CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Applicant's letter date:	02/25/2015
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Product:	OTO-201
Indication:	Intra-operative treatment for middle ear effusion in pediatric subjects requiring tympanostomy tube placement
Applicant:	Otonomy, Inc.
Review Division:	Division of Anti-infective Products
Reviewer:	James S. Wild, Ph.D.
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1 Executive Summary

1.1 Introduction

OTO-201 is a suspension of ciprofloxacin hydrate in buffered solution (pH 7-8) containing (b) (4)% poloxamer 407, (P407) a glycol polymer. P407 exists as a liquid at room temperature and gels quickly upon transitioning to body temperature. The drug is packaged in a sterile vial for injection. OTO-201 (with a 6% ciprofloxacin concentration) is intended for treatment of middle ear effusion at the time of tympanostomy tube (TT) placement. OTO-201 will be administered intra-operatively as a single, intratympanic dose of 6 mg ciprofloxacin ((b) (4) 6% OTO-201 per ear) bilaterally at the time of TT placement.

Currently a ciprofloxacin-containing marketed product, Ciprodex® (0.3% ciprofloxacin plus 0.1% dexamethasone), is used to treat acute otitis media in patients with tympanostomy tube placement. Another similar product Cetraxal® (0.2% ciprofloxacin otic solution) is approved for the treatment of otitis externa. Ciprodex® and Cetraxal® are administered in ear drops BID for 7 days. Compliance may be a problem for Ciprodex® treatment of otitis media, and delivery of the product to the middle ear is potentially inconsistent. It is anticipated that a single-intratympanic injection of 6% OTO-201 will provide more consistent ciprofloxacin coverage in the middle ear than a treatment course of Ciprodex® over 7 days.

1.2 Brief Discussion of Nonclinical Findings

Otic and Systemic Toxicity Findings

- Ototoxicity that occurred with OTO-201 was limited to moderate hearing loss (changes in ABR responses measured at 3 frequencies) for guinea pigs receiving a single intratympanic injection of 6% OTO-201 in one out of three studies. However, the OTO-201 hearing loss in the single study was not associated with microscopic evidence of cochlear damage. Hearing loss and cochlear damage were associated with 7-day administrations of Ciprodex® and Cetraxal®. Also in the studies where it was measured, 6% OTO-201 produced a low incidence of ossicle immobility (4/20 ears in one study and 1/20 ears in another) which was similar to the effects of Cetraxal® in the same studies (ossicle immobility in 2/20 ears in the first study and 3/20 ears in the second study).
- Other middle ear findings resulting from single-intratympanic administrations of OTO-201 were fluid granulomatous inflammation, fibroplasia, foamy macrophages, and foreign material which occurred in a dose-dependent manner with greater severity and incidence for 6% OTO-201 compared to saline. These findings were considered to be consistent with a foreign body reaction with the foreign substance possibly being ciprofloxacin and/or P407 particulates. In a single intratympanic-dose study in rats with (b) (4)% P407 (Study No.: OTO-104-RSP-086), similar findings largely resolved over time.

- Consistent with very low plasma ciprofloxacin exposure, toxicologically relevant changes in systemic toxicity endpoints, including clinical signs, hematological and clinical chemistry parameters and organ weight measurements did not consistently occur in the single intratympanic-dose studies. The highest plasma concentrations of 20-33 ng/ml occurred 24 hours after intratympanic dosing with 6% OTO-201. In comparison, C_{max} plasma concentrations of ciprofloxacin associated with clinical oral administration of 250 to 1000 mg of ciprofloxacin are reportedly much higher, in the 1-5 μg/ml range.

Impurities/Degradants of Concern

- [redacted] produced as a result of an early [redacted] technique for the vehicle for OTO-201, [redacted]% P407, were associated with hearing loss (increased baselines for ABR values at three frequencies) and cochlear toxicity (inner ear sensory hair loss). As a result, the [redacted] technique for the [redacted]% P407 vehicle was changed to a [redacted] technique which substantially reduced the production of [redacted] Because [redacted] were shown to be associated with cochlear toxicity, their levels should be monitored in batches of OTO-201 drug product, and the acceptance criteria for each specific [redacted] and the total [redacted] will be determined by the highest [redacted] levels that were not associated with ototoxicity in nonclinical intratympanic-injection studies.

Pharmacokinetic Findings

- Multiple otic pharmacokinetic patterns were elucidated in several intratympanic-dose studies. The middle ear C_{max} levels of ciprofloxacin did not vary greatly with increasing concentrations of ciprofloxacin (0.6, 2.0, 6.0 and 12.0%) in OTO-201 preparations. However AUC and mean residence time increased with dose although in a less than dose-proportional manner. Middle ear C_{max} levels following a single-intratympanic injection of 6% OTO-201 (C_{max} of approximately 100 μg/ml) were approximately 4-fold higher than middle ear C_{max} levels achieved with 7 days of dosing with Ciprodex® or Cetraxal® (C_{max} values for both of approximately 25 μg/ml).
- At the site of bacterial infection, middle ear epithelium, ciprofloxacin C_{max} values following a single-intratympanic dose of 6% OTO-201 were approximately 4-fold higher than middle ear lumen values and followed a dose-responsive pattern. In contrast middle ear epithelium concentrations following a single dose of Cetraxal® were approximately 5-fold less than middle ear lumen concentrations for Cetraxal® and approximately 100-fold less than the maximal epithelial concentrations that occurred after 6% OTO-201 administration.
- Ciprofloxacin concentrations in the inner ear, a potentially important site of toxicity, after a single dose of 6% OTO-201, followed a similar pattern compared to the middle ear in terms of retention time, but C_{max} values were much less (up to 12-fold less) than the middle ear values. Inner ear concentrations of ciprofloxacin produced by a single intratympanic administration of Cetraxal® was

similar to that produced by a single administration of 6% OTO-201 with both values equal to approximately 7-8 μg/ml.

- The single intratympanic-administration studies in guinea pigs indicated that OTO-201 remained in the middle ear longer than single doses of Cetraxal® and Ciprodex Otic®. Administration of 6.0% OTO-201 resulted in moderate concentrations (approximately 8 μg/ml) of ciprofloxacin remaining in the middle ear 21 days after intratympanic injection. This concentration is somewhat higher than the approximately 3 μg/ml middle ear concentrations of ciprofloxacin that resulted 21 days after the administration of Ciprodex® or Cetraxal® over a 7-day course of treatment in guinea pigs. The middle ear concentration-time curve for ciprofloxacin following a single administration of OTO-201 compared to that of multiple administrations of Ciprodex Otic® or Cetraxal® indicates that a single administration OTO-201 provides similar ciprofloxacin coverage in the middle ear compared to multiple administrations of the other products.

Genetic Toxicity

- Labeling information for several approved ciprofloxacin products including Cipro®, Ciprodex® and Cetraxal® indicates that ciprofloxacin has been tested in multiple *in vitro* and *in vivo* genotoxicity assays. The weight of evidence suggests ciprofloxacin has a limited potential to produce genotoxicity in humans in association with its approved administration.
- The vehicle for OTO-201, (b) (4)% P407, is a component in several approved products. However, the genotoxicity potential of P407 has not been reported. P407 rapidly forms a gel at (b) (4) and this characteristic restricts easy evaluation of P407 in traditional genotoxicity assays. For the purposes of this NDA, the restricted systemic exposure, single-dose application, and prior use in previously approved products supports the safety of poloxamer 407 for genotoxicity.

Reproductive Toxicity

- Because of the limited systemic exposure associated with clinical administration of OTIPRIO, this product is expected to be of minimal risk for maternal and fetal toxicity when administered to pregnant women**.**

Safety Evaluation

In nonclinical studies in guinea pigs, a single intratympanic dose of 6% OTO-201 provided similar if not better middle-ear exposure to ciprofloxacin compared to two other ciprofloxacin-containing otic products, Ciprodex® and Cetraxal®. Also the otic toxicity associated with administration of 6% OTO-201 in guinea pigs was consistently no worse and generally better than the toxicity profiles for Ciprodex® and Cetraxal® as indicated by otic toxicity endpoints including hearing loss (ABR elevations) and cochlear hair-cell loss. Based on these results, and the low potential for systemic toxicity, genotoxicity, and reproductive and developmental toxicity, OTIPRIO is considered approvable from a Pharmacology/Toxicology perspective.

1.3 Recommendations

1.3.1 Approvability

The product is approvable from a pharmacology/toxicology perspective

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Sponsor's Proposed Labeling Language for Section 8

8 Use in Specific Populations

8.1 Pregnancy

(b) (4)

Animal reproduction studies have not been conducted with OTIPRIO. No adequate and well-controlled studies have been performed in pregnant women. (b) (4)

(b) (4)

Proposed New Labeling Language for Section 8

8 Use in Specific Populations

8.1 Pregnancy

Risk Summary

Animal reproduction studies have not been conducted with OTIPRIO. No adequate and well-controlled studies have been performed in pregnant women. Because of the negligible systemic exposure associated with clinical administration of OTIPRIO, this product is expected to be of minimal risk for maternal and fetal toxicity when administered to pregnant women.

(b) (4)

Ciprofloxacin is excreted in human milk with systemic administration. However, because of the negligible systemic exposure after otic application, nursing infants of mothers receiving OTIPRIO should not be affected.

Sponsor's Proposed Labeling for Section 13

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Eight *in vitro* mutagenicity tests have been conducted with ciprofloxacin, and the test results are listed below:

Salmonella/Microsome Test (Negative)

Escherichia coli DNA Repair Assay (Negative)

Mouse Lymphoma Cell Forward Mutation Assay (Positive)

Chinese Hamster V79 Cell HGPRT Test (Negative)

Syrian Hamster Embryo Cell Transformation Assay (Negative)

Saccharomyces cerevisiae Point Mutation Assay (Negative)

Saccharomyces cerevisiae Mitotic Crossover and Gene Conversion Assay (Negative)

Rat Hepatocyte DNA Repair Assay (Positive)

Thus, 2 of the 8 in vitro tests were positive, but results of the following 3 in vivo test systems gave negative results:

Rat Hepatocyte DNA Repair Assay

Micronucleus Test (Mice)

Dominant Lethal Test (Mice)

Long-term carcinogenicity studies in mice and rats have been completed for ciprofloxacin. After daily oral doses of 750 mg/kg (mice) and 250 mg/kg (rats) were administered for up to 2 years, there was no evidence that ciprofloxacin had any carcinogenic or tumorigenic effects in these species.

(b) (4)

(b) (4)

Fertility studies performed in rats at oral doses of ciprofloxacin up to 100 mg/kg/day revealed no evidence of impairment. This would be (b) (4) times the maximum recommended clinical dose of ototopical ciprofloxacin based upon body surface area, assuming total absorption of ciprofloxacin from the ear of a patient treated with OTIPRIO.

Proposed New Labeling for Section 13

13 Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

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Micronucleus Test (Mice)

Dominant Lethal Test (Mice)

Long-term carcinogenicity studies in mice and rats have been completed for ciprofloxacin. After daily oral doses of 750 mg/kg in mice and 250 mg/kg in rats (for mice and rats respectively, approximately 300 and 200 times the maximum recommended clinical dose of ototopical ciprofloxacin based upon body surface area, assuming total absorption of ciprofloxacin from the ear of a patient treated with

OTIPRIO) were administered for up to 2 years, there was no evidence that ciprofloxacin had any carcinogenic or tumorigenic effects in these species.

Fertility studies performed in rats at oral doses of ciprofloxacin up to 100 mg/kg/day revealed no evidence of impairment. This is approximately 80 times the maximum recommended clinical dose of ototopical ciprofloxacin based upon body surface area, assuming total absorption of ciprofloxacin from the ear of a patient treated with OTIPRIO.

13.2 Animal Toxicology and/or Pharmacology

Guinea pigs dosed in the middle ear with OTIPRIO exhibited no drug-related structural or functional changes of the cochlear hair cells.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 85721-33-1

Generic Name: Ciprofloxacin

Code Name: OTO-201

Chemical Name(s)

a) 3-Quinolinecarboxylic acid, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-.

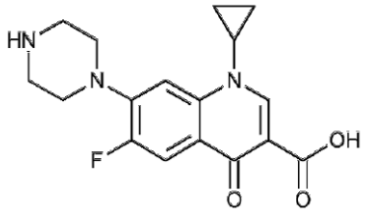
b) 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid

c) 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

d) 3-Quinolinecarboxylic acid, 1,4-dihydro-1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-.

Molecular Formula/Molecular Weight: $C_{17}H_{18}FN_3O_3$/331.34

Structure or Biochemical Description



Pharmacologic Class

Quinolone Antimicrobial

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND (b) (4)
IND 110244
NDA 19537

2.3 Drug Formulation

The excipients included in the OTO-201 drug product are shown in Table 1 and the proposed specifications for the OTO-201 drug product are shown in Table 2.

Table 1: Formulation of the OTO-201 Drug Product. (Sponsor's Table)

Ingredient	Quality Standard	Function	% w/w	Composition (mg/mL)
Ciprofloxacin	USP	Active ingredient	(b) (4)	60
Poloxamer 407	NF			(b) (4)
Sodium Chloride	USP			
Tromethamine	USP			
Hydrochloric Acid	NF			
Water for Injection (WFI)	USP			
Total			100.0	(b) (4)

(b) (4)

Table 2: Proposed Specification for OTO-201 Drug Product (Sponsor's Table)

Test	Test Method	Acceptance Criteria
Appearance	M9384 (visual)	White (b) (4) viscous flowing liquid suspension essentially free of visible foreign particulate matter
Identification by HPLC Retention Time	M9388	Conforms
Identification by UV (Photodiode array detector)		Conforms
Assay by HPLC		90.0% to 110.0% of Label Claim
Chromatographic Impurities		
(b) (4)		NMT (b) (4) %
Other individual impurities		NMT %
Total Impurities		NMT %
Uniformity of Dosage Units (release only)	M9388 (USP <905>)	Conforms
Particle Size Distribution	M9381	
D_{10}		NMT (b) (4)
D_{50}		NMT
D_{90}		NMT
pH	M9385 (USP <791>)	7.0-8.0
Temperature of Gelation	M9387	(b) (4)
Osmolality (5 x diluted sample)	M9386 (USP <785>)	50-70 mOsm/kg
Poloxamer 407 Content (% w/w)	M9383	(b) (4)
Dissolution	M11959	Q = (b) (4) at 60 min
(b) (4)		
Bacterial Endotoxins	MTM-200033 (USP <85>)	NMT (b) (4)
Sterility	MTM-200609 (USP <71>)	Sterile

2.4 Comments on Novel Excipients

All of the excipients other than poloxamer 407 (P407) and tromethamine have been used in previously approved otic products at higher concentrations than those included in OTO-201. P407 and tromethamine have not been used in any marketed otic products. P407 has been used in oral, topical, ophthalmic and periodontal products at lower concentrations. The periodontal products contained (b) (4) % P407. For the

purposes of this NDA application, the use of [(b)(4)]% P407 [(b)(4)] for OTO-201 is qualified by its safe use in the guinea pig and rat intratympanic dose studies and in the clinical trials supporting this NDA.

Tromethamine has been used in many different kinds of approved products including IV, IM, intrathecal, oral, and topical products. In approved IV and ophthalmic products tromethamine is included in higher percent concentrations than in the [(b)(4)]% concentration included in the current product. This prior use is considered adequate to qualify the use of [(b)(4)]% tromethamine in OTO-201.

2.5 Comments on Impurities/Degradants of Concern

[(b)(4)] concentrations in the drug product are considered of potential concern due to an apparent association with cochlear toxicity in nonclinical studies. In two guinea pig studies associated with IND [(b)(4)] (OTO-104-RSP-024 and OTO-104-RSP-025) the [(b)(4)] form of the [(b)(4)]% P407 vehicle with relatively high total [(b)(4)] concentrations (approximately [(b)(4)]) produced cochlear toxicity following intratympanic injections into the round window niche. In contrast, in other studies in guinea pigs and rats using [(b)(4)] P407 vehicle injected through the tympanic membrane into a site anterior to the round window niche as opposed to directly at the round window niche, inner ear toxicity did not occur. These conflicting results appear to implicate the tympanic injection site as a major determinate for the occurrence of [(b)(4)] related cochlear toxicity. Given the potential for cochlear toxicity associated with drug-product [(b)(4)] content, an acceptable course of action is to qualify the acceptance criteria for total [(b)(4)] in the drug product at concentrations shown to be safe in nonclinical and/or clinical studies.

Based on the results of a guinea pig study (Study No.: OTO-201-RSP-008) where a single-intratympanic injection of [(b)(4)] treated P407 did not produce cochlear toxicity, [(b)(4)] concentrations are qualified up to the total [(b)(4)] NOAEL/individual [(b)(4)] NOAELs in this study. Unfortunately [(b)(4)] content was not measured in the batch of [(b)(4)]% P407 that was used in Study No. OTO-201-RSP-008 (Batch No.: FG-10-0016), and thus total [(b)(4)] NOAEL/individual [(b)(4)] NOAELs cannot be determined. However, another [(b)(4)] batch of [(b)(4)]% P407 (Batch No.: 045-71) was reported to contain [(b)(4)] ppm of total [(b)(4)] with individual [(b)(4)] concentrations of [(b)(4)]. The human equivalent concentrations for the total and individual [(b)(4)] are modified based on the comparative dose volumes and perilymph (inner ear) volumes for guinea pigs and humans with the results shown in Table 3.

In another study conducted in rats (Study No.: OTO-104-RSP-086), a batch of P407 with elevated [(b)(4)] levels resulting from a forced degradation process [(b)(4)] for 90 days) was injected in a single intratympanic dose into the round window niche. The forced degraded batch of [(b)(4)]% P407 that was used in this study contained [(b)(4)] ppm of total [(b)(4)] with individual [(b)(4)] concentrations of [(b)(4)]. In this study as in Study No.: OTO-201-RSP-008 in guinea pigs, injections of [(b)(4)]% P407 including the

forced degradation batch of P407 did not increase the incidence or severity of cochlear toxicity compared to the saline control. Thus the (b) (4) concentrations in the forced degradation batch of P407 can be considered to be the NOAEL values for (b) (4) in this study and are useful for qualifying the acceptance criteria for (b) (4) in the drug product. The human equivalent concentrations for total and individual (b) (4) in the forced degradation batch of P407 based on comparative dose volumes and perilymph volumes for rats and humans are shown in Table 3.

Table 3: Qualified Limits for Total and Individual (b) (4) Levels Based on Nonclinical NOAEL Values and Dose and Perilymph Volume Conversions.

Study Type/ Study No.	(b) (4) Type	P407 (b) (4) Levels (ppm)	Concentration Normalized to Perilymph Volume[b]	Qualified Limit (ppm)[c]
Single IT Dose Guinea Pig Study/ OTO-201-RSP-008	TA	(b) (4)		
	FA			
	AA			
	PA			
Single IT Dose Rat Study/ OTO-201-RSP-086	TA			
	FA			
	AA			
	PA			

[a] TA = total (b) (4) .

Human equivalent exposure is calculated by dividing the animal injection volumes ((b) (4) for guinea pig and (b) (4) for rat) by the inner ear volume (perilymph) for each animal ((b) (4) for guinea pigs; (b) (4) for rats) and multiplying the correction factor by the (b) (4) concentrations. This calculation assumes that the total dose of (b) (4) will enter the perilymph.

[c] The qualified limit is calculated by multiplying the concentration normalized to perilymph in animals to the quotient of the human perilymph volume (b) (4) divided by the human injection volume per ear ((b) (4) This calculation assumes that the total dose of (b) (4) will enter the perilymph.

