Effects of cyclophosphamide and cyclosporine on primary IgM and IgG antibody responses to KLH in the dog using a species-specific ELISA assay

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Introduction

Limited nonclinical immunotoxicity data are available in the dog, although this is a major non-rat rodent species in regulatory safety studies. The aim of the present study was to measure primary anti-KLH IgM and IgG responses in Beagle dogs treated with the reference immunosuppressive drugs cyclophosphamide and cyclosporine, using a dog-specific sandwich ELISA assay jointly developed in-house by CiToxLAB France and Atlabio.

Materials and methods

Treatment

Groups of 3 male and 3 female Beagle dogs were treated orally for 4 weeks with 2 mg/kg cyclophosphamide on 4 consecutive days each week, or 25 mg/kg cyclosporine daily, or the same volume of drinking water daily.

The animals were given one single IM injection of 3 mg KLH on day +11. Blood samples were collected pre-test and on days +18 and +23 to measure anti-KLH IgM, and pre-test and on days +23 and +28 to measure anti-KLH IgG levels using a dog-specific sandwich ELISA assay using a peroxidase-conjugated anti-dog IgM or IgG.

Dog-specific sandwich ELISA development and validation

Three dogs were immunized with 5 mg KLH intramuscularly and their sera obtained 7 and 12 days later were pooled. Specific anti-KLH polyclonal IgM and IgG solutions were prepared from pooled serum samples by KLH-coupled NHS sepharose chromatography and then protein A affinity chromatography. Anti-KLH antibodies were characterized by 2-D gel electrophoresis, by affinity chromatography. Anti-KLH antibodies were characterized by 2-D gel electrophoresis and quantified for preparation of the standard (STD) solutions, and quality control (QC) solutions for the calibration of the ELISA response curves. Standard curves, which ranged from 10.24 ng/mL to 10000 ng/mL of anti-KLH IgM or IgG, included 2 anchor points and were fitted to a 4PL non-linear regression model (figure 1). Standard curves from 3 independent assays were within acceptance criteria. The whole analytical procedure was validated for specificity, precision and accuracy, repeatability, sample dilution, and short-term, long-term and freeze-thaw cycle analytic stability (Table I).

Results

The concentration level of 20 ng/mL was validated as the lower limit of quantification (LLOQ) and the concentration level of 5000 ng/mL as the upper limit of quantification (ULOQ). No non-specific binding for IgG and minimal non-specific binding in a few animals for IgM were observed in dog serum samples taken before the start of treatment and KLH immunization.

Table I: GLP-validation of a dog-specific sandwich ELISA for quantifying anti-KLH IgM and IgG

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance Criteria</th>
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<tr>
<td>Specificity at 60 ng/mL</td>
<td>76.8-127.9%</td>
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<tr>
<td>STD accuracy</td>
<td>CV ≤ 10.1%</td>
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<tr>
<td>QC accuracy</td>
<td>CV ≤ 24.5%</td>
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Figure 1. Anti-KLH IgM assay: typical standard curve

Figure 2. Primary anti-KLH IgM responses measured 7 and 12 days after single immunization in dogs treated with drinking water (Co), cyclophosphamide (CP) or cyclosporine (CS).

Figure 3. Primary anti-KLH IgG responses measured 12 and 18 days after single immunization in dogs treated with drinking water (Co), cyclophosphamide (CP) or cyclosporine (CS).

Conclusion

These results show that this dog-specific sandwich ELISA assay is validated and can be used to assess anti-KLH antibody responses in the dog during regulatory toxicity studies. As expected, cyclophosphamide markedly decreased primary anti-KLH antibody response in contrast to cyclosporine.