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ORIGINAL ARTICLE

The Effect of Topical Cefepime Solution on Outer Hair Cell Function of Rats

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Objectives: The current study was designed to investigate the ototoxic effect of topical cefepime solution on the outer cell function of cochlea by measuring distortion product otoacoustic emission (DPOAE) amplitudes.

Study Design: Prospective controlled animal study.

Materials and methods: A 50 mg/ml cefepime solution was injected into the left middle ear space of 8 of the rats with dental needle via transtympanic route 2 times per day for 7 days (Group 1a), and a 1 ml %0.9 NaCl solution was injected to the right ear of the same rats 2 times per day for 7 days (Group 1b). An 80 mg/ml gentamycine solution was injected to the left middle ear space of 4 of the 12 rats of the same way (Group2a), and a 1 ml %0.9 NaCl solution was injected into the right ear of the same rats (Group 2b). DPOAE measurements were then obtained for both experimental and control ears before day 0 (baseline) and days 3, 7, and 28 after the initiation of treatment.

Results: DPOEA measurements on day 0 were not significantly different between Groups 1a and 2a. DPOAE measurements during 0–28 days were not significantly different between Group 1a and Group 1b. Among groups 2a and 2b, DPOEA measurements on day 28 were significantly lower within all frequencies compared to day 0. Overall, DPOAE measurements decreased only with gentamycine ($p<0,05$).

Conclusions: The present study demonstrates that a topical cefepime solution does not affect DPOAE amplitudes after application to the middle ear cavity of rats.

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Introduction

Patients with chronic otitis media (COM) usually have chronic inflammation of the middle ear and mastoid, with a persistent perforation of the tympanic membrane, which subsequently may cause purulent otorrhea.[1,2]

Due to inflammatory or infectious conditions, topical application of various eardrops to the external ear canal and middle ear is a frequent and effective treatment in otology. Topical antibiotics are considered for patients with chronic otitis media.[3]

In spite of the advantages, any drug that is applied to the external ear canal and middle ear cavity may cause adverse effects to the vestibular and cochlear apparatus through the round window.[4]

Cefepime is a fourth-generation cephalosporin with an in vitro extended-spectrum of activity against Gram-negative and Gram-positive pathogens such as Moraxella catarrhalis, Streptococcus pyogenes, Haemophilus influenzae, Neiserria meningitidis, Enterobacteriaceae, Neiserria gonorrhoeae, meticillin-sensitive Staphylococcus aureus and Streptococcus pneumoniae.[5]

To date, no studies have been published to determine whether a topical solution of cefepim is safe when used on a non-intact tympanic membrane. We designed the current study to investigate the ototoxic effect of topical cefepime solution on the cochlea by measuring distortion product otoacoustic emission (DPOAE) amplitudes after the topical application of the cefepime solution in the middle ear of rats.

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Materials and Methods

This study used 12 experimental rats (female Wistar albino rats, weighing 230–250 g, approximately 13 weeks of age). The study was done at the Istanbul University Institute for Experimental Medicine Research, Istanbul, Turkey.

The experimental protocol was conducted in accordance with guidelines published in the *Guide for the Care and Use of Laboratory Animals* (DHEW publication NIH 85-23, revised 1996, Office of Science and Health Reports, DRR/NIH, Bethesda, MD) and approved by the Committee on Animal Research of Istanbul University, Istanbul, Turkey.

All rats had normal-appearing external auditory canals and tympanic membranes, with positive Preyer's reflexes. None of the rats had been used in another study and all had been grown under similar conditions. All experiments were done in the same room under low-noise conditions and constant room temperature and environment. Before the initial procedure and during otoacoustic emission measurements, the rats were anesthetized by ketamine (80 mg/kg Ketalar; Pfizer Ltd., Vienna, Austria) and xylazine (6 mg/kg Rompun; Bayer Ltd., Leverkusen, Germany) through intraperitoneal injection.

