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Preliminary Findings on the Effects of Topical Photoactivated Antimicrobial Methyl-δ-Aminolevulinic Acid on Murine Hearing Thresholds

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract
This study evaluates the ototoxicity of a potential novel otopical antimicrobial, photoactivated methyl-δ-aminolevulinic acid (M-ALA). Ten CBA/J mice received intratympanic injections of 10 mM M-ALA and 640 nm light source illumination for 7 days, with contralateral ears receiving saline. Auditory-evoked brainstem response (ABR) thresholds (8, 16, 24, and 32 kHz) were determined preinjection and at 1, 30, and 90 days postinjection. Mean ABR thresholds were similar after intratympanic administration of M-ALA and saline (F ratio, 0.001; P = .971). ABR thresholds temporarily increased in both groups (F ratio, 28.52; P = .00) at day 1 postinjection of intratympanic treatments but returned to baseline at day 30 and 90. This temporary elevation was associated with tympanic membrane perforations and granulation tissue at the injection sites, which resolved by day 30 posttreatment. The preliminary findings indicate that intratympanic application of M-ALA with light activation over a 7-day course in a murine model does not produce measurable ototoxicity and is well tolerated.

Keywords
photodynamic therapy, 5-aminolevulinic acid, methyl-δ-aminolevulinic acid, ototoxicity, ototopical

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Increasing bacterial antibiotic resistance is creating a need for new antimicrobials for otitis externa and suppurative otitis media, as commonly used ototopical medications weaken in efficacy and potentially predispose patients to systemic quinolone resistance.¹ Photodynamic therapy (PDT) may provide useful additions to current ototopical antibiotics. 5-δ-aminolevulinic acid (5-ALA) is a photosensitive precursor used frequently in PDT, with better efficacy against gram-positive than gram-negative bacteria due to cell membrane permeability.² A lipophilic 5-ALA derivative, methyl-δ-aminolevulinic acid (M-ALA), also eliminates gram-negative bacteria with improved cell membrane permeability increasing levels of protoporphyrin IX, a photoactive porphyrin (PAP), in cells with high metabolic turnover. Light activation with 600- to 700-nm light releases highly cytotoxic oxygen species (O₂⁻) from PAPs, causing apoptosis from cell membrane and organelle damage. PDT is used in cancer chemotherapy and dermatologic and ophthalmologic applications, with recent studies evaluating antimicrobial potential due to increased antibiotic resistance.³

5-ALA and M-ALA have been evaluated for in vitro antimicrobial activity,²⁻⁴ but no ototoxicity studies currently exist. The main objective of this study is to measure the ototoxicity of M-ALA in a murine model to assess safety for ototopical use. If M-ALA is nonototoxic, it could provide an important addition to current antibiotics, without overlapping resistance mechanisms.

Methods
Following University of Connecticut Health Center Animal Care Committee approval and a determination of N (to detect 10% difference from control with coefficient of variability 5% for 95% power at P < 0.05),⁵ 10 CBA/J mice (female, 2 months old) were purchased (Charles River Laboratories, Wilmington, Massachusetts). General anesthesia was induced with 2% to 4% isofluorane with 2 to 3 L/min oxygen. ABRs were measured using real-time signal processing (Tucker-Davis Technologies, Alachua, Florida) with precalibrated...
open sound system transmitting through a TDT-FF1 loudspeaker 2 mm from the test ear with contralateral ear occlusion. Pure-tone bursts of 4 milliseconds duration (rise/fall times of 2 milliseconds) were delivered in 5-dB steps between 20 and 90 dB sound pressure level at 8, 16, 24, and 32 kHz. Waveforms were averaged over 512 repetitions presented at 21/s. Auditory testing was conducted preinjection and postinjection on days 1, 30, and 90 to evaluate nonacute ototoxicity.

