

ABSTRACT

Background: Amphotericin B (AmB), due to its fungicidal efficacy, broad spectrum and limited resistance, can be considered the “gold standard” antifungal treatment. However, use of AmB is limited by toxicity and intravenous administration. CAmB is a lipid-crystal, nano-particle formulation designed for targeted oral delivery of AmB to the infected tissue without the associated toxicity.

Methods: BALB/c mice (N=65) were infected on Day 0 with 5×10^5 cells of *Candida albicans*. After infection mice were treated for 14-days with control, DAmB (Amphotericin B deoxycholate) 2 mg/kg intraperitoneal, or CAmB 10 mg/kg oral. Untreated and uninfected mice were used as blank controls. Five mice from each treatment group were sacrificed on days 1, 3, 5, 7, 11, and 15. Plasma and tissues were collected for analysis of AmB concentrations.

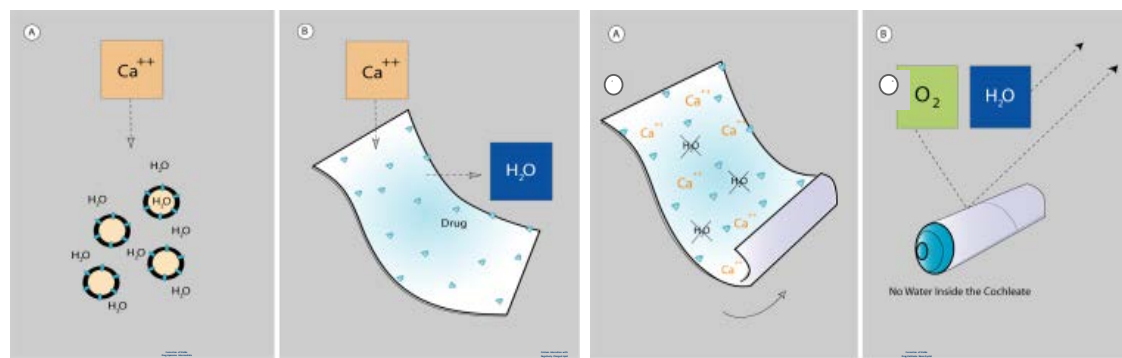
Results: Concentrations of AmB in plasma were undetectable in 61% of the CAmB and 44% of DAmB samples with no significant difference in plasma levels between groups. In the tissues however, quantifiable AmB levels were seen in all samples (See Figure), with CAmB reaching the MIC of 0.25 µg/ml in 1-2 days whereas DAmB takes 3-5 days to reach the MIC-level. At the efficacious dose, tissue levels levels for CAmB stay at 2-3x the MIC, whereas DAmB causes tissue levels to increase to 4-40x the MIC.

Conclusions: In *Candida*-infected mice, orally administered CAmB is taken up from the GI tract resulting in significant tissue concentrations above the MIC level. Contrary to DAmB, concentrations of AmB from CAmB accumulate rapidly in the tissues without escalating to extremely high levels during the second week of treatment, potentially mitigating side effects and toxicities.

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. 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.

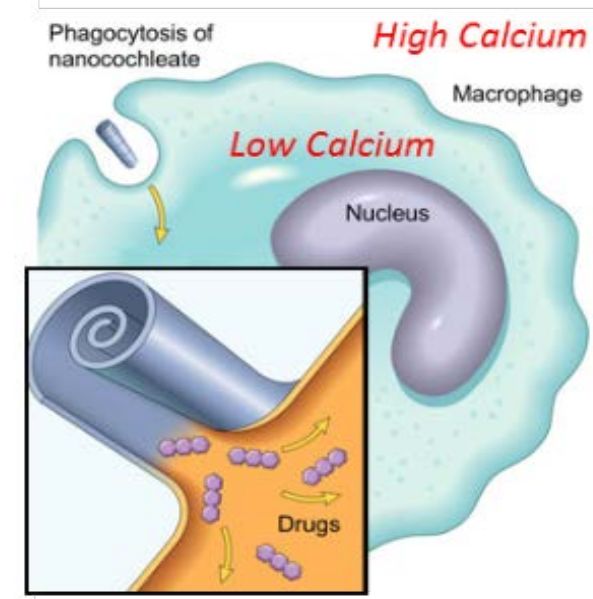


- Formation of Stable Drug-Liposome Intermediate
 - Calcium Interaction with Negatively Charged Lipid
 - Formation of Stable Drug-Cochleate Nano-Crystal
- The API is associated with the negatively charged lipid.
 - The addition of calcium creates a calcium-phospholipid anhydrous crystal.
 - Nano-crystals 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