Ten minutes following the induction of anesthesia, the external auditory canal and tympanic membrane were examined under a microscope, and the cerumen was removed. An Echoport ILO292 and ILOv6 software (Otodynamics Ltd, Hatfield, Herts, UK) were used for DPOAE recordings. Prior to testing, acoustic calibration was done. Following appropriate configuration of stimuli waveform, DPOAEs were measured in diagnostic mode by giving non-linear clicks. The ratio of f2/f1 was kept at 1.22. Stimuli intensities were L1 and L2 for f1 and f2 frequencies, respectively, and L1–L2 was kept at 10 dB SPL (L1=65 dB SPL, L2=55 dB SPL). DPOAEs were created by presenting two stimuli to the external ear canal from two different speakers (for f1 and f2, respectively), and emissions were recorded by a microphone in the probe placed in the canal. Measurements were done for f2 frequencies (3–8 kHz) without removing the probe from the ear.

While the ears of the rats were positioned parallel to the ground, DPOAE was measured using the smallest newborn probe attached to the Echoport ILO292 machine; animals with negative measurements or those having ear pathology/abnormality were excluded.

Measurements were positive in 12 Wistar albino rats. A 1 ml 50 mg/ml cefepime solution was injected into the left middle ear space of 8 of the rats with a fine calibrated dental needle via the transtympanic route 2 times per day for 7 days (Group 1a), and a 1 cc % 0.9 NaCL solution was injected to the right ear of the same rats 2 times per day for 7 days (Group 1b). An 80 mg/ml gentamycine solution was injected into the left middle ear space of 4 of the 12 rats with a fine calibrated dental needle via the transtympanic route 2 times per day for 7 days (Group2a), and a 1cc %0.9 NaCL solution was injected into the right ear of the same rats 2 times per day for 7 days (Group 2b).

DPOAE measurements were then obtained for both experimental and control ears before day 0 (baseline) and days 3, 7, and 28 after the initiation of treatment. All measurements were recorded under anesthesia as per the protocol described previously.

Statistical Analysis

Data were presented as mean ± standard deviation (SD) using an SPSS package 17.0 (SPSS Inc., Chicago, Illinois, USA). Differences between independent groups were assessed by the Friedman-test for quantitative data (median, min-max) and the Wilcoxon signed-ranked test for qualitative variables and for variables without normal distribution. ANOVA was used for repeated measurements. A two-tailed p value < 0.05 was considered significant.

Results

All 12 rats completed the study without complications or ear infections. During 0–28 days, DPOAE mesurements within all frequencies did not significantly differ between Group 1b and Group 2b (Table 1). While baseline DPOEA measurements on day 0 were not significantly different between Groups 1a and 2a, DPOEA measurements on days 3, 7, and 28 were significantly lower in Group 2a than Group 1a (Table 2).

DPOAE measurements during 0–28 days did not significantly differ between Group 1a and Group 1b (Table 3–4). Among Groups 2a and 2b, DPOEA measurements on day 28 were significantly lower within all frequencies compared to day 0 (Table 1). Overall, DPOAE measurements decreased only with gentamycine ($p<0,05$).

