Procedure refinement and reduced restraint enables extended 6 hour daily inhalation dosing in beagle dogs

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Introduction

Extended duration of dosing for preclinical assessment of inhaled pharmaceuticals is considered advantageous when testing products of expected low toxicity. Technical limitations have often determined the maximum time of exposure in non-rodents such as Beagle dogs. Increased exposure time can improve the chance and accuracy of determining the maximum tolerated dose rather than being limited to a maximum feasible dose.

Study design

A study was undertaken to confirm whether reduced restraint (platform) combined with procedural refinements would enable routine 4 hour daily dosing (5 days) and even 6 hour daily dosing (3 days). Prior to delivery from the supplier, a number of dogs were presented to a platform restraint and inhaled test aerosol for 4 hours to demonstrate acceptance were selected. Following receipt, the four dogs (2 males and 2 females) were gradually acclimated for increased periods of time (up to 4 hours) to the inhalation exposure equipment over 13 consecutive days.

During the acclimation period, the dogs were monitored for behavioral changes (excessive salivation, trembling, vocalization, struggling and increases in respiratory rate). Reinforcement through positive behavior by voice commands and minimal animal contact during the sham dosing was emphasized. Verbal rewards, pit pat, and treats were provided after completion of each session.

Table 1: Beagle dogs individual body weights and estimated respiratory minute volume (RMV) at the start of the dosing

<table>
<thead>
<tr>
<th></th>
<th>Male 1001</th>
<th>Male 1002</th>
<th>Female 1501</th>
<th>Female 1502</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>5.9</td>
<td>6.4</td>
<td>7.0</td>
<td>7.4</td>
</tr>
<tr>
<td>RMV (L min-1)</td>
<td>2.76</td>
<td>2.96</td>
<td>3.19</td>
<td>3.35</td>
</tr>
</tbody>
</table>

Note: Calculated using RMV (L min-1) = 0.608 x Body weight (kg)^0.852 (Ref 2.)

Assessments included clinical signs, body weights and food consumption measurements. Animal behavior was also monitored during dosing for excessive salivation, trembling, vocalization, struggling and increases in respiratory rate. Reinforcement through positive behavior by voice commands and minimal animal contact during the sham dosing was emphasized. Verbal rewards, pit pat, and treats were provided after completion of each session.

Animal Exposure System

The animals were exposed to air using a TSE stainless-steel flow-pass exposure chamber (TSE Systems Inc, Charleston, MO, USA) comprising of two sections each with 10 ports. The fresh air was introduced in the top section of the chamber and carried through the animal’s breathing zone. The spent air was returned to the bottom section from which it was exhausted. Airflows within the system were monitored and controlled to ensure a constant flow of fresh air throughout the system.

Results

Four Beagle dogs underwent the 4 hour daily sham dosing except one female for which the sham dosing had to be interrupted on Days 2 to 4. The 6 hour sham dosing was initiated and successfully conducted using the remaining 3 dogs (2 males and 1 female). There were no changes in clinical signs, body weights or food consumption throughout the study.

Behavioral changes recorded during acclimation, documented acceptance of the reduction. When recorded during treatment, they sometimes can supplement clinical observations used for toxicity assessment and explain systemic exposure outliers.

Conclusion

Extended daily inhalation dosing up to 6 hours in Beagle dogs was achieved by combining animal screening at the supplier, extensive acclimation, positive behavior re-enforcement and reduced restraint. This enables to more accurately determine the NOEL and improve the determination of a MTD while improving animal welfare by reducing the level of restraint and stress.

References