Oral Delivery of Amikacin-Lipid NanoCrystal Formulations Safely and Effectively Treats Macrolide Resistant *Mycobacteria* Infections in a Mouse Model of Cystic Fibrosis

Ruying Lu[1], Raphael Mannino[1] and Diane Ordway[2] ; [1]Matinas BioPharma, Inc. , [2]Colorado State University

ABSTRACT

Background

In cystic fibrosis, intracellular pathogens, such as Mycobacteria, are problematic. The buildup of thick layers of mucous and/or biofilms, accentuates the difficulty for many anti-microbial agents, such as amikacin, in penetrating into infected cells. Lipid NanoCrystal (LNC) formulations provide oral bioavailability for injectable drugs, reduce toxicity, and significantly enhance intracellular delivery to infected cells. In a previous study we demonstrated that oral administration of amikacin-LNCs safely and effectively treats macrolide sensitive Mycobacteria infections in a mouse model of Cystic Fibrosis.

Methods

The oral efficacy of Amikacin-LNC (AmK-LNC) was evaluated in a chronic cystic fibrosis mouse model (B6CFTR^{tm1UNC}/CFTR^{tm1UNC}) against each of the three NTM strains having high resistance to macrolide antibiotics (*M. avium subsp intracellulare* 25292, *M. abscesus ssp abscessus* 1513, and *M. abscessus ssp bolletii* 1948). Mice were infected with an aerosol of 1x10^8 CFUs of tested strains and treated - starting on day 28 - for a total of 8 weeks with saline control, oral LP-4 CAmK Lyophilized 50 mg/kg BID, oral LP-4 CAMK Lyophilized 100 mg/kg BID, IP Amikacin (AMI) 150 mg/kg QD, and oral Clarithromycin 250 mg/kg QD. Bacterial burden was measured on day 1, 27, 42, 56 and 84 after infection by plating serial dilutions of organ homogenates on nutrient 7H11 and charcoal agar and counting CFUs after 25-30 days incubation at 32°C. Results represent the average of six experiments (n=5 mice per experiment) expressed as the average Log_{10} CFU (± SEM) cells (± SEM).

Results

Orally administered AmK-LNC safely and effectively treated all three macrolide resistant Mycobacteria infections, with CFU lung, spleen and liver counts lower than IP amikacin or oral clarithromycin. Lung pathology also showed smaller and less numerous lesions after treatment with oral AmK-LNC than with clarithromycin or IP amikacin.

Conclusions

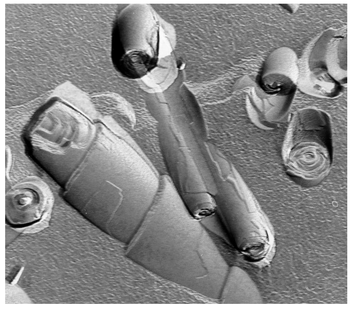
- **Orally administered amikacin-LNC represents a novel, targeted agent that provides effective treatment for macrolide-resistant Mycobacteria infections in a chronic mouse model of cystic fibrosis.**
- **These data support further study of amikacin-LNC formulations as a potential important advance in the treatment of these difficult-to-treat lung infections.**

PROTOCOL

- 6-8 week old B6CFTRtm1UNC/CFTRtm1UNC mice - rested one week before infection.
- Pulmonary infection with 1x10^8 CFU/mouse (M. avium intracellulare or M. avium ssp. hominissuis)
- i.v. infection (via tail vein) with 1x10^6 CFU/mouse (M. abscessus ssp. massiliense).
- 5 mice sacrificed at day 1 and day 27 post-infection. Pre-treatment bacterial loads determined by lung, spleen, and liver homogenates plated on 7H11 and charcoal agar. And placed in 32°C dry-air incubator for ~10 days (M. abscessus ssp. massiliense) or for ~25-30 days (M. avium intracellulare and M. avium ssp. hominissuis).
- Treatment begun day 28 post-infection - continued for 8 consecutive weeks.
  - ***Saline control - IP***
  - ***LP-4 CAmK Lyophilized 50 mg/kg BID - oral***
  - ***LP-4 CAMK Lyophilized 100 mg/kg BID - oral***
  - ***Amikacin (AMI) 150 mg/kg QD - IP***
  - ***Clarithromycin 250 mg/kg QD - oral***
- Five mice from each group sacrificed (day 84); post-treatment bacterial loads determined by plating of lung, spleen and liver homogenates, with similar incubations.
- Log 10 protection values of >0.60 indicate activity is statistically significant.
- Statistical analysis by one-way ANOVA followed by a multiple comparison analysis of variance by a one-way Tukey test.

