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OTO-201: Nonclinical Assessment of a Sustained-Release Ciprofloxacin Hydrogel for the Treatment of Otitis Media

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Hypothesis: OTO-201 can provide sustained release to the middle ear and effectively treat otitis media, when compared with FDA-approved ciprofloxacin otic drop formulations.
Background: There is an unmet medical need for antibiotic therapy that can provide a full course of treatment from a single administration by an otolaryngologist at the time of tympanostomy tube placement, obviating the need for twice daily multiday treatment with short-acting otic drops.
Methods: Studies in guinea pigs and chinchillas were conducted. OTO-201 was administered as a single intratympanic injection and compared with the twice daily multi-day treatment with Ciprodex or Cetraxal otic drops.
Results: OTO-201 demonstrated sustained release of ciprofloxacin in the middle ear compartment for days to approximately 2 weeks depending on the dose. The substantial C_{max} values and steady drug exposure yielded by OTO-201 were in contrast to the pulsatile short lasting exposure seen with Ciprodex and Cetraxal. OTO-201 was also effective in a preclinical chinchilla model of *Streptococcus pneumoniae*–induced otitis media. The degree of cure was comparable to that afforded by Ciprodex and Cetraxal. There was no evidence of middle or inner ear pathology in guinea pigs treated with OTO-201, unlike Ciprodex and Cetraxal, which both demonstrated mild cochlear ototoxicity. No adverse effects of the poloxamer 407 vehicle were noted.
Conclusion: Intratympanic injection of OTO-201 constitutes an attractive treatment option to twice daily multiday dosing with ciprofloxacin ear drops for the treatment of otitis media, as evidenced by superior middle ear drug exposure, efficacy in an acute otitis media model, safety of administration, and convenience of a single dose regimen. **Key Words:** Ciprofloxacin—Hydrogel—Sustained release—Otitis media.
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Otitis media (OM) is the most common condition for which antibiotics are prescribed to children in the United States (1,2). OM is considered a disease continuum that includes both acute otitis media (AOM) and otitis media with effusion (OME). Treatment of AOM typically focuses on bacterial eradication via the use of oral antibiotics. The most frequently found bacterial pathogens in OM are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* (3). OME is defined by the presence of fluid in the middle ear without signs or symptoms or acute ear infection, and watchful waiting is initially recommended. However, it is believed that 30% to 40% of children will develop persistent OME, and a significant proportion of AOM patients will experience recurrent episodes (1,2). In these individuals, OM can result in irreversible hearing loss and delays in speech, language, and learning development. In extreme cases, infection can spread and lead to severe conditions including mastoiditis and meningitis. Children with persistent or recurrent OM are frequently referred for surgical placement of tympanostomy tubes (TTs) in an effort to clear the infection/effusion and avert these complications.

It is estimated that, in the United States alone, approximately one million surgeries for the insertion of TT are performed each year in pediatric patients (4). However, a significant proportion of children treated with TT develop postsurgical otorrhea. For this reason, otolaryngologists

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Supplemental digital content is available in the text.

routinely administer topical antibiotic ear drops off-label during TT placement surgery and prescribe administration of these drops postoperatively for 7 to 10 days typically. Commonly available antibiotics for otic use are ciprofloxacin, ofloxacin, corticosporin, and neomycin. Ciprofloxacin is the most commonly used topical antibiotic in patients with OM requiring TT, largely because of its high degree of effectiveness and reported lack of ototoxicity (5–7).

Although relatively effective, current topical antibiotic regimens face several challenges. First, the necessity of a twice daily multiday regimen presents a significant compliance challenge for parents and caregivers of young children. Second, the difficulty in administering the otic drops through the tympanostomy tube can result in limited and variable exposure. Third, these aqueous solutions are rapidly eliminated from the middle ear compartment, thus providing short lasting relief. Finally, failure to adhere to a twice daily multi-day regimen may promote selection and proliferation of resistant pathogens.

There is thus an unmet medical need for a sustained-release antibiotic therapy that can provide a full course of treatment from a single administration by an otolaryngologist at the time of TT placement, thereby obviating the need for twice daily multiday treatment by the caregiver and providing superior drug exposure compared to short-acting otic drops. Previous studies have demonstrated that the formulation of a different therapeutic agent in a poloxamer hydrogel (OTO-104, dexamethasone suspension in poloxamer 407) can provide sustained release to the otic compartment for weeks to months after a single intratympanic injection (8–10). Furthermore, its administration was found to be safe and well tolerated in preclinical toxicology studies (8) as well as demonstrating clinically meaningful benefit in a Phase 1b clinical trial in Ménière's disease patients (11).

Taking advantage of the sustained release properties afforded by the poloxamer hydrogel, OTO-201, a suspension of ciprofloxacin in poloxamer 407, was thus developed. The pharmacokinetic profile and toxicologic potential of OTO-201 in the middle and inner ear compartments was characterized after a single administration in guinea pigs, a species traditionally used for otic evaluation. In addition, OTO-201 was also evaluated in a chinchilla model of *Streptococcus pneumoniae*–induced otitis media, the chinchilla being a common and accepted model to evaluate otitis media. Finally, the overall profile of OTO-201 was benchmarked to the FDA-approved ciprofloxacin containing otic drops Ciprodex Otic (a suspension of 0.3% ciprofloxacin and 0.1% dexamethasone) (12,13) and Cetraxal Otic (a solution of 0.2% ciprofloxacin) (14,15).

MATERIALS AND METHODS

This section can be found in the Supplemental Digital Content, (http://links.lww.com/MAO/A197).

RESULTS

Pharmacokinetics

The study, conducted in guinea pigs, compared the administration of a single intratympanic injection anterior to the round window membrane (IT-ANT) of OTO-201, a suspension of ciprofloxacin in poloxamer 407 hydrogel, with that of a twice daily for 7-day treatment course of Ciprodex or Cetraxal. Guinea pigs were chosen for the pharmacokinetic studies as that species constitutes a standard and well-accepted model for evaluation of middle and inner ear pharmacokinetics, especially in light of the ease of access to the cochlear structure and large middle ear compartment (16). A summary of the treatment regimens is presented in Table 1. A single intratympanic injection of OTO-201 provided high C_{max} and steady dose, with progressive decline over time (Fig. 1A). Depending on the OTO-201 dose given (0.06%–12%), ciprofloxacin levels peaked between 45.4 ± 22.1 and 99.9 ± 5.6 μg/ml, a less than 2-fold difference between doses despite a 200-fold dosing regimen span. Disappearance from the middle ear compartment was strongly dependent on the OTO-201 dose. The administration of Ciprodex resulted in a contrasting profile (Fig. 1B). After a single application of Ciprodex drops, ciprofloxacin peak levels reached 22.6 ± 5.9 μg/ml and declined sharply within hours of administration. During the twice daily for 7-day regimen, ciprofloxacin trough levels varied from 2.6 ± 0.4 μg/ml at Day 1, immediately before the third dose, to 8.6 ± 0.4 μg/ml at treatment completion. By compiling the data from peak and trough levels, the predicted middle ear free ciprofloxacin profile after Ciprodex treatment was derived. The pulsatile nature of Ciprodex administration was quite evident,