The Sponsor expressed a preference for using the (b) (4) levels in the forced degradation batch of P407 tested in the rat study to qualify the acceptance criteria for (b) (4) concentrations in the drug product. In addition, the Sponsor advocates applying more than 5-fold safety margins to the qualified limit values in the rat study to arrive at much lower acceptance criteria than what are qualified by the (b) (4) NOAEL values in the rat nonclinical study. These lower acceptance criteria (total (b) (4)) are well supported and qualified by nonclinical safety data, employ safety margins, and are acceptable from a pharmacology/toxicology perspective. Notably stability studies with the drug product indicate that (b) (4) concentrations after one year of storage at (b) (4) C are much lower than the acceptance

criteria as are the (b) (4) ppm total (b) (4) concentrations projected to be in the drug product after (b) (4) months of refrigerated storage.

2.6 Proposed Clinical Population and Dosing Regimen

Clinical Population: Pediatric patients with otitis media undergoing tympanostomy tube placement.

Dosing Regimen: A single intratympanic administration of 0.1 ml of 6% ciprofloxacin suspension into each affected ear to be administered through the tympanostomy tube after suctioning of middle ear effusion.

2.7 Regulatory Background

OTO-201 has been reviewed in association with IND 110244 in the following submissions:
James S. Wild, Nonclinical Review, 12/15/2011
James S. Wild, Nonclinical Review, 12/20/2011
James S. Wild, Nonclinical Review, 12/5/2012

Also, the vehicle for OTO-201, (b) (4) % poloxamer 407 (P407) has been used in another product (b) (4) submitted in IND (b) (4) and studies submitted for this IND were reviewed to inform decisions for the current NDA application.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacokinetics

1. Middle Ear Residence Time of Poloxamer 407 Following Intratympanic Administration in Guinea Pigs. Study No.: OTO-100-RSP-038-01.
2. Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. Study NO.: OTO-201-RSP-002-01.
3. Volume Range Pharmacokinetics of 2.0% OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-004-01.
4. Middle Ear Pharmacokinetics of OTO-201 in “Wet Ear” Conditions in Guinea Pigs. Study No.: OTO-201-RSP-005-01.
5. Pharmacokinetics of Cetraxal Otic® in Guinea Pigs. Study No.: OTO-201-RSP-011-01.
6. Low Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-014-01.
7. Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs (Middle Ear Epithelium). Study No.: OTO-201-RSP-019-01.
8. Pharmacokinetics of Cetraxal Otic® in Guinea Pigs (Study No.: OTO-201-RSP-014).
9. Pharmacokinetics of Cetraxal Otic® in Guinea Pigs (Middle Ear Epithelium). Study No.: OTO-201-RSP-025-01.
10. Pharmacokinetics of Sequential Administration of OTO-201 and Ciprodex Otic ® in Guinea Pigs. Study No.: OTO-201-RSP-033-01.

11. Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-034-01.
12. Pharmacokinetics of Ciprodex Otic in Guinea Pigs. Study No.: (b) (4)
13. Pharmacokinetics of Co-Administration of OTO-201 Vehicle and Ciprodex Otic® in Guinea Pigs. Study No.: (b) (4).

Toxicology

1. A One Month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-008.
2. Co-administration of OTO-201 and Ciprodex® Otic: A One-Month GLP Ototoxicity Study in Guinea Pigs. Study No.: OTO-201-RSP-010.
3. A One Month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-036.
4. A One-Month GLP Acute Ototoxicity Study Evaluating the Sequential Administration of OTO-201 and Ciprodex in Guinea Pigs. Study No.: OTO-201-RSP-037.
5. Ototoxicity and Pharmacokinetic Evaluation of OTO-201 Following a Single Administration in Guinea Pigs. Study No.: OTO-201-RSP-035-01.
6. Middle Ear Recovery Study Following a Single Dose of Poloxamer 407 or Forced-degraded Poloxamer 407 in Rats with 4-Week and 12-Week Recovery Periods. Study No.: OTO-104-RSP-086.

Special toxicity

1. An Acute Dermal Toxicity Study of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-009.
2. Ventilation Tube Patency Following Intratympanic Administration of (b) (4)% Poloxamer 407 (b) (4) in Guinea Pigs. Study No.: OTO-200-RSP-022-01.
3. Ototoxicity and Pharmacokinetic Evaluation of OTO-201 Following a Single Administration in Guinea Pigs. Study No.: OTO-201-RSP-035-01.

3.2 Studies Not Reviewed

1. Validation of an LC-MS/MS Method for the Analysis of Ciprofloxacin in Guinea Pig Sodium Heparin Plasma. Study No.: ET-0011-RB-BV.

3.3 Previous Reviews Referenced

IND 110244: James S. Wild, Nonclinical Review, 12/15/2011
IND 110244: James S. Wild, Nonclinical Review, 12/20/2011
IND 110244: James S. Wild, Nonclinical Review, 12/5/2012

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology information submitted with this NDA will be evaluated in the microbiology review.

4.2 Secondary Pharmacology

No secondary pharmacology information for OTO-201 was submitted with this NDA application.

4.3 Safety Pharmacology

No safety pharmacology information for OTO-201 was submitted with this NDA application.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Middle Ear Pharmacokinetics of OTO-201

The Sponsor conducted multiple pharmacokinetic studies in guinea pigs in an effort to characterize total middle ear, middle ear epithelium, and inner ear concentrations of ciprofloxacin under varying conditions. These studies, summarized below, examined ciprofloxacin pharmacokinetics associated with different ciprofloxacin concentrations in OTO-201 preparations (Study Nos.: OTO-201-RSP-002-01, and OTO-201-019-01), variable OTO-201 injection volumes (OTO-201-RSP-004-01), and OTO-201 injections in wet middle ear conditions (OTO-201-RSP-005-01). The studies were originally evaluated in the IND 110244 Nonclinical Review by James Wild dated 12/15/2011.

Study Title: Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. (Study No.: OTO-201-RSP-002-01)

Brief Methods: Guinea pigs received a single bilateral intratympanic injection of either 0.6, 2.0, 6.0, or 12.0% ciprofloxacin suspended in (b)(4)% P407 (b)(4) (OTO-201) in the inferior anterior quadrant of the tympanum on Study Day 0. Animals were followed for 28 days and middle and inner ear samples were collected on Days 1, 3, 7, 14, 21, and 28. Ciprofloxacin concentrations in both the middle and inner ear cavities were assessed with a LC-MS/MS technique. Middle ears were lavaged with two 100 μl volumes then washes gels and fluid were collected in a single tube for analysis. The inner ear perilymph space was accessed through a microhole drilled through the cochlear capsule adjacent to the round window membrane and 5 μl of perilymph was collected using a microcapillary.

Results: Substantial levels of ciprofloxacin were observed in the middle ear for all groups (Table 4). C_{max} levels did not substantially differ between the 2.0, 6.0, and 12.0% OTO-201 groups with values that were not much higher than the C_{max} for the low dose (0.6%). After 28 days, middle ear concentrations for the 6.0 and 12.0% groups were approximately 6.0 μg/ml compared to approximately 3 and 0.5 μg/ml for the 2.0 and 0.6% groups respectively. The AUC values increased in a less than dose-proportional manner by more than four-fold between the 0.6% dose and the 12% dose. The mean residence time also increased with dose but in a less than dose-proportional manner ranging from 6 days to 12.2 days. C_{max} ciprofloxacin concentrations were 5- to 25-fold

lower in the inner ear compared to the middle ear, and inner ear concentrations decreased 2- to 10-fold over 14 days.

Table 4: Summary of Ciprofloxacin Pharmacokinetic Parameters in the Middle Ear. (Sponsor's Table)

	Cmax µg/ml	AUC µg.h/ml	AUC_{0-24} µg.h/ml	MRT h	T>MIC h	Cmax/MIC ratio	AUC_{0-24}/MIC h
OTO-201							
0.6	77.4	7663	1858	143	322	39	929
2.0	96.9	11025	2326	200	413	48	1163
6.0	91.7	23921	2200	246	715	46	1100
12.0	99.9	32026	2398	293	721	50	1199

Study Title: Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs (Middle Ear Epithelium). Study No.: OTO-201-RSP-019-01

Brief Methods: Female guinea pigs (4/group) received a single bilateral intratympanic injection of 0.6%, 2.0%, 6.0% or 12.0% of the (b) (4) suspension (OTO-201) in the inferior anterior quadrant of the tympanum (directed away from the round window niche). Middle ear epithelium samples were obtained at Days 1, 3, 7, and 14.

Results: The C_{max} levels (Mean ± SEM) of ciprofloxacin in the middle ear epithelium following single intratympanic doses of 0.6% and 2% OTO-201 were similar (37.0 ± 10.2 and 62.5 ± 12.5 µg/ml respectively) and approximately 10-fold lower than the C_{max} values for the 6.0% and 12.0% doses of OTO-201 (393.3 ± 82.0, and 585.9 ± 309.2 µg/ml respectively). After 14 days, the middle ear epithelium concentrations of ciprofloxacin normalized to similar levels (55.5 ± 5.4, 27.3 ± 17.1, and 103.8 ± 72.4 µg/ml respectively) for the 2, 6, and 12% doses of OTO-201, while the concentration for the 0.6% dose was substantially lower (7.0 ± 2.1 µg/ml) (Table 5).

Reviewer Comment: *The ciprofloxacin C_{max} value in middle ear epithelium for 6% OTO-201 was approximately 4-fold higher than the ciprofloxacin C_{max} value in middle-ear lumen for 6% OTO-201 in Study No.: OTO-201-RSP-002-01.*

Table 5: Ciprofloxacin Concentrations in Middle Ear Epithelium Following Single Intratympanic Injections of 0.6%, 2.0%, 6.0%, and 12.0% OTO-201 in Guinea Pigs.
(Sponsor's Table)

0.6% OTO-201	Days	#1	#2	#3	#4	Mean	SEM
	1	29	21.9	N/A	60.1	37.0	10.2
	3	16.6	40.2	16.6	13.4	21.7	6.2
	7	9.3	4.4	7.8	6	6.9	1.1
	14	5.4	10.2	10.6	1.6	7.0	2.1
2.0% OTO-201	**Days**	**#1**	**#2**	**#3**	**#4**	**Mean**	**SEM**
	1	53.3	41	98.7	56.9	62.5	12.5
	3	54.3	67.8	23.2	78.3	55.9	12.0
	7	99.6	4.3	30	7.5	35.4	22.2
	14	N/A	63.2	N/A	47.8	55.5	5.4
6.0% OTO-201	**Days**	**#1**	**#2**	**#3**	**#4**	**Mean**	**SEM**
	1	473.5	391.9	165.3	542.4	393.3	82.0
	3	295.9	N/A	155.3	491.1	314.1	84.3
	7	81.9	43.1	128.7	N/A	84.6	21.4
	14	9.6	78.5	10.7	10.3	27.3	17.1
12.0% OTO-201	**Days**	**#1**	**#2**	**#3**	**#4**	**Mean**	**SEM**
	1	246.1	456.1	147.9	1493.3	585.9	309.2
	3	325.1	76.4	166.8	17.8	146.5	67.0
	7	10.7	N/A	79.6	460.8	183.7	121.2
	14	N/A	26.4	14.1	270.9	103.8	72.4

Study Title: Volume Range Pharmacokinetics of 2.0% OTO-201 in Guinea Pigs.
(Study No.: OTO-201-RSP-004-01)

Brief Methods: Female guinea pigs (4/group) received single unilateral intratympanic injections of 25, 50, or 75 μl of 2.0% OTO-201 into the inferior quadrant of the tympanum and the animals were followed for 14 days. The middle ear was sampled by lavaging twice with 100 μl of sterile water, then pooling the lavages. Middle ear samples were collected on Days 1, 3, 7, and 14 and ciprofloxacin was measured using an HPLC technique.

Results: Intratympanic injection of three different volumes of 2.0% OTO-201 resulted in substantial levels of middle ear ciprofloxacin. The middle-ear C_{max} values followed a general trend of dose-dependent increase with values of 56.0 ± 3.5, 70.8 ± 7.1, and 94.3 ± 5.7 μg/ml for the 25, 50, and 75 μl dosage volumes respectively. Over a 14-day period post injection, the differences in the dosage curves for the three volumes merged to some degree suggesting dosage volume had less effect on the overall drug elimination profile.

Study Title: Middle Ear Pharmacokinetics of OTO-201 in "Wet Ear" Conditions in Guinea Pigs. (Study No.: OTO-201-RSP-005-01)

Brief Methods: Guinea pigs received a trans-bulla injection which in guinea pigs triggers a transient trauma of the middle ear associated with an inflammatory reaction and fluid exudation typically lasting for 3-4 days (wet ear environment). Guinea pigs received a single bilateral trans-bulla injection of either 0.6% or 2.0% OTO-201 in the inferior anterior quadrant of the tympanum. The guinea pigs were followed for 21 days after dosing and samples from the middle ear were obtained on Days 1, 3, 7, 14, and 21.

Results: The middle ear C_{max} values for the 0.6 and 2.0% OTO-201 injections were both approximately 70 μg/ml. However, the middle ear concentrations decreased more rapidly for the 0.6% group to <1 μg/ml by Day 7 as compared to > 6 μg/ml for the 2.0% group on Day 7. While the middle ear ciprofloxacin concentrations continued to decrease to approximately 0.1 μg/ml by Day 21 for the 0.6% group, the Day 21 middle ear ciprofloxacin concentrations were still about 6 μg/ml for the 2.0% group. In another experiment (OTO-201-RSP-002-01) where 2.0% OTO-201 was injected in the absence of wet ear conditions, middle ear ciprofloxacin concentrations 21 days after injection were approximately 3.5 μg/ml. These results suggest ciprofloxacin retention in guinea pig middle ears may be prolonged in "wet ear" conditions.

Pharmacokinetics of Ciprodex® and Cetraxal®

The middle ear pharmacokinetics of Ciprodex® alone and in the presence of (b) (4)% P407 was examined (Study Nos.: (b) (4) as well as the pharmacokinetics following sequential administration of OTO-201 and Ciprodex® (OTO-201-RSP-033). These studies are summarized below and were first reviewed in the IND 110244 nonclinical reviews by James Wild, dated 12/20/2011 and 12/15/2012. In addition, studies examining the pharmacokinetics of Cetraxal® in guinea pig middle ear (OTO-201-RSP-011) and middle ear epithelium (OTO-201-RSP-025-01) are reviewed below.

Study Title: Pharmacokinetics of Ciprodex Otic® in Guinea Pigs (Study Number: (b) (4)).

Brief Methods: Female guinea pigs (N = 4/group) were fitted with tympanostomy tube placement, then administered Ciprodex® in 10 ml volumes into the tubes with a single dose or twice daily for 7 days. Middle ear lavages (twice with 100 μl of sterile volume) were obtained at 1, 3, or 6 hours after the single dose administration of Ciprodex® and on Days 1, 3, 5, 7, 14, 21, and 28 in conjunction with the 7 day course of administration. Lavages were collected and ciprofloxacin was measured using a LC-MS/MS method.

Results: Female guinea pigs (N = 4/group) were fitted with tympanostomy tube placement, then dosed with Ciprodex® Otic in 10 μl volumes in a single dose or twice daily for 7 days. Ciprofloxacin middle ear concentrations were highest at the first and

second sampling timepoints (1 and 3 hours) with values of approximately 20 μg/ml after a single dose of Ciprodex® then decreased approximately 3 fold 6 hours after dosing. During the 7-Day course of BID Ciprodex®, ciprofloxacin middle ear concentrations (trough levels) peaked at approximately 5-9 μg/ml by Day 3 and remained at this level until Day 14 before falling to 1.3 μg/ml by Day 28.

Study Title: Pharmacokinetics of Co-Administration of OTO-201 Vehicle and Ciprodex® in Guinea Pigs. (Study No.: (b) (4))

Brief Methods: Female guinea pigs (4/group) received a single intratympanic injection of OTO-201 vehicle ((b) (4)% P407) followed 3 days later by either a single dose of Ciprodex® or a treatment course of Ciprodex® (BID dosing for 7 days from Day 3 to Day 10). Ciprodex® was administered through a tympanostomy tube. Middle ear sampling by lavage (two 100 μl lavages) occurred at 1, 3, and 6 hours following the single Ciprodex® administration, or on Days 4, 10, 14, 21, and 28 for the 7-day treatment of Ciprodex®. Middle ear ciprofloxacin concentrations were assessed using a LC-MS/MS method.

Results: Three days after guinea pigs received intratympanic injections of OTO-201 vehicle, middle ear concentrations of ciprofloxacin were highest at 1 (≈ 70 μg/ml) and 3 (≈ 50 μg/ml) hours after a single dose of Ciprodex® then decreased approximately 4 fold by 6 hours after dosing (≈ 12 μg/ml). During the 7-Day course of BID Ciprodex®, ciprofloxacin middle ear concentrations (trough levels) peaked at approximately 6-8 μg/ml and remained at this level until the last day of Ciprodex® dosing (Day 14) before falling to 1.0 μg/ml by Day 28. The trough levels and the rate of elimination of ciprofloxacin from guinea pig middle ears was very similar to what occurred in a study ((b) (4)) that did not include the co-administration of OTO-201 vehicle. In contrast, C_{max} levels were increased approximately 3-fold relative to Ciprodex® administered in the absence of (b) (4)% poloxamer 407. These results suggest the presence of (b) (4)% poloxamer 407 in the middle ear does not increase the persistence of ciprofloxacin derived from a 7-day BID treatment course of Ciprodex®, but peak middle ear ciprofloxacin concentrations are increased.

Study Title: Pharmacokinetics of Sequential Administration of OTO-201 and Ciprodex Otic in Guinea Pigs. (Study No.: OTO-201-RSP-033-03)

Brief Methods
Anesthetized guinea pigs (female; N = 4/group/time point) received a single intratympanic injection of OTO-201 vehicle (poloxamer 407) or 2.0% or 6.0% OTO-201 to a tympanic site anterior to the round window membrane followed 3 days later by either A) a single dose of Ciprodex® and monitoring for 6 hours or B) BID Ciprodex® for 7 days (mirroring a clinical treatment course) and monitoring for up to 28 days. Ciprodex® was administered via a tympanostomy tube place in the tympanic membrane. Middle ear lavage samples (two 100 μl lavages combined) were collected at 1, 3, and 6 hours following the single administration of Ciprodex® and at 3, 10, 14, 21,

and 28 days after the 7-day course of BID Ciprodex®, and ciprofloxacin concentrations were determined using an HPLC technique.

Results
In the presence of a single IT-ANT administration of (b)(4)% P407 vehicle administered 3 days prior, a single administration of Ciprodex® resulted in approximate middle ear ciprofloxacin concentrations of 55 μg/ml one hour after Ciprodex® administration with rapid decrease to approximately 8 μg/ml after 6 hours. Repeated administration of Ciprodex® for 7 days did not result in middle-ear accumulation of ciprofloxacin with trough drug levels of approximately 8 μg/ml occurring on the first day of Ciprodex® administration as well as on Day 10 (the last day of Ciprodex® administration). Drug levels gradually diminished to below quantification limits on Day 21.

A single IT-ANT administration of OTO-201 (2.0 and 6.0%) yielded substantial ciprofloxacin levels in the middle ear peaking at approximately 91 and 104 μg/ml respectively on Day 1, and levels were similar on Day 3 (≈ 84 and 108 μg/ml respectively).

