INTRODUCTION

Immunogenicity of biopharmaceutical products is a major concern in both pre-clinical and clinical studies. In addition to the specific detection of anti-drug antibodies (ADA), regulatory bodies are increasingly expecting detailed characterization of any drug elicited immune response, including isotype determination and affinity evaluation. There are several approaches available to measure the affinity of an antibody molecule for its antigen, but most of these require either excess to expensive equipment (e.g. Surface Plasmon Resonance) or are not generally applicable (e.g. dialysis). An ELISA based approach was developed to determine the relative affinity of ADAs against a therapeutic protein product and validated in our laboratory.

ANALYTICAL METHODS

Evaluation of ADA response against a therapeutic protein product in human plasma was conducted using ELISA based techniques in our laboratory as follows:

- Screening for positive ADA samples, then confirmation of specificity for the drug of ADA response followed by sample titration
- Determination and titration of immunoglobulin species (IgG, IgM, and IgE) present in confirmed ADA positive samples
- Determination of relative affinity on confirmed ADA positive samples

The hallmark of the relative affinity ELISA is the use of a gradient of coated drug concentration row wise across the plate (Fig. 1). ADA present in the samples forms a bridge between the drug adsorbed onto the plate (used as capture reagent) and the labeled drug in solution (used as detection reagent). The complex is detected by specific binding of the enzyme horseradish peroxidase (HRP) via the biotin-streptavidin interaction and revealed using a chromogenic substrate for quantification by absorbance readout (Optical Density, OD).

VALIDATION OF A RELATIVE AFFINITY ELISA

The following parameters were evaluated as part of the validation study for the ELISA assay:
- Inter and intra assay precision for rh determination using a positive control (PC; plasma pool spiked with one level of reference ADA preparation) and ADA positive clinical samples (CS1 and CS2)
- selectivity in individual ADA naïve plasma spiked at PC level with reference ADA preparation
- range of acceptable OD at highest coated drug concentration used to establish the sample dilution factor in the assay
- stability of control samples and ADA positive clinical samples

A typical response obtained in the affinity ELISA from an ADA positive clinical sample is shown in Figure 2, left panel. The addition of free drug to the level of an ADA naïve plasma sample at all coated drug concentrations used (Fig. 2, left panel) demonstrates that the assay is able to identify individuals that show a shift of their ADA response over time that would be indicative of an apparent change in affinity. Eighty three percent of the samples measured showed an ADA response at higher affinity antibodies over time that would be indicative of an apparent change in affinity. Eighty three percent of the samples measured showed an ADA response.

CASE STUDY

Thirty six healthy individuals were submitted to a single dose of serine protease drug with sampling time of Day-1, Day-7, Day-14, and Day 30 for a total of 108 samples. Following screening and confirmatory assays, 27 samples were confirmed to be positive for specific ADAs and analyzed in the relative affinity ELISA.

REFERENCES

3. G. Lemaillet, S. Wood, P. Struwe

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