TABLE 1. *Summary of OTO-201, Ciprodex, and Cetraxal treatment regimens*

			OTO-201					
	Ciprodex	Cetraxal	0.06%	0.2%	0.6%	2%	6%	12%
Ciprofloxacin (mg/ml)	3.0	2.0	0.6	2.0	6.0	20.0		
Guinea pigs								
Regimen	bid, 7 days 10 μl AU	bid, 7 days 15 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU
Cumulative dose (mg)	0.42	0.42	0.03	0.10	0.30	1.00	3.00	6.00
Chinchillas								
Regimen	bid, 3 days 10 μl AU	bid, 3 days 15 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU	Single IT 50 μl AU
Cumulative dose (mg)	0.18	0.18	0.03	0.10	0.30	1.00	3.00	6.00

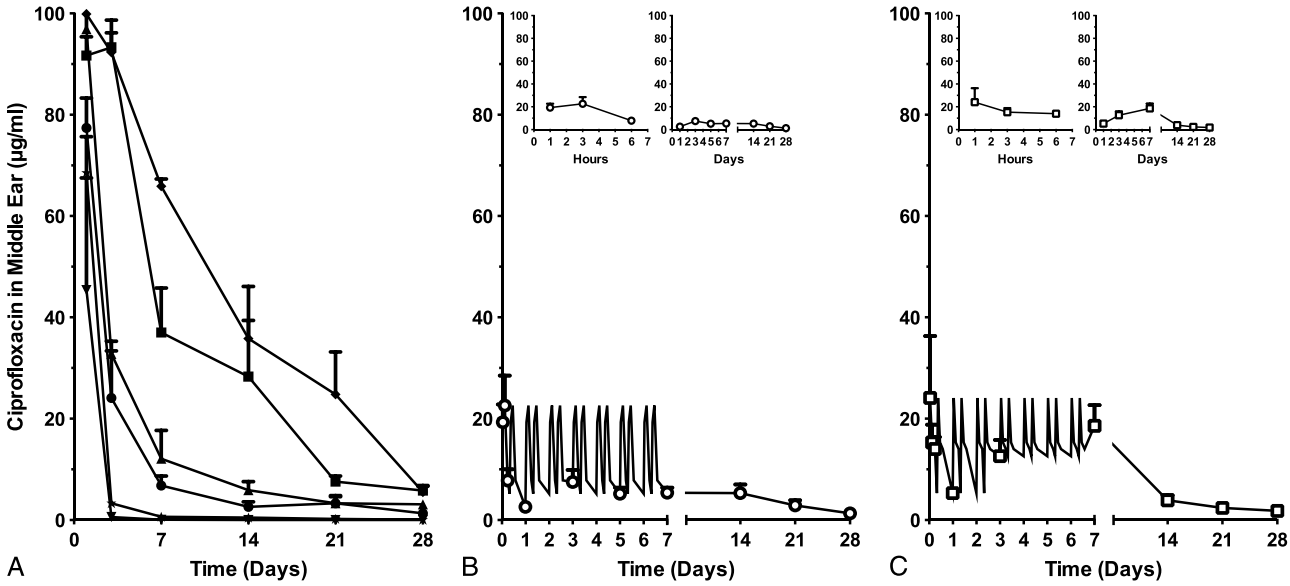

FIG. 1. Middle ear free ciprofloxacin levels after administration of OTO-201, Ciprodex or Cetraxal. *A*, Female guinea pigs received a single IT-ANT injection of various doses of OTO-201: 0.06% (*closed inverted triangles*), 0.2% (*stars*), 0.6% (*closed circles*), 2% (*closed triangles*), 6% (*closed squares*), and 12% OTO-201 (*closed diamonds*). A twice-daily for 7 days course of Ciprodex (*B*) or Cetraxal (*C*) administered through a tympanostomy tube was given. Free drug levels of ciprofloxacin, obtained by lavaging the middle ear, were determined at the indicated times. (*B* and *C*) Predicted profile of ciprofloxacin by combining peak and trough levels. Left inset: peak levels; right inset: trough levels. Data are presented as mean ± SEM (n = 4 ears per group per time point).

with free ciprofloxacin levels in the middle ear cycling rapidly between each otic drop application by almost 10-fold, evidence of drug accumulation overtime and slow drug elimination after the completion of treatment. A similar pulsatile profile with slow drug elimination was noted with the administration of Cetraxal (Fig. 1C). The administration of OTO-201 yielded significantly higher C_{max} values, ranging from 45.4 to 99.9 µg/ml, than either Ciprodex or Cetraxal (22.6 and 24.1 µg/ml, respectively) (Table 2), translating into a higher degree of exposure (as measured by AUC). Measures of predicted antimicrobial clinical efficacy (based upon a MIC of 2 µg/ml, which defines the breakpoint for bacteria of intermediate susceptibility to ciprofloxacin (17,18)) revealed a comparable T>MIC for both Ciprodex and Cetraxal (601 and 611 h, respectively) with OTO-201 bracketing these values depending upon the dose (63–721 h). For ciprofloxacin, the C_{max}/MIC and $AUC_{0\text{-}24}$/MIC ratios need to display values greater than 10 and greater than 100, respectively, for optimal clinical efficacy (19,20). Both Ciprodex and Cetraxal exhibit good predicted clinical efficacy values, typically 1- to 2-fold above the proposed limits. OTO-201 values are excellent with C_{max}/MIC ratios 2- to 5-fold and $AUC_{0\text{-}24}$/MIC 5-12 fold above the recommended values, respectively.

Ciprofloxacin levels in the middle ear epithelium indicated that a single IT-ANT injection of OTO-201 resulted in tissue-bound drug levels reaching 37.0 ± 10.2 to 586 ± 309 µg/ml (Fig. 2A), values 1- to 6-fold higher than the free ciprofloxacin concentrations, associated with limited elimination. After

TABLE 2. *Comparison of middle ear pharmacokinetic parameters of ciprofloxacin after administration of OTO-201, Ciprodex, or Cetraxal*

	C_{max} µg/mL	AUC µg.h/mL	$AUC_{0\text{-}24}$ µg.h/mL	MRT h	T>MIC h	C_{max}/MIC ratio	$AUC_{0\text{-}24/MIC}$ h
Ciprodex	22.6	3078	303	188 (11)	601 (22)	11	152
Cetraxal	24.1	5411	405	217 (16)	611 (29)	12	203
OTO-201							
0.06%	45.4	2288	1088	34	63	23	544
0.2%	68.5	3728	1645	37	90	35	823
0.6%	77.4	7663	1858	143	322	39	929
2%	96.9	11025	2326	200	413	48	1163
6%	91.7	23921	2200	246	715	46	1100
12%	99.9	32026	2398	293	721	50	1199

A MIC of 2 µg/ml was defined based upon the breakpoint for bacteria of intermediate susceptibility to ciprofloxacin.
AUC indicates area under the curve; MRT, mean residence time; MIC, minimum inhibitory concentration.

