Efficacy of Oral Cochleate-Amphotericin B for the Prevention of Invasive Candidiasis Caused by *Candida albicans* in Mice.

Ruying Lu[1], Raphael Mannino[1], Douglas Kling[1], J Carl Craft[1], Steven Park[2], Yanan Zhao[2], David Perlin[2]

INTRODUCTION

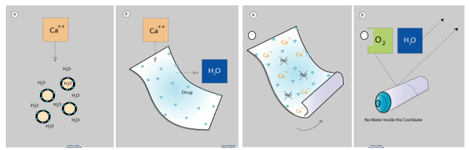
Introduction: Amphotericin B (AmB), due to its fungicidal efficacy, broad spectrum and limited resistance, can be considered the "gold standard" antifungal treatment and remains the principal therapeutic option for deep mycoses. However, its application is currently limited by toxicity and administration requiring slow intravenous injection. MAT2203, (AMB cochleates; CAMB), a novel lipid-crystal formulation of AMB, demonstrates oral bioavailability, significant efficacy, low toxicity, and shelf-life stability.
In animal models, CAMB demonstrates antifungal activity with similar efficacy as intraperitoneal AMB deoxycholate, without the associated toxicity. Oral administration of CAMB has shown strong efficacy in mouse models of cryptococcal meningitis, disseminated candidiasis and disseminated aspergillosis.
In a Phase 2a human clinical study being conducted at the National Institutes of Health Clinical Center in Bethesda, MD, under the direction of Dr. Alexandra Freeman, MAT2203 (CAMB) has shown efficacy, safety, and tolerability in predominantly hereditary immunodeficient patients with a recurrent or chronic mucocutaneous candidiasis infection (esophageal, oropharyngeal, vaginal) who are refractory or intolerant to standard non-intravenous therapies.
Background: MAT2203 (CAMB) is being developed for the prevention of invasive fungal infections due to immunosuppressive therapy, particularly in patients with acute lymphoblastic leukemia (ALL). In patients being treated for ALL the risk for invasive fungal infections (IFIs) is high, with an associated high risk of lethality. Currently, there is no standard of care for preventing these high risk IFIs in ALL patients.
The established treatment regimens for ALL are highly sensitive to liver-metabolized drug-drug interactions, causing serious concerns for drug-drug interaction induced side-effects. Amphotericin B is not liver metabolized and when incorporated in the lipid-crystal nano-particle structure of MAT2203, (CAMB) this otherwise toxic IV only compound can now be safely orally administered (providing patient convenience over ~12 weeks prophylactic treatment duration), without the typical kidney and liver toxicity associated with other Amphotericin B formulations.
Purpose: This study evaluated the efficacy of orally delivered CAMB for the prevention of invasive candidiasis caused by a virulent *C. albicans* WT strain SC5314 in mice.
Results: All mice treated with placebo cochleates died or turned moribund and were euthanized by day 5. In contrast, mice treated with CAMB had 100% survival regardless of the dosage. Fungal burdens in major organs were largely reduced in all CAMB treated mice in a dose-dependent manner. Organ sterilization was achieved for all CAMB dosing regimens at various levels. No significant toxicity was observed with CAMB treatment by gross assessment.
Conclusion: CAMB is highly effective for the prevention of invasive candidiasis in mice caused by a virulent *C. albicans* WT strain SC5314.

1. Matinas BioPharma, Inc. Bedminster, NJ, USA
2. Rutgers Publ. Health Research Institute, Newark, NJ, USA

COCHLEATE TECHNOLOGY

How Cochleates Encapsulate Drugs

Cochleate delivery vehicles have been shown to mediate **oral bioavailability for injectable drugs**, **reduce toxicity**, and significantly **enhance intracellular drug delivery**. Cochleates are stable, lipid-crystal, nano-particles 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. Cargo molecules 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.



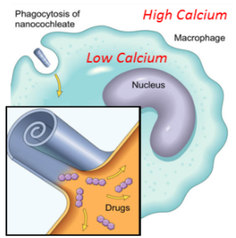
Formation of Stable Drug-Liposome Intermediate | Calcium Interaction with Negatively Charged Lipid | Formation of Stable Drug-Cochleate Nano-Crystal

- 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

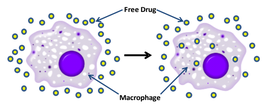
- **Macrophage readily engulf cochleates and their cargo**
- **Once inside the macrophage, the low level of calcium in the cytoplasm causes the cochleate to open, releasing the cargo molecule**

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.



