International Journal of Pediatric Otorhinolaryngology 78 (2014) 2136–2139

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Assessment of ototoxicity of tea tree oil in a chinchilla animal model

Aren Bezdjian [a,b], Mario A. Mujica-Mota [a], Michelle Azzi [a], Sam J. Daniel [a,b,*]

[a] McGill Auditory Sciences Laboratory, Montreal Children's Hospital, Montreal, Quebec, Canada
[b] Department of Otolaryngology-Head and Neck Surgery, McGill University, Montreal Children's Hospital, Montreal, Quebec, Canada

ARTICLE INFO

Article history:
Received 23 July 2014
Received in revised form 21 September 2014
Accepted 22 September 2014
Available online 2 October 2014

Keywords:
Ototoxicity
Tea tree oil
Olive oil
Intratympanic injection
Chinchilla
ABR

ABSTRACT

Objective: The aim of the present study is to examine the effects of tea tree oil on hearing function and cochlear morphology after intratympanic administration in a chinchilla animal model.
Methods: Nine chinchillas received intratympanic injection of 3% tea tree oil dissolved in olive oil in one ear, whereas the contralateral control ear received olive oil only. Outcome measures included auditory brainstem responses conducted before treatment and at 10 days and 30 days following the injection. Post-mortem cochlear morphology was assessed using scanning electron microscopy.
Results: At 10 and 30 days following the injection, there was no significant change in auditory brain response thresholds at 8, 16, 20 or 25 kHz. Scanning electron microscopy imaging showed no damage to auditory hair cells.
Conclusion: Tea tree oil (3%) does not appear to be ototoxic in a chinchilla animal model. Future preclinical and clinical studies are required to establish the effectiveness of TTO in treating otitis.

1. Introduction

Otitis media is the most frequent diagnosis in children and also the most common infection for which antibiotics are prescribed for Refs. [1–3]. A tympanic membrane perforation is a well-known complication observed in 4.5–7% of children diagnosed with otitis media [4,5]. In the presence of such perforation the risk of solutions administered in the ear canal entering into the middle ear greatly increases, thus raises the concern for ototoxicity.

Non-antibiotic approaches for infection treatments and pain reduction are becoming increasingly common and favored by parents and some health professionals [6]. When appropriate, alternatives to antibiotics are being considered to treat otitis, especially in children to prevent bacterial resistance. For many years, essential oils have been used as substitutes to medication [7]. Tea tree oil (TTO) is an essential oil derived from the leaves of *Melaleuca alternifolia* found in Australia. The medicinal properties of TTO were first reported by Penfold in the 1920s and since then have been thoroughly examined by numerous *in vitro* studies and *in vivo* [8–20]. These studies provide conclusive evidence supporting the antibacterial, antifungal, antiviral and anti-inflammatory properties of TTO.

Given that many of the pathogenic organisms responsible for otitis externa could fall within the TTO antimicrobial spectrum, Farnan et al. conducted an *in vitro* study assessing the susceptibility of organisms to TTO using swabs taken from patients' ears with otitis externa [21]. The study concluded that TTO might be an efficient alternative choice for the treatment of otitis externa.

Since TTO does not require a prescription, it can be used by individuals who may be unaware of the condition of their tympanic membrane. This study was conducted to assess the ototoxicity of TTO after intratympanic administration in a chinchilla model.

2. Materials and methods

2.1. Animals

The study was approved and monitored by the McGill University Health Centre Animal Care Committee in accordance with the Canadian Council of Animal Care Guidelines. Nine female chinchillas (*Chinchilla lanigera*) weighing 400–600 g served as the subjects of this study. The animals were kept in the animal care research facilities of the Montreal Children's Hospital Research Institute. The animals were closely monitored for signs of ear

* Corresponding author at: Department of Otolaryngology, Head and Neck Surgery, McGill University, Montreal Children's Hospital, 2300 Tupper Street, Room B240, Montreal, Quebec, Canada H3H1P3.
Tel.: +1 514 412 4246; fax: +1 514 412 4342.
E-mail address: sam.daniel@mcgill.ca (S.J. Daniel).

http://dx.doi.org/10.1016/j.ijporl.2014.09.023
0165-5876/

infection, vestibular toxicity, head tilts or significant weight loss. The nine animals used in this study had no hearing abnormalities.

2.2. Myringotomy

Under inhalational anesthesia with 2% isoflurane and oxygen, animals were positioned to allow visualization of the tympanic membrane. Guided by an operating microscope (Carl Zeiss, West Germany), myringotomy incisions were made in the lower anterior quadrant of the tympanic membrane of both ears.

2.3. Dilution of TTO

There is no consensus concerning the recommended percent dilution of TTO needed to treat otitis media. A study conducted by Zhang et al., used a 2% dilution of TTO dissolved in saline which proved to be non ototoxic when injected in the middle ear in a very short-term evaluation [22]. The producer's handbook recommends mixing 5 drops of TTO in 65 ml of olive oil, resulting in a 0.4% dilution of TTO in olive oil [23]. Other sources suggest the mixing of 5–10 drops of TTO in 15 ml of olive oil, resulting in 1.6–3.3% solutions [24]. It was decided to use a 3% dilution of TTO in olive oil in this study. Since our group has already demonstrated that olive oil by itself is not ototoxic when administered into the middle ear in a chinchilla animal model, we decided to use olive oil as control in our study [25].