Table 1. Statistical evaluation results according to the days in all groups

Group 1a (cefepime)							Group 1b [Saline (Cefepime)(SC)]						Group 2a (Gentamicin)						Grup 2b [Saline (Gentamicin)(SG)]					
	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz
Day 0	8.5(3-20)	10(6-20)	11.5(5-16)	10(7-14)	11(7-16)	10(5-15)	6(2-9)	7.5(2-10)	10(4-14)	10(3-15)	8(4-14)	5.5(5-15)	12.5(7-15)	12(6-15)	10(8-12)	9.5(8-11)	10(6-11)	7.5(5-13)	11.5(8-13)	10(9-15)	10(7-11)	9.5(8-11)	10(6-14)	8.5(5-13)
Day 3	7(3-12)	7(4-15)	9.5(6-15)	9(4-20)	9.5(4-15)	9.5(3-10)	5(3-15)	5.5(3-10)	7(4-20)	8(3-23)	9(3-20)	8(2-20)	0(0-0)	1.5(0-3)	0(0-3)	0(0-2)	0(0-2)	0(0-0)	5.5(4-10)	7(5-11)	9.5(7-10)	10.5(7-15)	8(5-10)	5(4-10)
Day 7	5.5(3-15)	6.5(4-15)	9(5-18)	9(5-16)	8(5-21)	7(0-20)	6(4-12)	7(4-13)	8(4-16)	8.5(5-10)	8(6-15)	5(5-15)	1(0-3)	0(0-2)	0(0-0)	0(0-0)	0(0-0)	0(0-0)	6.5(4-10)	6.5(5-11)	8(6-10)	13(8-20)	12(6-20)	10(5-15)
Day 28	8(3-15)	10(4-10)	10(5-15)	12.5(4-20)	10(5-23)	8(4-20)	7(3-14)	6(4-15)	9(6-10)	10(4-15)	10(4-20)	5.5(4-16)	1.5(0-5)	1.5(0-4)	0(0-3)	0(0-3)	0(0-0)	0(0-0)	6.5(4-8)	6.5(4-9)	9(6-10)	13.5(5-15)	15(10-20)	12.5(8-20)
p	0,944	0,309	0,269	0,950	0,966	0,687	0,492	0,914	0,638	0,976	0,843	0,975	0,009*	0,020*	0,019*	0,013*	0,012*	0,007*	0,096	0,326	0,572	0,468	0,353	0,319

Table 2. Comparison of the DPOAE results between Group 1a (Cefepime) and Group 2a (Gentamicin) in 0 and 28. days

	Group 1a (Cefepime)						Group 2a (Gentamicin)					
	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz
DAY 0	8.5(3-20)	10(6-20)	11.5(5-16)	10(7-14)	11(7-16)	10(5-15)	12.5(7-15)	12(6-15)	10(8-12)	9.5(8-11)	10(6-11)	7.5(5-13)
DAY 28	8(3-15)	10(4-10)	10(5-15)	12.5(4-20)	10(5-23)	8(4-20)	1.5(0-5)	1.5(0-4)	0(0-3)	0(0-3)	0(0-0)	0(0-0)
p	0,944	0,309	0,269	0,950	0,966	0,687	0,009*	0,020*	0,019*	0,013*	0,012*	0,007*

Table 3. Comparison of the DPOAE results in Group 1a (Cefepime) between 0 and 28. days

	Group 1a (Cefepime)					
	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz
DAY 0	8.5(3-20)	10(6-20)	11.5(5-16)	10(7-14)	11(7-16)	10(5-15)
DAY 28	8(3-15)	10(4-10)	10(5-15)	12.5(4-20)	10(5-23)	8(4-20)
p	0,944	0,309	0,269	0,950	0,966	0,687

Table 4. Comparison of the DPOAE results in Group 1b [Saline (Cefepime) (SC)] between 0 and 28. days

	Group 1b [Saline (Cefepime) (SC)]					
	3kHz	4kHz	5kHz	6kHz	7kHz	8kHz
DAY 0	6(2-9)	7.5(2-10)	10(4-14)	10(3-15)	8(4-14)	5.5(5-15)
DAY 28	7(3-14)	6(4-15)	9(6-10)	10(4-15)	10(4-20)	5.5(4-16)
p	0,492	0,914	0,638	0,976	0,843	0,975

