Inhalation Toxicity Profile of a 2′-MOE Modified Antisense Oligonucleotide in Mice

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Abstract
Antisense oligonucleotides (AOs) have a broad range of therapeutic targets. The toxicity profile of first-generation phosphorothioate and second-generation (2′-MOE, phosphorodiamidate) antisense oligos have been characterized after general i.v. or s.c. administration. However, little information exists on the local and systemic toxicity of these compounds after aerosolized administration to the lung. For the current study, a 2′-MOE antisense oligo was administered by nose-only inhalation to male and female C57BL/6 mice for 5 hr every other day, for a total of 9 exposure days. Antisense oligo solutions were aerosolized by individual PARI LC PLUS™ nebulizers plumbed to In-Tox Predators™ 34-port radial nose-only inhalation exposure chamber with a Positive Flow-by™ modulated resistance. The system was operated at slightly negative pressure vs. room air. Mouse exposure was designed to deliver 0, 1.3, and 10 mg/kg total dose of oligo deposited on the lung per exposure day. Chamber concentrations were 2% of target at all exposure levels on all exposure days, with mean particle sizes of 2.5 or less than 1.5 μm MMAD at all levels. Aerosolized product was deposited at the buccal mucosa by capillary gel electrophoresis. Concentrations ranged from 400 to 800 pg/μl wash to well lung weight from 1 to 100 mg/kg exposure levels. In general, exposure effects were mild. No exposure-related changes were observed in mortality, weight gain, food, water intake, clinical signs, serum chemistry, hematology, organ weight, or gross pathology. Changes in liver weights in some exposed males and females were not clearly attributed to oligo treatment. The most pronounced findings were exposure-related minimal to moderate increases in pulmonary macrophage hyperplasia and hyperplasty and mild histopathologic perivascular cuffing. Consistent with high concentrations of inhaled compounds, some 10 mg/kg male mice demonstrated minimal to moderate dysplasia with minimal of the oxyphilic epithelium and minimal to moderate hyalinization degeneration of the nasal epithelium in both genders at all exposure levels, including control.

These observations are consistent with the mild proinflammatory effects of both first- and second-generation antisense oligos administered to rodents by other routes of administration, with a somewhat milder systemic profile of general mononuclear cell infiltrates.

Introduction

Exposure Assessment
Test Article: 2′-MOE modified antisense oligonucleotide
Exposure System: In-Tox, 34 port cylindrical nose-only chamber, PARI LC PLUS™ nebulizer
Test System: Male and female C57BL/6 mice
Exposure: Nose-only inhalation
Exposure Levels: 0 μg/kg, 3, 10 μg/kg
Duration: 9 total exposure days, 2.5 hours per day
Health Endpoints:
- Mortality and morbidity
- Clinical signs
- Clinical pathology
- Bodyweight
- Histopathology (respiratory tract in all dose groups; liver, kidney and spleen in high-dose and control exposure groups)

Target and Actual Exposure and Dose Values

<table>
<thead>
<tr>
<th>Exposure Level</th>
<th>Target Aerosol (mg/kg)</th>
<th>Actual Mean Aerosol (mg/kg)</th>
<th>Dose Target (mg/kg)</th>
<th>Calculated Average Deposited Dose (50 g mouse and 2.5 h exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0.00</td>
<td>0.00</td>
<td>1</td>
<td>1 mg/kg (range 0.85–1.15)</td>
</tr>
<tr>
<td>Med</td>
<td>0.11</td>
<td>0.11</td>
<td>3</td>
<td>3.18 mg/kg (range 2.53–3.73)</td>
</tr>
<tr>
<td>High</td>
<td>0.5</td>
<td>0.49 (0.020)</td>
<td>10</td>
<td>5.3 mg/kg (range 4.5–6.2)</td>
</tr>
</tbody>
</table>

*Calculated deposited dose not based on individual mouse body weight or exposure group mean body weight at any exposure interval.

Conclusions
- Repeated exposure of male and female C57BL/6 mice up to 10 mg/kg of antisense 2′-MOE antisense oligos were associated with mild health responses.
  - Drug delivery by inhalation was feasible as indicated by the retained oligo present in the lung.
  - Increases in cytoplasmic volume of macrophages were consistent with uptake of oligo.
- Minimal to moderate macrophage hyperplasia and hyperplasty.
- These observations are consistent with the mild proinflammatory effects of both first- and second-generation antisense oligos administered to rodents by other routes of administration, with a somewhat milder systemic profile of general mononuclear cell infiltrates.
- Overall, inhaled oligonucleotide therapy is feasible and unlikely to be associated with clinically significant side effects of pulmonary administration.