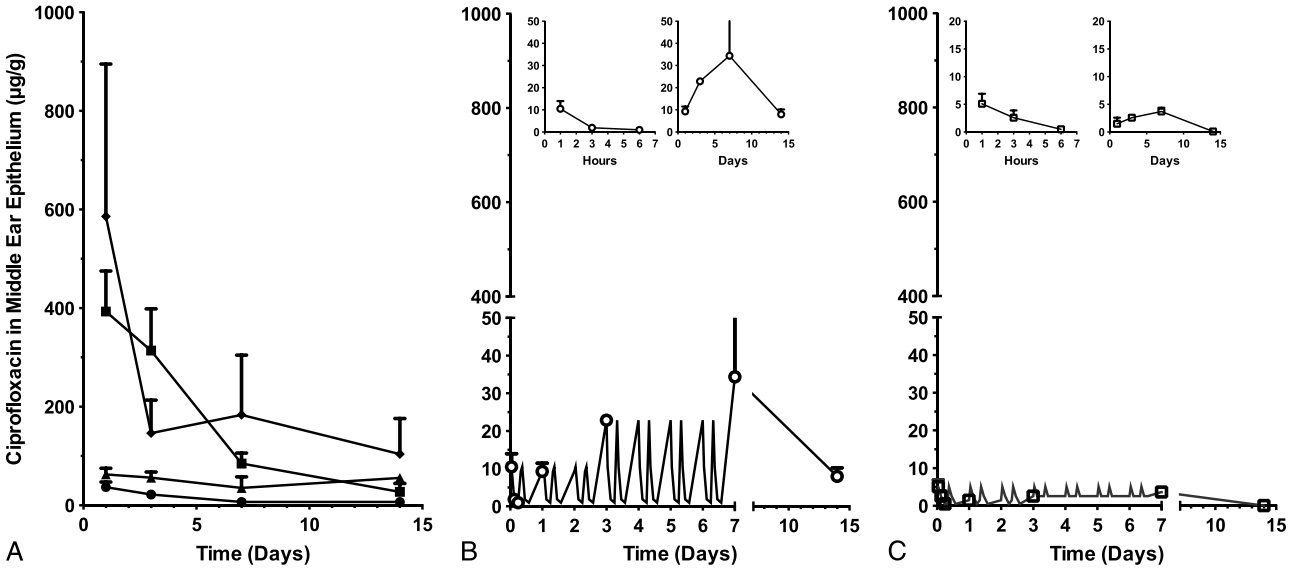

FIG. 2. Middle ear epithelium (tissue-bound) ciprofloxacin levels following administration of OTO-201, Ciprodex or Cetraxal. *A*, Female guinea pigs received a single IT-ANT injection of various doses of OTO-201: 0.6% (*closed circles*), 2% (*closed triangles*), 6% (*closed squares*), or 12% (*closed diamonds*). A twice-daily for 7 days course of Ciprodex (*B*) or Cetraxal (*C*) administered through a tympanostomy tube was given. Tissue-bound levels of ciprofloxacin, obtained by harvesting the middle ear epithelium, were determined at the indicated times. (*B* and *C*) Predicted profile of ciprofloxacin by combining peak and trough levels. Left inset: peak levels; right inset: trough levels. Data are presented as mean ± SEM (n = 4 ears per group per time point).

administration of Ciprodex, tissue-bound ciprofloxacin also exhibited a pulsatile profile, with marked drug accumulation peaking at 34.4 ± 16.3 µg/ml, and sharp elimination after treatment completion (Fig. 2B). A similar pulsatile profile was observed with administration of Cetraxal (Fig. 2C).

Drug penetration to the inner ear compartment was determined. After administration of OTO-201 (Fig. 3A), perilymph peak concentrations of ciprofloxacin were 5- to 25-fold lower than the levels observed in the middle ear. Elimination from this compartment was relatively rapid.

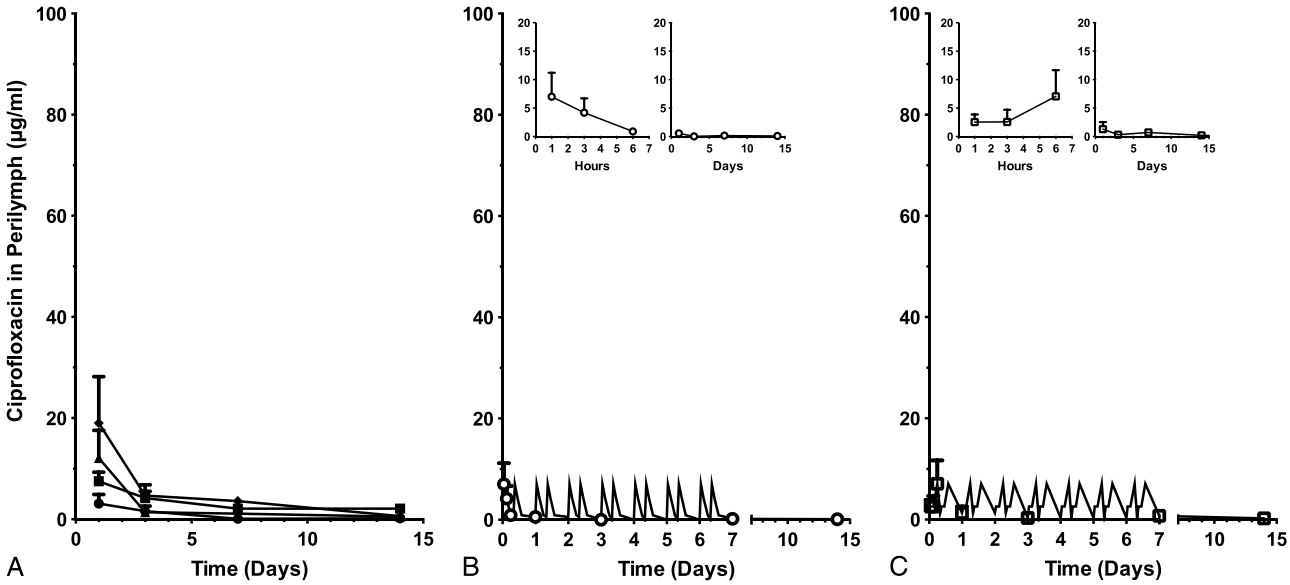

FIG. 3. Inner ear ciprofloxacin levels following administration of OTO-201, Ciprodex or Cetraxal. *A*, Guinea pigs received a single IT-ANT injection of various doses of OTO-201: 0.6% (*closed circles*), 2% (*closed triangles*), 6% (*closed squares*), or 12% (*closed diamonds*). A twice-daily for 7 days course of Ciprodex (*B*) or Cetraxal (*C*) administered through a tympanostomy tube was given. Perilymph levels of ciprofloxacin were determined at the indicated times. (*B* and *C*) Predicted profile of ciprofloxacin by combining peak and trough levels. Left inset: peak levels; right inset: trough levels. Data are presented as mean ± SEM (n = 4 ears per group per time point).