1. Rutgers New Jersey Medical School, Newark NJ, USA/ Matinas BioPharma, Bedminster, NJ, USA
2. Rutgers Publ. Health Research Institute, Newark, NJ, USA

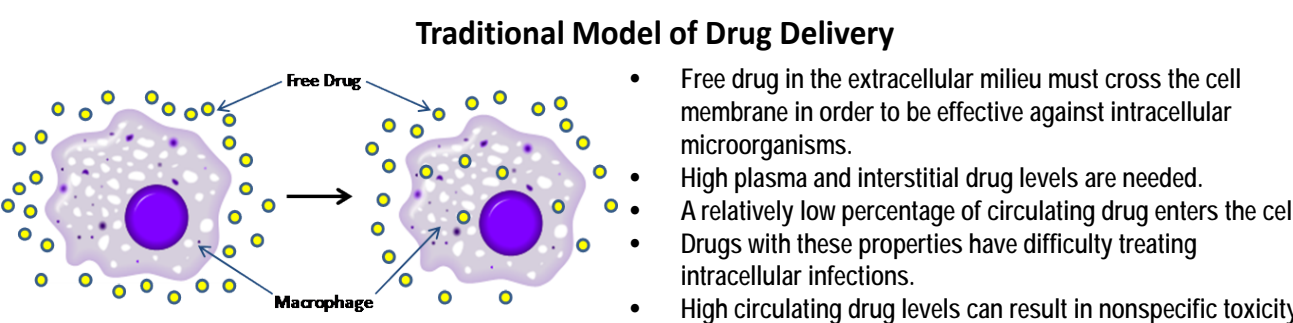
Cell-Targeted Delivery

- 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



Model of Drug Delivery - The “Trojan Horse” Hypothesis

- 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.
- 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 the cochleates.
 - Lower plasma levels are required to reach efficacious intracellular drug concentrations.
 - These lower plasma levels may result in less systemic toxicity.

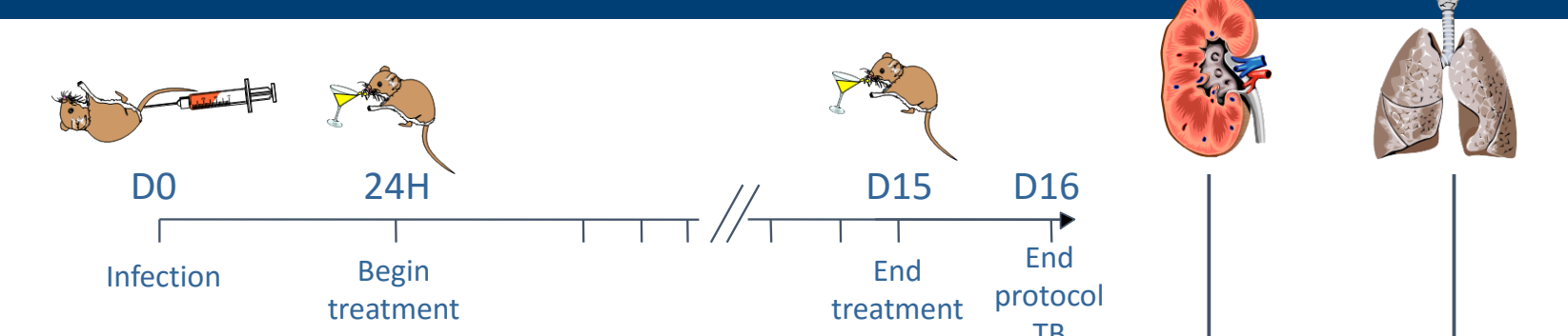
ORAL DELIVERY OF AMPHOTERICIN B COCHLEATES IS EFFICACIOUS IN A MOUSE MODEL OF DISSEMINATED CANDIDIASIS

- 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.
- A phase 2a efficacy study is targeted to begin in October, 2015.

