BACKGROUND: Cryptococcal meningoencephalitis (CM) is an important infection in HIV/AIDS, responsible for an estimated half million deaths annually. Amphotericin B deoxycholate is a standard treatment for cryptococcal disease; however, its use is limited by toxicities and intravenous administration. To help mitigate these limitations a novel orally available lipid-crystal nano-particle, cochleate, formulation of amphotericin B has been developed (CAMB) that has been shown to be effective for oral delivery of amphotericin B.

METHODS: Groups of 5 mice each were inoculated with 10^8 of C. neoformans strain JRF/ATCC 20881 into intravenously. In 10 ml. Therapy was delayed 72 hours and then daily treatment commenced with Fluconazole + Fluconazole (JS-C), CAMB + SJF, SJF + CAMB, or Fluconazole, or for 28 days and mice were followed for 70 days and sacrificed when moribund. In a second study, to study cochleate delivery to the brain, three mice were infected, as above, 2 days later 2 were treated once daily 3 d by CAMB + fluconazole, or fluconazole for 28 days and mice were followed for up to 150 days and sacrificed when moribund. In addition, to study cochleate delivery, the brain, three mice were infected as above. 5 days later 2 were treated once daily 1 d by orogal with a Rh-CAMB solution equivalent to 10mg/kg of CAMB. The fourth group of 5 mice remained untreated. Mice were then sacrificed at 7 d and brain material recovered and observed for fluorescence.

RESULTS: Mortality study: Two mice died as follows; vehicle control: 19 d; 10 mg/kg/d PO + 5-FC: 49 d; Rh-CAMB 25 mg/kg/d PO + SJF: 53 d; SJF: 49 d; CAMB 25 mg/kg/d PO + SJF: 53 d; Rh-CAMB 25 mg/kg/d PO + 5-FC: 47 d; SJF: 52 d; CAMB 25 mg/kg/d PO + SJF + 5-FC: 47 d; SJF 25 mg/kg/d PO: 47 d. The CAMB formulation led to a significantly increased survival over untreated, infected mice (19 vs. 58 d; p = 0.003). Additional to study cochleate delivery to the brain, three mice were infected as above. 5 days later 2 were treated once daily 1 d by oral gavage with a Rh-CAMB solution equivalent to 10mg/kg of CAMB. The fourth group of 5 mice remained untreated. Mice were then sacrificed at 7 d and brain material recovered and observed for fluorescence.

CONCLUSION: CAMB is an effective oral anti-fungal agent equivalent to systemic fungicide SJF in an intracranial model of Cryptooccoca neoformans brain infections and delivery of CAMB was evident by imaging of CAMB fluorescently labelled particles.

How Cochleates Encapsulate Drugs

Model of Drug Delivery - The “Trojan Horse” Hypothesis

• Microphage-modify engulf cochleates and their cargo
• Cochleates enter the circulatory system, diffuse into tissues and are taken up by “activated” or infected cells.
• High circulating drug levels can result in nonspecific toxicity
• High plasma and interstitial drug levels are needed to target intracellular infections.

The drug product is associated with the negatively charged lipid.
• The addition of calcium creates a calcium-phospholipid complex.

Cochleates change the Pharmacokinetics and Biodistribution of Drugs

• Cell targeted delivery
• Improved uptake by infected or activated cells
• Reduced Diverse ion concentrations in serum and mucosal secretions are such that the cochleate is a near physiological delivery system.
• Diverse Divalent cation concentrations in serum, mucosal secretions and tissues are such that the cochleate is a near physiological delivery system.
• In the presence of the cochleate, the drug cargo is released as the cochleate opens, releasing the cargo molecule.

Enzymatic and metabolic degradation of cochleates in the gastrointestinal tract.

Cochleates deliver their vehicles have been shown to mediate oral bioavailability for injectable drugs.

• Delivery of CAMB to the brain in mice infected with C. neoformans, strain JRF has been shown to be efficacious in a delayed-therapy model of cryptococcal meningitis.

SUMMARY AND FUTURE STUDIES

• Oral CAMB + SJF exhibits equivalent efficacy as fungizone injection + oral fluconazole in a mouse model of cryptococcal meningitis.

• Delivery of CAMB to the brain in mice infected with C. neoformans was demonstrated using fluorescently labelled CAMB particles.

• Experiments using CAMB in a model of cryptococcal meningitis in an alternate species will be conducted.

• Studies for evaluation of CAMB in human cryptococcal meningitis are warranted.

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