR containing region for Bevacizumab quantitation (Fig. S1).

3.2. Decision of the Bevacizumab signature peptide for assay validation

The analytical interference of 3 selected Bevacizumab peptides in plasma were analyzed by nSMOL proteolysis coupled with LC-MS/MS (data not shown). The peptide FTFSLDTSK was demonstrated as most significant peptide with no interference from human plasma, and in good correlation with Bevacizumab concentration. The optimized MRM transition of FTFSLDTSK for quantitation and structure confirmation were shown in Table 1. Following assay validation, we used molecule's unique peptide FTFSLDTSK.

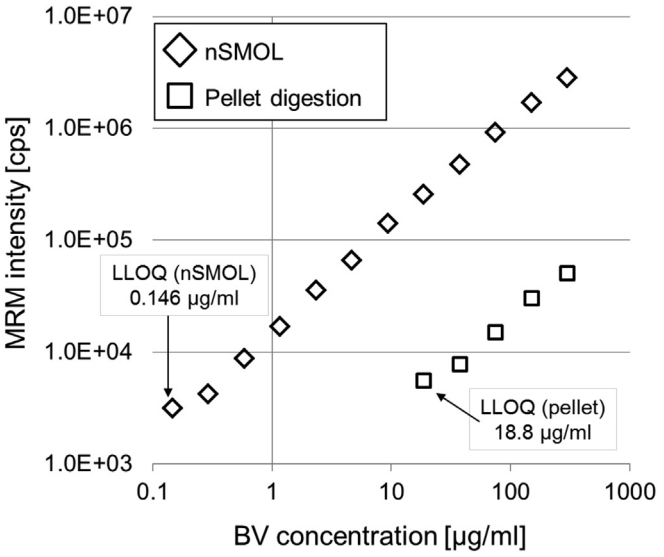

Fig. 1. Advantage of nSMOL bioanalysis in human plasma compared with normal LCMS method by calibration curve verifications. Bevacizumab bioanalysis by nSMOL and pellet digestion method were shown. The horizontal axis shows the Bevacizumab concentration in human plasma, and the vertical shows the intensity of MRM chromatogram with logarithmic indication. Bevacizumab bioanalysis was performed by using the sample of 2-fold serial dilutions from the Bevacizumab concentration of 300 µg/ml.

3.3. Comparison between nSMOL and another LCMS bioanalysis

The advantage of bioanalytical validation of nSMOL was demonstrated compared with common LCMS bioanalysis called pellet digestion [7] in Fig. 1. Calibration test in human plasma bioanalysis showed that remarkable improvement of LLOQ and ion intensity were available because of minimizing sample complexity by the successful Fab-selective proteolysis (Fig. S2). Using pellet digestion method, excess peptides would be caused the ionization suppression in LCMS ion path and decreased dynamic range compared with nSMOL chemistry.

3.4. Assay validation

3.4.1. Selectivity

Any interference peaks in the MRM chromatograms of the signature peptide and internal standard P14R were not detected from each plasma sample of male and female, suggesting that no interference was existed on the retention time of Bevacizumab peptide. The typical nSMOL assay selectivity by the MRM chromatogram of Bevacizumab peptide and $P_{14}R$ internal standard is shown in Fig. 2.

3.5. Matrix effect

The each plasma sample, 6 samples of males and females, was analyzed for matrix effect test at the concentration of LQC and HQC.

Table 1
Optimized MRM transition of Bevacizumab signature peptide for validation. The parameters are defined as follows: Selected peptide; peptide sequence for Bevacizumab quantitation, Region; region of selected peptide, Transition mass filter; fragment ion *m/z* for quantitation from the parent ion *m/z*, Q1 [V]; voltage condition of the quadrupole cell Q1, Collision; electrode voltage of collision cell Q2, Q3 [V]; voltage condition of the quadrupole cell Q3, Role; purpose of each ion *m/z*.

Selected peptide	Region	Optimal MRM condition				Role
		Transition mass filter [*m/z*]	Q1 [V]	Collision [V]	Q3 [V]	
FTFSLDTSK	H-chain of CDR2	523.3 → 797.4 ($y7^+$)	−38	−18	−30	Quantitation
		523.3 → 898.5 ($y8^+$)	−38	−20	−34	Structure confirmation
		523.3 → 650.3 ($y6^+$)	−38	−19	−34	Structure confirmation