CAmB Suspension



EXPERIMENTAL DESIGN

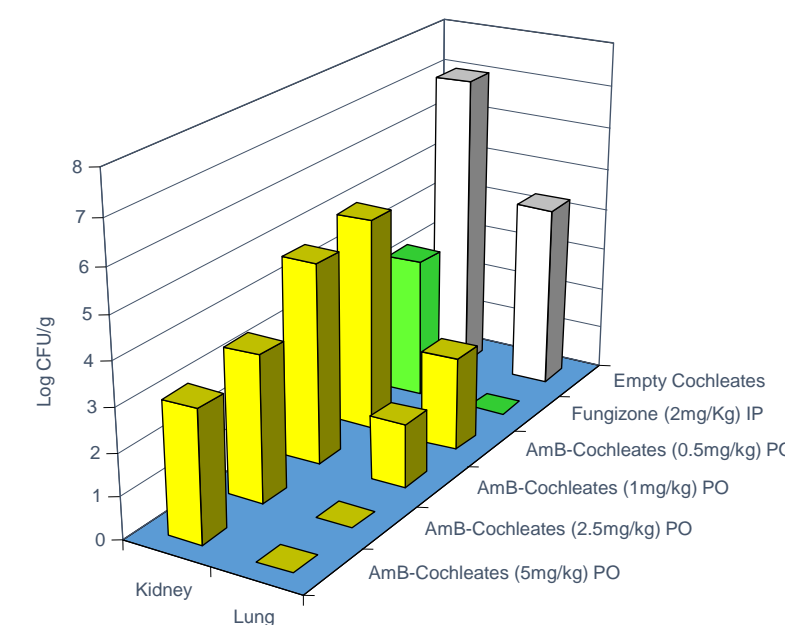


Mice: Balb/C, 25g, 10 mice/group
Infection: 1×10^5 CFU/ml, 0.2 ml/mouse, tail vein
Treatment: 15 daily doses in 0.1 ml of:
• AmB – Cochleate Intra-gastric at 5, 10, 20 mg AmB/kg
• AmBisome Intra-gastric at 10 mg AmB/kg
• Fungizone IP at 1 mg AmB/kg, (Positive Control)
• Empty Cochleates, Intergalactic (Negative Control)
Tissue Burden: Kidneys and lungs in saline, serial 10x colony count

