Platform Drug Delivery Technology that Creates Oral Bioavailability for Existing Injectable Antimicrobials

Aquarius Biotechnologies

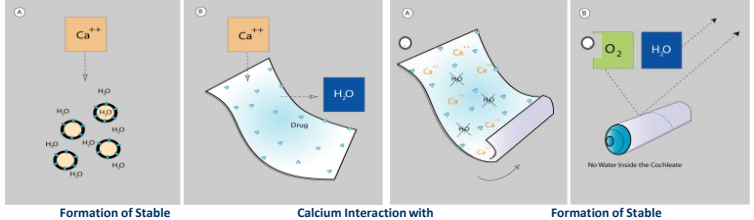
Unmet Need

Infectious diseases caused by microorganisms are an increasingly serious problem throughout the world. Many of the most serious and difficult to treat pathogens have evolved strategies that enable these microorganisms to invade cells of the immune system and subvert or even utilize the immune cell physiology to multiply and cause disease. A major challenge to the effective treatment of intracellular microorganisms is the difficulty in achieving highly efficient delivery of antimicrobial agents across the plasma membrane of infected cells. Many existing, highly effective antimicrobials can currently only be delivered by injection, demonstrate potential toxic side effects, and inefficient intracellular delivery.

Solution: Cochleate Technology

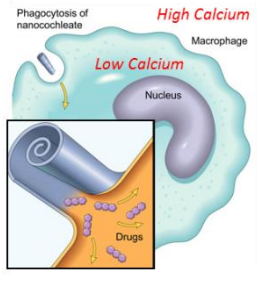
The proposed solution is to reformulate existing anti-infective drugs using cochleate technology. Cochleate delivery vehicles have been shown to mediate oral bioavailability for injectable drugs, reduce toxicity, and significantly enhance intracellular drug delivery. Cochleates are stable, crystalline 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 to get the drug into the cell, and the drug enters by diffusion across the membrane.
  - By contrast, in the azithromycin model, the drug is taken up by phagocytosis, leading to more efficient delivery, lower doses, and less systemic toxicity.

Divalent cation concentrations *in vivo* in serum and mucosal secretions are such that the cochleate structure is maintained. Hence, the majority of cochleate associated molecules are present in the inner layers of a solid, stable, impermeable structure. Once within the interior of a cell, however, the low calcium concentration results in the opening of the cochleate crystal and release of the entrapped API.

Applications to Antimicrobials

Amphotericin B Cochleates

The lead product in development using cochleate technology is amphotericin B (AmB).

- Oral administration of AmB-cochleates has been shown to be as effective as equivalent, injectable doses of the leading AmB formulation (Fungizone) in mouse models of systemic candidiasis and aspergillosis
- AmB-cochleates also demonstrate substantially lower toxicity than existing commercial AmB products
- AmB-cochleates showed good safety in rats and dogs in 7 and 28 day toxicity studies
- A commercially viable and cost effective manufacturing process for AmB-cochleates has been developed, and scaled-up 100 liter GMP batches of AmB-cochleates have been produced
- An IND for AmB-cochleates is open, and data from a phase Ia human clinical trial were supportive of further studies

Liquid AmB

Dry Powder AmB

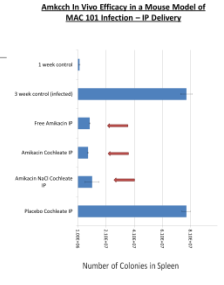
Amikacin Cochleates

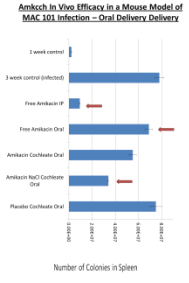
Amikacin cochleates have also been developed. The in vivo efficacy of amikacin cochleates against Mycobacterium avium complex (MAC) was evaluated using C57BL/6 black mice.

- 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.

Number of organisms (CFU) in the spleen of C67BL/6 mice after 2 weeks of treatment

Experimental group	Treatment route of administration	CFU/spleen	p value
1 week (control)	---	2.1 ± 0.4 × 106	---
3 weeks (control infected)		7.8 ± 0.4 × 107	
0 NaCl AK	oral	5.5 ± 0.3 × 107	---NS
0 NaCl AK	I.P.	8.1 ± 0.4 × 106	(1) (2)
high NaCl AK	oral	3.4 ± 0.2 × 107	(1) (3)
high NaCl AK	I.P.	1.1 ± 0.5 × 107	(1)
placebo + cochleate	oral	7.5 ± 0.5 × 107	NS
placebo + cochleate	I.P.	7.8 ± 0.3 × 107	NS
free AK	oral	6.9 ± 0.3 × 107	NS
free AK	I.P.	9.3 ± 0.4 × 106	(1)





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.

Commercialization

Portfolio of Oral Aminoglycosides

Use of aminoglycosides has been limited due to their inconvenient route of administration (intravenous or intramuscular), toxicity and inefficient intracellular delivery. In addition to amikacin, gentamicin and paromomycin cochleate formulations have been created. 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.

	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

Development Partners

Given the broad applicability of the cochleate technology, multiple types of collaborations are possible.

- *Pharmaceutical partners*
  - Partnerships with pharma companies will be sought for later-stage development of amphotericin cochleates, amikacin cochleates, and any other product developed using cochelate technology
  - In addition, cochleates can be used to formulate proprietary molecules. This will generate non-dilutive revenue that can be used to fund overhead and R&D
- *NGOs* – A collaboration has begun with DNDi to continue development of amphotericin cochleates, given the application to diseases in the developing world, including *visceral leishmaniasis*
- *Federal Government*
  - Cooperative Research and Development Agreement with WRAIR
  - $750k Phase II SBIR contract for the development of amikacin cochleates

Two development paths:

Preclinical	IND	Clinical Trials: Phase I	Clinical Trials: Phase II	Clinical Trials: Phase III	NDA	Marketing & Sale
Develop In-House				Completed by Licensing Partner		
Provide Formulation Services to Pharma Partners						

Next Steps

- Continue development of CAmB through Phase I and Phase II clinical trials, and license the product to a major pharma company post Phase II.
- Continue preclinical development of amikacin cochleates, including toxicity studies, prior to filing an IND.
- Develop collaborative alliances with biotech and major pharmaceutical companies.