LB-176

Aminoglycocide Formulations with Oral Bioavailability

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Abstract

Use of aminoglycosides has been limited due to their inconvenient route of administration (intravenous or intramuscular), toxicity and inefficient intracellular delivery. New aminoglycoside formulations with **oral bioavailability** and **cell targeted intracellular delivery** have been developed using cochleates, an innovative drug delivery platform technology with potential uses in the DEVELOPING WORLD.

Cochleate delivery vehicles are stable, crystalline phospholipid-cation precipitates composed of soybean phosphatidylserine (PS) and calcium with a unique multilayered structure and no internal aqueous space. Encochleated molecules within the interior of the cochleate structure remain intact resulting in **oral bioavailability, lower toxicity, and intracellular targeting**.

We report that amikacin, gentamicin, and paromomycin cochleate formulations were optimized for encochleation efficiency by varying the type of PS used, PS:drug ratio, PS:Ca ratio, and NaCl concentration.

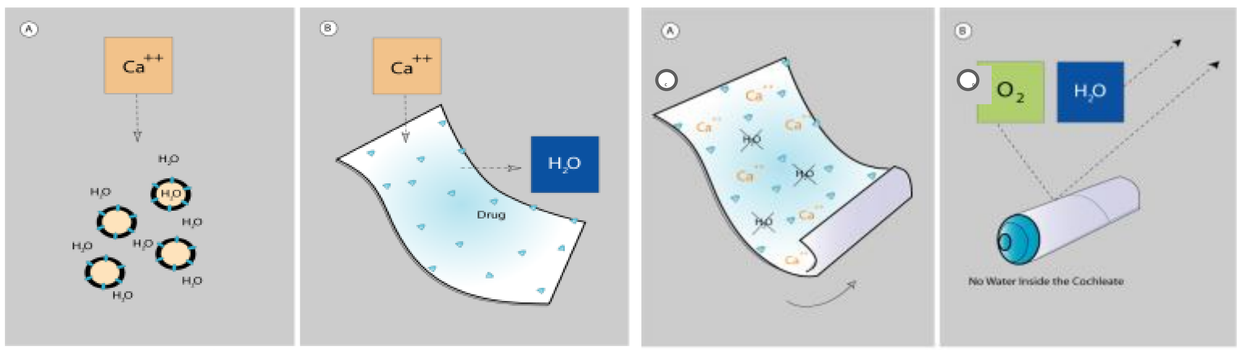
- In *in vitro* macrophage assays, when compared to free drug, amikacin cochleates have shown **enhanced efficacy** against infections by Mycobacterium avium (10x – 20x), Mycobacterium tuberculosis (7x), and Francisella tularensis LVS (3x).
- *In vivo*, using C57BL/6 black mice infected with Mycobacterium avium complex (MAC), amikacin cochleates given orally showed **activity comparable to IP free drug**, reducing the bacterial load in the spleen.
- In *in vitro* macrophage assays, when compared to free drug, gentamicin cochleates have shown **enhanced efficacy** against infections by Mycobacterium avium (10x), Mycobacterium smegmatis (50x), Mycobacterium tuberculosis (2x), Francisella tularensis LVS (2x) and Francisella tularensis type A (4x).
- *In vitro* evaluation of paromomycin cochleates has begun.

A portfolio of aminoglycoside-cochleate formulations with **oral bioavailability**, **reduced toxicity**, **enhanced efficacy** and **no cold chain requirement** could provide significant new opportunities for the treatment of serious human diseases, particularly in the 3rd world.

Introduction: Cochleate Technology

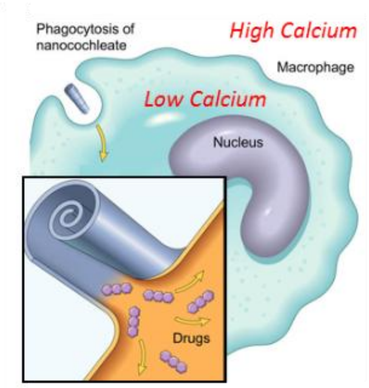
- Cochleate delivery vehicles have been shown to mediate **oral bioavailability** for injectable drugs, **reduce toxicity**, and significantly **enhance intracellular drug delivery**.
- Cochleates are stable, crystalline phospholipid-cation precipitates composed of **simple, naturally occurring materials**: phosphatidylserine and calcium. They have a unique multilayered structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in a spiral or as stacked sheets, with no internal aqueous space.
- This unique structure provides **protection from degradation** for "encochleated" molecules. Components within the interior of the cochleate remain intact, even though the outer layers of the cochleate may be exposed to harsh environmental conditions or enzymes.

How Cochleates Encapsulate Drugs



Formation of Stable Drug-Liposome Intermediate | **Calcium Interaction with Negatively Charged Lipid** | **Formation of Stable Drug-Cochleate Nano-Crystal**

- The API is associated with the negatively charged lipid.
- The addition of calcium creates a calcium-phospholipid anhydrous crystal.
- Nano-crystals are composed of layers of a lipid-calcium complex.
- The API is trapped in or between the layers protecting the API from harmful environmental elements



Cell-Targeted Delivery

- Macrophage readily engulf cochleates and their cargo
- Once inside the macrophage, the low level of calcium in the cytoplasm causes the cochleate to open, releasing the cargo molecule
- Specific macrophage delivery can change the PK profile of a drug.
  - For example, in the penicillin model, high plasma levels are needed since the drug does not readily cross the plasma membrane and get into the cell.
  - By contrast, in the azithromycin model, the drug is taken up by phagocytosis, leading to more efficient intracellular delivery, enhancing the effectiveness against intracellular pathogens, and higher drug concentrations at the site of infection.

