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Original Article

D-Methionine reduces tobramycin-induced ototoxicity without antimicrobial interference in animal models

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Abstract

Background: Tobramycin is a critical cystic fibrosis treatment however it causes ototoxicity. This study tested D-methionine protection from tobramycin-induced ototoxicity and potential antimicrobial interference.
Methods: Auditory brainstem responses (ABRs) and outer hair cell (OHC) quantifications measured protection in guinea pigs treated with tobramycin and a range of D-methionine doses.

In vitro antimicrobial interference studies tested inhibition and post antibiotic effect assays. *In vivo* antimicrobial interference studies tested normal and neutropenic *Escherichia coli* murine survival and intraperitoneal lavage bacterial counts.
Results: D-Methionine conferred significant ABR threshold shift reductions. OHC protection was less robust but significant at 20 kHz in the 420 mg/kg/day group.

In vitro studies did not detect D-methionine-induced antimicrobial interference. *In vivo* studies did not detect D-methionine-induced interference in normal or neutropenic mice.
Conclusions: D-Methionine protects from tobramycin-induced ototoxicity without antimicrobial interference. The study results suggest D-met as a potential otoprotectant from clinical tobramycin use in cystic fibrosis patients.
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Keywords: D-Methionine; Aminoglycoside; Otoprotection; Ototoxicity; Antimicrobial interference

1. Introduction

Since first introduced in 1942, aminoglycoside antibiotics have increased our ability to treat gram-negative bacterial infections that are not responsive to penicillin or other conventional antibiotics [25]. However, aminoglycoside clinical use in the United States is limited by toxic side effects that include cochleo-, vestibulo-, and/or nephrotoxicities. Preferential damage to tissue types varies by the specific aminoglycoside employed [8,30,64]. Nonetheless,

Abbreviations: ABR, auditory brainstem response; AG, aminoglycosides; ANOVA, analysis of variance; ATCC, American Type Culture Collection; BUN, blood urea nitrogen; CAMHB, cation-adjusted Mueller–Hinton broth; CFU, colony forming unit; D-Met, D-methionine; DP-OAEs, distortion product otoacoustic emissions; HMDS, hexamethyldisilazane; IHC, inner hair cell; IP, intraperitoneal; MIC, minimum inhibitory concentration; FIC, fractional inhibitory concentration; OHC, outer hair cell; PAE, post-antibiotic effect; ROS, reactive oxygen species.

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because of their low cost, high efficacy, and low incidence of resistance, aminoglycoside antibiotics are commonly used in many parts of the world today including South Africa and the United States [2,7,29,35]. Aminoglycosides have been commonly prescribed for sepsis, meningitis, complicated urinary tract and respiratory infections because they were highly effective against gram-negative infections [52].

The prevalence of aminoglycoside-induced ototoxicity has been documented to be high in populations where they are monitored for possible hearing loss. Studies have reported an incidence range of 6–41% [13,54,74]. This incidence does not appear to be decreasing. Fausti et al. [26] monitored 370 patients receiving aminoglycoside antibiotics in the Veteran's Administration system and found a 33% occurrence of aminoglycoside-induced ototoxicity. The incidence is higher in developing countries because audiologic monitoring for early ototoxicity detection is less common, dosing is less controlled than in the United States, and antibiotic options may be limited [48].

Aminoglycoside antibiotics exhibit *in vitro* activity against a variety of clinically significant gram-negative bacilli such as *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Citrobacter* spp., *Acinetobacter* spp., *Proteus* spp., *Klebsiella* spp., *Serratia* spp., *Morganella* spp., and *Pseudomonas* spp. as well as gram positive *Staphylococcus aureus* and some streptococci. As with other antibiotic classes, significant differences in the spectrum exist among the various aminoglycosides [25].

Each aminoglycoside induces different overall and organ-specific side effects; with particular aminoglycoside vestibulo- and/or cochleotoxicities restricting clinical use [5,45,47]. Tobramycin generally causes equal cochlear and vestibular damage [40,51,66]. Tobramycin first damages cochlear outer hair cells (OHCs) from base to apex of the organ of Corti, progressing to inner hair cells with continued use [36], and causes irreversible hearing damage [41,70].