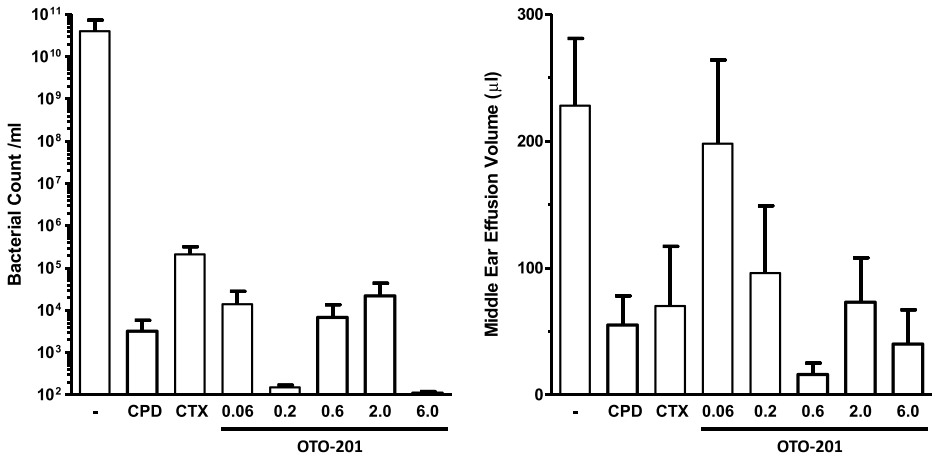

FIG. 4. Middle ear bacterial load and effusion volume in chinchillas with otitis media treated with OTO-201, Ciprodex, or Cetraxal. Otitis media was induced by middle ear inoculation of *S. pneumoniae*. Immediately before drug administration (at Day 3 postinoculation), the middle ear was drained of effusion and a tympanostomy tube placed. Chinchillas received either a single IT-ANT injection of various doses of OTO-201, or a twice daily for 3 days treatment course of Ciprodex (CPD) or Cetraxal (CTX). The bacterial titer and effusion volume in the middle ear were determined. Data are presented as mean ± SEM (n = 6–13 ears).

In contrast, after the administration of Ciprodex (Fig. 3B) and Cetraxal (Fig. 3C), perilymph levels of ciprofloxacin in the perilymph cycled rapidly and extensively between each dose, with values 2- to 3-fold lower than the ones observed in the middle ear.

Pharmacology

The ability of OTO-201, Ciprodex and Cetraxal to alleviate *S. pneumoniae*–induced OM in chinchillas was investigated (Fig. 4 and Table 1). The chinchilla represents a preferred species for evaluation of otitis media because (1) a similar sensitivity to pathogens known to induce otitis media in humans and (2) the disease progression mimics that observed in the human population (21). Both Ciprodex and Cetraxal treatment courses reduced both the bacterial load in the middle ear (by more than 5–6 log orders of magnitude relative to untreated subjects) and the extent of the middle ear effusion. All OTO-201 doses (0.06%–6%) were effective in reducing the middle ear bacterial load by 6 to 8 log orders of magnitude, as well as reducing the middle ear effusion to the levels seen with either Ciprodex and Cetraxal (with the exception of the lowest OTO-201

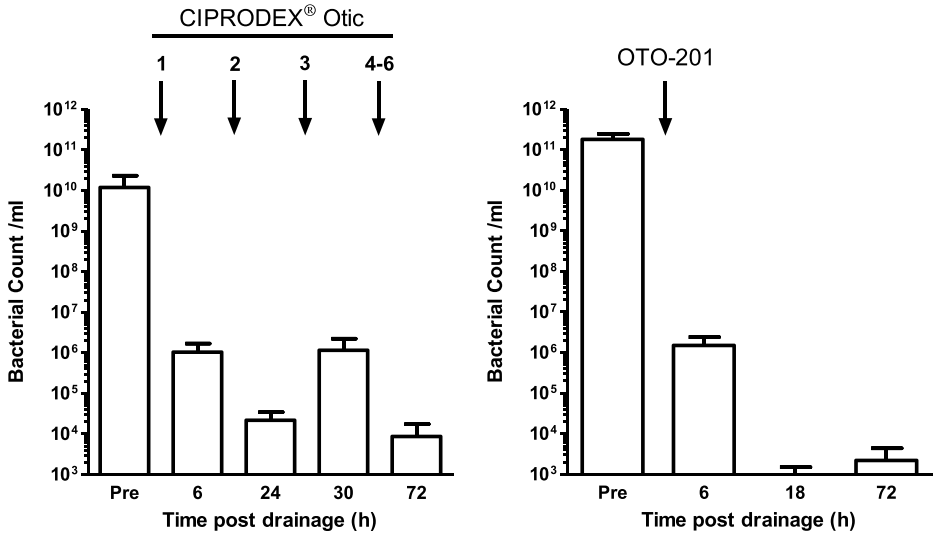

FIG. 5. Time to clinical cure in chinchillas with otitis media treated with OTO-201 or Ciprodex. Otitis media was induced by middle ear inoculation of *S. pneumoniae*. Immediately before drug administration (at Day 3 postinoculation), the middle ear was drained of effusion, and a tympanostomy tube was placed. Chinchillas received either a single IT-ANT injection of various doses of OTO-201, or a twice daily for 3 days treatment course of Ciprodex. The bacterial titer was determined at the indicated times. Data are presented as mean ± SEM (n = 6–10 ears). *Arrows* refer to the time of administration of OTO-201 or Ciprodex.