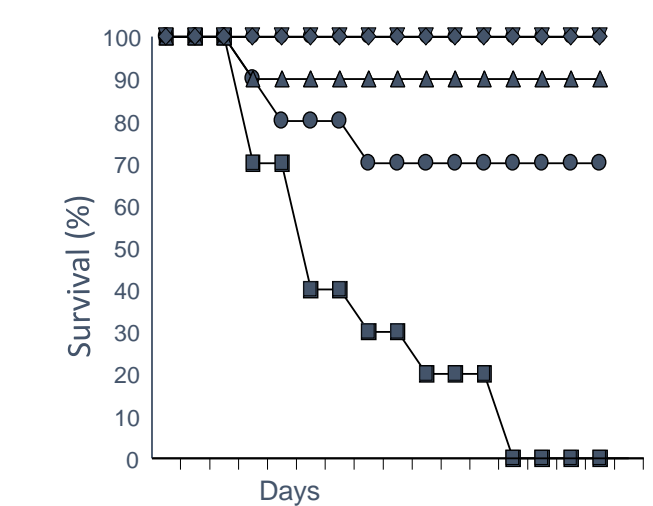
RESULTS

Oral CAmB Efficacy

Candida Tissue Burden Study

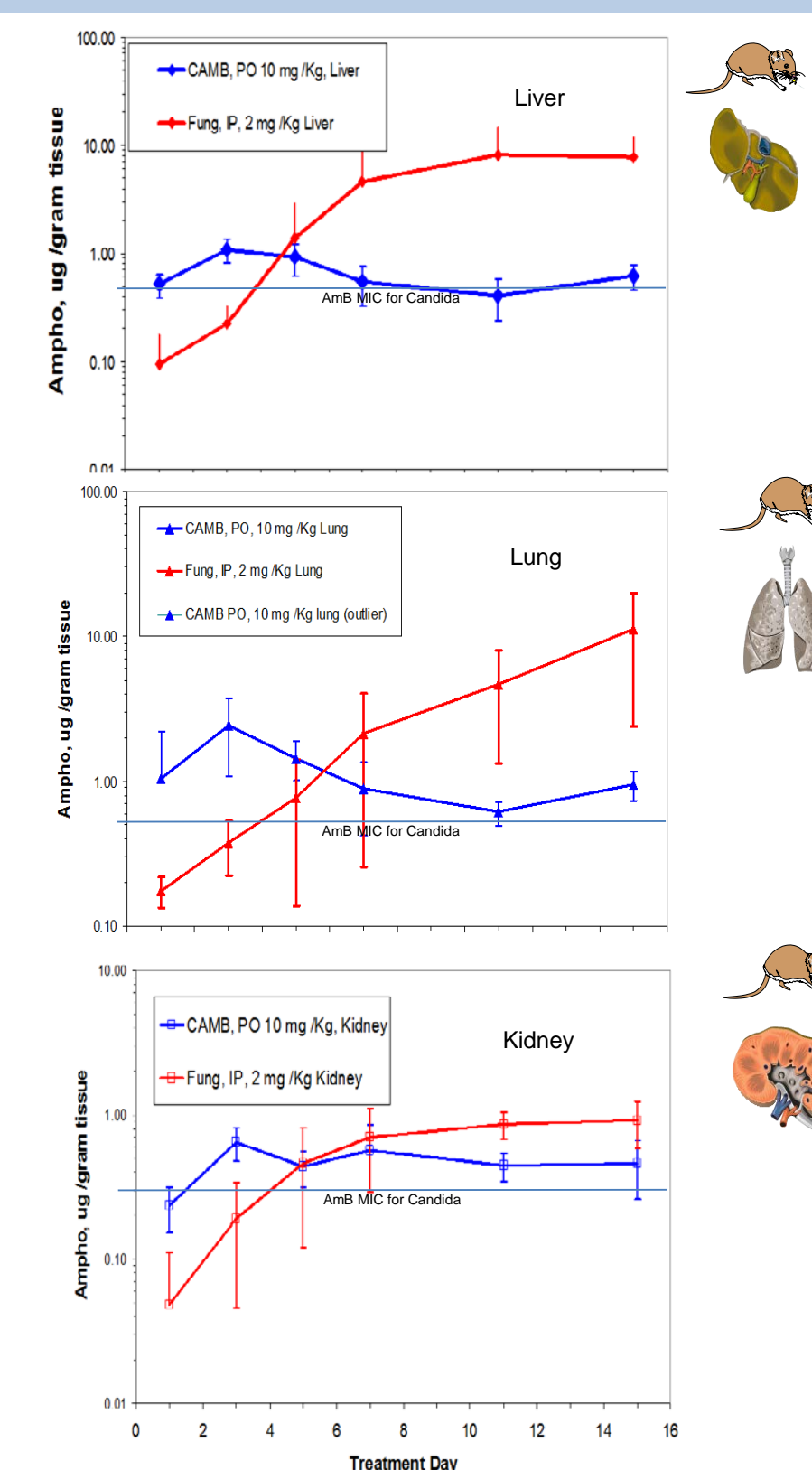


Survival



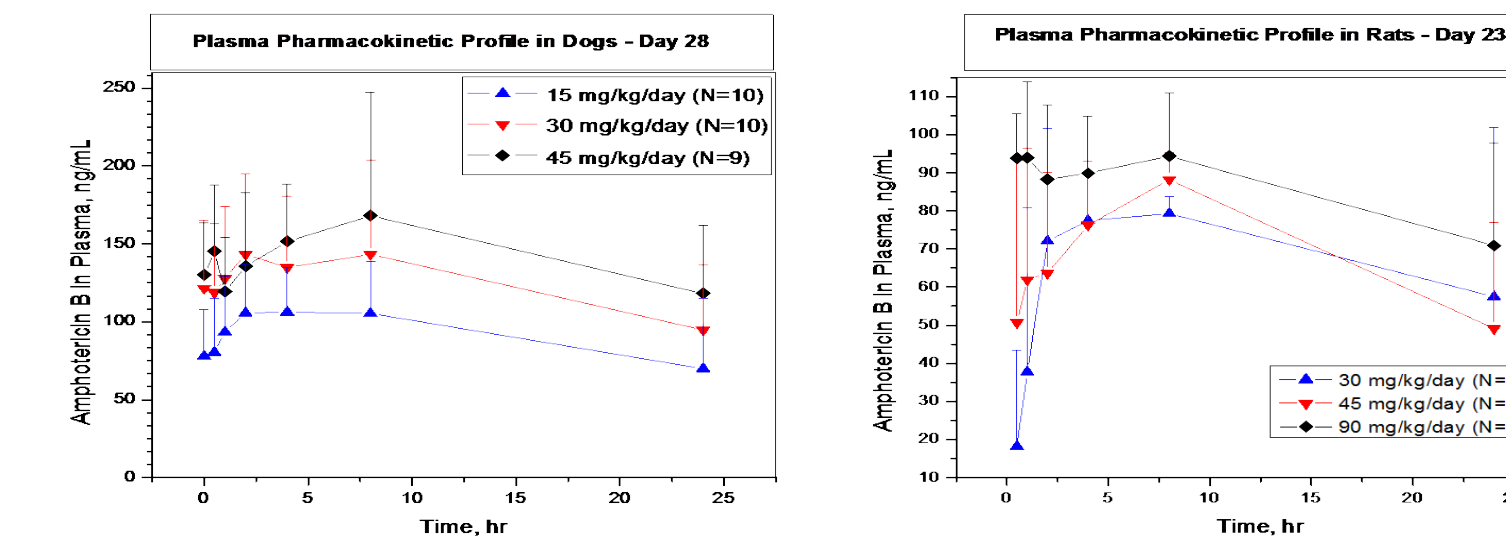
- No drug → 0%
 - DAMB (1mg/kg IP) → 70%
 - DAMB (2 mg/kg IP) → 100%
 - LAMB (10 mg/kg PO) → 90%
 - CAmB (0.5 mg/kg)
 - CAmB (2.5 mg/kg)
 - CAmB (5 mg/kg PO)
 - CAmB (10 mg/kg PO)
 - CAmB (20 mg/kg PO)
- 100% Survival