Reviewer Comment: *These results are similar to those shown in a previous study (Study No.: OTO-201-RSP-002-01) where single IT injections of 2.0 and 6.0% OTO-201 resulted in respective middle ear levels of ≈96 and 92 μg/ml on Day 1 and 35 and 100 μg/ml on Day 3. The only clear difference is that the Day 3 middle ear levels of ciprofloxacin decreased more dramatically for the 2.0% OTO-201 concentration in the earlier study compared to the present study.*

In the presence of OTO-201 (2.0 and 6.0%) administered 3 days prior, a single administration of Ciprodex® administered on Day 4 resulted in lower peak levels than those measured for OTO-201 alone on Days 1 and 3. Drug levels peaked at 3 hours ranging from ≈ 75 to 94 μg/ml and decreased to ≈48 to 57 μg/ml by 6 hours. Repeated administration of Ciprodex® (BID for 7 days) beginning on Day 4 following a single IT-ANT injection of OTO-201 (2 and 6 %) on Day 1, did not result in accumulation of middle ear ciprofloxacin with trough levels remaining relative static at respective levels of ≈28 and 57 μg/ml on Day 4 and ≈ 29 and 55 μg/ml on Day 10. Following completion of the Ciprodex® treatment, middle ear ciprofloxacin levels gradually decreased to ≈ 4 and 12 μg/ml respectively on Day 28. The trough levels of ciprofloxacin during the Ciprodex® treatment were substantially higher following a single injection with 6% OTO-201 (≈ 55 μg/ml) compared to the levels during Ciprodex® treatment following a single injection of (b)(4)% P407 alone (≈ 8 μg/ml).

Reviewer Comment: *C_{max} middle ear drug levels were not measured following repeated administration of Ciprodex®. Thus it is not clear if the C_{max} levels increased with repeated dosing. However, the similar trough levels measured throughout Ciprodex® administration suggest an absence of ciprofloxacin accumulation.*

Study Title: Pharmacokinetics of Cetraxal Otic® in Guinea Pigs. (Study No.: OTO-201-RSP-011-01)

Methods

This non-GLP study was conducted by Otonomy Inc. in 2011. Anesthetized guinea pigs (females; N = 4/group/time point) received either A) a single dose of Cetraxal® followed by monitoring for 6 hours or B) a 7-Day BID course of Cetraxal® (mirroring the clinical treatment course) followed by monitoring for 28 days. Cetraxal® was administered via a tympanostomy tube placed in the tympanic membrane to the middle ear compartment. Middle ear and inner ear lavage samples were collected at 1, 3, and 6 hours after the single administration of Cetraxal®, and at 1, 3, 7, 14, 21, and 28 days in conjunction with the 7-day, BID course of Cetraxal®. Middle ear ciprofloxacin concentrations were determined using an HPLC technique, and inner ear (perilymph) samples were analyzed using HPLC-MS.

Results

Following a single administration of Cetraxal®, middle ear C_{max} concentrations for ciprofloxacin occurred at 1 hour after administration (≈ 24 μg/ml) and declined slowly to ≈ 14 μg/ml at 6 hours after administration (Figure 1).

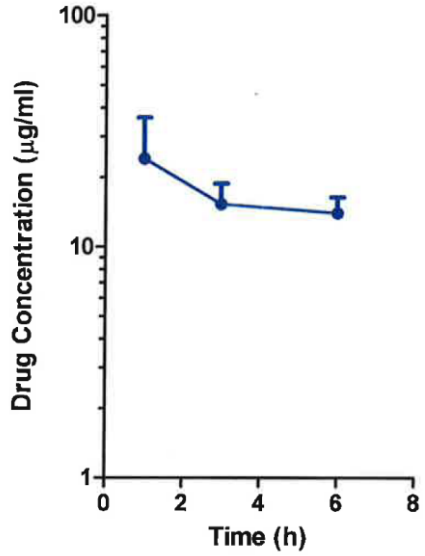


Figure 1: Middle Ear Ciprofloxacin Concentrations Following a Single Intratympanic Administration of Cetraxal®.

In conjunction with the 7-Day BID administration, ciprofloxacin accumulated in the middle ear based on trough levels which peaked on Day 7 with ≈ 19 μg/ml compared to ≈ 5 μg/ml on Day 1 of dosing (Figure 2). Following cessation of dosing, ciprofloxacin elimination was rapid from Day 7 to 14 then more gradual from Day 14 to 28 with approximately 1 μg/ml concentrations remaining on Day 28.

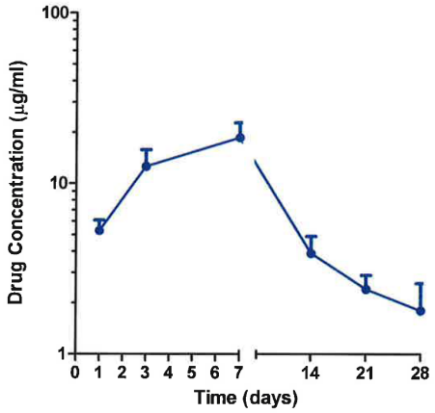


Figure 2: Trough Ciprofloxacin Concentrations in the Middle Ear Following a 7-Day BID Course of Treatment with intratympanic Cetraxal®. (Sponsor's Figure)

In the inner ear, perilymph concentrations of ciprofloxacin were much lower than in middle ear and no evidence of inner ear accumulation was observed. Perilymph concentrations peaked at 6 hours at ≈ 7 μg/ml after the single administration of Cetraxal® (Figure 3). In conjunction with the 7-Day administration of Cetraxal®, perilymph trough concentrations peaked at approximately 1.5 μg/ml on the first day of dosing then remained approximately the same on Day 3 and Day 7 (≈ 0.3 and 0.7 μg/ml respectively) followed by a rapid decline in levels up to Day 21 (≈ 0.1 μg/ml) with no further decline on Day 28 (Figure 4).

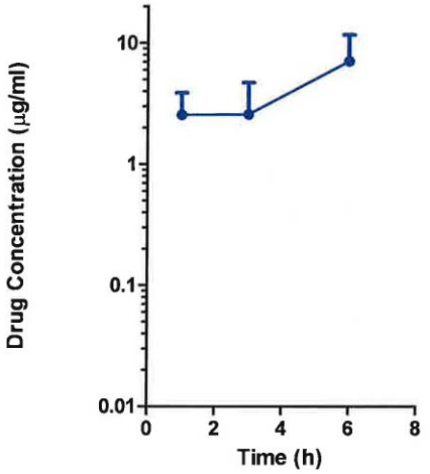


Figure 3: Perilymph Ciprofloxacin Levels Following a Single Intratympanic Administration of Cetraxal®. (Sponsor's Figure)

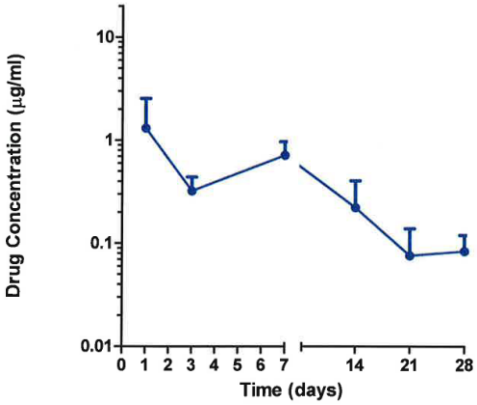


Figure 4: Perilymph Ciprofloxacin Trough Levels Following a 7-Day BID Course of Treatment with intratymanic Cetraxal®. (Sponsor's Figure)

Study Title: Pharmacokinetics of Cetraxal Otic® in Guinea Pigs (Middle Ear Epithelium). (Study No.: OTO-201-RSP-025-01)

Methods

This non-GLP study was conducted by Otonomy Inc., in 2011. Anesthetized guinea pigs (females; N = 4/group/time point) received either A) a single dose of Cetraxal® followed by monitoring for 6 hours or B) a 7-Day BID course of Cetraxal® (mirroring the clinical treatment course) followed by monitoring for 14 days. Cetraxal® was administered via a tympanostomy tube placed in the tympanic membrane to the middle ear compartment. Middle ear epithelium samples were collected at 1, 3, and 6 hours after the single Cetraxal® administration and at 1, 3, 7, and 14 days after the 7-day treatment course.

Results

Much lower levels of ciprofloxacin were observed in middle ear epithelium compared to the approximate 25 µg/ml C_{max} values in middle ear lavage samples from previous studies (Figure 5). C_{max} values in middle ear epithelium occurred at 1 hour after intratympanic administration with values of ≈ 5 µg/ml which rapidly decreased approximately 10-fold to ≈ 0.5 µg/ml at 6 hours after administration.

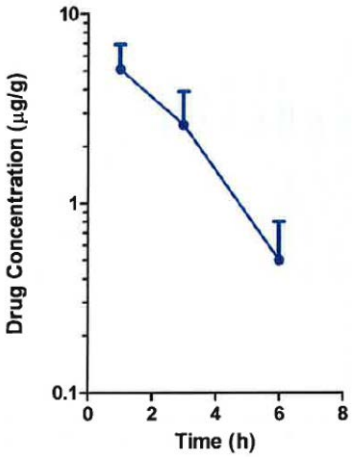


Figure 5: Ciprofloxacin Peak Concentrations in Middle Ear Epithelium Following a Single Administration with Intratympanic Cetraxal®. (Sponsor's Figure)

Ciprofloxacin concentrations in middle ear epithelium increased with repeated Cetraxal Otic® dosing with accumulation occurring on Day 3 and increasing further by the last day of dosing on Day 7. Upon cessation of dosing ciprofloxacin levels rapidly declined more than 10 fold by Day 14 (Figure 6).

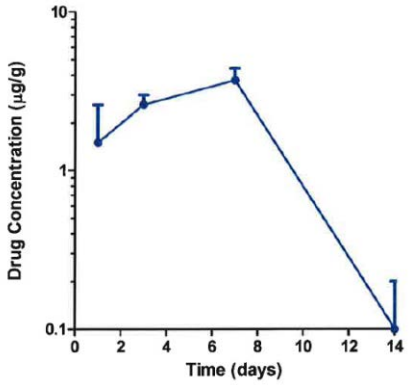


Figure 6: Ciprofloxacin Trough Concentrations in Middle Ear Epithelium Following a 7-Day BID Treatment Course with Intratympanic Cetraxal®. (Sponsor's Figure)

Additional Pharmacokinetic Studies

Additional studies examining the mean residence time of poloxamer 407 in guinea pig middle ear and middle ear epithelium (Study No.: OTO-100-RSP-038-01) and the pharmacokinetics of a range of doses of OTO-201 in middle ear (Study Nos.: OTO-201-RSP-014-01 and OTO-201-RSP-034-01) are reviewed below.

Study Title: Middle Ear Residence Time of Poloxamer 407 Following Intratympanic Administration in Guinea Pigs. (Study No.: OTO-100-RSP-038-01)

Methods

In this non-GLP study conducted by Otonomy Inc. in 2013, female guinea pigs (4/group) were anesthetized and received 50 μl intratympanic injections of (b) (4)% poloxamer 407 (P407). Middle ear lavage and middle ear epithelium were collected as samples for analysis before intratympanic administration and at 1 hour, and 1, 7, and 14 days after administration. P407 concentrations in the samples were analyzed using the cobalt thiocyanate colorimetric method. The assay limit of detection was 100 μg/ml with a linear range of 100 to 7500 μg/ml and poloxamer 407 concentrations were interpolated based on calibration curves.

Middle Ear Lavage Collection: The lower anterior and posterior potion of the tympanic membrane was removed and the middle ear cavity was lavaged twice with 100 μl of sterile water. The washes containing lavage fluid, gel, and middle ear fluid were collected in a single tube, centrifuged, and the supernatant was analyzed for P407 content.

Middle Ear Epithelium Collection: The lower anterior and posterior portions of the tympanic membrane were removed and the middle ear cavity was lavaged extensively with sterile water. Middle ear epithelium was collected by removing the mucosal lining of the middle ear. Tissues were extracted by incubating the samples in acetonitrile-water (1:1 v/v) with sonication and grinding with a tissue shredder. Following centrifugation, the supernatant was collected for analysis.

Results

One hour after administration maximum middle ear concentrations of P407 were observed (C_{max} of 9367 ± 2512 μg/ml), but concentrations rapidly decreased to 35 ± 3 μg/ml by Day 14 after administration (Table 6). Based on these results, a mean residence time of 28 hours and an elimination half-life of 42 hours were calculated (Table 7). Much lower concentrations of P407 were measured in middle-ear epithelium ranging from 0 to 190 μg/ml with almost all of the measurements below the limit of quantification.

Table 6: Middle Ear P407 Levels at Collection Time-points Before and After Intratympanic Administration of (b)(4)% P407 (Sponsor's Table)

Middle Ear						
Days	**#1**	**#2**	**#3**	**#4**	**Mean**	**SEM**
0	10000	10500	10200	3800	8625.0	1611.6
1h	9400	13700	5000		9366.7	2511.5
1	190	50	170	6600		
	50	650	290	4700	1587.5	907.0
7	50	180	30	30	72.5	36.1
14	40	40	30	30	35.0	2.9

Middle Ear Epithelium						
Days	**#1**	**#2**	**#3**	**#4**	**Mean**	**SEM**
0	BQL	BQL	BQL	BQL	0.0	0.0
1h	BQL	BQL	BQL	BQL	0.0	0.0
1	BQL	BQL	BQL	BQL		
	BQL	BQL	BQL	190	23.8	23.8
7	BQL	BQL	4.4	BQL	1.1	1.1
14	6.4	3.3	8	0.9	4.7	1.6

Guinea pigs (n=4-8 per group) were sampled at the indicated times in the middle ear. Values (mean ± SEM) are expressed as µg.
BQL: Below quantification level. A value of 0 was used in the mean calculation for any sample listed as BQL.

Table 7: Summary of Middle Ear Pharmacokinetic Parameters for P407 in Middle Ear Lavages. (Sponsor's Table)

	Cmax µg	AUC µg.h	MRT h	T1/2 h
ME	9367	265651	28	42

Study Title: Low Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs (Study No.: OTO-201-RSP-014-01)

Methods

This non-GLP study was performed by Otonomy Inc. in 2011. Anesthetized female guinea pigs (N = 4/group/time point) weighing 200-300g received a single injection of low concentrations of OTO-201 (0.06 and 0.20%) into the inferior anterior quadrant of the tympanum (intratympanic injection directed away from the round window niche) followed by monitoring for up to 28 days. Animals were sacrificed and middle ears were lavaged with sterile water at the following time points: Days 1, 3, 7, 14, 21, and 28. Ciprofloxacin concentrations in middle ear lavages were determined using an HPLC method.

Results

As in other dose-ranging studies, C_{max} values were similar for both ciprofloxacin concentrations (Table 8). Mean retention times were also similar, but elimination profile for the higher dose (0.2%) was prolonged compared to the lower, 0.06% dose, with <1 μg/ml middle ear concentrations after 7 days occurring with the lower dose and middle ear concentrations of between 0.1 and 1 μg/ml remaining for the high dose for up to 25 days (Figure 7).

Table 8: Summary of Middle Ear Pharmacokinetics for Study No.: OTO-201-RSP-014-01. (Sponsor's Table)

	Cmax μg/ml	AUC μg.h/ml	AUC_{0-24} μg.h/ml	MRT h	T>MIC h	Cmax/MIC ratio	AUC_{0-24}/MIC h
OTO-201							
0.06	45.4	2288	1088	34	63	23	544
0.2	68.5	3728	1645	37	90	35	823

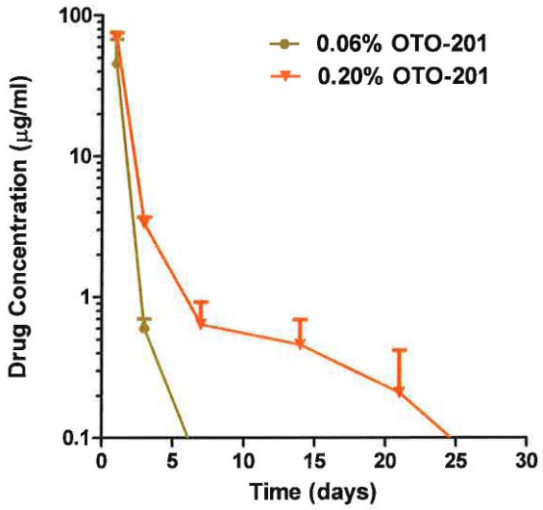


Figure 7: Middle ear ciprofloxacin levels following a single intratympanic injection of low doses (0.06 and 0.2%) of Ciprofloxacin. (Sponsor's Figure)

Study Title: Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. (Study No.: OTO-201-RSP-034-01)

Methods

This non-GLP study was conducted by Otonomy Inc. in 2012. Anesthetized female guinea pigs (N =4 per timepoint) received single bilateral intratympanic injections of 2.0% and 6.0% OTO-201 in the inferior quadrant of the tympanum (directed away from the round window niche). Animals were followed for 28 days and at specific timepoints, the middle ears of animals were lavaged twice with 100 μl of sterile water. Wash fluid and gel and fluid from the middle ears were collected from specific animals on Days 1, 3, 7, 14, 21, and 28 after injection and centrifuged for analysis of ciprofloxacin content. Ciprofloxacin concentrations were determined using an HPLC method.

Results

Middle Ear C_{max} values for ciprofloxacin on Day 1 were similar for both concentrations of OTO-201. The mean residence time at both concentrations was approximately 8-9 days increasing by about a day with the higher dose (Table 9, Figure 8).

Table 9: Summary of Middle Ear Ciprofloxacin Pharmacokinetic Parameters. (Sponsor's Table)

	Cmax µg/ml	AUC µg.h/ml	AUC_{0-24} µg.h/ml	MRT h	Cmax/MIC ratio	AUC_{0-24}/MIC h
OTO-201						
2.0	83.4	13403	1459	193	42	730
6.0	79.2	22158	1840	225	40	920

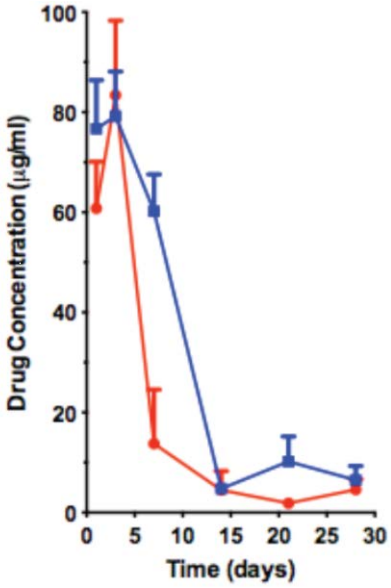


Figure 8: Middle Ear Ciprofloxacin Levels Following a Single IT-ANT Injection of 2.0% (red circles) and 6.0% (blue squares) OTO-201. (Sponsor's Figure)

6 General Toxicology

6.1 Single-Dose Toxicity

Several general toxicology studies examined the effects of a single dose of OTO-201 on the auditory system and as well as systemic toxicity with and without the combination administration of a treatment course of Ciprodex® (Study Nos.: OTO-201-RSP-008 and OTO-201-RSP-010, OTO-201-RSP-036, OTO-201-RSP-037, and OTO-201-RSP-035). Only summaries of these studies are presented below as all of the studies have been previously reviewed in conjunction with an IND 110244 reviews by James Wild dated 12/20/2011 and 12/5/2012. An additional rat study submitted in conjunction with IND (b) (4) is fully reviewed below.