In Vitro Assays

Methods

Macrophages and infection. Cell lines: mouse peritoneal macrophage cell line (Raw 246.7), and/or THP-1, a human macrophage cell line. Cells were cultured in DMEM and RPMI-1640, respectively, supplemented with 5% heat-inactivated fetal bovine serum. Macrophage monolayers were established by adding 10^5 macrophages to a 24-well tissue culture plate. After 24 hours, monolayers were infected and the infection was allowed to happen for 1 hour, and then the extracellular bacteria were removed by washing. Some of the well contents were lysed and plated onto Middlebrook 7H10 agar plate, to determine the intracellular inoculum of the bacterium. The remaining wells were treated daily with drug. After treatment, cell monolayers were lysed and the lysate plated into 7H10 agar to quantify the intracellular load.

Results

	Organism Used and Enhanced Efficacy vs. Free Drug
Amikacin	•Mycobacterium avium (10x – 20x) •Mycobacterium tuberculosis (7x) •Francisella tularensis LVS (3x)
Gentamicin	•Mycobacterium avium (10x) •Mycobacterium smegmatis (50x) •Mycobacterium tuberculosis (2x) •Francisella tularensis LVS (2x) •Francisella tularensis type A (4x)
Paromomycin	Formulations developed, *In vitro* assays underway

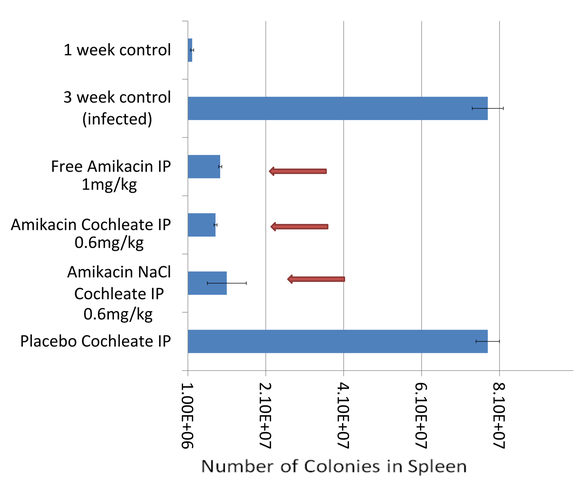
In Vivo System

A formulation of amikacin cochleates has been developed. The *in vivo* efficacy of amikacin cochleates against Mycobacterium avium complex (MAC) was evaluated using C57BL/6 black mice.

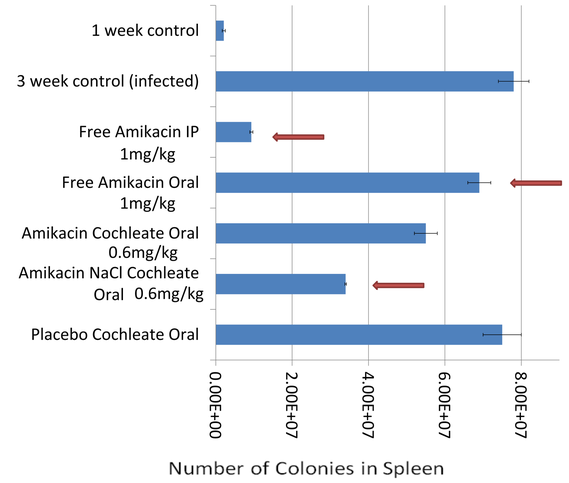
- Mice, 12/group, were infected with M. avium 101, (8.1 × 107 bacteria/ mouse) by tail vein injection.
- After 7 days, 6 mice were harvested and the number of MAC in spleen was quantified to establish the baseline bacterial load (Time 0).
- Mice were treated with various amikacin preparations (indicated below) at 1.0 mg amikacin/day for 2 weeks.
- Mice were harvested at week 3 and 2 days later (after 2 weeks of treatment), and spleens homogenized and plated onto 7H10 agar.
- Colonies on plates were counted and the data analyzed.

In Vivo Testing - Results

Amkcch In Vivo Efficacy in a Mouse Model of MAC 101 Infection – IP Delivery



Amkcch In Vivo Efficacy in a Mouse Model of MAC 101 Infection – Oral Delivery Delivery



Cochleate preparations, given I.P. or orally, were active, reducing the number of bacterial load in the spleen. The amikacin cochleate preparation with high salt concentration dosed orally was significantly more active than free amikacin.

Conclusion and Next Steps

Conclusion

- Cochleate preparations of aminoglycocides have shown increased efficacy compared to free drug in multiple *in vitro* models. *In vivo* models of amikacin cochleates, delivered orally or I.P., showed efficacy similar to free drug.
- In addition, Amphotericin B cochleate formulations (CAmB), the leading cochleate product, has shown oral efficacy comparable to injectable formulations and low toxicity in mouse models of fungal infections. CAmB has begun Phase I human clinical trials.

Next Steps

- Based on these data showing oral and I.P. efficacy for amikacin cochleates, a two-year $750k NIH SBIR contract has been awarded. These funds will be used to continue research on the optimal formulation for amikacin cochleates, ultimately leading to an IND filing and initiation of human trials.
- A Cooperative Research and Development Agreement with WRAIR has been established to continue development of CAmB. Through a collaboration with DNDi, additional CAmB efficacy studies are planned.
- Given the growing issue of antimicrobial resistance, and the difficulty delivering existing antimicrobials, there is strong market demand for new technologies such as cochleates. The recently passed Generating Antibiotic Incentives Now ("GAIN") Act helps create a preferential regulatory environment.

A portfolio of orally available aminoglycocides, with reduced toxicity and no cold chain, could provide significant new opportunities for the treatment of serious diseases, especially in the developing world.