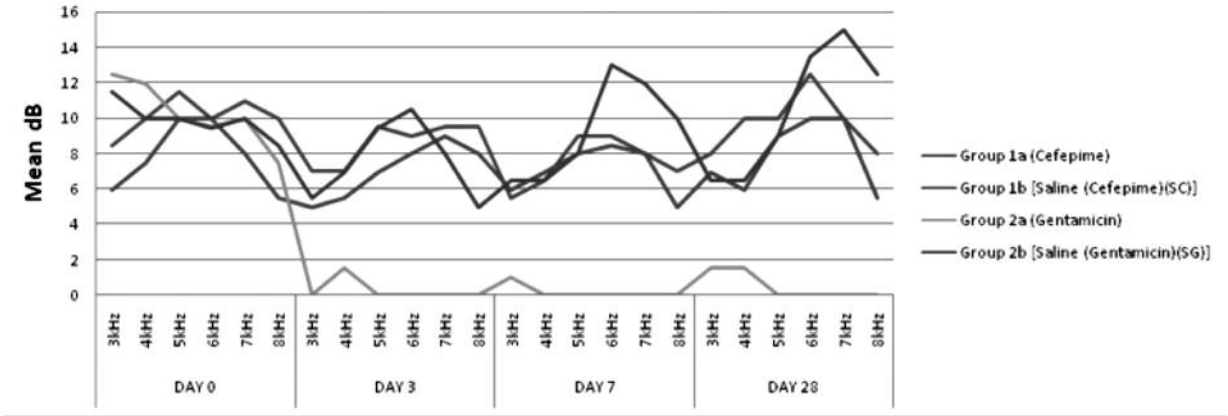

Figure 1. Results of mean DPOAE measurements according to the days in all study groups

Discussion

Many otolaryngologists use topical antibiotic solutions for chronic otitis media.[6] In light of increased resistance to various eardrops with greater clinical use,[13] a safe alternative is needed for patients with chronic otitis media. Many investigations have been published about ototoxicity based on experimental studies in animals. Several different types of animals have been used for this purpose.[3,24] There are no available topical cefepime solutions and no animal investigations on the ototoxicity of the drug. Application of cefepime solution to the middle ear of humans or animals has not yet been studied. We conducted this study as an experimental model in which the solution was applied to the transtympanic membranes of the rats.

In chronic otitis media, bacterial infection is often a cause of exacerbation during the clinical course. Aerobic pathogens such as Pseudomonas aeruginosa and Staphylococcus aureus were the most frequently found microorganisms in chronic otitis media. The most common isolates were Staphylococcus aureus, 42.9%; and Pseudomonas aeruginosa, 19.8%; Acinetobacter spp., 4.1%; Proteus mirabilis, 37%; Proteus vulgaris, 3.3%; and methicillin-resistant Staphylococcus, 1.5% of bacterial isolates.[12]

In the treatment of patients with chronic otitis media, use of topical medications has many potential advantages over systemic treatments. Oral and intravenous antibiotics have potential complications, are costly, and exhibit increasing resistance.[3,17] Topical antibiotics are applied directly to the site of inflammation or infection, and tissue concentrations result in higher drug concentrations. They are mostly free of systemic side effects, such as vomiting, rash, diarrhea, and anaphylaxis. However, they have minimal local side effects like local irritation.[3,6] In accordance with previous studies,[7,14] the present study found that an application of 0.9% sodium chloride to the middle ear did not significantly decrease DPOAE amplitudes. This means it has no effect on the outer cell function of the inner ear.

Topical medication may enter the middle ear through a perforated tympanic membrane and can reach the inner ear by crossing through the round window membrane,[3] effecting the cochlear and vestibular apparatus.[19,20] The safety of various components of otic drugs used in this way, particularly corticosteroids, aminoglycosides, and solvents, has been questioned in light of their ototoxic potential.[7,21]

For early detection of the ototoxic effects of drugs, high-frequency audiometry, transient evoked otoacoustic emission (TEOAE) and DPOAEs are highly sensitive.[24] DPOAE studies are used to investigate this ototoxicity. This is a noninvasive method for early diagnosis of cochlear impairment caused especially by some drug solutions, which are usually detected first in the outer hair cells, the primary source of OAE.[3,16]