Study Title: A One-Month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs (Study No.: OTO-201-RSP-008)

Methods: In this study, six groups of male and female Albino Hartley guinea pigs (7/sex/group) were administered the (b) (4)% P407 vehicle, 0.6, 2.0, and 6.0% OTO-201, or the positive control articles, Cetraxal®, and Ciprodex®. The vehicle and OTO-201 doses were administered once on Day 1 by intratympanic injection to both ears in 50 µl volumes. The vehicle ((b) (4)% P407) was (b) (4) technique for this study. The positive control drugs were administered as drops twice daily for 7 consecutive days via a tympanostomy tube (15 µl dose volume for Cetraxal® and 10 µl dose volume of Ciprodex®). All of the surviving animals except those designated for Eustachian tube removal (2/sex/group) were euthanized on Day 30. Animals were assessed for clinical signs, body weights, auditory brain stem responses (ABR), hematology and serum chemistry, auditory system histopathology including an ossicle mobility assessment and cytocholeogram analysis, systemic gross pathology, organ weights, systemic histopathology and plasma toxicokinetics.

Results

1. No effect of treatment on the auditory brainstem responses (ABR) was noted for either gender in the vehicle control group (Group 1) or any of the OTO-201 treatment groups (Groups 2-4) receiving 0.6, 2.0, or 6.0% OTO-201. Males, but not females receiving Cetraxal® demonstrated a mild threshold elevation in both left and right ears suggesting mild hearing loss.
2. In the 6.0% OTO-201 group, the presence of mild focus was noted in one ear (out of 20), as well as a small potential for minimal-moderate tissue reaction in the middle ear, at times associated with minimal fibrosis. In the Cetraxal® group, the presence of deformation/perforation/white foci were noted in 3 ears, and in 7 ears in the Ciprodex® group. Ossicle immobility occurred in 4/20 ears in the 6% OTO-201 group, 2/20 ears in the Cetraxal® group, and in 3/20 ears in the Ciprodex® group.
3. Administration of OTO-201 and Cetraxal® was not associated with pronounced inner ear hair loss. In contrast, 60% of the animals were deleteriously affected in the Ciprodex® group.
4. In the middle ear, the presence of foreign material was noted at about the same incidence in all the groups including the vehicle control group.
5. Foamy macrophages were present in a small percentage of eustacian tubes from all of the groups receiving (b) (4)% P407 suggesting foamy macrophages may represent uptake of the vehicle.
6. Significant OTO-201-related reductions in total blood platelets, eosinophils, basophils, and MCHC were not thought to be toxicologically relevant due to a lack of dose response, similar effects in the Cetraxal® group, and/or inconsistent effects between the genders.
7. The NOAEL for OTO-201 was considered to be the highest administered dose of 6% OTO-201.
8. Toxicokinetic measurements for plasma ciprofloxacin indicate only low plasma levels (Table 10).

Table 10: Plasma Ciprofloxacin Levels Following Intratympanic OTO-201 Injection or Treatment Courses of Cetraxal® or Ciprodex®.

Sampling Time	Plasma Concentrations (ng/ml)					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	BQL	10.1 ± 3.0	17.1 ± 8.9	22.9 ± 7.4	BQL – 2.3	BQL – 13.9
Day 7	BQL	BQL – 2.2	4.1 ± 1.5	16.2 ± 4.9	5.8 ± 2.2	6.0 ± 1.6
Day 14	BQL	BQL – 1.2	BQL – 8.0	12.1 ± 2.7	BQL	BQL
Day 29	BQL	BQL	2.3 ± 1.3	10.5 ± 4.0	BQL	BQL

BQL = below quantification level (< 1.0 ng/ml)
Group 1: (b)(4)% P407; Group 2: 0.6 % OTO-201; Group 3: 2.0% OTO-201; Group 4: 6.0% OTO-201; Group 5: Cetraxal®; Group 6: Ciprodex®.

Study Title: Co-Administration of OTO-201 and Ciprodex®: A One-Month GLP Ototoxicity Study in Guinea Pigs. (Study No.: OTO-201-RSP-010).

Methods: Groups of guinea pigs (7/sex/group) were administered vehicle ((b)(4)% P407) or OTO-201 (0.6 and 6.0%) during the study as a single intratympanic (bilateral) injections (50 μl) immediately prior to undergoing tympanostomy tube placement on study Day 1. The (b)(4)% P407 vehicle was (b)(4) technique for this study. The co-article (Ciprodex®) was administered topically BID for 7 consecutive days beginning on Day 4 at a dose of 10 μl per ear. In this dosing procedure, animals were immobilized, the tympanostomy tubes were visualized and the doses were administered bilaterally via pipette. Delivery to the middle ear was facilitated by pumping the tragus. Animals were assessed for clinical signs, body weights, auditory brain stem responses (ABR), hematology and serum chemistry, auditory system histopathology including ossicle mobility assessment and cytocholeogram analysis, systemic gross pathology, organ weights, systemic histopathology and plasma toxicokinetics.

Results

1. Co-administration of OTO-201 and Ciprodex® did not produced systemic toxicity (mortality, clinical signs, changes in body weights, hematology and clinical chemistry parameters, or organ weights) 29 days after OTO-201 dosing.
2. Auditory brain response thresholds were elevated in all groups suggesting the effect was mediated by Ciprodex® treatment.
3. Most of the auditory-system gross pathology including mild deformation/white foci/perforation of the tympanum and middle ear ossicle immobility occurred at a similar incidence in the vehicle control group and the OTO-201 treatment groups.
4. Foreign material associated with the tympanum and in the middle ear occurred only in the OTO-201-treatment groups in a ciprofloxacin dose-responsive manner suggesting the foreign material may have been ciprofloxacin.
5. The histological determination of cochlear hair loss occurred at a similar low incidence in the OTO-201 treatment and vehicle control groups suggesting the effect was mediated by treatment with Ciprodex®.
6. Toxicokinetic analysis of plasma ciprofloxacin concentrations indicated limited distribution to plasma with the highest concentrations of approximately 33 ng/ml measured the day after intratympanic injection.

Study Title: A One-month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs. (Study No.: OTO-201-RSP-036)

Methods: Six groups of male and female Albino Hartley guinea pigs (5/sex/group) were administered saline, gentamicin (400mg/ml), (b) (4)% P407 vehicle, 2.0, and 6.0% OTO-201, or the positive control article, Cetraxal Otic®. The saline, vehicle, gentamycin, and OTO-201 doses were administered once on Day 0 by intratympanic injection to both ears in 50 μl dose volumes. In this study, the vehicle, (b) (4)% P407, was (b) (4) technique. Cetraxal® was administered twice daily for 7 consecutive days via a tympanostomy tube in a fixed volume of 15 μl/ear/dose. All surviving animals were euthanized on Day 28. Animals were assessed for clinical signs, body weights, auditory brain stem responses (ABR), and auditory system histopathology including ossicle mobility assessment and cytocholeogram analysis and microscopic evaluation of middle and inner ear structures.

Results

1. Auditory brainstem response (ABR) assessments were similar in the saline, P407, and 2.0% OTO-201 groups. Cetraxal® and 6.0% OTO-201 produced similar moderate increases in ABR values (hearing loss) at all three measured frequencies, while gentamycin produced more severe hearing loss.
2. Gentamycin produced the greatest incidence and severity of middle ear edema, fluid, and fibrosis. OTO-201 at both concentrations produced mild-moderate middle ear edema and fluid at a somewhat greater incidence than the saline group and the Cetraxal® group. P407 administration was associated with mild middle ear edema and fluid in 1/20 ears. However, the middle ear edema, fluid, and fibrosis results were not corroborated upon microscopic analysis.
3. Ossicle immobility was impaired in 1/20 ears in the 6% OTO-201 Group, 3/20 ears in the Cetraxal® group, and 3/20 ears in the gentamycin group.
4. The cochleae of all the animals in the saline, P407, 2.0 and 6.0% OTO-201 groups were within normal variability in terms of inner and outer hair patency. Gentamycin administration resulted in a large loss of inner and outer hair cells in all treated animals, and administration of Cetraxal Otic® produced mild to moderate hair cell loss.
5. Middle ear histopathology results indicated all of the saline-treated right ears exhibited minimal to mild subacute inflammation of the middle ear in conjunction with the presence of scattered aggregates of macrophages. The P407 and Cetraxal Otic® groups were indistinguishable from the saline group with the addition of foamy macrophages. Additional findings in the OTO-201 groups included minimal to mild granulomatous inflammation which was considered to be consistent with foreign body reaction possibly to ciprofloxacin particulates. Gentamycin produced a marked chronic active inflammatory response in all right middle ears with evidence of fibroplasia and bone loss/remodeling.

Study Title: A One-Month GLP Acute Ototoxicity Study Evaluating the Sequential Administration of OTO-201 and Ciprodex in Guinea Pigs. (Study No.: OTO-201-RSP-037)

Methods: Six groups of male and female Albino Hartley guinea pigs (5/sex/group) were administered saline, (b)(4)% P407 vehicle, and OTO-201 doses once on Day 0 by intratympanic injection to both ears in 50 µl volumes. In this study, the vehicle, (b)(4)% P407 was (b)(4) technique. Ciprodex® was administered twice daily for 7 consecutive days (Days 3-9) via a tympanostomy tube in a fixed volume of 10 µl/ear/dose. All surviving animals were euthanized on Day 28. Animals were assessed for clinical signs, body weights, auditory brain stem responses (ABR), and auditory system histopathology including an ossicle mobility assessment and cytocochleogram analysis and microscopic evaluation of middle and inner ear structures.

Results

1. All treatment groups including the saline group experienced mild to moderate hearing loss suggesting the effects were caused by the Ciprodex® treatment. OTO-201, 2% or 6% did not worsen the hearing loss.
2. The incidence of middle ear edema, fluid and fibrosis occurring in the OTO-201 groups was increased compared to the saline and (b)(4)% P407 groups. However, the pathology report indicated that these effects were not corroborated by histopathology.
3. Cytocochleograms demonstrated similar incidences of mild to moderate cochlear hair-cell loss in all treatment groups suggesting the cause was attributable to Ciprodex Otic® administration.
4. Middle ear histopathology for the saline group included minimal to mild subacute inflammation of the middle ear in conjunction with the presence of scattered aggregates of foamy macrophages and a low incidence of minimal granulomatous inflammation. P407 animals exhibited the same findings except a greater incidence of foamy macrophages. Both OTO-201 groups demonstrated a greater incidence of minimal to mild granulomatous inflammation and foreign material. The Sponsor indicated that the findings in the OTO-201 group were characteristic of a minimal to mild foreign body reaction with the foreign body hypothesized to be ciprofloxacin particulates.

Study title: Ototoxicity and Pharmacokinetic Evaluation of OTO-201 Following a Single Administration in Guinea Pigs (Study No.: OTO-201-RSP-035-01)

Methods: For this study, all of the (b)(4)% P407 preparations were (b)(4) Female guinea pigs (n = 8/group) received a single bilateral intratympanic (IT) injection of 50 µl of saline, (b)(4)% poloxamer 407 (P407), 2% and 6% OTO-201 or gentamicin to the anterior quadrant of the tympanum (ANT; directed away from the round window niche). Animals were followed for 28 days with auditory brainstem response (ABR) assessments performed on Days 7 and 28.

Other female guinea pigs (n = 4/time point) receiving 2 or 6% OTO-201 also underwent a terminal middle ear lavage on Days 1, 3, 7, 14, 21, and 28. Two 100 µl middle ear

lavages were performed and total wash volumes of 200 μl containing washes, gel, and fluid were collected in a single tube for analysis. Ciprofloxacin concentrations were determined using a HPLC method with a limit of detection of 100 ng/ml.

Results: Single IT-ANT injections of (b) (4)-P407, and 2 and 6% OTO-201 did not substantially affect auditory function in guinea pigs as assessed with ABR in three frequencies (4, 10, and 20 kHz). The positive control ototoxicant, gentamicin produced substantial hearing loss across all three frequencies.

Substantial middle ear concentrations of ciprofloxacin were measured following a single IT-ANT administration of 2 and 6% OTO-201 (Figure 5, Table 3). C_{max} levels were similar between groups (78.5 and 89.3 μg/ml respectively) and also similar to those observed following IT-RWM injection of 2% and 6% OTO-201 (97 and 92 μg/ml respectively) in a previous pharmacokinetic study (OTO-201-RSP-002-01). Middle ear half-life values were long, on the order of 100 hours, and similar to previous values.

Study title: Middle Ear Recovery Study Following a Single Dose of Poloxamer 407 or Forced-degraded Poloxamer 407 in Rats with 4-Week and 12-Week Recovery Periods.

Study no.:	OTO-104-RSP-086
Study report location:	Electronic transmission
Conducting laboratory and location:	Otonomy, Inc., 6275 Nancy Ridge Drive, Suite 100, San Diego, CA 92121
Date of study initiation:	October 11, 2013
GLP compliance:	Yes, with some deficiencies
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) % P407, batch # W0006474 stored at 2-8°C; forced-degraded (b) (4) % 407 Type II, batch # W0006474 stored at 40°C for 10 months then at 2-8°C.

Key Study Findings

- Minimal to mild inflammation and foamy macrophages were observed in animals dosed with P407 and forced-degraded P407 at 4 weeks after injection. The incidence and severity of these findings were notably lower at 12 weeks after injection, suggesting reversibility.
- Treatment with P407 or forced-degraded P407 did not result in changes in ABR thresholds at 3 frequencies (4, 10, and 20 kHz) compared to saline control values.
- There was no test article-related hair cell loss in the cochlea of animals dosed with P407 or forced-degraded P407.
- The positive control agent, gentamicin, resulted in substantial ABR shifts particularly at frequencies of 10 and 20 kHz and a marked loss of cochlear hair cells.

Table 11: [redacted] Concentrations in the P407 and Forced-degraded P407 (Type II) Test Articles. (Sponsor'sTable)

	P407	Forced-degraded P407 (Type II)
Description	Stored 2-8°C	Stored 40°C
[redacted]		

Methods

Doses:	See Table 12
Frequency of dosing:	Single dose
Route of administration:	Intratympanic injection directly toward the round window niche
Dose volume:	20 µl
Formulation/Vehicle:	[redacted]% Poloxamer 407 was the test article
Species/Strain:	Sprague Dawley rats
Number/Sex/Group:	12 females/group (see Table 12)
Age:	1 to 3 months old
Weight:	Approximately 250 to 400 grams
Satellite groups:	none
Unique study design:	Saline, P407, forced-degraded P407 (type II), and a positive control for ototoxicity, gentamycin were administered in single intratympanic injections into the right ear of anesthetized Sprague Dawley rats once on Day 1 in 20 µl volumes. Using a surgical microscope, Injections were directed toward the round window niche and solutions were slowly injected at a rate of 1 µl/second using an injection pump. Following the injections animals were monitored during recovery from anesthesia, then assessed for mortality
Deviation from study protocol:	Deviations from the study protocol were noted. However, none was considered to have altered the results or compromised the integrity of the study.

Table 12: Study Design for Study No: OTO-104-RSP-086

Group #	Dose Level	Number
A	Saline	24
B	Poloxamer 407	24
C	Forced-degraded Poloxamer 407 (Type II)	24
D	Gentamicin ((b) (4) mg/mL)	24

Note: Groups A, B, C, and D are also referred to as Groups 1, 2, 3, 4 respectively.

Observations and Results

Table 13: Observation Schedule for Study No.: OTO-104-RSP-086

Observations	Schedule
Mortality	All animals were observed daily for morbidity, mortality and signs of injury.
Clinical signs	All animals received a complete physical pretest, and again prior to termination (Day 28 or 29 for Groups 1-4 and Day 84 or 85 for Groups 5-8. Otoscopic examinations were performed during the physical exams.
Body weights	Body weights were recorded pretest and weekly during the study.
ABR evaluations	Bilateral ABR evaluations at three different frequencies (4, 10, and 20 kHz) were conducted on all animals pretest and prior to termination.
Postmortem evaluations	The tissues shown below were collected on the scheduled termination days.

Tissue	Collected and Preserved	Microscopic Examination (Dose Group)
Cochlea for cytocochleograms:		
6 ears, 4-week recovery (Day 28)	X	All groups
6 ears, 12-week recovery (Day 84)	X	All groups
Middle ear histology:		
6 ears, 4-week recovery (Day 28)	X	All groups
6 ears, 12-week recovery (Day 84)	X	All groups

Mortality

All animals in each of the treatment groups (saline, gentamicin, P407 and forced degraded P407 (Type II) survived to their scheduled necropsies at 4 weeks and 12 weeks.

Otoscopic Examinations

There was no evidence of middle ear inflammation or middle ear fluid in any of the treated and untreated ears in any of the saline, P407 and forced-degraded P407 Type II treatment groups, following 4 and 12 weeks recovery. Middle ear inflammation and fluid

was observed in the treated ear of one animal dosed with gentamicin following a 12-week recovery.

Body Weights

There were no significant changes in body weight in the P407 treatment group. A mild effect was noted with forced-degraded P407 Type II and gentamicin when compared to saline. Following a 4-week recovery period, saline and P407 animals had a mean weight gain of 15%, while forced-degraded P407 animals had a mean weight gain of 8%, and animals treated with gentamicin had a mean weight gain of 13%. Following a 12-week recovery period, P407 animals had a mean weight gain of 30%, compared to 31% for the saline control group while animals treated with forced-degraded P407 had a mean weight gain of 23%, and animals treated with gentamicin had a mean weight gain of 25%.

Auditory Brainstem Response Evaluations

There was no treatment effect on ABR after a single IT-RWM injection in rats, of saline, the vehicle P407 and forced-degraded P407 Type II following recovery periods of 4 and 12 weeks. Administration of the known ototoxicant gentamicin resulted in a mild hearing loss, which was maintained throughout the recovery period of 12 weeks. There was no evidence of hearing loss in any of the untreated ears in any of the treatment groups. The positive control agent, gentamycin, produced hearing loss (significant shifts from baseline) primarily at the 10 and 20 kHz frequencies. The ABR results for the treated right ears at 4, 8, and 12 weeks after intratympanic injections for all three of the tested frequencies are shown below in Figure 9, Figure 10, and Figure 11.