Intratympanic (IT) applications of M-ALA solution and saline with light activation occurred daily for 7 days. Five to 10 μL of 10 mM M-ALA solution (1 g of M-ALA; Sigma Aldrich, St Louis, Missouri) dissolved in 550 mL of sterile isotonic saline under minimal light conditions, chosen for optimal bacterial killing efficacy and low dark toxicity,2 was injected IT under operating microscope guidance. Five to 10 μL of sterile isotonic saline was injected into contralateral ears as controls with the M-ALA ear chosen randomly. Each ear was light-activated transtympanically with a 640-nm LED light source (Ocean Optics, Florida) through an ear speculum (Figure 1).

Figure 1. Transtympanic illumination with 640-nm wavelength light under snout mask anesthesia.

ABR thresholds were statistically compared using 3-way repeated-measures analysis of variance and Student t test.

Results

Figure 2 shows mean ABR thresholds of M-ALA- and saline-treated ears as a function of days from treatment and stimulus frequency, and differences between averaged M-ALA minus saline ABR thresholds. ABR thresholds increased temporarily on postinjection day 1 for all ears. ABR thresholds were influenced by stimulus frequency (F ratio, 59.65; P = .00) but not by IT saline versus M-ALA (F ratio, 0.001; P = .971). The main effect of day was statistically significant (F ratio, 28.52; P = .00) without an interaction with stimulus frequency (F ratio, 0.33; P = .803). Follow-up 2-tailed t tests showed a significant difference between day 8 and other test intervals for both M-ALA- and saline-treated ears (P < .05), with return of ABR threshold to baseline at 30 and 90 days posttreatment and no difference between M-ALA and saline (mean difference ± 95% confidence interval preinjection, −1 ± 0.122, P = .65; postinjection day 1, 0.25 ± 0.081, P = .91; day 30, −1.75 ± 0.089, P = .42; day 90, 1.75 ± 0.107, P = .39). Inspection of tympanic membranes suggested that transiently elevated ABR thresholds after 7 days of IT injections were associated with tympanic membrane perforations (100% of the ears) and granulation (45% of the ears) at the injection sites, which resolved by 14 to 30 days after treatment.

Discussion

Increases in drug-resistant bacterial infections are propelling the search for new antimicrobial therapies, and PDT has shown potential utility. Gram-positive bacteria, yeasts, and
mycoplasma are readily susceptible to 5-ALA PDT, and lipophilic M-ALA also additionally eliminates gram-negative bacteria effectively. Fotinos et al found high efficacy with 10-mM M-ALA correlating directly to increased PAP concentrations in Pseudomonas aeruginosa and methicillin-resistant Staphylococcus aureus, increasingly resistant otitis pathogens. In the clinical setting, ototopic drops could be administered in the external canal or through ventilation tubes to the middle ear, and photoactivated transcanal by handheld light sources. Hurdles to clinical application of PDT for ototopical use include unknown potential ototoxicity of these compounds and delivery methods. The utility of M-ALA as an ototopical antimicrobial hinges on this effectiveness against common otitis pathogens and a safe ototoxicity profile.

Our preliminary murine results using frequency-specific ABR thresholds showed no long-term ototoxicity of light-activated M-ALA after a 7-day IT exposure compared with saline controls. A temporary statistically significant threshold increase occurred immediately following both treatments. This transient increase likely represents a conductive hearing loss induced by granulation and tympanic membrane perforations observed after IT injections, which resolved by 30 days. The lack of ototoxicity in this murine transtympanic M-ALA model opens possibilities for further studies of potential applications as an ototopical antimicrobial. Tympanic membrane perforation rates observed here, related to the delivery technique, are comparable with our previous studies involving intratympanic injections. Future work will be directed at quantification of the transtympanic light activation and assessing bacteriocidal efficacy in vivo.

**Author Contributions**

Corrie E. Roehm, corresponding author, design, data acquisition, analysis, manuscript drafting, final approval; Tulio A. Valdez, design, analysis, manuscript drafting, final approval; Kourosh Parham, data acquisition, analysis, manuscript drafting, and final approval.

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**References**


