

***In Vivo* Biodistribution and Ototoxicity Evaluation of Noninvasive Trans-Tympanic Delivered Liposomal-Ceftriaxone for Otitis Media Treatment**

Shayan Shafiee¹, Wenzhou Hong², Julliette Lucas⁵, Pawjai Khamphang², Christina L Runge², Clive Wells³, Ke Yan⁴, Joseph E Kerschner², Amit Joshi¹.

Affiliations:

1. Department of Biomedical Engineering, Medical College of Wisconsin, Milwaukee, WI 53226, USA.
2. Department of Otolaryngology and Communication Sciences, Medical College of Wisconsin, Milwaukee, WI 53226, USA.
3. Department of Microbiology and Immunology, Medical College of Wisconsin, Milwaukee, WI 53226, USA.
4. Department of Pediatrics Quantitative Health Sciences, Medical College of Wisconsin, Milwaukee, WI 53226, USA.
5. Medical College of Wisconsin, Milwaukee, WI 53226, USA.

This study aims to assess the *in vivo* biodistribution of Cationic-Liposomal-Ceftriaxone via intact tympanic membrane in chinchillas and assess its ototoxicity for local treatment of Otitis media.

Ceftriaxone (CFX) was encapsulated in cationic-liposomes composed of DOTAP, DOPC, and DOPE lipids to achieve a hydrodynamic size of 121 nm and a zeta potential of +21.1 mV, with a drug concentration equivalent of 17.5 µg/mL (17.5 µg CFX / 12.5 mg Lipid). Chinchillas were treated trans-tympanically with liposomal or free CFX, or a systemic oral control, twice daily for 3 or 10 days. The amount of encapsulated CFX in the middle ear lavage fluid was determined by high-performance liquid chromatography. The auditory brainstem response hearing sensitivity test was conducted, and cochlear histology was performed to evaluate ototoxicity.

Liposomal-CFX demonstrated efficient drug delivery to the middle ear, with increased delivery rates as treatment duration increased from 3 to 10 days (0.87% vs. 5.8% of the injected dose). Free CFX and conventional oral treatment had minimal delivery to the middle ear (0.015% and 0.024%). Auditory brainstem response did not differ significantly between baseline and post-treatment at 7, 10, and 30 days. No differences were observed in outer or inner hair cell morphology between experimental groups and untreated controls.

Liposomal-Ceftriaxone proves efficacious as a non-invasive trans-tympanic nanoparticle-antibiotic delivery system for otitis media treatment. The optimized formulation allows effective drug delivery with low systemic exposure and off-target effects, and negligible ototoxicity, displaying promise as a treatment alternative.