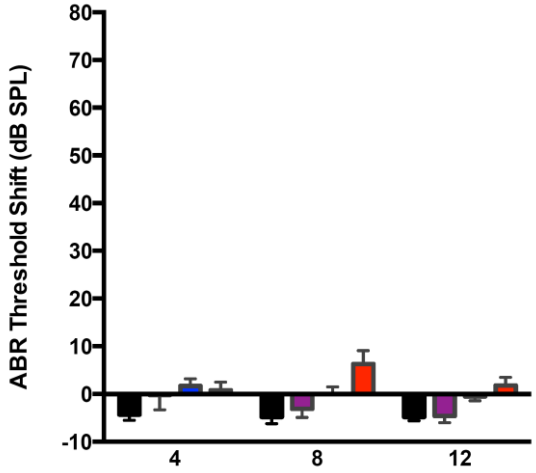


Figure 9: Right Ear Average Threshold Shifts from Baseline at 4 kHz for Control and Treated Animals at 4, 8, and 12 weeks after Single Intratympanic Dose Administration in Rats. The ABR threshold shifts are shown for each group: saline (black bars), P407 (purple bars), forced-degraded P407 type II (blue bars), and gentamycin (red bars). Values were determined at 4, 8, and 12 weeks. Data are presented as mean ± SEM. (Sponsor's Figure)

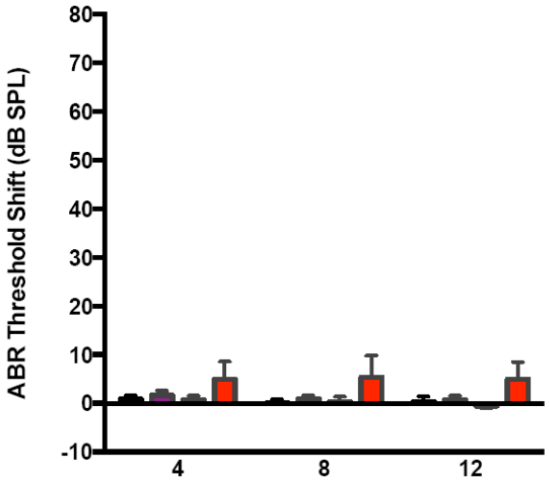


Figure 10: Right Ear Average Threshold Shifts from Baseline at 10 kHz for Control and Treated Animals at 4, 8, and 12 weeks after Single Intratympanic Dose Administration in Rats. The ABR threshold shifts are shown for each group: saline (black bars), P407 (purple bars), forced-degraded P407 type II (blue bars), and gentamycin (red bars). Values were determined at 4, 8, and 12 weeks. Data are presented as mean ± SEM. (Sponsor's Figure)

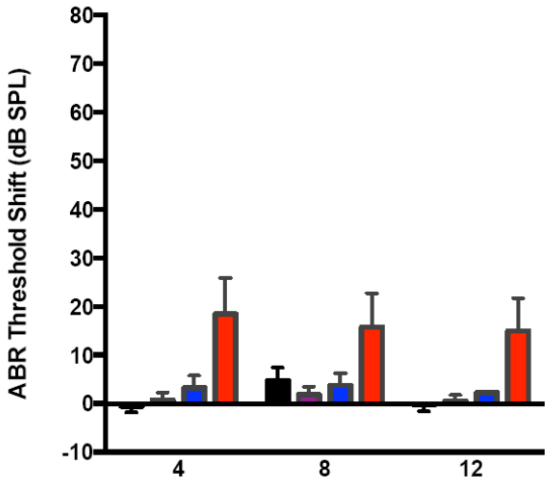


Figure 11: Right Ear Average Threshold Shifts from Baseline at 20 kHz for Control and Treated Animals at 4, 8, and 12 weeks after Single Intratympanic Dose Administration in Rats. The ABR threshold shifts are shown for each group: saline (black bars), P407 (purple bars), forced-degraded P407 type II (blue bars), and gentamycin (red bars). Values were determined at 4, 8, and 12 weeks. Data are presented as mean ± SEM. (Sponsor's Figure)

Histopathology

Adequate Battery: only cochlear and middle ear tissues were examined
Peer Review: No
Histological Findings

Middle ear histology

4-Week Recovery
Treatment with P407 (Group 2) and forced-degraded P407 (Group 3) produced microscopic findings in the middle ear that differed from treatment with saline. However, the two P407 groups did not produce changes that were substantially different from each other (Table 14). The changes were characterized by the presence of foamy macrophages in the middle ear and at the round window membrane and inflammation/fibroplasia in the middle ear.

The incidence and severity of the microscopic findings in the middle ear were greater near the ossicles, middle ear cavity lining, and tympanic cavity than at the round window injection site. Lesser effects were observed with the saline treatment which was associated with minimal foamy macrophages, minimal to mild fibroplasia at the round window injection site in 5/6 Group 1 animals (right ears) and minimal subacute/chronic inflammation in 1/6 right ears. The more severe microscopic findings in Groups 2 and 3 were considered reflective of a process of removal of the foreign material consistent with P407 having a longer residence time than saline. Also, procedure-related minimal to mild hemorrhage was observed in the middle ear and round window membrane in all groups.

The gentamicin administered ears had middle-ear changes consistent with that of an ototoxicant including increased incidence and/or severity of fibroplasia, epithelial hyperplasia, focal perforation, and subacute inflammation of the tympanic membrane.

Table 14: Microscopic Findings in Middle Ear – 4-Week Recovery. (Sponsor's Table)

Group:	1	2	3	4
Number Examined	6	6	6	6
Middle ear, right				
Fibroplasia	0	4	4	3
-minimal	0	2	2	1
-mild	0	2	2	2
Foreign material, hair/keratin				
-minimal	0	0	0	1
Hemorrhage	5	4	1	4
-minimal	3	3	1	4
-mild	2	1	0	0
Inflammation, acute				
-minimal	0	0	0	1
Inflammation, granulomatous				
-minimal	0	0	0	1
Inflammation, subacute/chronic	0	5	4	3
-minimal	0	2	1	2
-mild	0	3	3	1
Macrophages, foamy	3	6	5	5
-minimal	3	5	1	5
-mild	0	1	4	0
Multinucleated cells				
-minimal	0	1	0	0
Round window, right				
Fibroplasia				
-minimal	0	0	2	1
Hemorrhage	3	4	1	2
-minimal	3	2	1	2
-mild	0	2	0	0
Inflammation, subacute/chronic	0	4	4	1
-minimal	0	4	3	1
-mild	0	0	1	0
Macrophages, foamy	1	6	5	4
-minimal	1	6	4	4
-mild	0	0	1	0

Group 1 - Saline
Group 2 - Poloxamer 407
Group 3 - Forced-Degraded Poloxamer 407
Group 4 - Gentamicin

12-Week Recovery

At 12 weeks, middle ear findings were decreased for incidence and severity compared to the 4-week recovery findings. Animals from Groups 2 and 3 had increased numbers and/or severity of foamy macrophages in the middle ear and at the round window membrane injection site compare to saline controls. Middle ear inflammation was increased in Groups 2 and 3 compared to saline controls. However, both findings were partially recovered compared to the 4-week recovery measurements. Fibroplasia in the

middle ear was completely resolved in Groups 2 and 3 at the 12-week recovery measurement. Ototoxicity associated with gentamicin administration was also resolved at the 12-week recovery.

Table 15: Microscopic Findings in Middle Ear – 12-Week Recovery. (Sponsor's Table)

Group:	1	2	3	4
Number Examined	5	6	5	5
Middle ear, right				
Hemorrhage				
-minimal	1	0	1	0
Inflammation, subacute/chronic				
-minimal	0	2	0	0
Macrophages, foamy				
-minimal	2	6	3	1
Round window, right				
Hemorrhage				
-minimal	1	2	0	0
Macrophages, foamy				
-minimal	0	2	2	0

Group 1 - Saline
Group 2 - Poloxamer 407
Group 3 - Forced-Degraded Poloxamer 407
Group 4 - Gentamicin

Cytocochleogram Analysis

There were no effects that were considered adverse on cochlear sensory cells in the rat following a single IT-RWM injection with saline, P407, or forced-degraded P407 Type II, following recovery periods of 4 and 12 weeks with right cochleae within normal variability for scattered sensory outer hair loss. These results indicate that forced-degraded P407 had no direct treatment effect on sensory cells in the cochlea. Gentamicin had the expected ototoxic effect, with severe outer hair cell loss in all of the treated ears.

Dosing Solution Analysis

Levels of the individual (b) (4) were determined from the dosing vials immediately after administration. The results are shown below in Table 16.

Table 16: Duplicate Results for Dosing Solution Analysis. (Sponsor's Table)

Sample	Test Description	Acceptance Criteria	Post-Injection
Group B 4-8°C 11Oct13	(b) (4) Species Content	Report Results in µg/mL	(b) (4)
Group C 40°C 14Oct13	(b) (4) Species Content	Report Results in µg/mL	
Group B 4-8°C 15Oct13	(b) (4) Species Content	Report Results in µg/mL	
Group C 40°C 16Oct13	(b) (4) Species Content	Report Results in µg/mL	

6.2 Repeat-Dose Toxicity

Repeated-dose toxicology studies were not submitted with this NDA application.

7 Genetic Toxicology

Genetic Toxicology Studies were not performed with OTO-201.

The Ciprodex® and Cetraxal® product labels include the following paraphrased information regarding ciprofloxacin genetic toxicity.

Eight *in vitro* mutagenicity tests have been conducted with ciprofloxacin and the test results are listed below:

1. *Salmonella*/Microsome Test (Negative)
2. *Escherichia coli* DNA Repair Assay (Negative)
3. Mouse Lymphoma Cell Forward Mutation Assay (Positive)
4. Chinese Hamster V79 Cell HGPRT Test (negative).
5. Syrian Hamster Embryo Cell Transformation Assay (Negative).
6. *Saccharomyces cerevisiae* Point Mutation Assay (Negative)
7. *Saccharomyces cerevisiae* Mitotic Crossover and Gene Conversion Assay (Negative).
8. Rat Hepatocyte DNA Repair Assay (Positive).

Three In vivo tests have been conducted with ciprofloxacin, and the test results are listed below:

1. Rat Hepatocyte DNA Repair Assay (Negative)
2. Micronucleus Test in Mice (Negative)
3. Dominant Lethal Test in Mice (Negative)

While a summary evaluation will not be included on the product label for OTO-201, the weight of evidence based primarily on the negative results in the *in vivo* tests indicates

that ciprofloxacin is considered to have a low potential for genotoxicity when administered clinically.

8 Carcinogenicity

Carcinogenicity studies were not performed with OTO-201, and because OTO-201 is not administered in a chronic manner for 6 months or longer, carcinogenicity studies are not recommended.

The information included on the Ciprodex® and Cetraxal® product labels indicates that "Long-term carcinogenicity studies in mice and rats have been completed for ciprofloxacin. After daily oral doses of 750 mg/kg (mice) and 250 mg/kg (rats) were administered for up to 2 years, there was no evidence that ciprofloxacin had any carcinogenic or tumorigenic effects in these species."

9 Reproductive and Developmental Toxicology

Reproductive and Developmental Toxicology Studies were not performed with OTO-201 and no adequate and well-controlled studies have been performed in pregnant women. However, because of the negligible systemic exposure expected with clinical administration of OTO-201, this product is expected to be of minimal risk for maternal and fetal toxicity when administered to pregnant women**.**

Nonclinical embryo-fetal, fertility and pre-postnatal studies have been conducted with ciprofloxacin. The available data from the product labels of approved ciprofloxacin products is shown below. In general, based on body surface area comparisons, the ciprofloxacin NOAEL values from the nonclinical studies and their associated human equivalent dose (HED) values provide substantial safety margins compared to the ciprofloxacin in the proposed clinical doses of OTIPRIO (Table 17).

Regarding pregnancy and teratogenic effects, the Cipro®, Ciprodex® and Cetraxal® product labels indicate: "Pregnancy Category C: Reproduction studies have been performed in rats and mice using oral doses of up to 100 mg/kg and intravenous (IV) doses up to 30 mg/kg and have revealed no evidence of harm to the fetus as a result of ciprofloxacin. In rabbits, ciprofloxacin (30 and 100 mg/kg orally) produced gastrointestinal disturbances resulting in maternal weight loss and an increased incidence of abortion, but no teratogenicity was observed at either dose. After intravenous administration of doses up to 20 mg/kg, no maternal toxicity was produced in the rabbit, and no embryotoxicity or teratogenicity was observed."

Regarding fertility effects, the Cipro®, Ciprodex®, and Cetraxal® product labels indicate: "Fertility studies performed in rats at oral dose of ciprofloxacin up to 100 mg/kg/day revealed no evidence of impairment."

Regarding pre-postnatal effects the product label for Proquin® XR, an extended release tablet form of ciprofloxacin includes the following information: "A peri/postnatal developmental toxicity study conducted in pregnant/lactating female rats exhibited no developmental effects in offspring at the highest dose level of 600 mg/kg/day."

Table 17: NOAEL Values for Nonclinical Developmental and Reproductive Toxicology Studies and Associated Safety Margins for the Maximum Clinical Doses of Ciprofloxacin in Adults and Children Administered OTO-201.

Study Type	Species	NOAEL (mg/kg/day)	HED (mg/kg/day)[a]	Safety Margin [b]
Embryo-Fetal Toxicity	Mouse	100	8.1	14 - 41
Embryo-Fetal Toxicity	Rat	100	16.1	27 - 81
Embryo-Fetal Toxicity	Rabbit	20	6.5	11 - 33
Fertility	Rat	100	16.1	27 - 81
Pre-postnatal Toxicity	Rat	600	96.8	161 - 484

[a] The human equivalent dose (HED) values based on body surface area comparison for individual species are arrived at by dividing the NOAEL values by 12.3, 6.2, and 3.1 for mice, rats, and rabbits respectively.
[b] The maximum clinical dose of ciprofloxacin following administration of OTIPRIO to two human ears for an adult human of average 60 kg weight is 100 μl times 2 ears times 6% ciprofloxacin = 12 mg divided by 60 kg = 0.2 mg/kg. For an average child weighing an average 20 kg, the dose is 12 mg divided by 20 kg = 0.6 mg/kg.

10 Special Toxicology Studies

Study title: An Acute Dermal Toxicity Study of OTO-201 in Guinea Pigs. (Study No.: OTO-201-RSP-009)

Methods: The comparator (Cetraxal®; 0.2% ciprofloxacin) and test article (OTO-201; 6.0% ciprofloxacin) were administered once to each animal in 1 ml dose volumes by topical dermal application. The comparator and test articles were administered on Day 1 and held in contact with the skin for 24 hours.

Results: No mortality occurred, and no dermal toxicity was observed in either the Cetraxal® or OTO-201 groups.

Study title: Skin Sensitization (Buehler Method) Study of OTO-201 in Guinea Pigs. (Study No.: OTO-201-RSP-007)

Methods: During the Induction Phase, vehicle, comparator, and test article formulations were administered to the left scapular region on Days 1, 8, and 15. Animals were not treated for 2 weeks prior to the Challenge Phase. During the Challenge Phase (Day 29), comparator or test article formulations were administered once to the left flank of the same animals used in the Induction Phase (including the designated control animals). On the day after the Challenge Phase dose (at least 6 hours before the first scoring), the animals were depilated with a short application of lotion hair remover. Dermal observations and scores for erythema and edema were recorded at approximately 24 and 48 hours post-challenge.

Results: No dermal irritation indicative of skin sensitization was observed in any animal dosed with the vehicle, Cetraxal®, or OTO-201 at any strength. These results were

compared to a historical positive control, hexyl cinnamic aldehyde (HCA) which induced slight to moderate erythema in 95% of treated animals from a previous study.

Study title: Ventilation Tube Patency Following Intratympanic Administration of (b)(4)% Poloxamer 407 (b)(4) in Guinea Pigs. (Study No.: OTO-200-RSP-022-01)

Methods: Four female guinea pigs received a single intratympanic injection (50 µl) of (b)(4)% poloxamer 407 while anesthetized. Subsequently, while still anesthetized, a ventilation tube was inserted through the tympanic membrane into the middle ear. The opening of the ventilation tube was visually assessed for clogging using a surgical microscope on Days 1 and 3.

Results: The study report included two photographs taken with a surgical microscope of the ear drum and the opening of the ventilation tube. At least for the Day 3 photograph, the opening to the ventilation tube does not appear to be occluded by the poloxamer 407 (dyed with Evan's Blue dye).

Reviewer Comment: *It is difficult to assess the patency and occlusion status of the ventilation tubes from the photographs that were provided. More comprehensive information could have been provided by assessing and photographing the entire length of the tubes following termination of the study animals and tube removal.*

11 Integrated Summary and Safety Evaluation

Inactive Ingredients
Inactive ingredients in the OTO-201 preparations include tromethamine, poloxamer 407 (P407), sodium chloride, hydrochloric acid (b)(4), and water. All of the excipients other than P407 and tromethamine have been used in previously approved otic products at higher concentrations than those included in OTO-201.

P407 and tromethamine have not been used in any marketed otic products. P407 has been used in oral, topical, ophthalmic, and periodontal products at lower concentrations. The periodontal products contained (b)(4)% P407. For the purposes of this NDA application, the use of (b)(4)% P407 (b)(4) for OTO-201 is qualified by its safe use in the guinea pig and rat intratympanic dose studies and in the clinical trials supporting this NDA.

Tromethamine has been used in many different kinds of approved products including IV, IM, intrathecal, oral, and topical products. In approved IV and ophthalmic products, tromethamine is included in higher percent concentrations than in the (b)(4)% concentration included in the current product. This prior use is considered adequate to qualify the use of (b)(4)% tromethamine in OTO-201.

Pharmacokinetics
Plasma ciprofloxacin exposure was measured following single doses of OTO-201 in guinea pigs in two toxicology studies (OTO-201-RSP-008 and OTO-201-RSP-010). Minimal plasma ciprofloxacin concentrations were measured the day after OTO-201

intratympanic administration (22.9 and 33.3 ng/ml for the 6.0% OTO-201 administrations). Plasma concentrations gradually decreased to approximately 10 ng/ml after 29 days. These values are approximately 100-fold lower than clinical plasma C_{max} values associated with the administration of oral ciprofloxacin tablets (1-5 μg/ml) and approximately 10-fold higher than clinical C_{max} plasma concentrations associated with administration of Ciprodex® (0.543 ng/ml to 3.45 ng/ml). Plasma concentrations associated with the clinical administration of OTIPRIO were not measured.

The Sponsor conducted multiple pharmacokinetic studies in guinea pigs in an effort to characterize middle and inner ear concentrations of ciprofloxacin under varying conditions. These studies examined variations associated with different ciprofloxacin concentrations in OTO-201 preparations, variable OTO-201 injection volumes, and OTO-201 injections in wet middle-ear conditions. In addition, the middle ear pharmacokinetics of Ciprodex® alone and in the presence of (b)(4)% P407 was examined. Several patterns were apparent in the findings.

The middle ear C_{max} levels of ciprofloxacin did not vary greatly with increasing concentrations of ciprofloxacin (0.6, 2.0, 6.0, and 12.0%) in OTO-201 preparations. However AUC and mean residence time increased with dose although in a less than dose-proportional manner. Inner ear concentrations followed a similar pattern in terms of retention time, but C_{max} values were much less (up to 25-fold less) than the middle ear C_{max} values. Thus the highest concentrations of ciprofloxacin occurred in the middle ear following intratympanic injection of OTO-201. The results indicate that maximum middle ear concentrations of ciprofloxacin are not greatly increased by increasing the dose (ciprofloxacin concentration) of OTO-201, but higher ciprofloxacin concentrations are maintained longer depending on the dose of OTO-201.

Other variables were shown to influence middle ear C_{max} and AUC values following intratympanic administration of OTO-201. In guinea pigs, ciprofloxacin C_{max} values increased with dose volume, but the effect of dose volume on the overall ciprofloxacin elimination profile was less pronounced suggesting dose volume will not greatly affect ciprofloxacin retention in the middle ear. The human-dose volume will be 100 μl per injection. Increases in middle ear AUC and retention time were observed in guinea pig "wet ears" for the 2.0% ciprofloxacin concentration in OTO-201 compared to the same dose in dry guinea pig ears. Because post-surgical otorrhea occurs at a high frequency in the ears of patients receiving tympanostomies, the guinea pig "wet ear" model probably simulates the actual disease conditions best.

