Safety of Transtympanic Application of Probiotics: An Animal Model

Carol Nhan, Aret Bezdjian, Shyamali Saha, Satya Prakash, Lily H.P. Nguyen, Sam J. Daniel

McGill Auditory Sciences Laboratory, McGill University, Montreal, QC, Canada.
Department of Otolaryngology-Head and Neck Surgery, The Montreal Children’s Hospital, Montreal, QC, Canada.

INTRODUCTION

Chronic suppurative otitis media (CSOM) can be particularly challenging to treat, particularly when complicated by antibiotic resistance or secondary otomy. It is the leading cause of childhood hearing impairment in the developing countries and can have serious long-lasting implications for a child’s speech and language development. Complications of these infections can be severe and include meningitis, intracranial abscess, facial palsy, and lateral sinus thrombosis.

The majority of infections are polymicrobial and involve chronic inflammation of the middle ear. Pathogens most commonly associated with CSOM are Pseudomonas, Staphylococcus, Peptostreptococcus, Fusobacterium, Prevotella, and Porphyromonas.

Although initially investigated for gastrointestinal (GIT) diseases, several recent non-GIT applications of probiotics have been studied. It has been established that children with recurrent otitis media have reduced levels of nasopharyngeal commensals such as alpha-hemolytic streptococci. Therapeutic approaches such as nasal spray applications restoring this microbial population appear to reduce rates of recurrent acute otitis media and otitis media effusion.

The first-line treatment for uncomplicated CSOM in immunocompetent patients involves antibiotics and anti-inflammatory agents applied topically to the ear since that is the best way to achieve the highest dose delivery at the site of infection with the least secondary effects. However, overuse of antibiotics has resulted in resistant pathogens and prolonged use of antibiotics has been associated with development of otomy.

Lactobacillus plantarum is a probiotic that is shown to prevent both S. aureus and P. aeruginosa, the main organisms found in CSOM, from establishing wound infections. It makes it a potential good candidate for the treatment of CSOM, however, its ototoxicity potential remains to be established. Thus, the objective of our study is to investigate whether L. plantarum applied to the middle ear is ototoxic in an animal model.

METHODS AND MATERIALS

Animal care and ethics: The study received approval by the Animal Care Committee of the McGill University Health Centre Research Institute. Eight female chinchillas with normal baseline auditory brainstem-evoked response (ABR) thresholds were included in the study.

Hearing Evaluation: Performed at three different times: at baseline, early (7-10 days) and late (28 days) after application of probiotic. ABRs were done on chinchilla anesthetised by 5% Isoflurane and maintained with 3% Isoflurane. Acoustic stimuli of 8,000, 20,000, and 25,000 Hz pure tone bursts were presented to the chinchilla through insert earphones starting at 80 dB intensity and decreasing by 5 dB until a threshold was reached.

Probiotic Bacteria Preparation: L. plantarum ATCC 10241 was plated using MRS agar from an 80% (v/v) frozen MRS-glycerol stock. The plate was incubated for 24 hrs at 37°C with 5% CO2 to ensure purity. A single colony from the MRS-agar plate was incubated for 24 hrs at 37°C in 10 mL of MRS broth. A standard curve derived using the overnight culture of the bacteria was used to make a solution of 10^6 CFU/mL.

RESULTS

Probiotic Preparation: Standard colony counting of an aliquot of the probiotic solution used gave a count of 1.5 x 10^9 CFU/mL. The solution had a neutral pH of 7.0, Na+ was 156 mmol/L, K+ was 1.7 mmol/L, and Cl- 148 mmol/L.

Hearing Evaluation: The results from our study demonstrate that a 1.5 x 10^9 CFU/mL solution of L. plantarum applied intratympanically is not ototoxic. Based on in vitro, animal, and burn wound studies we suspect that this probiotic bacteria could potentially be effective in CSOM caused by P. aeruginosa and S. aureus. Hence, our safety evaluations and the pathogen inhibitory effects of L. plantarum demonstrated by other groups, presents this bacteria as a candidate for treating recalcitrant CSOM. Further pre-clinical and clinical investigations to understand the mechanism of actions responsible for such effects and efficacy studies will be invaluable for determining whether L. plantarum could be used therapeutically in CSOM.

CONCLUSIONS

Since L. plantarum has demonstrated ability to limit growth of P. aeruginosa and S. aureus, it may be a candidate for therapeutic use in recalcitrant CSOM. Recolonization of the nasopharynx with commensal bacteria has been suggested as a strategy to treat recurrent otitis media, as demonstrated by several studies. It is hypothesized that probiotic bacteria inhibit colonization by pathogenic bacteria via several mechanisms: (1) competition for nutrients and epithelial adhesion sites, (2) production of bacteriocins and inhibitory substances, (3) immune modulation, and (4) production of organic acids that lower pH and inhibit growth of pathogens.

Inhibition of P. aeruginosa growth, as well as inhibition of the production of biofilm and elastase by L. plantarum has been demonstrated both in vitro and in vivo. In a burned-mouse model where burn wounds were infected with P. aeruginosa, L. plantarum applied topically to wounds led to decreased pathogen growth and improved healing. A study of burn patients suggested that topical L. plantarum was as effective as silver sulfadiazine in decreasing bacterial load and promotion of wound healing.

Our study is the first to test the ototoxicity of a probiotic in vivo. This is particularly relevant for an organism that is not pro-inflammatory, such as L. plantarum, since these bacteria tend to permeate the round window membrane more easily and are at greater risk of causing ototoxicity.

The transient significant difference in threshold shift between experimental and control ears seen at 25 kHz during the early (day 7-10) ABR was likely due to the greater viscosity of our probiotic solution compared to PBS. It was noted on physical examination that the test ear demonstrated more residual effusion at the time of early ABR. Even then this threshold shift was not considered clinically significant at only 11 dB, and most importantly was resolved by day 28.