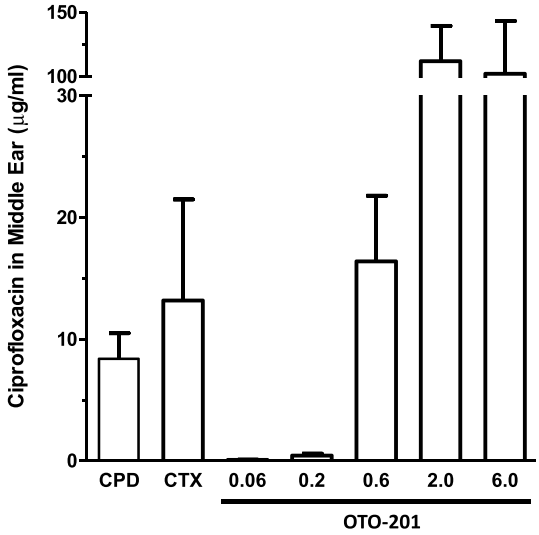

FIG. 6. Middle ear ciprofloxacin levels in chinchillas with otitis media treated with OTO-201, Ciprodex or Cetraxal. The levels of free ciprofloxacin in the middle ear of treated chinchillas with OM was determined. Three days posttreatment initiation, middle ear samples were collected, and the concentration of ciprofloxacin was determined. Data are presented as mean ± SEM (n = 6–13 ears). CPD: Ciprodex, CTX: Cetraxal.

dose). No differences between OTO-201 and Ciprodex or Cetraxal were evident, probably because of the high susceptibility to ciprofloxacin of the *S. pneumoniae* used in this study with a determined MIC of approximately 0.2 μg/ml (data not shown). Examination of the time to clinical cure (Fig. 5) revealed that following the first application of Ciprodex, the bacterial titer dropped by approximately 4 logs within the first 6 hours of treatment, but evidence of intermittent bacterial growth was noted in between Ciprodex applications. After the completion of the Ciprodex treatment course (72 h), clinical cure was evidenced by a 6-log reduction in bacterial load compared with pretreatment levels. In contrast, the single IT-ANT administration of OTO-201 yielded rapid clinical cure, within 18 hours of treatment.

Levels of free ciprofloxacin in the middle ear were determined at study termination (Fig. 6). After the twice daily for 3-day treatment course of Ciprodex or Cetraxal, ciprofloxacin levels were significant with values of 8.4 ± 2.1 μg/ml and 13.2 ± 8.3 μg/ml, respectively. After the single IT-ANT administration of OTO-201, ciprofloxacin concentrations ranged from 0.1 ± 0.0 μg/ml to 112.0 ± 27.4 μg/ml, depending on the dose. The concentrations observed were 2- to 13-fold higher than the ones reached with administration of Ciprodex or Cetraxal.

Toxicology

A 1-month acute ototoxicity study was conducted to compare the toxicologic potential of OTO-201 to that of Ciprodex and Cetraxal. At termination, functional and anatomic assessments of the middle and inner ear compartments were conducted.

Hearing evaluation was evaluated using auditory brainstem responses (ABR) (Fig. 7). Baseline ABR thresholds were similar between guinea pigs assigned to the different treatment groups at each tested frequency (data not shown). ABR thresholds in the saline control group increased marginally from baseline upon study completion (≤10 dB SPL), and IT-ANT administration of the known

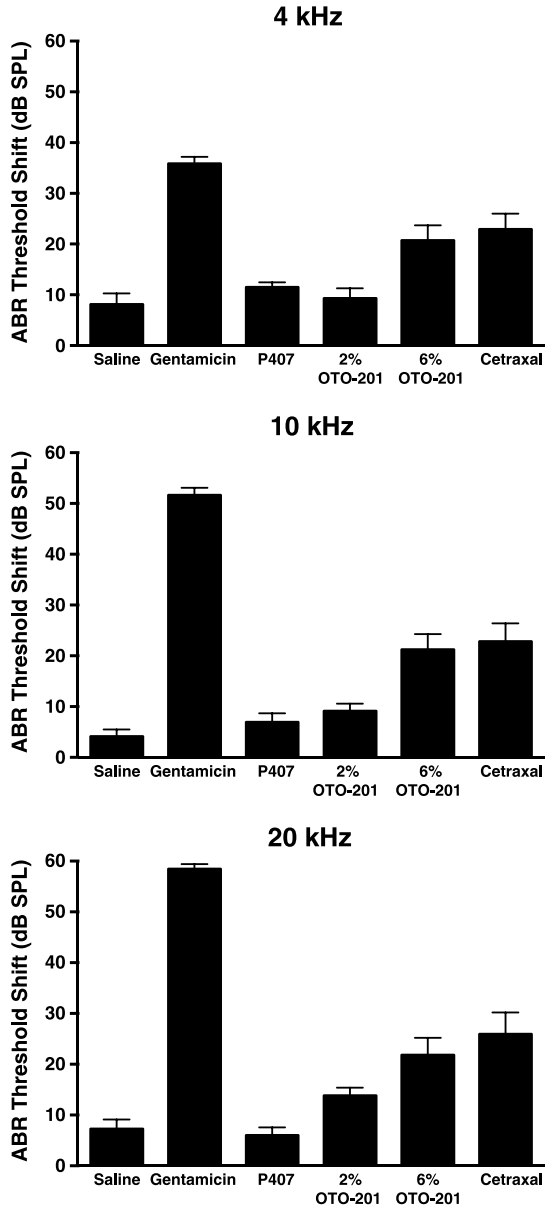

FIG. 7. Auditory function following administration of OTO-201 or Cetraxal. The auditory function of male and female guinea pigs was monitored using ABRs at baseline and termination (28 d). Animals received a single IT-ANT injection of poloxamer 407 vehicle, 2% or 6% OTO-201, or a twice daily for 7 days treatment course of Cetraxal. Hearing threshold shifts were reported at low (4 kHz), medium (10 kHz), and high (20 kHz) frequencies. Data are presented as mean ± SEM (n = 5 per sex per group).

ototoxicant gentamicin resulted in severe ABR threshold shifts (≥40–60 dB SPL). Administration of the vehicle P407 resulted in ABR threshold shifts that were comparable to that seen with saline. In the OTO-201 treatment groups, a dose-dependent mild ABR threshold shift was evident at termination (10–20 dB SPL), with the 2% OTO-201 dose exhibiting ABR shifts comparable to saline. Cetraxal treatment course (twice daily for 7 d) resulted in mild-to-moderate hearing loss at termination (20–30 dB SPL). Overall, OTO-201 treatment was associated with minimal-to-mild ABR threshold shift across the frequencies tested and compared favorably to Cetraxal treatment, which was associated with mild to moderate hearing loss.

Histologic analysis of middle ear plastic sections was conducted after termination. Histology of the middle ear revealed the presence of minimal subacute inflammation in the vast majority of animals treated with saline, poloxamer 407 vehicle, OTO-201 (2% and 6%), Ciprodex, and Cetraxal (Fig. 8). The known ototoxicant gentamicin caused moderate chronic inflammation in all treated ears and associated moderate-to-severe fibroplasia, mild hemorrhage, and bone remodeling. The findings observed in all treatment groups, with the exception of gentamicin, were considered secondary to the intratympanic injection procedure and tympanostomy tube placement.

The integrity of the sensorineural epithelium was examined in a quantitative assessment of inner and outer hair cells from surface preparations of the cochlear spiral generating cytocochleograms (Fig. 9). IT-ANT administration of saline or the poloxamer 407 vehicle was not associated with hair cell loss in any of the treated animals, whereas administration of the known ototoxicant gentamicin resulted in profound to complete hair cell loss. OTO 201 administration did not produce otopathology. In contrast, both Ciprodex and Cetraxal produced mild-to-moderate outer hair cell loss, primarily confined to the apical half of the cochlea. Possibly, the reasons for the apical loss relate to placement of the materials away from the round window, diffusion across the thin cochlear bone, and increased susceptibility to ototoxicity of the apical versus basal sensory epithelium (Mikulec et al., 2009).

