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Research Paper

Factors affecting sound energy absorbance in acute otitis media model of chinchilla

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ABSTRACT

Acute otitis media (AOM) is a rapid-onset infection of the middle ear which results in middle ear pressure (MEP), middle ear effusion (MEE), and structural changes in middle ear tissues. Previous studies from our laboratory have identified that MEP, MEE, and middle ear structural changes are three factors affecting tympanic membrane (TM) mobility and hearing levels (Guan et al., 2014, 2013). Sound energy reflectance or absorbance (EA) is a diagnostic tool increasingly used in clinical settings for the identification of middle ear diseases. However, it is unclear whether EA can differentiate these three factors in an AOM ear. Here we report wideband EA measurements in the AOM model of chinchilla at three experimental stages: unopened, pressure released, and effusion removed. These correspond to the combined and individual effects of the three factors on sound energy transmission. AOM was produced by transbullar injection of *Haemophilus influenzae* in two treatment groups: 4 days (4D) and 8 days (8D) post inoculation. These time points represent the relatively early and later phase of AOM. In each group of chinchillas, EA at 250–8000 Hz was measured using a wideband tympanometer at three experimental stages. Results show that the effects of MEP, MEE, and tissue structural changes over the frequency range varied with the disease time course. MEP was the primary contributor to reduction of EA in 4D AOM ears and had a smaller effect in 8D ears. MEE reduced the EA at 6–8 kHz in 4D ears and 2–8 kHz in 8D ears and was responsible for the EA peak in both 4D and 8D ears. The residual EA loss due to structural changes was observed over the frequency range in 8D ears and only at high frequencies in 4D ears. The EA measurements were also compared with the published TM mobility loss in chinchilla AOM ears.

1. Introduction

Wideband sound energy absorbance (EA) describes the sound power transmitted into the middle ear over a broad frequency range (Feeney et al., 2003; Keefe et al., 2012). EA is sensitive to middle ear diseases, and the patterns of EA or energy reflectance (ER = 1-EA) have been characterized for various pathological conditions. These include tympanosclerosis (Rosowski et al., 2012), ossicular discontinuity (Feeney et al., 2003, 2009; Nakajima et al., 2012; Voss et al., 2012), and otosclerosis (Allen et al., 2005; Shahnaz et al., 2009a, 2009b; Sanford et al., 2012; Nakajima et al., 2012; Voss et al., 2012).

Otitis media, defined as inflammation of the middle ear, is the most common disease in young children (Gould and Matz, 2010; Hoberman et al., 2011). Two major types of otitis media occur: otitis media with effusion (OME), and acute otitis media (AOM). In OME, middle ear effusion (MEE) accumulates in the ear cavity in the absence of signs of infection (Bluestone and Klein, 1983; Paradise 1987). EA associated with OME are characterized by low absorbance amplitudes at both low and middle frequencies, and by a single peak at high frequencies (f > 2 kHz), though sharpness and value of the high-frequency peak varies considerably (Piskorski et al., 1999; Allen et al., 2005; Beers et al., 2010; Feeney et al., 2003; Ellison et al., 2012; Hunter and Margolis, 1997). The mechanism of such changes was further illustrated by Voss et al. (2012). In contrast, AOM arises from rapid infection onset within the

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middle ear. Such infections are accompanied by MEE production, middle ear pressure (MEP) reduction, and structural changes in middle ear tissues (Guan and Gan, 2013; Guan et al., 2014). While EA in OME has been well studied, the effects of AOM on EA remain unclear.

The mechanoacoustic properties of the middle ear that accompany AOM differ from those occurring in OME. Bacterial infection can produce distinctive changes in the middle ear including profound structural alterations of the tympanic membrane (TM) (Guan et al., 2015; Grote and van Blitterswijk, 1984; Larsson et al., 2003; Magnuson and Hellstrom, 1994; Schmidt and Hellstrom, 1993), mucosal thickening (Lim and Klainer, 1971; Fulghum et al., 1982), and ossicular adhesions (Guan et al., 2014; von Unge et al., 1997; Caye-Thomasen and Tos, 2000). In guinea pig and chinchilla models of AOM, we identified that MEP, MEE, and middle ear structural changes are three factors contributing to the loss of umbo mobility and hearing (Guan et al. 2014, 2015; Guan and Gan, 2013). However, it remains unclear whether sound ER or EA, an increasingly used clinical tool for diagnosis of middle ear diseases (Allen et al., 2016; Pitaro et al., 2016; Hunter et al., 2010), can differentiate these three factors in infected ears from patients with AOM.

In this paper, we measured wideband EA in the AOM model of chinchilla. Here we report EA instead of ER values since sound absorption is more directly relevant to hearing. EA in the chinchilla ear with AOM was measured sequentially at three stages: the unopened ear, the pressure-released ear, and the ear after effusion removal. This approach allowed us to assess both the combined and individual effects of these three factors on sound energy absorption, and permitted us to investigate whether MEP, MEE, and structural changes induced EA changes in the ear with AOM could be differentiated in the same ear specimen. Two AOM disease time courses, 4 and 8 days post inoculation with *Haemophilus influenzae* (Guan et al., 2014), were established in chinchillas. EA was measured across these two disease courses at three experimental stages and the measurements were compared with the published TM mobility loss in chinchilla AOM ears.

