



Source of bacterial RNA in chronic otitis media with effusion

P.-Z. Li, L. Cheng and M.-L. Qiu

Department of Otolaryngology, Huai'an First People's Hospital,
Nanjing Medical University, Huai'an, Jiangsu, China

Corresponding author: L. Cheng
E-mail: leichengcn@163.com

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ABSTRACT. The purpose of this study was to investigate whether the bacterial RNA detected by polymerase chain reaction (PCR) and reverse transcription (RT)-PCR methods in middle ear effusion (MEE) for pediatric chronic otitis media with effusion (OME) originated from live bacteria. Degradation of RNA was observed by spectroscopic analysis; we also investigated the effect of MEE on the digestive activity of RNase. The optical density of RNA solution was stable within 3 h. MEE could not degrade the RNA, while RNase could rapidly digest the RNA. MEE significantly inhibited the digestive activity of RNase, and the inhibitory effect was correlated with MEE concentration. The bacterial DNA and RNA detected by PCR and RT-PCR methods may not originate from live bacteria, but might instead originate from residues from previous bacterial infection(s). Chronic OME is not an infection of live bacteria, and therefore, antibiotics should be used with caution for clinical treatment of pediatric chronic OME.

Key words: Chronic otitis media with effusion; Middle ear effusion; RNA; Optical density

INTRODUCTION

Chronic otitis media with effusion (OME) is defined as the presence of fluid in the middle ear without symptoms or signs of infection and is the most common cause of acquired hearing loss in childhood. Thus, this condition can result in the developmental impairment of linguistic, behavioral, motor, and social skills (de Miguel Martinez and Macias, 2008; Serbetcioglu et al., 2008; Eser et al., 2009). The causes of OME include bacterial and viral infections, allergic reaction, and Eustachian tube dysfunction (Keles et al., 2005). It has been confirmed by standard bacterial culture methods that bacteria can be cultivated in middle ear effusion (MEE) in only 40% of children with OME. However, some studies using polymerase chain reaction (PCR) and reverse transcription (RT)-PCR methods found at least one type of pathogen in most MEE cases, and that OME is a type of bacterial infection (Tano et al., 2008; Guvenc et al., 2010).

The Gram-positive bacteria *Alloicoccus otitidis* is thought to play an important role in the etiology of otitis media and OME. Using a multiplex PCR method, the detection rate of *A. otitidis* is higher than that of *Haemophilus influenzae* and *Streptococcus pneumoniae* (Kalcioğlu et al., 2002; Leskinen et al., 2002; Harimaya et al., 2006a,b; Kaur et al., 2010). However, the PCR system is very sensitive and only requires small DNA fragments for amplification. Thus, detecting bacterial DNA and RNA in MEE does not necessarily indicate infection with live bacteria. These DNA and RNA may instead represent previous bacterial infection(s) or their residues.

In this study, the degradation of RNA in MEE was evaluated and the inhibitory effect of MEE on RNase was investigated. The goal of this study was to clarify whether RNA detected in MEE is from live bacteria.

MATERIAL AND METHODS

Preparation of MEE

One hundred Chinese children (74 males and 26 females) with chronic OME were enrolled in this study at Huai'an First People's Hospital, Nanjing Medical University from July 2010 through December 2011. Their ages were 1-14 years, with an average age of 4.5 years. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Huai'an First People's Hospital.

The course of disease in each patient was more than 3 months. The external auditory canal was cleansed with 75% ethanol solution and then myringotomy was performed with a paracentesis knife under general anesthesia. The MEE specimen was obtained and was stored at -20°C until use. When conducting the following experiments, the specimen was melted at room temperature and then flushed out of the collection tube using 0.1 M acetate buffer solution, pH 5.0. The mixture was homogenized for 1 min in Silvenson homogenizer to obtain a uniform liquid.

Observation of RNA degradation

The optical density of the RNA solution was determined at 25°C and 300 nm in a PU 8625 UV/Vis spectrophotometer (Philips Company, Cambridge, England). Acetate buffer solution and distilled water were used as blank controls. A decrease in optical density represented RNA degradation.

MEE digestive activity on RNA

To evaluate the MEE digestive activity on RNA, 0, 0.5, and 1 mL homogenized MEE was added to 3, 2.5, and 2 mL RNA solution (RNA, 2 mg; solvent, acetate buffer solution), respectively. The change in optical density in the mixture within 3 h was observed.

RNase digestive activity on RNA and MEE

To determine the RNase digestive activity on RNA and MEE, 0.5 mL acetate buffer solution was added to 2 mL RNA solution. In addition, 0.1, 0.2, 0.3, 0.4, and 0.5 mL homogenized MEE was added to 2 mL acetate buffer solution, followed by addition of 2 mL RNase (5 U). The total volume of the mixture was adjusted to 4.5 mL with acetate buffer solution. The change in optical density in the mixture within 3 h was recorded.

Effect of MEE on the digestive activity of RNase

To determine the effect of MEE on the digestive activity of RNase, 0.1, 0.2, 0.3, 0.4, and 0.5 mL homogenized MEE was added to 2 mL RNA solution, followed by addition of 2 mL RNase. The change in optical density in the mixture within 3 h was recorded.

