INTRODUCTION

- Treatment with intravenous amikacin can result in inadequate targeted drug exposure to the site of lung infection and toxicities attributable to high systemic exposure.1
- Liposomes encapsulating antibiotic delivered to the airways may maximize drug delivery:
  - By penetrating and diffusing through mucus and bacterial biofilm layers to direct drug to deep bacterial colonies2
  - By directing drug to specific cells, including pulmonary macrophages, which is especially beneficial in the treatment of intracellular infections such as those caused by nontuberculous mycobacteria3
- By maintaining active concentrations for a prolonged time
- Previously, we demonstrated in multiple-dose studies that liposomal amikacin for inhalation (LAI) does not have an impact on the phagocytic function of pulmonary macrophages and does not induce inflammatory cytokines4
- This study of LAI in rats investigated lung deposition, distribution, and clearance of drug and liposomes deposited throughout the various lobes of the lungs.
- Amikacin and liposomes (ie, lipid) were tracked simultaneously using a fluorescent dual-labeling approach. Previously, it was demonstrated that the fluorescent probes remained with the liposomes and did not affect amikacin encapsulation or release.
- Both global and local distribution and clearance were assessed.
- Cellular distribution was also assessed through microscopic analysis of lung tissue and through cells retrieved by bronchoalveolar lavage.

OBJECTIVE

- To evaluate the pulmonary distribution, clearance, and macrophage uptake of LAI administered in rats once daily for 28 days at 2 dose levels

METHODS

- The amikacin concentration of all lung homogenates and biologic fluids was measured by immunopolarization using the Abbott TDX automated fluorescence polarization analyzer (Abbott Laboratory, Abbott Park, IL).
- The dual-labeled LAI Test Article was prepared by mixing 4 parts LAI with 1 part dual-labeled LAI. The dual-labeled LAI contained 1 mol% DiIC18(5)DS and approximately 1 mol% amikacin-TAMRA (~1.2 mg amikacin-TAMRA and 120 mg amikacin) (Figure 1).
- After approximately 20 minutes of aerosol generation, each nebulizer was disconnected from the compressor, the residual contents (~2 mL) were discarded, and the nebulizer was refilled with 5 mL of fresh Test Article. This nebulizer-refilling procedure was repeated every 20 minutes until the animals were exposed to the desired dose of Test Article.
- Fluorescent micrographs of lung tissues after 27 daily doses of 90 mg/kg LAI showed that amikacin-TAMRA (white) and DiIC18(5)DS (red) co-localized in lung tissues (Figure 8).
- Fluorescent micrographs also showed that lipid is co-localized with amikacin in BALF macrophages (Figure 9).
- The macrophages in BALF from the high-dose group (Figure 9B) are significantly larger and more numerous than those from the low-dose group (Figure 9A).

RESULTS

- The ratios of area under the concentration-time curve to maximum concentration of amikacin-TAMRA were equivalent in the lungs, sana, and urine of rats regardless of whether the concentrations of amikacin-TAMRA were measured by immunopolarization or determined by fluorescence (Table 1).
- Amikacin-TAMRA and liposomes (ie, lipid) were tracked simultaneously using a fluorescent dual-labeling approach. Previously, it was demonstrated that the fluorescent probes remained with the liposomes and did not affect amikacin encapsulation or release.
- Both global and local distribution and clearance were assessed.
- Cellular distribution was also assessed through microscopic analysis of lung tissue and through cells retrieved by bronchoalveolar lavage.

Animal Treatment and Evaluation

- 76-CD® IGS female rats (Charles River) were randomized into 2 treatment groups (90 mg/kg/day or 10 mg/kg/day LAI) of 36 each and in a control group. Test article was administered for 27 days (Figure 2).
- On day 28, twelve (12) rats in each dosing group received a single 90 mg/kg dose of LAI via inhalation; the remaining 24 rats in each group received a single 90 mg/kg LAI via inhalation.
- Lungs were sectioned and examined microscopically. A cohort of rat lungs were separated into lobes and homogenized to determine drug and lipid levels.

Nose-Only Inhalation

- Test Articles were administered via the Jaeger-NYU Nose-Only Directed-Flow Inhalation Chambers (CH Technologies, Westwood, NJ) using PARI LC® Star nebulizers (PARI, Monterey, CA).
- Three, 12-port Jaeger-NYU Nose-Only Directed-Flow Inhalation Chambers were used to expose animals to the Test Articles (Figure 3). DeVilbiss® compressors (Sunrise Medical, Somerset, PA) and PARI LC® Star nebulizers were used to generate the aerosols. The compressors were set to operate at 30 PSI, and each nebulizer was filled with 5 mL of Test Article.

RESULTS

- Amikacin deposition is the same regardless of lung lobe evaluated or tissue sectioned, or the dose administered (Figure 5 A, B).
- Amikacin-TAMRA deposition is the same regardless of lung lobe evaluated or tissue sectioned, or the dose administered (Figure 5 C, D).
- There were no statistically significant differences in the mean DiIC18(5)DS deposition, regardless of lung lobe evaluated or tissue sectioned, or the dose administered (Figure 5 E, F).
- After day 7, and through day 28 post-dosing, the liposomal fraction was likely engulfed by intestinal, bronchial, and alveolar macrophages.
- In vivo, amikacin is released from the liposomes and macropinocytosed and is cleared.
- Similar results were seen for rats that received 27 daily doses of 10 mg/kg LAI followed by a single dose of 90 mg/kg fluorescently dual-labeled LAI.
- Fluorescent micrographs show that amikacin-TAMRA (white) and DiIC18(5)DS (red) co-localize in lung tissues (Figure 8).
- Fluorescent micrographs also showed that lipid is co-localized with amikacin in BALF macrophages (Figure 9).
- The macrophages in BALF from the high-dose group (Figure 9B) are significantly larger and more numerous than those from the low-dose group (Figure 9A).

CONCLUSIONS

- Exposure to LAI, regardless of dose, results in uniform deposition of amikacin and liposomes in all lung lobes followed by uniform clearance of amikacin from the lung of normal rats.
- There is substantial uptake of LAI by pulmonary macrophages, which serve as reservoirs for LAI.
- Repeated daily administration for 27 days of low- or high-dose LAI did not affect LAI clearance 28 days post-dosing, suggesting that deposition and clearance mechanisms appear to be unaffected by dose or repeat dosing.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Scott Liu, Dr. J-K Lee, Dr. Eric Dady, Crystal Arissi, and Bill Alsio for their contributions to this work. The authors also acknowledge Connex Ion Healthcare (Newtown, PA) for providing editorial, layout, and design support. Insmed Incorporated (Bridgeport, NJ) provided funding to Connex Healthcare for these services.

REFERENCES