Pseudomonas aeruginosa and anaerobes are most frequently isolated organisms and aminoglycosides such as tobramycin and gentamicin, neomycin sulphate, are commonly used in patients with otitis media.[11] Ciprofloxacin is one of the fluoroquinolones, and it is effective against P. aeruginosa.[12] Application of the ototopical ciprofloxacin solution has also increased. The literature has previously reported on ciprofloxacin resistant P. aeruginosa (CRPA) in patients with chronic otitis media. Due to the development of fluoroquinolone resistance during therapy and fluoroquinolone resistance of P. aeruginosa, selecting an optimal treatment for patients suffering with this pathogen is complicated. Ciprofloxacin-resistant P. aeruginosa (CRPA) is still sensitive to cefepime, ceftazidime, imipenem, piperacillin, and piperacillin-tazobactam.[13,14,15]

Despite their topical ototoxicity, aminoglycoside-containing eardrops are frequently used in clinical practice for patients with otitis externa, otitis media with or without perforations, and infected mastoid cavities.[21] Various investigations have looked at topical gentamicin-containing eardrops' ototoxicity when used for longer than 7 days in patients with a tympanic membrane defect. After the discharge has stopped, these eardrops should be discontinued in the presence of healthy middle ear mucosa.[10,19,20,21] In this study, there have been statistically significant reductions for hearing thresholds before or after application of gentamicin-containing eardrops. Overall, DPOAE measurements decreased only with gentamycine ($p<0,05$).

Moxifloxacin is a third-generation fluoroquinolone available as an ophthalmic solution. Daniel et al. applied topical moxifloxacin to a nonintact tympanic membrane of chinchillas and found that moxifloxacin causes a significant decrease in the DPOAE response at high frequencies.[18]

Cefepime is a semi-synthetic, fourth-generation cephalosporin with an in vitro extended-spectrum of activity against Gram-negative and Gram-positive pathogens including meticillin-sensitive Staphylococcus aureus and Streptococcus pneumoniae. It is approved for the treatment of moderate-to-severe infections, such as uncomplicated and complicated urinary tract infections, pneumonia, soft tissue and skin infections, febrile neutropenia, and intra-abdominal infections.[5]

Cefepime's superior activity is attributed to more rapid penetration into bacteria, and it works well against Gram-negative organisms, including Pseudomonas aeruginosa, similar to ceftazidime. It is more active than cefotaxime or ceftazidime against Enterobacteriaceae. Streptococcus pneumoniae; Streptococcus pyogenes, Haemophilus influenzae, Neiserria meningitidis, Neiserria gonorrhoeae, and Moraxella catarrhalis are highly susceptible to cefepime.[8,9,22,23]

Topical cefepime solution is ideal to use against the most common organisms in patients with otitis media. Its ototoxic potential has been ignored, especially when applied topically in the presence of tympanic membrane perforation. In this study, we investigated the effect of topical cefepime solution on the hearing of rats that were exposed to the solution twice a day for a week. DPOAE mesurements during 0–28 days were not significantly different between Group 1a and Group 1b.

Our investigation found no significant decrease of DPOAE amplitudes after the application of the cefepime solution topically to the middle ear of rats. The data derived from this present study suggest that cefepim solution can be safely used for the topical treatment of otorrhea in patients who suffer from chronic suppurative otitis media.

Conclusion

Topical treatment is often more effective than oral antibiotics in the eradication of chronic otitis media in patients. One should be cautious in the use of potentially ototoxic drugs in patients with open middle ears. The present study demonstrates that there have been statistically significant differences in hearing thresholds before or after application of gentamicin-containing eardrops. İn the presence of a perforation on the tympanic membrane, topical cefepime solution has no ototoxic effect after application of the drug into the middle ear cavity of rats. Therefore, further studies are needed to evaluate the effects of the solution on human hearing.

References

1. Mostafa BE, El Fiky LM, El Sharnouby MM. Complications of suppurative otitis media: Still a problem in the 21st century. ORL J Otorhinolaryngol Relat Spec. 2009;71:87-92.