2. Methods

2.1. Animal preparation

Fifteen adult chinchillas (*Chinchilla lanigera*) weighing between 600 and 780 g were included in this study. These animals were purchased from Moulton Chinchilla Ranch (Rochester, MN). The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Oklahoma and met the guideline of the National Institutes of Health. All animals were free from middle ear disease (as evaluated by otoscopic examination) at the beginning of the study.

The animals were divided into uninfected controls ($n = 6$) and two AOM groups, one evaluated at 4 days (4D) post bacterial challenge ($n = 5$) and the other evaluated at 8 days (8D) post challenge ($n = 4$). AOM was produced by transbullar injection of a *H. influenzae* 86-028NP suspension into both ears following the procedure described by Morton et al. (2012). Under general anesthesia [ketamine (10 mg/kg) plus xylazine (2 mg/kg)], 0.3 ml bacterial suspension containing 3000 colony-forming units (CFU) was injected into the superior bulla bilaterally using a 1 cc syringe equipped with a 26 gauge needle. Control animals were untreated.

On the 4th or 8th day post-inoculation, animals were anesthetized with a mixture of ketamine (10 mg/kg) plus xylazine (2 mg/kg). Additional anesthesia was administered as needed to maintain areflexia. To expose the entrance of the ear canal, the pinna and the skin covering the ear canal were removed surgically. Then the TM was examined microscopically to identify signs of AOM such as inflammation or MEE. In each animal, the experiment was conducted bilaterally. Body temperature of both control and AOM animals was maintained throughout the experiment at ~38 °C by placing the animal in a prone position on a thermo-regulated surgical heating blanket.

2.2. EA measurements

EA was measured with a wideband tympanometer (Model AT235h, Interacoustic, MN) with Reflwin PC software. The measurement probe with commercial tips (outer diameter of 8 mm) was pressed to the bony rim of the entrance of the ear canal and held by hand to achieve a pressure seal. Using click stimuli, the EA was measured at 60 frequencies between 0.25 and 8 kHz while the ear canal air pressure was swept from −300 to +200 daPa in the descending direction. EA values at ambient pressure were extracted from these measurements at 0 daPa. Instead of simply measuring EA at ambient pressure in the ear canal, this type of EA tympanometry measurement was performed to estimate the MEP of both the control and the AOM ears. The surface area of the probe within the ear canal was used for calculation of EA (Keefe et al., 1993). In this study, the diameter at the entrance of the bony part of chinchilla ear canal was determined to be 4–6 mm. The system was calibrated with a set of two rigidly terminated tubes with an inner diameter of 4.5 mm.

EA measurements in ears with AOM were performed in three experimental stages: OM-1, unopened bulla containing the MEP and MEE; OM-2, pressure released from the middle ear; and OM-3, effusion removed from the middle ear. At each experimental stage, the EA measurement was conducted bilaterally in a given animal.

After completion of the EA measurements in stage OM-1, further surgery was performed following the procedure by Guan et al. (2014). Briefly, the skin of the superior temporal bone was removed to expose the middle ear bony wall on top of the temporal bone. Then, a 1 mm diameter hole was drilled into the roof of the middle ear to release the middle ear pressure. After sealing the hole with dental cement (PD-135, Pac-Dent, CA), the OM-2 test was performed.

Upon completing stage OM-2 EA measurements, the hole on top of the temporal bone was re-opened and enlarged with a drill to a diameter of 3–4 mm. Assisted by microscopic visualization, a silicone tube then was inserted to the bottom of the middle ear cavity through this hole. The middle ear effusion was then aspirated manually from the cavity with a 1 ml syringe. The aspiration process was repeated as necessary until no additional fluid could be drained from the tympanic cavity. The total effusion volume obtained from each ear was recorded. Ossicular adhesions were frequently found on the malleus head and between the manubrium and the cochlear promontory when the top cavity was opened for stage OM-3. These ossicular adhesions were not disturbed during the aspiration of the effusion. Thereafter, the opening on top of the temporal bone was covered by a thin glass sheet, and sealed with dental cement. Then, measurement of EA was conducted (stage OM-3). After measurements were completed at the three OM stages, the bulla was harvested. To confirm total MEE removal from the middle ear, another hole, diameter ~4 mm, was opened in the posterior area from the medial side to allow microscopic examination of the middle ear.

Control ears were prepared in the same manner as described above for ears with AOM. To exclude the effect of middle ear pressure in anesthetized animals (Guinan and Peake, 1967), a 1 mm diameter hole was drilled through the top of the upper cavity of the bulla to release any pre-existing pressure. EA measurements then were performed.