Poster

[P25-7] P25-7: Immunosuppressive drugs (2): Monoclonal antibody and genotyping

Chair: Toru Hashida, Japan

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[P25-7-5] Assay development toward Infliximab therapeutic monitoring by LCMS: new strategy using CDR-peptide selective proteolysis nSMOL

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Background

Infliximab (IFX) is a chimeric antibody against TNF-alpha, and widely used as a therapeutic treatment for rheumatoid arthritis, Crohn disease, ulcerative colitis and so on. IFX is known for the formation of anti-drug antibodies (ADA) in treatment. Therefore adjustment of IFX dosage for individual patients may be an effective treatment by drug monitoring. The method for IFX monitoring has been generally analyzed by ligand binding assay (LBA). LBA may have some significant limitation such as the indispensable of anti-idiotypic antibodies and interference by ligand, biological matrix, or ADA.

Methods

For the IFX monitoring in serum, LCMS is alterative approach for the viewpoint of the high-selectivity and sensitivity. However reproducible assay for regulated LCMS bioanalysis cannot obtain by traditional proteomic method for some intimate reasons. Recently, Iwamoto et.al. reported the novel method for antibody drug quantitation and method validation for several antibodies focusing on the CDR-targeting quantitation named nSMOL (nano-surface and molecular-orientation limited proteolysis). nSMOL chemistry is achieved by limited reaction fields, the surface of trypsin-immobilized nanoparticle and immunoglobulin in pore. Here we have developed and validated the LCMS assay for IFX using nSMOL application. The structure and ion series of IFX tryptic peptides were confirmed by LC-TOF-MS/MS and ClustalW sequence alignment. The candidate signature peptides was further verified the no interference in human serum after nSMOL reaction.

Results

Five signature peptides (GLEWVAEIR, SINSATHYAESVK, SAVYLQMTDLR from H-chain, and ASQFVGSSIHWYQQR, YASESMSGIPSR from L-chain) for IFX bioanalysis were obtained. And each multiple reaction monitoring (MRM) transitions were set as one for quantitation and two for structural confirmation. The range of quantitation was set from 0.1 to 200 g/ml in human serum. And partial method validation was succeeded in accordance with the Guideline on Bioanalytical Method Validation in Pharmaceutical Development for low molecular weight drug compounds.

Conclusions

We are now performing the IFX monitoring using clinical samples of ulcerative colitis. And the nSMOL data correlation in the presence of ADA are also verified using some experimental models. We have concluded that nSMOL strategy would be an optimal and accurate assay method for IFX therapeutic monitoring.