Oral CAmB PK/PD Study – Tissue AmB Levels



ORAL DELIVERY OF CAmB – PHARMICOKINETICS IN RATS AND DOGS

Toxicokinetic Parameters of Amphotericin B on Day 1 and Day 28 after Oral Administration of BAmB in Male and Female Dogs



Dose/ Gender	Day 1				Day 28			
	t _{max} (h)	C _{max} (ng/mL)	AUC _{last} (ng.h/mL)	t _{1/2} (h)	t _{max} (h)	C _{max} (ng/mL)	AUC _{last} (ng.h/mL)	t _{1/2} (h)
15 mg/kg Males	8.0 (8.9)	51.9 (20.0)	304.6 (117.6)	NC ^a	6.6 (9.8)	118.2 (20.6)	2295.4 (701.6)	32.0 (26.5)
15 mg/kg Females	7.2 (1.8)	67.3 (36.1)	887.2 (678.1)	NC ^a	8.4 (9.0)	131.2 (35.6)	1953.6 (879.2)	10.5 (NA ^b)
30 mg/kg Males	14.4 (8.8)	80.4 (26.9)	1389.4 (596.8)	14.0 (NA ^b)	3.6 (4.0)	187.3 (52.4)	3311.5 (1074.1)	13.0 (NA ^b)
30 mg/kg Females	17.6 (8.8)	81.8 (14.6)	1299.6 (161.2)	NC ^a	8.0 (8.9)	146.1 (55.5)	2521.8 (881.3)	29.8 (29.6)
45 mg/kg Males	14.4 (8.8)	114.5 (51.9)	2062.4 (919.9)	59.0 (NA ^b)	4.9 (3.2)	198.8 (96.1)	3865.7 (1606.7)	83.0 (NA ^b)
45 mg/kg Females	13.6 (9.6)	67.5 (10.9)	1242.4 (113.1)	28.5 (NA ^b)	4.3 (2.9)	155.0 (26.1)	2836.4 (376.3)	40.5 (20.5)

^a NC = not calculated. t_{1/2} values could not be calculated for some animals due to limited number of plasma concentrations after t_{max} that were > LLOQ or r-value of best-fit line was < 0.8.
^b NA = not applicable. t_{1/2} was calculated for only 1 or 2 animals in these groups.