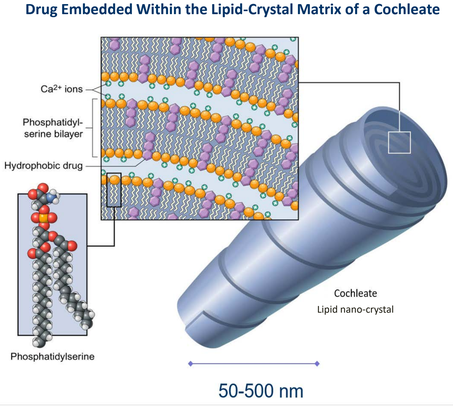
All procedures approved by the Colorado State University Animal Care and Use Committee

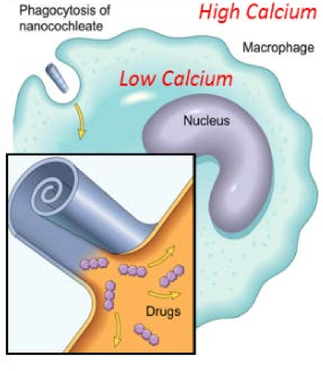
LIPID NANOCRYSTAL (LNC) TECHNOLOGY



- **Cochleate delivery vehicles allow oral delivery of drugs that would otherwise be given systemically (i.v.), and have been shown to reduce toxicity and significantly enhance intracellular drug delivery**
- **Cochleates are stable, crystalline phospholipid-cation precipitates composed of phosphatidylserine and calcium.**
- **Their multilayered structure consists of large, continuous, solid, lipid bilayer sheets with no internal aqueous space.**
- **This unique structure provides protection from degradation for therapeutic "cargo", which remains intact in the interior, even when outer layers are exposed to harsh environmental conditions or enzymes.**

COCHLEATE DELIVERY ALTERS THE PHARMACOKINETICS AND BIODISTRIBUTION OF DRUGS





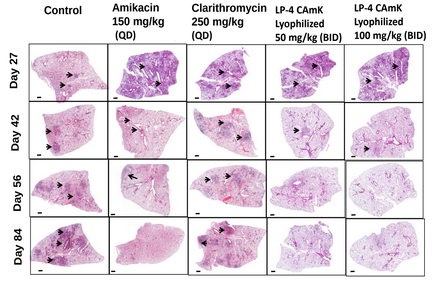
- **High Ca^{++} concentrations in GI secretions and extracellular fluids maintain stability of LNC crystals**
- **Orally administered cochleates are absorbed from the gut and enter the circulation via the lymphatics, bypassing first-pass hepatic metabolism**
- **Macrophages and other activated and/or infected cells readily engulf cochleates and their cargo**
- **Once inside cells, the low levels of cytoplasmic Ca^{++} cause cochleates to open, releasing the cargo molecules intracellularly**
- **Macrophages may also migrate to areas of active infection, further augmenting local drug delivery**
- **Because therapy is delivered ***within*** the cells, high plasma levels are not necessary to deliver adequate amounts of drug to infected tissues**
- **With much lower plasma levels, the risk of systemic toxicity can be substantially reduced**

BACTERIAL COUNTS IN LUNG, SPLEEN, AND LIVER

Group	Lung Log10 CFU ±SEM	Spleen Log10 CFU ±SEM	Liver Log10 CFU ±SEM
Day 1 Pretreatment Control (n=5)	5.15±0.60	0.0±0.0	0.0±0.0
Day 27 Pretreatment Control (n=5)	5.29±0.11	4.56±0.21	3.87±0.11
Day 42 Control (n=5)	6.35±0.04	5.36±0.04	5.04±0.15
Amikacin (AMI), 150 mg/kg QD (n=5)	4.73±0.13	4.05±0.18	4.17±0.04
Clarithromycin 250 mg/kg QD (n=5)	5.02±0.25	4.69±0.21	4.68±0.18
LP-4 CAmK Lyophilized 50 mg/kg BID (n=5)	4.43±0.01	4.31±0.01	4.30±0.01
LP 1-4 CAmK 100 mg/kg BID (n=5)	2.72±0.04	3.75±0.05	3.65±0.06
Day 56 Control (n=5)	6.93±0.04	5.35±0.19	5.01±0.10
Amikacin (AMI), 150 mg/kg QD (n=5)	4.43±0.23	3.94±0.17	3.90±0.06
Clarithromycin 250 mg/kg QD (n=5)	4.96±0.03	4.48±0.21	5.01±0.06
LP-4 CAmK Lyophilized 50 mg/kg BID (n=5)	3.72±0.06	4.26±0.03	4.29±0.04
LP 1-4 CAmK 100 mg/kg BID (n=5)	2.93±0.10	3.57±0.06	3.81±0.06
Day 84 Control (n=2)	7.21±0.03	6.14±0.05	5.99±0.03
Amikacin (AMI), 150 mg/kg QD (n=4)	3.76±0.03	3.73±0.06	3.93±0.07
Clarithromycin 250 mg/kg QD (n=5)	5.11±0.05	4.13±0.02	4.13±0.07
LP-4 CAmK Lyophilized 50 mg/kg BID (n=5)	3.68±0.08	4.01±0.04	4.15±0.01
LP 1-4 CAmK 100 mg/kg BID (n=4)	2.97±0.10	3.23±0.08	3.48±0.06

Results represent the average of 6 experiments (n=5 mice per experiment)
Bacterial load in each group expressed as average Log10 CFU (± SEM) cells (± SEM).
ANOVA, saline control compared to drug-treated groups - p<0.05.
Red highlighting indicates treatment group with the greatest response

LUNG PATHOLOGY



Sections of lung tissue on days 27, 42, 56 and 84 after drug treatment.

Lesions (arrows) were less numerous and smaller in infected mice treated with **oral** LP-4 CAmK (50 mg/kg BID) and **oral** LP-4 CAmK (100 mg/kg BID), compared with oral clarithromycin, and IP amikacin.