Effect of ethylenediaminetetraacetic acid (EDTA) on the digestive activity of RNase

For this assay, 0.05, 0.1, and 0.5 mm EDTA was added to 2 mL bacterial RNA solution, followed by addition of 2 mL RNase. The change in optical density in the mixture within 3 h was recorded.

Statistical analysis

Data were analyzed using SPSS ver. 19 (SPSS, Inc., Chicago, IL, USA), and the difference between the groups was compared using the Student *t*-test. A P-value less than 0.05 was considered to be statistically significant.

RESULTS

MEE digestive activity on RNA

The optical density of RNA solution was relatively stable within 3 h, showing only a 0.95% decrease. After addition of 0.05 and 1 mL MEE, the optical density remained stable, showing decreases of 1.5 and 7.6%, respectively. This indicates that the RNA solution was stable within 3 h.

After adding RNase to the bacterial RNA solution, the optical density decreased rapidly by 87.4% within 3 h, indicating that RNase could degrade the bacterial RNA. However, after 0.1-0.5 mL homogenized MEE were added to the bacterial RNA solution, the optical density remained stable, showing decreases of only 12.6, 9.1, 6.6, 4.5, and 2.2% within 3 h, respectively. This suggests that MEE could not degrade RNA and it had no RNase activity

(Table 1). Statistical analysis revealed that the difference between groups was not significant ($P > 0.05$).

Table 1. Middle ear effusion (MEE) digestive activity on RNA.

MEE	0.1 mL	0.2 mL	0.3 mL	0.4 mL	0.5 mL
Digestive activity	12.6%	9.1%	6.6%	4.5%	2.2%

Effect of MEE on the inhibition activity of RNase

When RNase was added to the bacterial RNA solution containing different amounts of MEE, the optical density of the mixture also decreased, but the degree of the decrease was significantly less than that of the RNA solution without MEE. In addition, 0.1 mL MEE showed no inhibitory activity on RNase, but 0.2-0.5 mL MEE inhibited RNase activity by 37.8, 44.3, 63.4, and 83.8%, respectively. After dialysis, inhibition was 24.5, 46.1, 62.7, 72.6, and 89.3%, respectively, which were similar to the non-dialysis values. This indicates that MEE significantly inhibited the digestion activity of RNase and that the inhibitory effect was related to MEE concentration. In addition, EDTA showed a clear inhibitory effect on RNase; 0.5 mM EDTA inhibited activity by 87.7%, which was similar to the effect of 0.5 mL MEE (Table 2). Statistical analysis showed that the difference between groups was very significant ($P < 0.01$).

Table 2. Effect of middle ear effusion (MEE) on inhibition activity of RNase.

MEE	0.1 mL	0.2 mL	0.3 mL	0.4 mL	0.5 mL
Inhibition activity	0%	37.8%	44.3%	63.4%	83.8%

DISCUSSION

OME is an MEE disease without acute infection in the tympanum or mastoid. Pediatric OME can result in hearing loss, thus affecting language learning (Post et al., 1995). OME occurs in more than 50% of infants overall during the first year of life (Rosenfeld et al., 2004). PCR is a sensitive method for detecting bacterial DNA, requiring only small DNA fragments for amplification. It is thought that detection of bacterial DNA by PCR only represents the presence of live bacteria (Post et al., 1996; Hendolin et al., 2000; Holder et al., 2012). Studies involving PCR have shown that bacterial DNA exists in some MEE samples. Thus, OME is considered to be a bacterial infection (Paradise et al., 1997). Rayner et al. (1998) confirmed the existence of bacterial mRNA in MEE using RT-PCR. This is considered as evidence of a bacterial infection.

A. otitidis can be detected in the external auditory canal, nasopharynx, and maxillary sinus of normal individuals, whereas it is also detected in the middle ear cavity of patients. This indicates that *A. otitidis* may be part of the normal flora found in the external auditory canal. *A. otitidis* in the middle ear cavity may originate in the external auditory canal that be-

comes polluted during myringotomy, and is likely not the pathogen causing OME (Durmaz et al., 2002; Kalcioğlu et al., 2003; De Baere et al., 2010).

In this study, we found that the bacterial RNA solution was stable within 3 h. After adding 0.05 and 1 mL MEE, the optical density in the bacterial RNA solution showed only a slight decrease. This is in contrast to the traditional view that the half-life of RNA is only a few seconds to several minutes. Thus, MEE appears to have no RNase activity. However, after RNase was added to the bacterial RNA solution, the optical density decreased rapidly, showing an 87.4% decrease within 3 h. This indicates that RNase can quickly degrade RNA. When RNase was added to the bacterial RNA solution containing MEE, the decrease in optical density was less than that observed in the RNA solution without MEE. The inhibitory effect of 0.5 mL MEE was similar to that of 0.5 mM EDTA, indicating that MEE could significantly inhibit the digestive activity of RNase and that the inhibitory effect is related to MEE concentration. Bacterial mRNA in MEE, similar to DNA, may be present from a previous bacterial infection. This RNA is protected from degradation by MEE.

The bacterial DNA and RNA detected by PCR and RT-PCR methods may not originate from live bacteria, but are rather the residues from previous infection. These results indicate that chronic OME is not an infection of live bacteria, and antibiotics should be used with caution for clinical treatment of pediatric chronic OME.

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