2. Salomonsen RL, Hermansson A, Cayé-Thomasen, P. Ossicular bone modeling in acute otitis media. Otol Neurotol. 2010;31:1109-14.

3. Gültekin E, Yener M, Ozdemir I. The effect of topical Castellani solution on outer haircell function of rats. Laryngoscope. 2010;120:808-12.

4. Yang TH, Liu SH, Young YH. A novel inner ear monitoring system for evaluating ototoxicity of gentamicin eardrops in guinea pigs. Laryngoscope. 2010;120:1220-26.

5. Endimiani A, Perez F, Bonomo RA. Cefepime: A reappraisal in an era of increasing antimicrobial resistance. Expert Rev Anti Infect Ther. 2008;6:805-24.

6. Pappas S, Nikolopoulos TP, Korres S, et al. Topical antibiotic ear drops: Are they safe? Int J Clin Pract. 2006; 60:1115-9.

7. Haynes DS, Rutka J, Hawke M, et al. Ototoxicity of ototopical drops—an update. Otolaryngol Clin North Am. 2007;40:669-83.

8. Chapman TM, Perry CM. Cefepime: A review of its use in the management of hospitalized patients with pneumonia. Am J Respir Med. 2003;2:75-107.

9. Grassi GG, Grassi C. Cefepime: Overview of activity in vitro and in vivo. J Antimicrob Chemother. 1993;32:87-94.

10. Bath AP, Walsh RM, Bance ML, et al. Ototoxicity of topical gentamicin preparations. Laryngoscope. 1999;109:1088-93.

11. Force RW, Hart MC, Plummer SA, et al. Topical ciprofloxacin for otorrhea after tympanostomy tube placement. Arch Otolaryngol Head Neck Surg. 1995;121:880-84.

12. Ikeda K, Takasaka T. In vitro activity of ototopical drops against middle ear pathogens. Am J Otol. 1993;14:170-71.

13. Sheng WH, Chen YC, Wang JT, et al. Emerging fluoroquinolone-resistance for common clinically important gram-negative bacteria in Taiwan. Diagn Microbiol Infect Dis. 2002;43:141-47

14. Jang CH, Park SY. Emergence of ciprofloxacin-resistant pseudomonas in pediatric otitis media. Int J Pediatr Otorhinolaryngol. 2003;67:313-16.

15. Aslam MA, Ahmed Z, Azim R. Microbiology and drug sensitivity patterns of chronic suppurative otitis media. J Coll Physicians Surg Pak. 2004;14:459-61.

16. Pouyatos B, Campo P, Lataye R. Use of DPOAEs for assessing hearing loss caused by styrene in the rat. Hear Res. 2002;165:156-64.

17. Jang CH, Park H, Cho YB, et al. Evaluating the ototoxicity of topical piperacillin-tazobactam. Int J Pediatr Otorhinolaryngol. 2008;72:1815-21.

18. Daniel SJ, Duval M, Sahmkow S, et al. Ototoxicity of topical moxifloxacin in a chinchilla animal model. Laryngoscope. 2007;117:2201-5.

19. Rutka J. Topical aminoglycosides? No. The case against using these agents in chronic ear disease. Ear Nose Throat J 2003;82:9–12.

20. Tan CT, Lee SY, Yao CJ, et al. Effects of gentamicin and pH on [Ca2+] in apical and basal outer hair cells from guinea pigs. Hear Res.2001;154 : 81-87.

21. Marais J, Rutka JA. Ototoxicity and topical eardrops. Clin Otolaryngol Allied Sci. 1998;23:360-67.

22. Sanders CC. Cefepime: The next generation? Clin Infect Dis 1993;17: 369–79.

23. Yahav D, Paul M, Fraser A, et al. Efficacy and safety of cefepime: A systematic review and meta-analysis. Lancet Infect Dis. 2007:7:338-48.

24. Palomar García V, Abdulghani Martínez F, Bodet Agustí E, et al. Drug-induced otoxicity: Current status. Acta Otolaryngol. 2001;121:569-72.