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

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Abstract

Antisense oligonucleotides (oligo) have a broad range of therapeutic targets. The toxicity profiles of first- (phosphorothioate) and second-generation (2´-MOE phosphorothioate) 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 CD-1 mice for 2.5 h every other day, for a total of 9 exposure days. Antisense oligo saline solutions were aerosolized by individual PARI LC PLUS™ nebulizers plumbed to In-Tox Products' 24-port radial nose-only inhalation exposure chamber with a Positive Flow-By™ rodent restraint. The system was operated at slightly negative pressure vs. room air. Mouse exposures were designed to deliver 0, 1, 3, and 10 mg/kg total dose of oligo deposited in the lung per exposure day. Chamber concentrations were within 2% of target at all exposure levels on all exposure days, with mean particle sizes at or less than 1.6 µm MMAD at all levels. The presence of oligo in the lungs of exposed mice was confirmed by capillary gel electrophoresis. Concentrations ranged from 400 to 800 µg/g oligo to wet lung weight from the 1 to 10 mg/kg exposure levels. In general, exposure effects were mild. No exposure-related changes were observed in morbidity, mortality, body weight, clinical signs, serum chemistry, hematology, organ weight, and gross pathology. Changes in liver weights in some exposed male and female mice were not clearly attributed to oligo treatment. The most pronounced findings were exposure-related minimal to moderate increases in pulmonary macrophage hyperplasia and hypertrophy and mild histolymphocytic perivascular cuffing. Consistent with high concentrations of inhaled compounds, some 10 mg/kg males demonstrated minimal to moderate degeneration with necrosis of the olfactory epithelium and minimal to moderate hyaline degeneration of the nasal epithelium in both genders at all exposure levels, including control.

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

Introduction

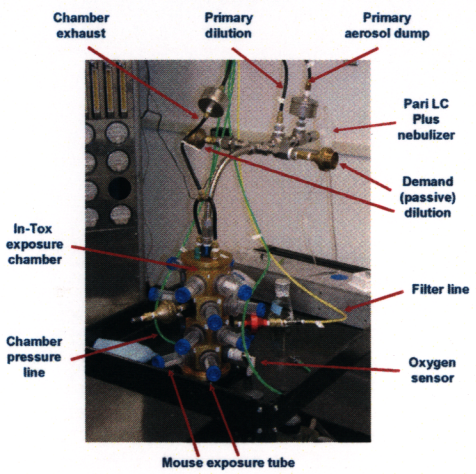
Exposure:

- **Test Article:** 2´-MOE modified antisense oligonucleotide
- **Exposure System:** In-Tox, 24-port cylindrical nose-only chamber, PARI LC Plus™ nebulizer
- **Test System:** Male and female CD-1 mice
- **Exposures:** Nose-only inhalation
- **Exposure Levels:** 0 (clean air), 0.05, 0.15, 0.5, mg/L
- **Duration:** 9 total exposure days, 2.5 hours per day

Health Endpoints:

- Morbidity and mortality
- Clinical signs
- Clinical pathology
- Body/organ weight
- Histopathology (respiratory tract in all dose groups; liver, kidney and spleen in high-dose and control exposure groups)

Exposure System

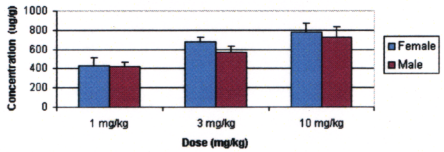


Deposition Assumptions

- Mouse, minute volume (Vm): 0.04 L/min
- Particle size ~1.5 µm
- Pulmonary deposition fraction (Df): 0.10
- Concentration exposure chamber (Ce): e.g., high dose = 0.5 mg/L
- Time of exposure (T): 2.5 h
- Deposited dose (DD): 10, 3, 1 mg/kg (0.03 kg mouse = 0.3, 0.09, 0.03 mg)

where: DD = Ce (mg/L) x Vm (L/min) x Df x T (min)

Gender Comparison of Total Oligonucleotide Detection in Pulmonary Tissue Samples



Study Design

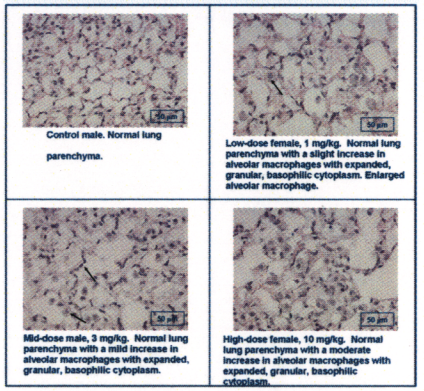
Group	Treatment	Interval
Control	Vehicle	Every other day x 18 days (9 exposure days)
Low	1 mg/kg	
Mid	3 mg/kg	
High	10 mg/kg	

Target and Actual Exposure and Dose Values

Exposure Level	Target Aerosol [ ]	Actual Mean Aerosol [ ] Mean ± (SD)	Dose Target (mg/kg)	Calculated Average Deposited Dose (30 g mouse and 2.5 h exposure)[a]
Low	0.05	0.051 (0.005)	1	1 mg/kg (range 88–116%)
Mid	0.15	0.149 (0.016)	3	2.98 mg/kg (range 82–115%)
High	0.5	0.49 (0.029)	10	9.8 mg/kg (range 90–109%)

[a]Calculated deposited dose not based on individual mouse body weight or exposure group mean body weight at any exposure interval

Summary Pulmonary Histopathology



Control male. Normal lung parenchyma.

Low-dose female, 1 mg/kg. Normal lung parenchyma with a slight increase in alveolar macrophages with expanded, granular, basophilic cytoplasm. Enlarged alveolar macrophage.

Mid-dose male, 3 mg/kg. Normal lung parenchyma with a mild increase in alveolar macrophages with expanded, granular, basophilic cytoplasm.

High-dose female, 10 mg/kg. Normal lung parenchyma with a moderate increase in alveolar macrophages with expanded, granular, basophilic cytoplasm.

No Exposure-Related Effects

- Morbidity and mortality
- Body weight (M/F) for all study animals
- Clinical observations for all study animals
- Gross pathology for all study animals
- Clinical pathology

Conclusions

- Repeated exposures of male and female CD 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 hypertrophy.
- These observations are consistent with the mild proinflamatory 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 monocuicear cell infiltrates.
- Overall, inhaled oligonucleotide therapy is feasible and unlikely to be associated with clinically significant side effects of pulmonary administration.