Tympanostomy tube patency was examined. After IT-ANT administration of poloxamer 407 vehicle to the middle ear immediately before ventilation tube placement,

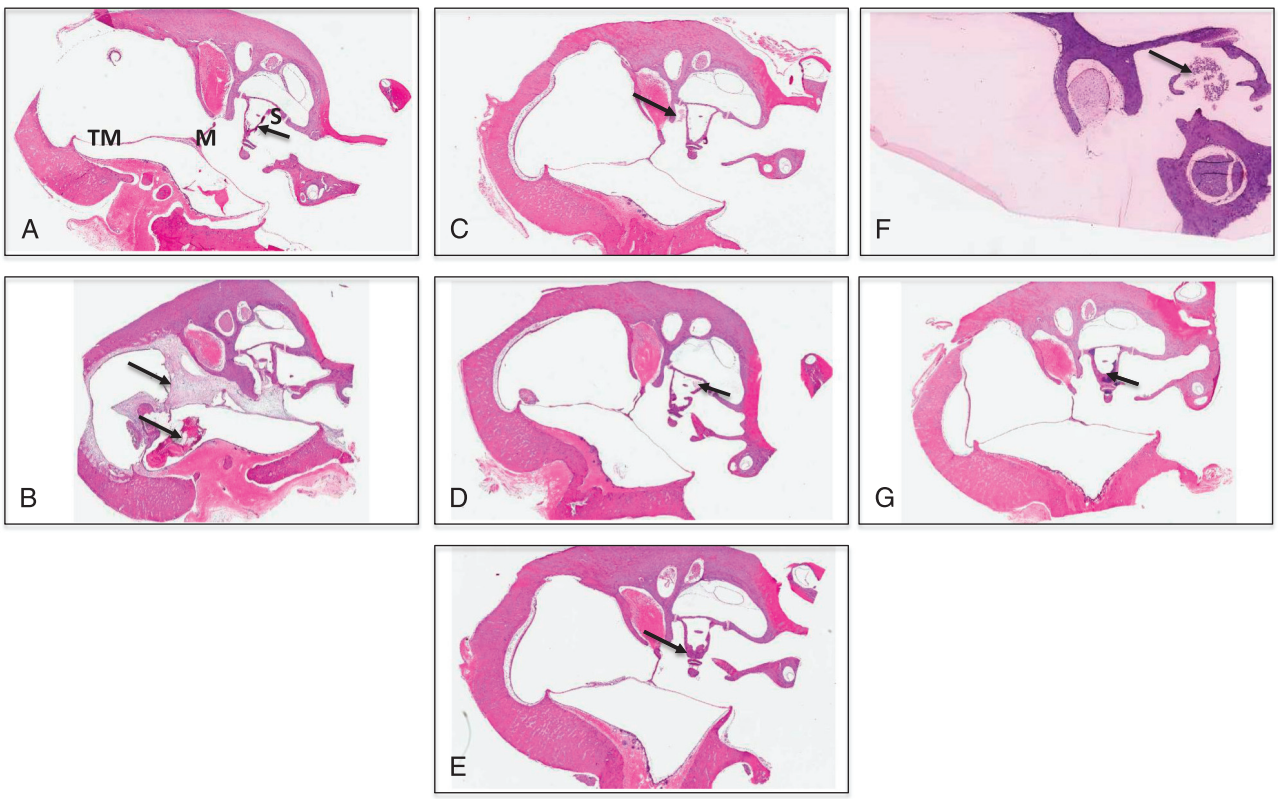

FIG. 8. Middle ear histology after administration of OTO-201, Ciprodex, or Cetraxal. Male and female guinea pigs (n = 5 per sex, per group) received a single IT-ANT injection of poloxamer 407 vehicle, 2% or 6% OTO-201, or a twice daily for 7 days treatment course of Ciprodex or Cetraxal. Representative tissue sections of the middle ear (at termination, 28 d) from guinea pigs treated with saline (*A*), gentamicin (*B*), P407 vehicle (*C*), 2% OTO-201 (*D*), 6% OTO-201 (*E*), Ciprodex (*F*), and Cetraxal (*G*) are presented. Legend: M: malleus, S: stapes, TM: tympanic membrane. *Arrows* make references of the following: (*A*) foamy macrophages; (*B*) *upper arrow*: fibroplasia and inflammation, *lower arrow*: mixed cellular and proteinaceous debris; (*C*) foamy macrophages; (*D*) foamy macrophages; (*E*) granulomatous inflammation; (*F*) reactive cells; (*G*) basophilic foamy macrophages.