In general, aminoglycosides, including tobramycin, induce reactive oxygen species (ROS) production as demonstrated in the cochlea [18,39]. These ROS can damage cochlear tissues and lead to apoptosis, loss of cochlear sensory cells, and resultant hearing loss. ROS production may be secondary to mitochondrial ribosome malfunction by causing protein mistranslation and impaired resistance to oxidative stress in the cochlea [50].

Several otoprotective agents have been proposed to prevent aminoglycoside-induced hearing loss with therapeutic targets at various steps of the proposed ototoxic pathway. However, none of the otoprotectants under development are currently FDA-approved for this purpose. Effective antioxidants, such as D-methionine (D-met), may be able to mitigate aminoglycoside-induced oxidative stress, prevent hair cell death, and reduce or alleviate subsequent hearing loss. If D-met reduces the toxicity profile of any, and hopefully all, aminoglycosides without compromising antimicrobial efficacy, a wider range of infections could be safely treated without the risk of inducing hearing loss.

D-Met has effectively protected against gentamicin- and amikacin-induced ototoxicity [13,68] by a presumptive free radical-detoxifying mechanism, prevention of hair cell death in the organ of Corti, and indirect cochlear mitochondrial glutathione level increases [13,73] and may also advantageously be delivered orally [13]. This translational study tested the antioxidant D-met as an otoprotective agent against tobramycin ototoxicity and tested whether D-met administration interferes with *in vitro* or *in vivo* aminoglycoside antimicrobial efficacy.

2. Materials and methods

2.1. D-Methionine otoprotection studies

2.1.1. Subjects

Six groups of 10 male albino guinea pigs (250–350 g; approximately 5–7 weeks old) were treated with daily injections of tobramycin sulfate (100 mg/kg/day subcutaneously) for 21 days. Group 1 served as a control and received tobramycin and an equivalent-volume saline intraperitoneal injection twice daily for 21 days. Groups 2–6 received subcutaneous tobramycin and two equal intraperitoneal D-met doses totaling 240, 300, 360, 420 or 480 mg/kg each day. The D-met or saline control doses were delivered 15 min before and 7 h after the daily tobramycin injection. Campbell et al. [14] demonstrated no functional or histological change of the inner ear in animals treated only with D-met. Thus, a D-met-only-treated group was not included in this study.

All animals were maintained on a regular diet and given free access to water. After arriving at the laboratory animal facilities, animals were allowed to acclimate one week before experiments began. Animal weight was recorded prior to each ABR assessment and once per week during drug administration. All animal protocols were reviewed and approved by Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee.

2.1.2. Treatments

D-Methionine powder (99 + % pharmaceutical grade, Acros Organics; St. Louis, Mo) was diluted into sterile normal saline at 30 mg/ml.

A 40 mg/ml tobramycin injectable solution was purchased from APP Pharmaceuticals (Schaumberg, IL).

Animals were fully anesthetized throughout ABR procedures and at sacrifice with 86.21 mg/kg ketamine (Fort Dodge; Madison, NJ) and 2.76 mg/kg xylazine (Lloyd Laboratories; Shenandoah, IA), which was supplemented as needed with half doses.

2.1.3. Electrophysiology

All subjects underwent auditory brainstem response (ABR) testing prior to treatment and 2, 4, and 6 weeks after initiation of drug administration. ABR monitoring used an Intelligent Hearing System evoked potential unit in a double-walled IAC sound booth. ABR thresholds were measured in both ears in response to tone-bursts with 1 ms rise/fall and 0 ms plateau gated by a Blackman envelope and centered at the frequencies of 4, 8, 14, and 20 kHz presented at 30/s. Threshold was analyzed by readers blinded to condition and defined as the lowest intensity capable of eliciting a replicable, visually detectable response at the fourth