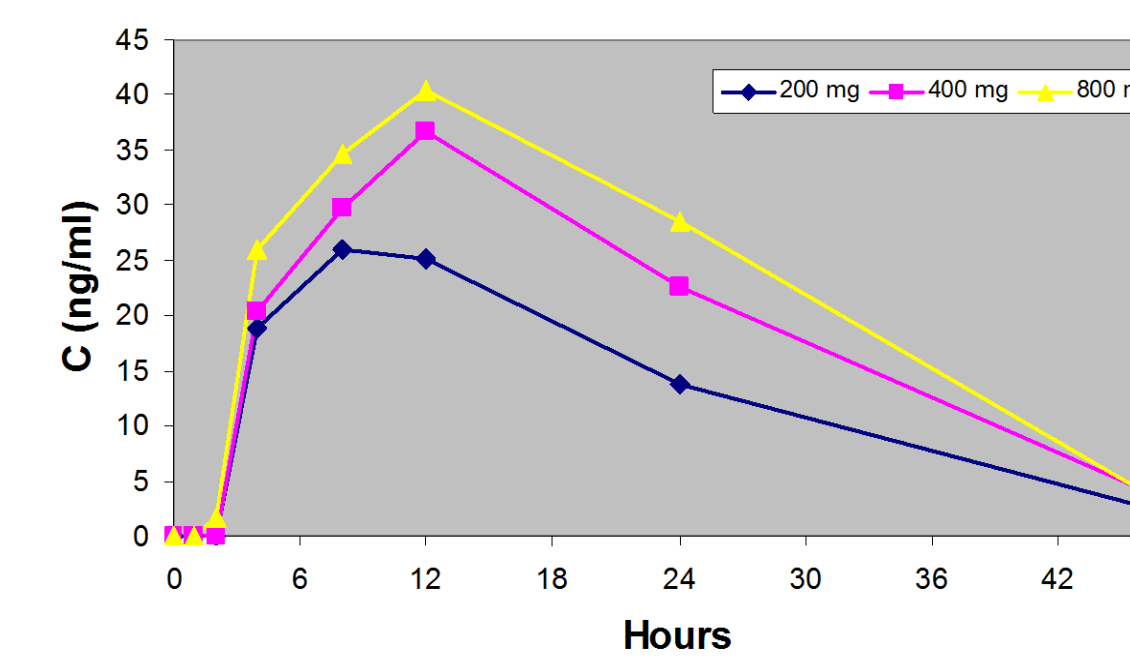
Comparison of Tissue Levels in Healthy vs. Infected Animals

Parameter	Healthy Rats 15mg/kg	Healthy Dogs 15mg/kg	Infected Mice 10mg/kg
Plasma C _{max}	0.06 µg/ml	0.062 µg/ml	BLQ
Urine	0.83 µg/ml	0.52 µg/ml	ND
Lung	0.056 µg/gm	0.035 µg/gm	0.7 – 1.0 µg/gm
Liver	0.077 µg/gm	0.069 µg/gm	0.6 – 1.0 µg/gm
Kidney	0.094 µg/gm	0.142 µg/gm	0.5 - 0.8 µg/gm

Conclusion:
AmB at least 5-10 fold higher in infected tissues.

ORAL DELIVERY OF CAmB – HUMAN PHASE I CLINICAL TRIAL: PHARMICOKINETICS

Oral Administration of CAmB in Humans: A Phase I Study of Tolerability and Pharmacokinetics



	200 mg	400 mg	800 mg
C _{max} (ng/ml)			
Mean	28.11	37.09	40.76
SD	17.06	8.66	6.48
Median	30.8	40.1	39.45
Range	0 - 48.2	22.2 - 45.9	29.8 - 53.1
AUC ₀₋₂₄ (ng.h/ml)			
Mean	407.4	522.9	624.5
SD	302.3	274.0	294.9
Median	396.1	609.2	636.7
Range	0 - 853	0 - 816	0 - 1092
T _{max} (h)			
Median	8	12	12

- Preliminary pharmacokinetic assessment indicated increases in C_{max} and AUC with increasing dose.
- Preliminary C_{max} and AUC₀₋₂₄ are compatible with prior results from animal toxicology studies.

SUMMARY AND CONCLUSIONS

- In infected mice treated with oral CAmB at 10mg/kg, the liver, lung, and kidneys showed reproducible and quantifiable levels of AmB that achieved their maximal levels (~ 0.5-1 µg AmB/g tissue) early in the treatment schedule.
- Fungizone-treated mice showed lower tissue concentrations at early times, but nearly ten-fold higher AmB levels in liver and lung which were achieved by day 11.
- Plasma AmB levels were at or below the limit of quantitation (50ng/ml).
- In infected mice treated with oral CAmB at 2mg/kg, which results in 100% survival and a 4 log reduction in CFU in both the lung and kidney, AmB levels in plasma and tissue are undetectable.
- Rapid tissue penetration to above MIC occurred in the infected organs of animals with invasive Candidiasis, while no such accumulation occurred in the organs of healthy animals, consistent with the targeted therapy concept.

Oral Administration of Drugs Formulated into Cochleates Can Change the Pharmacokinetics, Biodistribution and Tolerability of the Drug