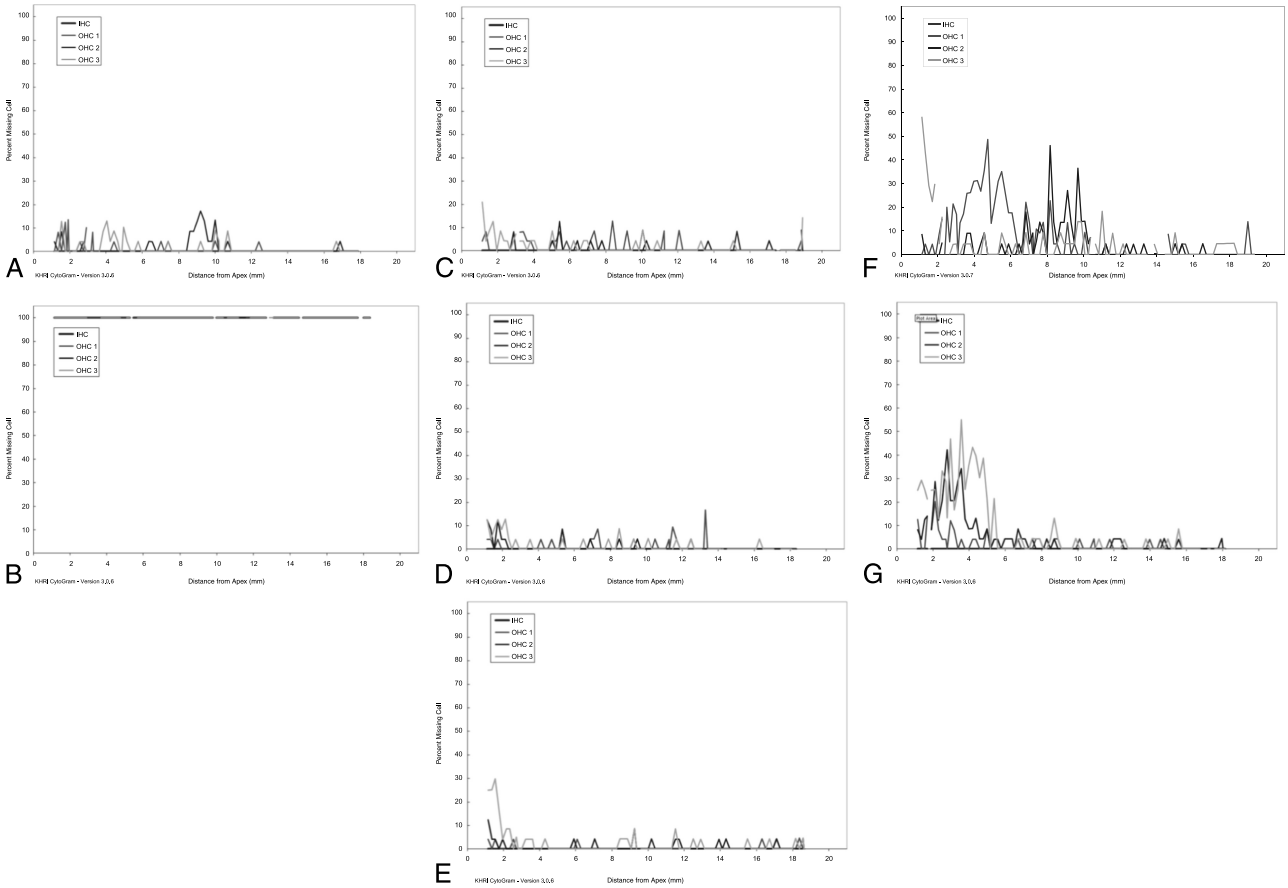

FIG. 9. Inner ear cytocochleogram after administration of OTO-201, Ciprodex, or Cetraxal. Male and female guinea pigs (n = 5 per sex, per group) received a single IT-ANT injection of poloxamer 407 vehicle, 2% or 6% OTO-201, or a twice daily for 7 days treatment course of Ciprodex or Cetraxal. Representative cytocochleograms (at termination, 28 d) mapping the presence or absence of inner hair cells (*black line*) and the 3 rows of outer hairs (Row 1, light gray line; Row 2, gray line; Row 3, dark gray line) by position along the cochlear spiral, with apex on the left and base on the right, from the cochleae of treated guinea pigs having received saline (*A*), gentamicin (*B*), P407 vehicle (*C*), 2% OTO-201 (*D*), 6% OTO-201 (*E*), Ciprodex (*F*), and Cetraxal (*G*).

there was no evidence of tube clogging at Days 1 and 3 posttreatment (Fig. 10). Thus, the presence of poloxamer 407 hydrogel in the middle ear does not affect the patency of ventilation tubes inlayed through the tympanic membrane.

DISCUSSION

There were significant inherent differences observed in the middle ear pharmacokinetic profile of Ciprodex or Cetraxal versus OTO-201. For instance, the twice daily

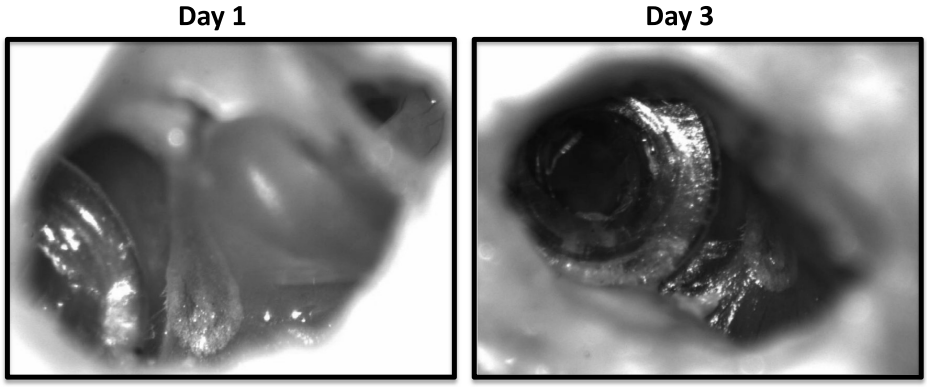

FIG. 10. Tympanostomy tube patency. Pictures of the tympanic membrane region depicting the patency at Days 1 and 3 after IT-ANT administration in guinea pigs of poloxamer 407 vehicle (dyed with Evans Blue), immediately before tympanostomy tube placement.

multiday regimen of otic drops resulted in pulsatile exposure with a large fluctuation in the magnitude of drug levels achieved between each application with ciprofloxacin free and tissue-bound levels typically cycling between 1 and 20 μg/ml. These findings provide the basis for the biological evidence in support of the effectiveness of ciprofloxacin otic drops in treating otitis media. However, it should be considered that because of the relatively limited drug levels reached in the middle ear compartment, Ciprodex and Cetraxal otic drops can only be effective against ciprofloxacin susceptible organisms, as intermediate and resistant bacterial strains typically exhibit a MIC significantly above 2 μg/ml (17,18). During the course of treatment, ciprofloxacin levels dipped within the MIC threshold, potentially limiting the efficacy of this class of concentration-dependent antibiotic and possibly potentiating the emergence of drug-resistant pathogens. In contrast, OTO-201 yielded high C_{max} values (50–100 μg/ml and up to 500 μg/ml for free and tissue-bound ciprofloxacin, respectively), steady drug levels declining gradually overtime. Consequently, OTO-201 provides exposure to high and stable ciprofloxacin concentrations in excess of 100 μg/ml, suggesting that traditionally resistant bacterial strains would be effectively targeted by OTO-201. The persistence of steady levels (i.e., without fluctuating drug concentrations) maximizes the clinical efficacy of the antibiotic and severely limits the potential for antibiotic resistance. Clinical efficacy of ciprofloxacin is best described by C_{max}/MIC and $AUC_{0\text{-}24}$/MIC pharmacokinetic param eters, with values expected to reach above 10 and 100, respectively (19,20). When considering ciprofloxacin susceptible bacterial strains (i.e., MIC <2 μg/ml) a treatment course of Ciprodex or Cetraxal is anticipated to provide adequate clinical cure (Table 2). However, clinical efficacy is lost when facing even mildly resistant strains: at a MIC of 4 μg/ml, C_{max}/MIC and $AUC_{0\text{-}24}$/MIC ratios dropped to 5 to 6 and 75 to 100 for both Ciprodex and Cetraxal, respectively. In contrast, the high predicted clinical efficacy values associated with OTO-201 suggest that ciprofloxacin formulated in poloxamer should provide adequate clinical cure against even resistant microorganisms.