2.4. Application of oils

After baseline hearing tests, one ear was randomly chosen as the experimental ear while the contralateral ear served as control. A surgical microscope (OPM I99; Carl Zeiss AG, Germany) allowed constant visualization of the tympanic membrane. TTO (Holista Health Inc., WN Pharmaceuticals Ltd., BC, Canada) diluted in olive oil (Pharmasystems Inc., HL healthcare Ltd., United Kingdom) was gently injected to fill the middle ear cavity by sterilized polyethylene catheter connected to a 1 ml syringe into the tympanic bulla of the experimental ear while the control received the same amount of olive oil. After each injection, the animals were held still in an upmost anatomical position for five minutes to ensure even distribution within the tympanic cavity. The animal model used has been previously validated in our laboratory for studying the ototoxicity of ear drops [25–28].

2.5. Hearing assessments

Auditory Brainstem Responses (ABR) were obtained using the SmartEP System (Intelligent Hearing Systems, Miami, FL). Responses were recorded from electrodes placed subdermally at the pinnas and vertex. Tone burst stimuli (8, 16, 20, and 25 kHz) with Blackman envelope were presented at 80 dB sound pressure level then decreasing to the threshold. The ABR threshold was determined at the lowest intensity where three reproducible waves were produced. Responses to stimuli were amplified, filtered and averaged over 1600 sweeps. Baseline ABR measurements were taken after the myringotomies, before injection. Prior to performing each ABR, the ears were examined to visualize the status of the tympanic membrane and cleaning of the ear canal was performed when required. The ABRs were repeated at 10 and 30 days following application of oils in the tympanic bulla. A threshold shift of 20 dB between baseline and post measurement was established as indicative of significant hearing loss.

2.6. Histology

Six pairs of cochleae were randomly selected and visualized under scanning electron microscopy. After decapitation, the temporal bones were removed, and the round and oval windows were opened and perfused with cold 2.5% glutaraldehyde. After 2 h, the inner ears were washed with 1 M pH 7.4 phosphate solution and left in buffer. Post-fixation with osmium tetroxide for 2 h was done the following day. After dehydration up to 70% ethanol, the cochleae were drilled and the organ of Corti removed. The samples were dehydrated to absolute ethanol and critical point dried. The samples were finally mounted on stubs and sputter coated with gold to be compared with a Hitachi S-3000N Scanning Electron Microscope (Hitachi Limited, Tokyo, Japan).

3. Results

3.1. Auditory brainstem responses

The differences between ABR thresholds between experimental and control groups were calculated using paired sample *t*-tests at each frequency tested. The highest threshold shifts were found following 10 days of injection in the experimental ear at 16 and 20 kHz (11.9 ± 3.9 dB, $p = 0.28$ and 9.7 ± 4.4 dB, $p = 0.49$ respectively). During the second measurement at 30 days post treatment, these thresholds shifts decreased to 8.1 ± 4.4 dB, $p = 0.42$ and 3.9 ± 3.3 dB, $p = 0.64$, respectively. The highest difference between experimental and control ears were noted at 16 kHz at day 10 (4.7 ± 3.4 dB, $p = 0.28$) and this remained stable at day 30 (4.2 ± 3.6 dB, $p = 0.42$). No significant hearing loss difference was observed at all frequencies and time points tested (Fig. 1, Table 1).

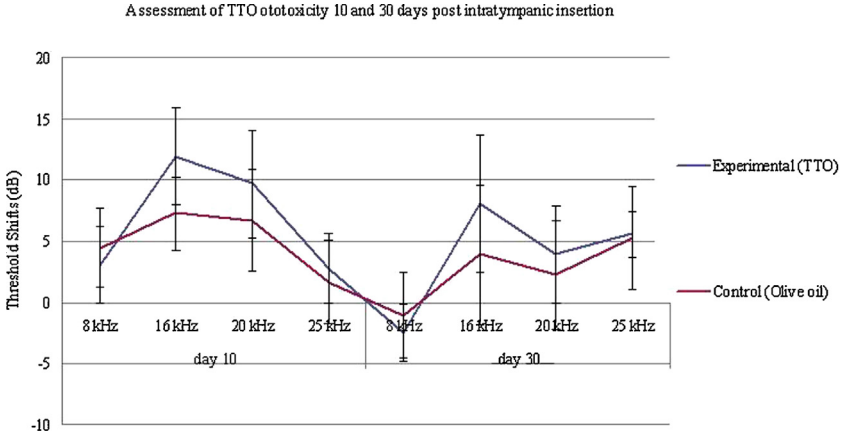

Fig. 1. Auditory brainstem response thresholds for the experimental ears at baseline and days 10 and 30 following application. Error bars = 1 standard deviation. $^{*}p < 0.05$.