The middle ear C_{max} concentrations of ciprofloxacin that resulted following a single dose or a 7-day course of BID Cetraxal® were similar to those produced by Ciprodex Otic® (single dose or 7 day course), approximately 25 μg/ml, and much lower than the C_{max} middle ear concentrations of ciprofloxacin associated with a single dose of 6% OTO-201, 90-100 μg/ml. However, inner ear perilymph concentrations of ciprofloxacin produced by a single intratympanic administration of Cetraxal® was similar to that produced by a single administration of 6% OTO-201 with both values equal to approximately 7-8 μg/ml. This is an interesting comparison, because in two of the

toxicology studies, intratympanic Cetraxal® produced mild to moderate increases in ABR values (hearing loss) as well as mild to moderate inner-ear hair cell loss in one study, while 6% OTO-201 was never associated with inner-ear hair loss, and was associated with moderately increased ABR values in only one study. Because Cetraxal® is administered for 7 days, the persistent inner-ear exposure to daily C_{max} concentrations of ciprofloxacin is prolonged compared to the inner-ear C_{max} concentrations associated with the single administration of 6% OTO-201 where inner-ear ciprofloxacin concentrations rapidly dissipate from the C_{max} concentration. This difference in the duration of inner-ear exposure may explain the less pronounced inner-ear toxicity produced by 6% OTO-201 in the guinea pig studies.

The site of action for ciprofloxacin is bacteria colonizing the middle-ear epithelium. In contrast to the middle-ear lumen, ciprofloxacin C_{max} values for the middle-ear epithelium for a single dose of 6% OTO-201 were approximately 4-fold higher and followed a dose-responsive pattern. Retention of ciprofloxacin in the middle-ear epithelium appeared slightly increased compared to the middle-ear lumen even at the lowest ciprofloxacin dose concentration of 0.6% suggesting that prolonged retention may be accentuated in middle ear epithelial tissue. In contrast middle-ear epithelium concentrations of ciprofloxacin following a single dose of Cetraxal® were approximately 5-fold less than middle-ear lumen concentrations following Cetraxal® administration and 100-fold less than the maximal epithelial concentrations of ciprofloxacin that occurred after 6% OTO-201 administration.

In order to achieve a ciprofloxacin therapeutic effect over a therapy period of one to two weeks, but minimize the potential for ciprofloxacin-related toxicity associated with prolonged middle-ear retention of high ciprofloxacin concentrations, an optimal ciprofloxacin concentration in OTO-201 is desirable, and the Sponsor determined this concentration to be 6.0% in humans. The guinea pig studies suggest that 6.0% concentrations of ciprofloxacin in OTO-201 result in moderate concentrations (approximately 8 μg/ml) of ciprofloxacin remaining in the middle ear 21 days after intratympanic injection. This concentration is somewhat higher than the approximately 3 μg/ml middle-ear concentrations of ciprofloxacin that resulted 21 days after the administration of Ciprodex® or Cetraxal® over a 7-day course of treatment in guinea pigs.

In a study where (b) (4) OTO-201, (b) (4)% P407, was administered alone, large amounts of P407 (approximately (b) (4)) were detected in the middle ear one hour after a (b) (4) injection of (b) (4)% P407, but levels rapidly decreased to approximately (b) (4) on Day 7 and (b) (4) on Day 14. Despite this pattern of rapid reduction of P407 in the middle ear, when ciprofloxacin was suspended in (b) (4)% P407 in OTO-201 in guinea pig studies, moderate concentrations (approximately 8-10% of C_{max}) of ciprofloxacin were found in the middle ear 21 days after injection suggesting P407 aids retention of middle-ear ciprofloxacin.

If treatment with a single intratympanic injection of 6% OTO-201 in patients is not effective, additional treatment with Ciprodex® may be prescribed. In a study where a 7-

day course of Ciprodex® was initiated 3 days after a single intratympanic dose of 2 or 6% OTO-201, C_{max} and trough levels for ciprofloxacin were increased relative to administration of Ciprodex® alone. In the combination study, ciprofloxacin C_{max} values were higher (≈ 94 μg/ml for 6% OTO-201 plus Ciprodex®) than the C_{max} ciprofloxacin values for Ciprodex® alone (C_{max} of ≈ 23 μg/ml), but similar to the middle ear C_{max} concentrations that occurred with a single injection of 6% OTO-201 in other studies (80-100 μg/ml). Trough ciprofloxacin values were substantially increased for the combination (≈ 57 μg/ml for 6% OTO-201 plus Ciprodex®) compared to Ciprodex® alone (≈5-9 μg/ml). These results indicate that the combination treatment with OTO-201 and Ciprodex® does not lead to higher middle-ear C_{max} concentrations for ciprofloxacin compared to a single injection of OTO-201 alone. However, the combination treatment can contribute to higher trough middle- ear ciprofloxacin concentrations thus providing higher sustained ciprofloxacin concentrations in the middle ear than when either treatment is applied individually.

A somewhat different pattern was apparent when (b)(4)% P407 was administered in a single intratympanic injection 3 days before a treatment course with Ciprodex®. In this study, C_{max} concentrations of ciprofloxacin (C_{max} of ≈ 67 μg/ml) were approximately 3-fold higher than C_{max} ciprofloxacin values when Ciprodex® was administered without co-administration of (b)(4)% P407 (C_{max} of ≈ 23 μg/ml). However, unlike when OTO-201 and Ciprodex® were combined, trough levels of ciprofloxacin measured daily during the 7-day course of Ciprodex® remained similar in the presence and absence of (b)(4)% P407 co-administration (≈ (b)(4)). These results suggest that co-administration of (b)(4)% P407 increases C_{max} ciprofloxacin levels in the middle ear, but has little effect on the persistence of ciprofloxacin administered in a 7-day course of Ciprodex®.

General and Otic Toxicology

Toxicologically relevant changes in systemic toxicity endpoints, including clinical signs, hematological and clinical chemistry parameters and organ weight measurements did not occur in the single-dose studies. This is consistent with the very low plasma concentrations of ciprofloxacin that resulted following single intratympanic injections. The highest plasma concentrations of 20-33 ng/ml occurred 24 hours after intratympanic dosing with 6% OTO-201. In comparison, C_{max} plasma concentrations of ciprofloxacin associated with clinical oral administration of 250 to 1000 mg of ciprofloxacin are reportedly much higher, in the 1-5 μg/ml range. Systemic effects that did occur were limited to slightly decreased body weights associated with administration of 6% OTO-201 in females in one study.

In addition to analysis of systemic toxicity parameters, several otic toxicity parameters were examined in the single-dose toxicology studies. These parameters included auditory brain stem responses (ABR), middle ear gross pathology and histopathology, measurements of middle ear ossicle immobility, and inner ear histopathology to detect hair-cell loss and cochlear toxicity.

In the four studies involving single intratympanic injections of (b)(4)% P407 and OTO-201, no changes in ABR or cochlear toxicity were observed to occur as a result of (b)(4)% P407

administration. In two of these studies (OTO-201-RSP-008 and OTO-201-RSP-010), the P407 was [redacted], a process associated with increased levels of [redacted] while in the two other studies (OTO-201-RSP-036 and OTO-201-RSP-037) the P407 was [redacted], the planned [redacted] method for P407 in the marketed product. In two guinea pig studies associated with another drug candidate also including the [redacted]% P407 vehicle, the [redacted] P407 vehicle was shown to produce hearing loss (increased baselines for ABR values at three frequencies) and cochlear toxicity (inner-ear sensory hair loss) in association with [redacted] produced via oxidative degradation of P407. However, in these studies the intratympanic injections were directed to the round window niche suggesting facilitated access to the inner ear. In fact, histological analysis suggested the round window membrane was disrupted in guinea pigs allowing unimpeded access for the injected substances to the inner ear. In the studies associated with OTO-201, the intratympanic injections were directed anterior to the round window membrane, and [redacted]% P407, even when [redacted], was not associated with cochlear toxicity. Also, in a later study in rats (OTO-201-RSP-086) where a single intratympanic injection into the round window niche did not disrupt the round window membrane, [redacted]% P407 did not cause cochlear toxicity or hearing loss. The results from the guinea pig studies suggest [redacted] degradants of P407 can cause hearing loss and cochlear toxicity, but also that the placement of the intratympanic injections is a primary determinant of whether toxicity will occur. Because [redacted] are associated with cochlear toxicity, their levels should be monitored in batches of OTO-201 drug product, and the acceptance criteria for each specific [redacted] and the total [redacted] will be determined by the highest [redacted] levels that were not associated with ototoxicity in nonclinical intratympanic-injection studies.

Ototoxicity that occurred with OTO-201 administered in the absence of concomitant Ciprodex Otic® in guinea pig intratympanic injection studies was limited to middle ear inflammation as well as moderate hearing loss for guinea pigs receiving a single intratympanic injection of 6% OTO-201 in one study (OTO-201-RSP-036), but not in two others (OTO-201-RSP-008 and OTO-201-RSP-035). The hearing loss was indicated by threshold shifts at three wavelengths in ABR analysis. The ABR threshold shifts produced by 6% OTO-201 in Study No.: OTO-201-RSP-036 was similar to that produced by 7 days of BID dosing with Cetraxal® in the same study, but Cetraxal® administration was associated with cochlear damage, while 6% OTO-201 was not. In all of the studies with single-dose administrations of OTO-201, findings of fluid granulomatous inflammation, foamy macrophages, limited fibroplasia and foreign material in the middle ears occurred in a dose-dependent manner with greater severity and incidence for 6% OTO-201 compared to the saline and [redacted]% P407 controls. These findings were considered to be consistent with a foreign body reaction with the foreign substance possibly being ciprofloxacin particulates. These results were identified 4-weeks after the intratympanic injection in the guinea pig studies, and a recovery study was not included in these studies. However, in a single intratympanic-dose study in rats (Study No.: OTO-104-RSP-086), similar findings substantially resolved after a 12-week recovery period.

In two of the single intratympanic dose studies (Study Nos.: OTO-201-RSP-008 and OTO-201-RSP-036) in guinea pigs where OTO-201 or Cetraxal® was administered without concomitant Ciprodex®, the middle ear temporal bones were removed at the end of the experiment and middle ear-ossicle immobility was assessed. In the first of these studies, single doses of 6% OTO-201 were associated with ossicle immobility in 4/20 ears, and 1/20 ears in the second study. These values were greater than those for the saline and the (b) (4)% P407 control groups and for the lower concentration doses (0.6 and 2.0%) of OTO-201, but about the same as for 7 days of dosing with Cetraxal® which was associated with ossicle immobility in 2/20 ears in the first study and 3/20 ears in the second study. In the single study where it was measured (Study No.: OTO-201-RSP-008), a 7-day course of Ciprodex® treatment resulted in ossicle immobility in 3/20 ears.

When Cetraxal® and Ciprodex® were administered in drops through tympanic tubes BID for 7 days, resulting measurements of otic toxicity were generally increased relative to those induced by 6% OTO-021 administration. Cetraxal® produced mild (only in males) or moderate (both sexes) increases in ABR values (hearing loss) in the two studies where it was examined as well as a corresponding mild to moderate hair cell loss in the inner ear in one of the studies. All of the groups receiving Ciprodex® administered in combination with a range of treatments including saline, P407, and different concentrations of OTO-201 in Study Nos.: OTO-201-RSP-010 and OTO-201-RSP-037 consistently demonstrated the same degree of mild to moderate elevations in ABR values (hearing loss) and mild to moderate inner-ear hair loss. Because the same effects were seen despite variable treatment combinations with Ciprodex®, the effects can be attributed to Ciprodex®.

Genetic Toxicity

According to the product labels for approved ciprofloxacin products, ciprofloxacin has been shown to be both positive (mouse lymphoma cell forward mutation assay, rat hepatocyte DNA repair assay) and negative (*Salmonella*/microsome test, *Escherichia coli* DNA repair assay, Chinese hamster V79 cell HGPRT test, Syrian hamster embryo-cell transformation assay, *Saccharomyces cerevisiae* point mutation assay, *Saccharomyces cerevisiae* mitotic-crossover and gene-conversion assay) for mutagenesis in a variety of *in vitro* genotoxicity assays. However, in three *in vivo* assays (rat hepatocyte DNA-repair assay, micronucleus test in mice, dominant-lethal test in mice), ciprofloxacin was determined to be negative for mutagenicity and clastogenesis. Thus the weight of evidence suggests ciprofloxacin has a limited potential to produce genotoxicity in humans in association with its approved administration.

The vehicle for OTO-201, P407 is a component in several approved products. However, information regarding the genotoxicity potential of P407 has not been characterized. P407 rapidly forms a gel (b) (4)C, and this attribute restricts its systemic distribution when administered intratympanically. Its characteristic gel formation at (b) (4)C also restricts easy evaluation of P407 in traditional genotoxicity assays. For the purposes of this NDA, the restricted systemic exposure, single-dose application, and prior use in previously approved products supports the safety of P407 for genotoxicity.

Reproductive and Developmental Toxicity
Because of the negligible systemic exposure expected with clinical administration of OTO-201, this product is expected to be of minimal risk for maternal and fetal toxicity when administered to pregnant women**.** Also in nonclinical embryo-fetal, fertility, and pre-postnatal studies, ciprofloxacin was not associated with significant adverse effects. The NOAEL ciprofloxacin values and equivalent HED in the nonclinical studies provide substantial safety margins compared to the maximum human dose of ciprofloxacin in OTIPRIO based on whole body surface-area comparisons. The summation of these factors: limited clinical ciprofloxacin exposure with OTIPRIO, no observations of teratogenicity or other adverse effects for ciprofloxacin in nonclinical studies, and substantial safety margins for the NOAEL ciprofloxacin values compared to the maximum clinical dose of ciprofloxacin in OTIPRIO supports the conclusion that OTIPRIO poses minimal risk for reproductive and developmental toxicity.

Safety Evaluation
In nonclinical studies in guinea pigs, a single intratympanic dose of 6% OTO-201 provided similar if not better middle-ear exposure to ciprofloxacin compared to two other ciprofloxacin-containing otic products, Ciprodex® and Cetraxal®. Also the otic toxicity associated with administration of 6% OTO-201 in guinea pigs was consistently no worse and generally better than the toxicity profiles for Ciprodex® and Cetraxal® as indicated by otic toxicity endpoints including hearing loss (ABR elevations) and cochlear hair-cell loss. Based on these results, and the low potential for systemic toxicity, genotoxicity, and reproductive and developmental toxicity, OTIPRIO is considered approvable from a Pharmacology/Toxicology perspective.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES S WILD
11/23/2015

WENDELYN J SCHMIDT
11/24/2015
i concur with Dr. Wild's assessment of the completeness and interpretation of the data. I agree that this NDA can be approved from the pharmacology/toxicology perspective.

MEMO: IND 110244, OTO-201 for the Intra-Operative Treatment of Middle Ear Effusion in Pediatric Subjects Requiring Tympanostomy Tube Placement. Sponsor: Otonomy Inc.

SUBMISSION DATE:	**November 2, 2012**
TO:	**Jane Dean, Project Manager, DAIP**
FROM:	**James Wild, Ph.D., Pharmacology Reviewer, DAIP**
THROUGH:	**Wendelyn Schmidt, Ph.D., Supervisory Pharmacology Reviewer, DAIP**
RE:	**Remove from Clinical Hold Response**

BACKGROUND

OTO-201 is a suspension of ciprofloxacin (b) (4) containing (b) (4)% poloxamer 407, a glycol polymer. OTO-201 exists as a liquid at room temperature and gels immediately upon transitioning to body temperature. The drug product is packaged in a sterile vial for injection.

The original IND 110244 for OTO-201 (ciprofloxacin in (b) (4)% poloxamer 407) was submitted on 8/26/2011. Based on the data submitted, no holds were placed on the initial clinical study, but design modifications were requested, and the clinical study design was modified. Another IND ((b) (4)) submitted (b) (4)

On November 23, 2011, in conjunction with IND (b) (4), but also referenced for IND 110244, the Sponsor submitted new nonclinical data that included description of cochlear toxicity associated with the (b) (4) form of the (b) (4)% poloxamer 407 (b) (4) in two guinea pig studies where injections were directly into the round window membrane (RWM). This toxicity was not noted for the original (b) (4) form (b) (4) However, the Sponsor had intended to use the new (b) (4) formulation in their planned clinical studies as the vehicle for the OTO-201 (b) (4) .

Based on the data from the two guinea pig studies, the Sponsor, in a 11/4/2011 letter submitted to IND (b) (4) IND 110244, indicated the following:

"Based on these findings, we have made the decision to suspend screening in…the proposed Clinical Study 201-201101 (IND 110,244) entitled 'A Prospective, Randomized, Double-blind, Placebo-controlled, Multicenter, Phase 1B Study of OTO-201 Given as a Single Intratympanic Injection for Intra-Operative Treatment of Middle Ear Effusion in Pediatric Subjects Requiring Tympanostomy Tube Placement.' Otonomy is thoroughly investigating the findings from this GLP toxicology study and planning additional investigations to further elucidate the observations. Otonomy will provide a complete analysis of these data to the Agency when available. No sites have been initiated for Study 201-201101, and no sites have received study drug. Therefore no subjects have been exposed to study drug (Ready to Dilute OTO-201). Until the findings from the toxicology study are clarified with the Agency, no subjects will be enrolled in any clinical studies of OTO-201 in the US."

It should be noted that the location of administration also seemed to affect the toxicity potential of P407 (b) (4) and OTO-201 (b) (4). In two previous guinea pig toxicology studies (OTO-201-RSP-008 and OTO-201-RSP-010), single doses of P407 (b) (4) and OTO-201 (b) (4) injected anterior to the round window membrane (ANT) did not produce ototoxicity beyond that seen with the approved dose of Cetraxal® and did not produce additive toxicity when co-administered with Ciprodex®.

In their 11/23/2011 submission, the Sponsor summarized their plan of action to better understand and characterize the toxicity potential of different preparations of P407 as follows:

"Otonomy is currently conducting an extensive cross-functional investigation into the toxicology findings reported above. First, P407 (b) (4) and P407 (b) (4), as well as several other differently processed P407 formulations, are being subjected to extensive analytical characterization to discern chemical differences. Second, we have developed *in vitro* and *in vivo* screening systems that may provide information as to the topical toxicity potential of differently processed P407 formulations. Finally, a large non-GLP study in guinea pigs given repeated intratympanic injections of P407 (b) (4), P407 (b) (4) or several other differently processed P407 formulations at different dosing intervals is also underway to confirm these findings and better understand the in vivo toxicity profiles."

A full clinical hold was placed on IND 110244 on 12/13/2011. In response to the Sponsor's plan of action, the Agency Pharmacology/Toxicology comments were as follows:

"We agree that your plan appears to be appropriate for characterizing the chemical entities underlying the P407-related ototoxicity and for providing information useful in determining which types of P407 processing are associated with ototoxicity. Please include negative (saline) and positive (chemicals or drugs known to produce cochlear hair cell loss) controls in the in vitro and in vivo studies where appropriate."

Preliminary results included in the materials submitted by the Sponsor in their 5/4/2012 Request of Comments and Advice suggest that (b) (4) treatment of P407 under (b) (4) conditions results in the production of degradation products ((b) (4) and (b) (4) that can contribute to ototoxicity in guinea pigs. The Sponsor has developed an alternative processing method (b) (4) for P407 and OTO-201. In preliminary studies, the (b) (4) preparations did not appear to produce biologically relevant ototoxicity in guinea pigs. Based on these results, the Sponsor intends to use the (b) (4) process to manufacture OTO-201 and P407 vehicle for several proposed non-GLP and GLP toxicology studies to support removal of the full clinical hold on IND 110244.

FORMULATION

Previously, OTO-201 Drug Product and Diluent were manufactured via a process including (b) (4). Products prepared with this process have been referred to as (b) (4). However, poloxamer 407 vehicle sterilized with this process was shown to produce substantial cochlear toxicity when injected into the round window membrane in two guinea pig studies (OTO-104-RSP-024 and OTO-104-RSP-025) submitted in association with

IND [redacted]. Subsequently, the [redacted] process was shown to produce degradation products including [redacted] and [redacted] which were thought to mediate the cochlear toxicity (Table 1, Figure 1).