In recent years, evidence has emerged that persistent otitis media is likely due to the presence of bacterial biofilms (22,23). Several factors have been proposed to contribute to the antibiotic resistance displayed by biofilms, including nutrient-depleted zones in the biofilm resulting in a stationary dormancy phase, limited and delayed diffusion of antimicrobials into the biofilm, and inactivation of antibiotics by components of the extracellular matrix (24). In vitro studies demonstrated biofilms have a much higher tolerance to ciprofloxacin than planktonic cultures, with MIC and minimal bactericidal concentration (MBC) values increased by 10 to 1,000-fold (25,26). Consistent with this, and as would be expected from the limited middle ear drug levels observed in the studies herein, ototopical antibiotic drops, including Ciprodex and Cetraxal, have inadequate efficacy against biofilms present on tympanostomy tubes (27). OTO-201, in contrast, would be expected to provide significant antimicrobial protection against biofilms, because ciprofloxacin levels reached in the middle ear are 100- to 2,500-fold above the MIC of susceptible bacteria strains (i.e., MIC of 0.2 μg/ml), and still greater than 100-fold for intermediate and resistant microorganisms and because of the known biofilm disruption properties of the poloxamer hydrogel matrix that forms OTO-201 (28). Thus, the combination of biofilm destruction and high drug levels at the site of action provides OTO-201 with a unique ability to manage middle ear infections.

In a preclinical model of otitis media, OTO-201 demonstrated clinical cure comparable to that of Ciprodex or Cetraxal Otic. Thus, a single intratympanic administration of OTO-201 provides an equivalent treatment effect against otitis media as a twice daily multiday regimen of Ciprodex or Cetraxal otic drops. Furthermore, the time to clinical cure is achieved significantly more rapidly with OTO-201 than with the topical drops (18 versus 72 h, respectively). Ciprodex time requirement to achieve optimal clinical cure was associated with intermittent bacterial growth in between drug application, consistent with the short duration of action of the otic drops.

Toxicologic evaluation following a 1-month recovery period was conducted in guinea pigs, a preferred model known to its sensitivity to ototoxicants and otic insults. Single IT-ANT administration of the P407 vehicle was associated with minimal functional changes in hearing. Histologically, the effects of P407 were limited to minimal subacute inflammation of the middle ear, and no evidence of cochlear pathology. Overall, the effects of P407 administration were indistinguishable from those of the saline control. The safety profile of P407 is in sharp contrast to the deleterious effects observed for several other polymers commonly administered intratympanically. For example, intratympanic administration to rats of a gelatin matrix (Gelfoam) results in severe acute inflammatory response leading to prominent tissue fibrosis (29). A hyaluronic acid polymer (Sepragel), although highly biocompatible, causes mild inflammatory reaction and fibrosis in the middle ear (29,30). In contrast, poloxamers (including P407) are largely nontoxic to animals, with LD_{50} values ranging from 5 to 35 g/kg (31), and their intratympanic administration has been shown repeatedly to be safe and well tolerated in animals (8,9) and humans (11).

Toxicologic evaluation after a 1-month recovery period of the comparators Ciprodex and Cetraxal demonstrated that a twice daily for 7 days treatment course resulted in mild-to-moderate hearing loss at termination (20–30 dB SPL), loss that could not solely be attributed to the tympanostomy tube placement procedure. The histologic analysis of the otic compartment revealed the presence of minimal subacute inflammation of the middle ear, comparable to that seen in the saline group, and considered secondary to the tympanostomy tube placement procedure. Both Ciprodex and Cetraxal administration adversely resulted in mild to moderate hair cell loss, primarily confined to the apical half of the cochlea. The observed cochlear pathology is indicative of mild ototoxicity after otic administration of Ciprodex and Cetraxal. These findings are in contrast to previously conducted studies in both chin-

chillas and guinea pigs showing a lack of ototoxicity of topical ciprofloxacin (32–34). Possibly, the observed differences could result from the relative sensitivities of guinea pig strains used in the various studies. A supportive finding is that the negative control saline yielded, in this study, a 10 dB SPL ABR threshold shift unlike in the cited studies. In addition, the presence of additives, such as benzalkonium in Ciprodex or povidone and glycerin in Cetraxal, combined with an acidic pH, could account for the observed mild ototoxicity (35,36).

OTO-201 IT-ANT administration resulted in dose-dependent minimal-to-mild changes in auditory function at termination (1 mo). The 2% OTO-201 dose yielded minimal hearing threshold shifts that were no different than the ones observed in the saline group and considered secondary to the intratympanic injection and tympanostomy tube placement procedures. 6% OTO-201 treatment exhibited mild changes in hearing function, comparable to that seen with the comparator Cetraxal. OTO-201 at doses up to 6% did not influence cochlear pathology or induce hair cell loss in any of the guinea pigs, in contrast to the mild ototoxicity observed with Ciprodex and Cetraxal.

A major aim of the present study was to compare the safety profile of OTO-201 with that of Ciprodex and Cetraxal. Under the current experimental conditions, the middle ear safety profile of OTO-201 at doses up to 6% is comparable to that of the approved dosage forms of both Ciprodex and Cetraxal. However, both Ciprodex and Cetraxal are associated with mild cochlear toxicity that is not observed with OTO-201. It is unlikely that the cochlear toxicity observed with the ciprofloxacin otic drops is due to a direct effect of ciprofloxacin, but rather is a consequence of the excipients present in the formulations, acidic pH, and repeated administration (35,36). These findings could also be species specific as Ciprodex has not demonstrated ototoxicity in patients with otitis media (12,13), clinical studies conducted to support the approval of Cetraxal did not report any ototoxicity in patients with acute otitis externa (14), and administration of ciprofloxacin otic solution is safe and well tolerated in populations with otitis media (5,37,38).

Maintaining tympanostomy tube patency in patients is important. Systematic reviews of clinical studies conducted in children with recurrent OM (39) and OME (40) suggested benefits in the placement and maintenance of tympanostomy tubes. These advantages, lasting for approximately 6 months, provided improved hearing and decreased incidence of acute OM episodes. Of interest in this work was whether the injection of a polymeric formulation could affect the patency of tympanostomy tubes. No such clogging was observed in the studies conducted, and preliminary studies conducted internally have demonstrated that OTO-201 can be injected into the middle ear directly through the tympanostomy tube without any incidence of clogging.

In conclusion, a single intratympanic injection of OTO-201 provided sustained release of ciprofloxacin to the middle ear that is superior and more convenient to that achieved with twice daily multiday dosing with Ciprodex or Cetraxal otic drops. The substantial C_{max} and steady drug exposure offer advantages to the pulsatile concentration profile resulting from the short-acting drops. OTO-201, Ciprodex, and Cetraxal all provided clinical cure in a preclinical model of otitis media. The formal ototoxicity evaluation demonstrated the safety of administering OTO-201, whereas Ciprodex and Cetraxal seem to be associated with mild cochlear toxicity. At the time of this writing, OTO-201 is being evaluated in a prospective, randomized, double-blind, placebo- and sham-controlled, multicenter, Phase 1b safety study in pediatric patients with bilateral middle ear effusion requiring tympanostomy tube placement (www.clinicaltrials.gov).

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