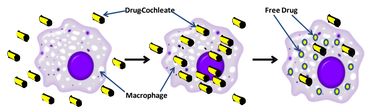
Cochleates Can Change the Pharmacokinetics and Biodistribution of Drugs

Traditional Model of Drug Delivery



- **Free drug in the extracellular milieu must cross the cell membrane in order to be effective against intracellular microorganisms.**
- **High plasma and interstitial drug levels are needed.**
- **A relatively low percentage of circulating drug enters the cell.**
- **Drugs with these properties have difficulty treating intracellular infections.**
- **High circulating drug levels can result in nonspecific toxicity.**

Model of Cochleate Mediated Drug Delivery The "Trojan Horse Hypothesis"



- **High calcium concentrations in gastrointestinal secretions, serum and interstitial fluid stabilize the drug cochleate crystal.**
- **Drug cochleates enter the circulatory system, diffuse into tissues and/or are taken up by "activated" and/or infected cells.**
- **Intracellular levels of drug cochleates increase and reach high levels.**
- **The low intracellular calcium concentration causes the drug cochleates to open releasing their cargo molecules.**
- **Lower plasma levels are required to reach efficacious intracellular drug concentrations.**
- **These lower plasma levels may result in less systemic toxicity.**

CAMB PREVENTION OF INVASIVE CANDIDIASIS IN MICE

Murine infection model and antifungal treatment.
An immunocompetent mouse model of invasive candidiasis was used in this study.
Animals. Female 8-week-old BALB/c mice weighing ~20g were used for this experiment.
Strain. *C. albicans* susceptible strain SC5314 were subcultured in liquid yeast extract-peptone-dextrose (YPD) medium at 37°C with shaking overnight. Cells were collected by centrifugation, washed twice with sterile phosphate-buffered saline (PBS), and counted with a hemocytometer. *Infection dose:* The infection dose was 7.7x105 CFU per mouse.
Drug. CAMB/MAT2203 (0.25, 0.5, and 1 mg/ml) and placebo cochleates were provided by Matinas.

Experiment Methods. CAMB at 2.5, 5, or 10 mg/kg, or placebo control was administered once daily via oral administration, starting from day -3 until day 4 or day 9. On day 0, mice were infected with 7.7x105 CFU of *C. albicans* SC5314 via retro- orbital injection. Except mice treated with placebo control (group 4 & 6) and quickly died after infection (12 out of 20 died at day 2, 6 died at day 3), the remaining control mice (n=2) and mice in groups 2~4 were sacrificed via CO2 inhalation at day 5 post-infection. Mice in group 5 were kept on 5 mg/kg CAMB treatment until sacrifice at day 10. Kidneys, lungs, liver, and spleen were aseptically removed from all mice in the study. Upon removal, 1/2 of left kidney and 1/3 of spleen were sectioned, snap-frozen, and stored at -80°C for drug level measurement. All remaining organs were homogenized in 5 ml of sterile PBS, and 100 µl of homogenate or proper dilutions were spread onto YPD agar plates for fungal burden counts.

Treatment	No. of survivors at sacrifice	Log_{10} CFU/g of tissue (sterilization %)			
		Kidney	Lung	Liver	Spleen
Placebo day 5	0	7.05±0.36	5.12±0.47	5.05±0.44	5.39±0.37
CAMB 2.5 mg/kg day 5	10	4.86±1.63*** (20%)	3.54±1.45*** (80%)	4.56±2.22* (60%)	4.47±2.06*** (80%)
CAMB 5 mg/kg day 5	10	2.99±1.39*** (70%)	Sterile (100%)	4.70±2.48 (60%)	BLQ (90%)
CAMB 10 mg/kg day 5	10	BLQ (90%)	Sterile (100%)	4.10±2.07** (70%)	Sterile (100%)
Placebo day 10	0	7.25±0.16	4.76±0.57	4.57±0.55	5.15±0.58
CAMB 5 mg/kg day 10	10	2.18±1.23*** (60%)	Sterile (100%)	3.72±1.94* (70%)	Sterile (100%)

Results: All mice treated with placebo cochleate died or turned moribund and had to be euthanized prior to the scheduled sacrifice time point, with average fungal burdens of 7.1 log10 CFU/g in kidney, 5.1 log10 CFU/g in lung, 5.1 log10 CFU/g in liver, and 5.4 log10 CFU/g in spleen. In contrast, mice treated with CAMB had 100% survival at both sacrifice time points, regardless of the dosage. Fungal burdens in major organs were also largely reduced in all CAMB treated mice in a dose-dependent manner. Organ sterilization was achieved for all CAMB dosing regimens at various levels. By day 5 post-infection, lung and spleen sterilization was observed in 10 of 10 mice with 10 mg/kg CAMB treatment, and the sterilization rate was 90% for kidneys and 70% for livers. The 5 mg/kg CAMB also resulted in 100% lung sterilization, and 90%, 70% and 60% sterilization for spleens, kidneys, and livers, respectively. The 2.5 mg/kg CAMB had the lowest organ sterilization rates, but significant burden reduction was still achieved in all organs with this regimen. Compared to 8 days (day -3 to day 4) treatment of 5 mg/kg CAMB, the continued 5 more days of treatment (day -3 to day 9) did result in further lowered kidney and liver burdens, but the differences were not statistically significant. Among all major organs assessed, kidneys have the highest starting fungal load, but liver seems to be the organ that yeast cells persist the most.

Conclusion: CAMB is highly effective for the prevention of invasive candidiasis in mice caused by a virulent *C. albicans* WT strain SC5314, as demonstrated by the 100% 5-day and 10-day survival of mice treated with CAMB in contrast to the 100% mortality of untreated mice. Burden reduction and organ sterilization efficacy of CAMB is dose-dependent.