To control the degradation of the poloxamer material, a manufacturing process using [redacted] of the poloxamer, ([redacted] process) was developed. This new sterilization process was shown to produce much less of the degradation products (Table 2, Figure 1) which remained low for up to 6-months under the recommended storage conditions of 2-8°C. At higher storage temperatures [redacted] and [redacted] increased in the [redacted] solutions although the levels were still lower that those observed in the [redacted] solution, indicating that degradant formation was temperature dependent.

The results suggest that the [redacted] process for the OTO-201 drug product and diluent sterilization limits the degradation previously associated with use of the [redacted] process resulting in lower levels of the impurities ([redacted] and [redacted] thought to be associated with cochlear toxicity for products stored at 2-8°C.

Table 1: Analytical Degradant Concentration in OTO-201-[redacted] stored at 2-8°C. (Sponsor's Table).

Formula	**OTO-201-[redacted] Diluent**			
Lot Number	**045-71**			
Dosage Strength	**0 mg/mL**			
Manufacturer	**Otonomy Pharmaceutical Development**			
Date of Manufacture	**March 6, 2012**			
Drug Substance Lot	**N/A**			
Use	**Research and Development Stability Studies: OTO-104-RSP-033**			
Tests	**Time Point (Months)**			
	0	**1**	**3**	**6**
[redacted]	[redacted]			
[redacted]	[redacted]			
[redacted]	[redacted]			
[redacted]	[redacted]			

Table 2: Analytical Degradant Concentration in OTO-201-(b) (4) stored at 2-8°C. (Sponsor's Table)

Formula	OTO-201-(b) (4) Diluent			
Lot Number	045-53			
Dosage Strength	0 mg/mL			
Manufacturer	Otonomy Pharmaceutical Development			
Date of Manufacture	March 6, 2012			
Drug Substance Lot	N/A			
Use	Research and Development Stability Studies: OTO-104-RSP-033			
Tests	Time Point (Months)			
	0	1	3	6
(b) (4) ontent (µg/mL)				(b) (4)
ontent (µg/mL)				
Content (µg/mL)				
[a] (µg/mL)				

[a]All measurements below the limit of quantification.

(b) (4)

Figure 1: (b) (4) Acetaldhyde and (b) (4) in OTO-201 Drug Product and Diluent Stored for Up to 6 Months at 2-8°C and 25°C. (Sponsor's Figure)

According the latest Sponsor information, the current manufacturing process for OTO-201 Drug Product and vehicle consists of the following four principal steps:

In section 3.2.P.5.4 of the 11/2/2012 (b) (4), the Sponsor submitted batch analyses for the clinical drug product and vehicle batch to be used in the proposed clinical study (Protocol No.: 201-201101) and similar (b) (4) batches that were used in the latest nonclinical toxicology studies (OTO-201-RSP-036 and OTO-201-RSP-037). The batch analysis profiles indicate that the (b) (4) levels in the clinical and nonclinical batches are similarly low (Appendix 1). The (b) (4) levels present in the (b) (4) drug product and diluent are qualified for initial clinical use by their safe use in the nonclinical studies.

CLINICAL PLAN

Study Title: **A Prospective, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Phase 1B Study of OTO-201 Given as a Single Intratympanic Injection for Intra-Operative Treatment of Middle Ear Effusion in Pediatric Subjects Requiring Tympanostomy Tube Placement.** Protocol No.: 201-201101

Study Population: The study population will be composed of male and female patients of ages 6 months to 12 years with a clinical diagnosis of bilateral middle ear effusion requiring tympanostomy tube placement.

Dosing regimen: Eligible subjects will be randomized to either OTO-201 or placebo in a 2:1 ratio. Two dose levels of OTO-201 will be evaluated and the cohorts will be stratified by age with cohort 1 composed of subjects 6 months to 2 years old and cohort 2 composed of subjects older than 2 years.

Cohort 1:

1. OTO-201 4 mg; single 200 µL intratympanic injection to each ear of 2% OTO-201.
2. Placebo (Vehicle) for OTO-201; single 200 µl intratympanic injection to each ear.

Cohort 2:

1. OTO-201 12 mg; single 200 µl intratympanic injection to each ear of 6% OTO-201.
2. Placebo (Vehicle) for OTO-201; single 200 µl intratympanic injection to each ear.

Subjects with visible otorrhea in the auditory canal on external examination by the blinded investigator will be considered treatment failures if otorrhea is observed on or after 3 days post-surgery (Day 4) through Day 15. Treatment failures will be eligible for rescue medication and will be treatment failures for the remainder of the study. The rescue treatment for subjects who fail study drug due to ottorhea is Ciprodex®. Enrollment for the second cohort is scheduled to begin when at least 12 subjects in the 4 mg dose cohort have completed the Day 8 visit and no safety issues have been identified.

Reviewer Comment: *Significantly, the latest written version of Protocol No.: 201-201101 indicates on page 28 in Section 6.3 Drug Study Administration that the recommended injection procedure for intratympanic administration of OTO-201, placebo or sham includes the following "Using a tuberculin syringe, direct 200 µL of OTO-201 or placebo anterior and inferior to the myringotomy site through the open tympanic membrane." This procedural direction stipulates anterior placement of the injection rather than injection into the round window niche which in animal studies has been shown to correlate with increased hearing loss and inner ear damage.*

PREVIOUS CLINICAL EXPERIENCE

The vehicle for OTO-201 ((b) (4) poloxamer 407) was recently tested in a clinical trial (Study No.: (b) (4)) for a different investigational product (b) (4) (IND (b) (4)), which is also administered by intratympanic injection into the middle ear. In this study, subjects received a single intratympanic injection of either vehicle ((b) (4) poloxamer 407) or one of two doses of (b) (4) in the affected ear. Subjects were followed for 3 months post injection. A total of 14 patients were administered vehicle. All enrolled subjects completed the study. There were no deaths on study, no serious adverse events and no adverse events of interest associated with a single intratympanic injection of the poloxamer 407 vehicle. There were no adverse findings based on laboratory measurements, physical examination, vital signs, or ECG.

Reviewer Comment: *Significantly, audiograms were conducted on all the study subjects multiple times during the study and no vehicle-related adverse events associated with altered audiometry were noted.*

NONCLINICAL

Title: Pharmacokinetics of sequential administration of OTO-201 and Ciprodex Otic in guinea pigs. (Study No.: OTO-201-RSP-033-01)

Objective: The aim of this study was to determine the pharmacokinetic profile of ciprofloxacin in the middle ear compartment following sequential administration of OTO-201 (vehicle, 2.0 and 6.0%) given as a single intratympanic injection (IT-ANT) and Ciprodex Otic® (BID for 7 days) administered to the middle ear through a tympanostomy tube.

Methods
Female guinea pigs received a single IT-ANT injection of vehicle or OTO-201 (2.0 and 6.0%) followed 3 days later by either a single dose of Ciprodex Otic® or 7-days administration of Ciprodex Otic®. Animals were observed for 6 hours following the single dose of Ciprodex Otic® and for up to 28 days following the treatment course of Ciprodex Otic®. The middle ear lavage procedure involved removal of the lower anterior and posterior portion of the tympanic membrane and lavage with a syringe with 100 µl of sterile water two times. Lavage fluids were collected and analyzed using HPLC with a limit of detection of 100 ng/ml.

Results
In the presence of a single IT-ANT administration of P407 vehicle administered 3 days prior, a single administration of CIPRODEX Otic® resulted in approximate levels of 55 µg/ml one hour after Ciprodex Otic® administration with rapid decrease to approximately 8 µg/ml after 6 hours. Repeated administration of Ciprodex Otic® did not result in middle-ear accumulation of ciprofloxacin with trough drug levels of approximately 8 µg/ml occurring on the first day of Ciprodex Otic® administration as well as on Day 10 (one day after the end of Ciprodex Otic® administration). Drug levels gradually diminished to below quantification limits on Day 21 (Figure 2).

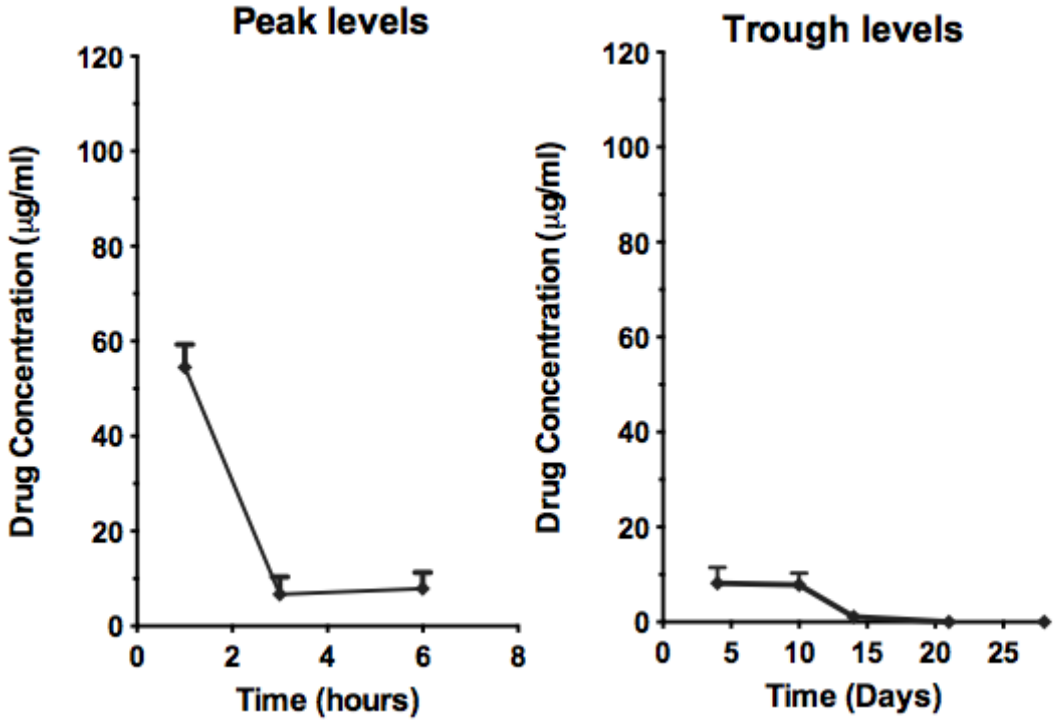


Figure 2: Middle Ear Ciprofloxacin Levels Following Administration of Ciprodex Otic® drops 3 days post IT-ANT injection of P407 Vehicle. (Sponsor's Figure)

Reviewer Comment: *C_{max} middle ear drug levels were not measured following repeated administration of Ciprodex Otic®. Thus it is not clear if the C_{max} levels increased with repeated dosing. However, the similar trough levels noted one day after initiation and one day after*

cessation of Ciprodex Otic® administration suggest unchanging patterns of daily Ciprodex Otic® accumulation and elimination.

A single IT-ANT administration of OTO-201 (2.0 and 6.0%) yielded significant ciprofloxacin levels in the middle ear peaking at approximately 91 and 104 µg/ml respectively on Day 1, and levels were similar on Day 3 (≈ 84 and 108 µg/ml respectively). These results are shown in Figure 3 (trough levels) as the Day 1 and Day 3 trough levels.

Reviewer Comment: *These results are similar to those shown in a previous study (Study No.: OTO-201-RSP-002-01) where single IT injections of 2.0 and 6.0% OTO-201 resulted in respective middle ear levels of ≈96 and 92 µg/ml on Day 1 and 35 and 100 µg/ml on Day 3. The only clear difference is that the Day 3 middle ear levels of ciprofloxacin decreased more dramatically for the 2.0% OTO-201 concentration in the earlier study compared to the present study.*

In the presence of OTO-201 (2.0 and 6.0%) administered 3 days prior, a single administration of Ciprodex Otic® administered on Day 4 resulted in lower peak levels than those measured for OTO-201 alone on Days 1 and 3 (Figure 3, peak levels). Drug levels peaked at 3 hours ranging from ≈ 75 to 94 µg/ml and decreased to ≈48 to 57 µg/ml by 6 hours. Repeated administration of Ciprodex Otic® (BID for 7 days) beginning on Day 4 following a single IT-ANT injection of OTO-201 (2 and 6 %) on Day 1, did not result in accumulation of middle ear ciprofloxacin with trough levels remaining relative static at respective levels of ≈28 and 57 µg/ml on Day 4 and ≈ 29 and 55 µg/ml on Day 10 (Figure 3, trough levels). Following completion of the Ciprodex Otic® treatment, middle ear ciprofloxacin levels gradually decreased to ≈ 4 and 12 µg/ml respectively on Day 28.

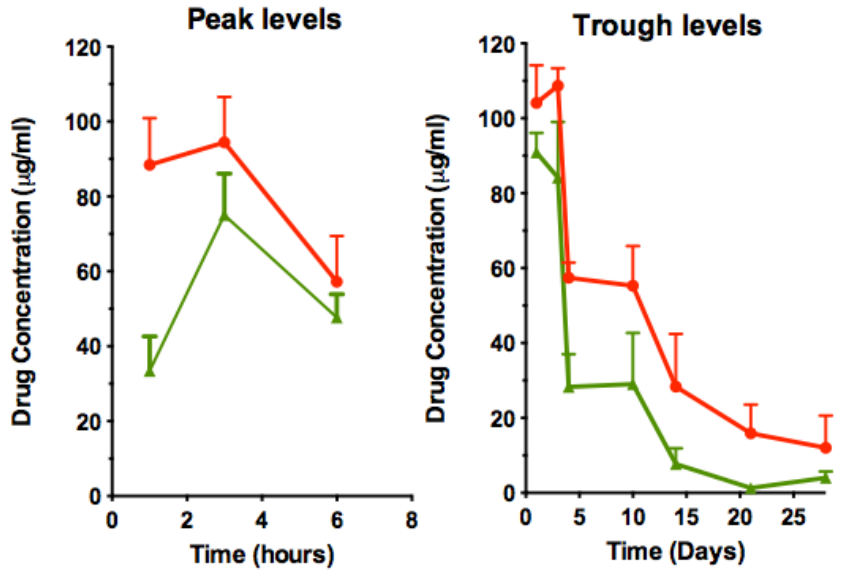


Figure 3: Middle Ear Ciprofloxacin (Peak and Trough Levels) Following Administration of Ciprodex Otic® Drops Three Days after IT-ANT Injections of 2.0% (Green Triangles) and 6.0% (Red Circles) OTO-201. (Sponsor's Figure)

Reviewer Comment: *C_{max} middle ear drug levels were not measured following repeated administration of Ciprodex Otic®. Thus it is not clear if the C_{max} levels increased with repeated dosing. However, the similar trough levels measured throughout Ciprodex Otic® administration suggest an absence of ciprofloxacin accumulation.*

Title: Ototoxicity and pharmacokinetic evaluation of OTO-201 following a single administration in guinea pigs (Study No.: OTO-201-RSP-035-01).

Methods

For this study, all of the P407 and OTO-201 preparations were sterilized using the (b) (4) technique. Female guinea pigs (n = 8/group) received a single bilateral intratympanic (IT) injection of 50 µl of saline, poloxamer 407 (P407), 2 and 6% OTO-201 or gentamicin to the anterior quadrant of the tympanum (ANT; directed away from the round window niche). Animals were followed for 28 days with auditory brainstem response (ABR) assessments performed on Days 7 and 28.

Other female guinea pigs (n = 4/time point) receiving 2 or 6% OTO-201 also underwent a terminal middle ear lavage on Days 1, 3, 7, 14, 21, and 28. Two 100 µl middle ear lavages were performed and total wash volumes of 200 µl containing washes, gel, and fluid were collected in a single tube for analysis. Ciprofloxacin concentrations were determined using a HPLC method with a limit of detection of 100 ng/ml.

Results

Single IT-ANT injections of (b) (4) P407, and 2 and 6% OTO-201 did not substantially affect auditory function in guinea pigs as assessed with ABR in three frequencies (4, 10, and 20 kHz). The positive control ototoxicant, gentamicin produced substantial hearing loss across all three frequencies (Figure 4).

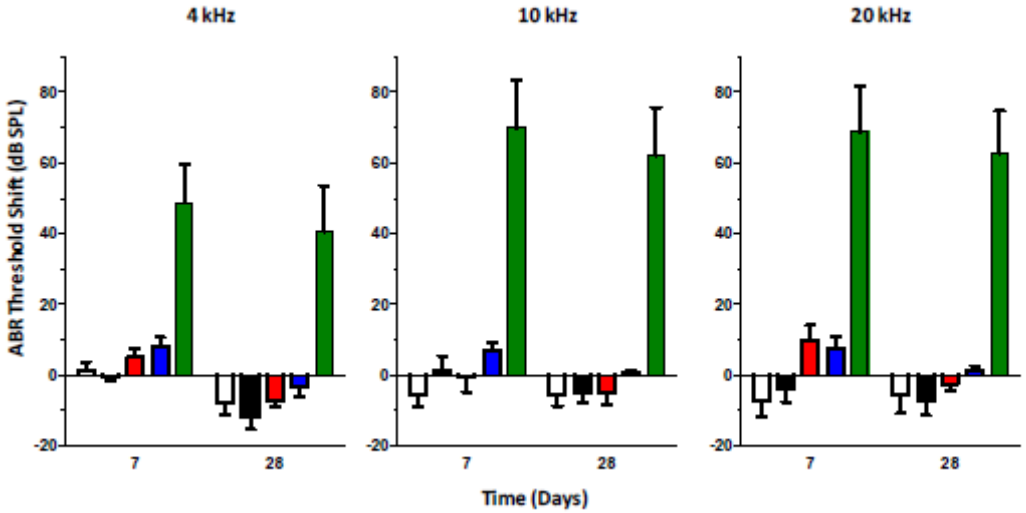


Figure 4: ABR measurements on Days 7 and 28 at three different wavelengths (4, 10, and 20 kHz) in guinea pigs (n = 8 ears) following single IT-ANT injections on Day 0. Treatment groups were saline (white bars), (b) (4) P407 (black bars), 2% (b) (4) OTO-201 (red bars), 6% (b) (4) OTO-201 (blue bars) and 20 mg gentamicin (green bars). Values are expressed as mean ± SEM. (Sponsor's Figure)

Substantial middle ear concentrations of ciprofloxacin were measured following a single IT-ANT administration of 2 and 6% OTO-201 (Figure 5, Table 3). C_{max} levels were similar between groups and also similar to those observed following IT-RWM injection of 2% (97 µg/ml) and 6% (92 µg/ml) OTO-201 in a previous pharmacokinetic study (OTO-201-RSP-002-01). Middle ear half-life values were long, on the order of 100 hours, and similar to previous values.

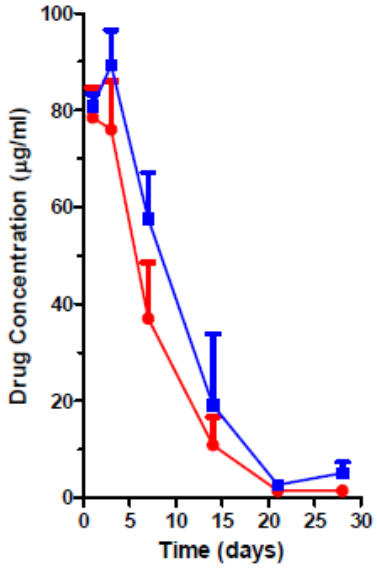


Figure 5: Middle Ear Ciprofloxacin levels following a single intratympanic injection of 2% (b) (4) OTO 201 (red circles) and 6% (b) (4) OTO 201 (blue squares). Ciprofloxacin levels in the middle ear (mean + SEM) were determined 1, 3, 7, 14, 21, and 28 days after injection. (Sponsor's Figure)

Table 3: Summary of the Middle Ear Pharmacokinetics of Single IT-ANT Doses of 2 and 6% OTO-201 in Guinea Pigs. (Sponsor's Table)

	Cmax µg/ml	AUC µg.h/ml	MRT h	T1/2 h	Cmax/MIC ratio	$AUC_{0\text{-}24}$/MIC h
2% OTO-201	78.5	16,560	140	117	39	942
6% OTO-201	89.3	22,829	175	100	45	1021

Title: Ototoxicity of Saline and Poloxamer P407 Materials Following Repeat Administration in Guinea Pigs (Study No.: OTO-104-RSP-030-01)

Methods

Guinea pigs received up to six bilateral intratympanic injections of 50 µl of test materials. Five of the test groups received injections in the superior posterior quadrant of the tympanum (directed toward the round window membrane niche). The last group receiving P407-RTD received injections anteriorly into the tympanum away from the round window niche. Animals were followed for up to 24 weeks and ABR assessment was performed every two weeks.

Table 4: Study Design for Study No.: OTO-104-RSP-030-01. (Sponsor's Table)

Treatment	IT Placement	Bi-weekly Dosing (Q2)	Monthly Dosing (Q4)	Bi-monthly Dosing (Q8)
		Every 2 weeks	Every 4 weeks	Every 8 weeks
Saline solution	RWM	6 injections	6 injections	3 injections
P407 – SPC	RWM	6 injections	3 injections	3 injections
P407 – RTD	RWM	4 injections	3 injections	2 injections
P407 – GMP	RWM	6 injections	3 injections	2 injections
P407 - RTD	ANT	4 injections	6 injections	2 injections

Results

All of the groups including the saline group exhibited a substantial incidence of hearing loss after a single IT-RWM injection (Table 5).

Table 5: The Percentage of Individual Ears with Biologically Relevant Hearing Loss (≥ 20dB SPL at the 20 kHz wavelength) Following a Single IT-RWM Injection. (Sponsor's Table)

	Bi-weekly Dosing (Q2)	Monthly Dosing (Q4)	Bi-monthly Dosing (Q8)
Saline solution	19	38	56
P407 – SPC	13	38	13
P407 – RTD	50	69	38
P407 – GMP	13	25	69

Following repeated IT-RWM injections, all the groups including the saline group experienced similar levels of hearing loss (Figure 6). For all but the P407-GMP group the least hearing loss was associated with the lowest bimonthly dosing.

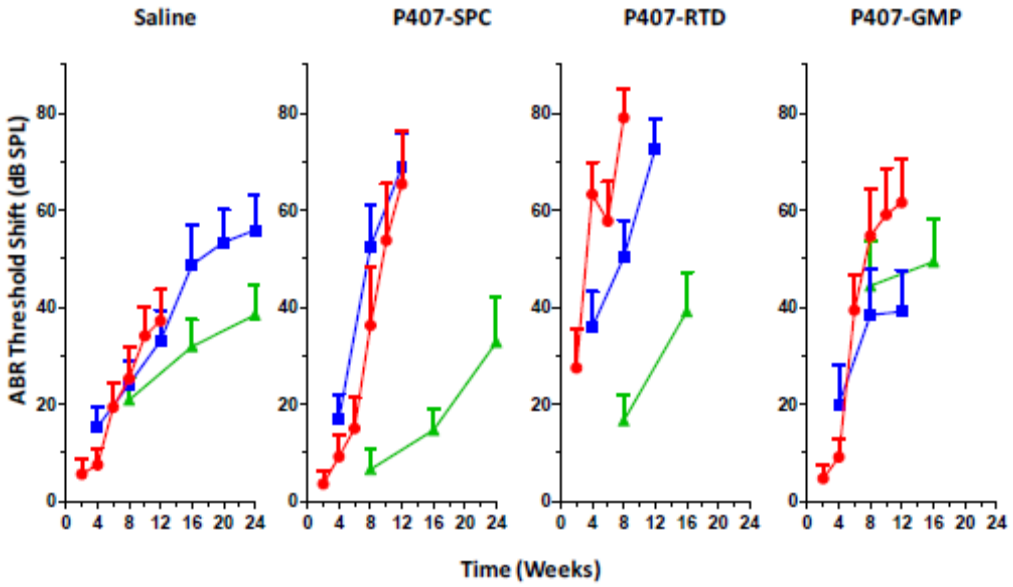


Figure 6: ABR Threshold Shifts (20 kHz Shown) in Guinea Pig Ears (n =16/group) Following Repeated IT-RWM injections. Biweekly dosing (red circles), monthly dosing (blue squares), bimonthly dosing (green triangles). Data are presented as the mean ± SEM. (Sponsor's Figure)

Administration of P407-RTD by the IT-ANT site of injection resulted in much less hearing loss as assessed by ABR (Figure 7). As with the IT-RWM injections, more injections resulted in the greatest degree of hearing loss, but even six biweekly IT-ANT injections of P407-RTD produced ABR threshold shifts of less than 20 dB SPL at all three of the measured frequencies (4, 8, and 20 kHz) compared to ABR threshold shifts of greater than 70 dB SPL for P407-RTD injections into the RWM.

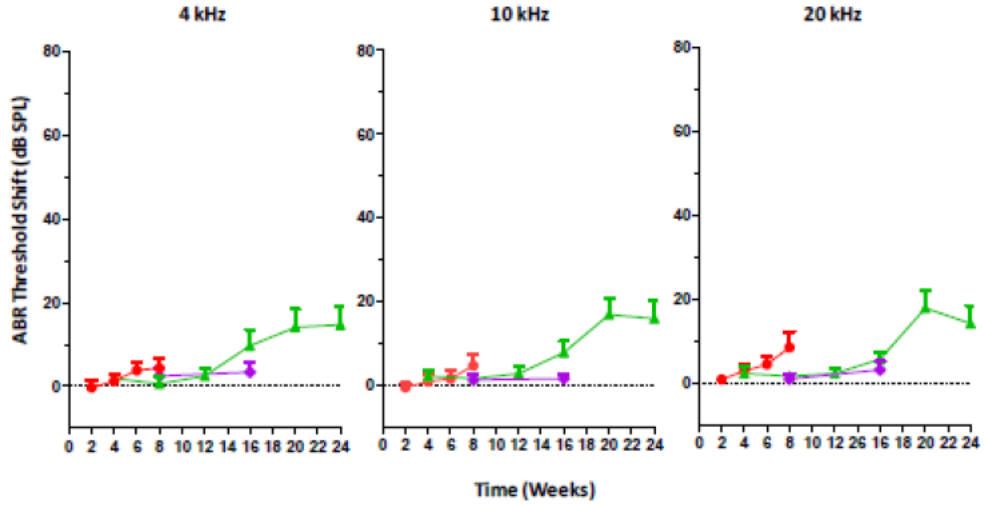


Figure 7: ABR threshold shifts (4, 8, and 20 kHz) in guinea pig ears (16 ears/group) following repeated-IT ANT injections of P407-RTD. Biweekly dosing; 4 injections (red circles), monthly dosing; 6 injections (green triangles), and bimonthly dosing; 2 injections (purple diamonds). Data are presented as mean ± SEM. (Sponsor's Figure)

Cytocochleograms were performed on a total of 49 ears. The results indicated a good correlation between the degree of hearing loss and inner ear integrity suggesting the hearing loss demonstrated in ABR assessments was due to inner ear damage.

The results suggest that repeated IT-RWM injections regardless of the treatment article results in hearing loss correlating with inner ear damage. Much less hearing loss resulted from repeated IT-ANT injections suggesting this route of injection better supports inner ear integrity.

Reviewer Comment: *The experimental P407 formulations (P407-SPC, P407-RTD, and P407-GMP) were not described beyond their route of administration. It is not clear how the differences in formulation influenced ototoxicity.*

Title: Ototoxicity of (b) (4) Poloxamer P407 Materials Following Repeat Administration in Guinea Pigs. (Study No.: OTO-104-RSP-033-01)

Methods

Poloxamer 407 (P407) preparations sterilized with two different techniques were examined in this non-GLP study (b) (4) .

Eight female guinea pigs/group received up to six bilateral intratympanic (IT) injections of saline or different poloxamer (b) (4) P407 preparations at a two-week dosing interval (Table 6).

Using an operating microscope, injections were consistently directed into the superior posterior quadrant covering the round window membrane (RWM) niche of the tympanic membrane. The guinea pigs were then assessed every two weeks over the next 24 weeks for auditory brain stem responses (ABR). All of the poloxamer 407 preparations contained (b)(4)% P407 except for the P407-(b)(4) pluromed formulation which contained (b)(4)% P407.

Table 6: Study Design for Study No.: OTO-104-RSP-033-01 (Sponsor's Table)

Treatment	Bi-weekly Dosing (Q2)
	Every 2 weeks
Saline solution	6 injections
P407 – (b)(4) 580B	5 injections
P407 – 580C	6 injections
P407 – Pluromed	6 injections
P407 – 580B	6 injections
P407 – 580C	5 injections

Reviewer Comment: *The precise (b)(4) formulations were not defined in the study report, and it is not clear how the differences in formulation influenced ototoxicity.*

Results

A single IT RWM injection in guinea pigs produced biologically relevant hearing loss characterized as ≥ 20 dB SPL determined by ABR in a percentage of animals in all the groups with the highest percentage (63%) in the P407-(b)(4)-580C (Table 7).

Table 7: Proportion of Individual Ears in Each Group with Biologically Relevant Hearing Loss (≥ 20 dB SPL) Following a Single IT RWM (n =16).

Groups	Percentage of Ears with Hearing Loss*
Saline solution	13%
P407 (b)(4) 580B	25%
P407 (b)(4) 580C	13%
P407 (b)(4) Pluromed	19%
P407 (b)(4) 580B	25%
P407 (b)(4) 580C	63%

Following repeated IT RWM administration, all treatment groups including the saline treatment group converged over time to biologically significant levels of hearing loss in the 40-70 dB SPL range at the three measured wavelengths (4, 10, and 20 kHz). A general trend was less hearing loss for the saline, P407-(b)(4) 580C and P407-(b)(4) Pluromed groups and more hearing loss for the P407-(b)(4)-580 B and C groups. These results suggest that IT administration into the round window membrane regardless of preparation results in biologically significant hearing loss and that the (b)(4) preparations result in the greatest hearing loss (Figure 8).

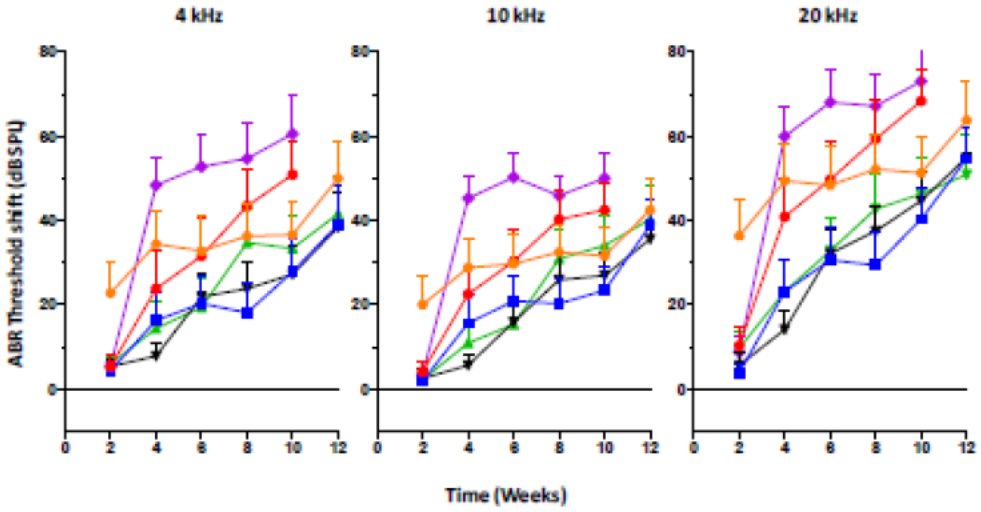


Figure 8: ABR threshold shift values at three different wavelengths for guinea pigs repeatedly administered saline or [(b) (4)] P407 preparations into the RWN. Treatment groups were as follows: saline (inverted black triangles), P407-[(b) (4)]580B (red circles), P407-[(b) (4)]580C (blue squares), P407-[(b) (4)]pluromed (green triangles), P407-[(b) (4)]-580B (purple diamonds) and P407[(b) (4)]-580C (orange circles). (Sponsor's Figure)

Study Title: A one-month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs

Study no.:	1740-013; OTO-201-RSP-036
Study report location:	Electronic transmission
Conducting laboratory and location:	[(b) (4)]
Date of study initiation:	June 27, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OTO-201, Lot # 059-30 (2%) and 059-50 (6%), 99.9% purity for ciprofloxacin. P407, Lot # 059-11 Cetraxal®, Lot # P09085. Gentamycin sulfate salt Lot # SLBB0442V

Reviewer Comment: *The P407 and OTO -201 lots that were used in this study were sterilized with the [(b) (4)] technique and shown to be comparable to the clinical batch intended for the initial clinical study including in terms of [(b) (4)] content.*

Key Study Findings

1. Auditory brainstem response (ABR) assessments were similar in the saline, P407, and 2.0% OTO-201 groups. Cetraxal Otic® and 6.0% OTO-201 produced similar moderate increases in ABR values (hearing loss) at all three measured frequencies, while gentamycin produced more severe hearing loss.
2. Gentamycin produced the greatest incidence and severity of middle ear edema, fluid, and fibrosis. OTO-201 at both concentrations produced mild-moderate middle ear edema and

fluid at a somewhat greater incidence than the saline group and Cetraxal Otic® group. P407 administration was associated with mild middle ear edema and fluid in only 1/20 ears. However, the middle ear edema, fluid, and fibrosis results were not corroborated upon microscopic analysis.

3. The cochleae of all the animals in the saline, P407, 2.0 and 6.0% OTO-201 groups were within normal variability in terms of inner and outer hair patency. Gentamycin administration resulted in a large loss of inner and outer hair cells in all treated animals, and administration of Cetraxal Otic® produced mild to moderate hair cell loss.
4. Middle ear histopathology results indicated all of the saline-treated right ears exhibited minimal to mild subacute inflammation of the middle ear in conjunction with the presence of scattered aggregates of macrophages. The P407 and Cetraxal Otic® groups were indistinguishable from the saline group with the addition of foamy macrophages. Additional findings in the OTO-201 groups included minimal to mild granulomatous inflammation which was considered to be consistent with foreign body reaction possibly to ciprofloxacin particulates. Gentamycin produced a marked chronic active inflammatory response in all right middle ears with evidence of fibroplasia and bone loss/remodeling.

Methods

Doses:	400 mg/ml gentamicin, 2.0, 6.0% OTO-201, Cetraxal® (0.2% ciprofloxaxin solution).
Frequency of dosing:	Bilateral single doses for vehicle and OTO-201. Bilateral twice daily for 7 days for Cetraxal®
Route of administration:	Intratympanic injection for OTO-201 and tympanostomy tube drops for Cetraxal®.
Dose volume:	50 µl/ear
Formulation/Vehicle:	Poloxamer 407
Species/Strain:	Crl:HA (Albino Hartley) Guinea Pigs
Number/Sex/Group:	5/sex/group
Age:	Approximately 4 weeks old at receipt.
Weight:	Males: 356 to 452 grams and females: 311 to 367 grams at the time of randomization
Unique study design:	Six groups of male and female Albino Hartley guinea pigs (5/sex/group) were administered saline, gentamicin, poloxamer 407 vehicle, 2.0, and 6.0% OTO-201, or the positive control article, Cetraxal Otic®. The saline, vehicle, gentamycin, and OTO-201 doses were administered once on Day 0 by intratympanic injection to both ears. Cetraxal® was administered twice daily for 7 consecutive days via a tympanostomy tube in a fixed volume of 15 µl/ear/dose. All surviving animals were euthanized on Day 28.
Deviation from study protocol:	Multiple protocol deviations were noted for this study. However, the deviations were not considered to have altered the results or the study integrity.

Table 8: Study Design for Study No.: OTO-201-RSP-036			
Group No.	**Dose Level[a]**	**Male**	**Female**
1	Saline	5	5
2	Gentamycin	5	5
3	Vehicle (P407)	5	5
4	2.0% OTO-201	5	5
5	6.0% OTO-201	5	5
6	Cetraxal® Otic	5	5
[a] Animals in Groups 1 and 5 were dosed once on Day 0 via an IT injection. Animals in Group 6 were dosed BID beginning on Day 0 for 7 days via ear drops.			

Observations and Results

Mortality

All animals were examined for morbidity and mortality twice daily throughout the duration of the study.

All animals survived to scheduled termination.

Clinical Signs

All animals were examined for clinical signs twice per day. In addition, a detailed clinical examination of each animal was performed for all animals prior to dosing on Day 0, once following each dose, and weekly thereafter during the study. Observations included evaluation of skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactions to handling and bizarre behavior.

No clinical signs considered to be related to P407 or OTO-201 administration were observed.

Body Weights

Animal body weights were measured once prior to randomization and daily throughout the study.

No P407- or OTO-201-related changes in body weight were noted over the course of the study.

Physical Examinations

In addition to the physical signs assessment, a complete physical examination was conducted on all animals prior to termination except for gentamicin animals. Otoscopic evaluations were performed during the examinations, and observations included any irritation and possible signs of infection.

No P407- or OTO-201-related changes in otoscopic evaluations.

Auditory Brainstem Response (ABR) Evaluations

ABR evaluations were performed on animals pretest and prior to necropsy. Examinations were conducted at each of three different frequencies (4, 10, and 20 kHz).

Auditory function was similar in the saline, P407, and 2.0% OTO-201 groups. Cetraxal Otic® and 6.0% OTO-201 produced similar moderate increases in ABR values (hearing loss) at all three frequencies, while gentamycin produced more severe hearing loss. ABR measurements were similar in males and females (Table 9).

Table 9: Summary of Auditory Brain Stem Responses (Sponsor's Table)

Summary of Auditory Brainstem Response Values, Left Ear, dB - MALE

		Saline			Gentamicin			Poloxamer 407			2.0% OTO-201			6.0% OTO-201			Cetraxal® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	58.6	2.30	5	62.0	5.66	5	59.0	2.55	5	65.0	3.16	5	61.4	5.68	5	63.4	6.11	5
	Terminal	71.4	11.33	5	99.4	1.34	5	71.2	4.09	5	74.0	2.74	5	87.8	7.19	5	79.4	12.52	5
10 kHz	Pretest	42.2	2.77	5	45.0	2.65	5	44.0	4.58	5	44.8	1.79	5	43.4	4.83	5	47.8	2.49	5
	Terminal	48.4	5.90	5	100.0	0.00	5	54.6	7.67	5	52.8	2.39	5	67.4	12.10	5	65.6	17.85	5
20 kHz	Pretest	35.0	5.48	5	38.2	3.27	5	40.0	3.87	5	39.4	2.88	5	41.2	4.97	5	37.2	3.83	5
	Terminal	45.2	7.36	5	99.6	0.89	5	44.2	7.40	5	51.8	7.60	5	65.6	9.99	5	68.0	23.03	5

Summary of Auditory Brainstem Response Values, Right Ear, dB - MALE

		Saline			Gentamicin			Poloxamer 407			2.0% OTO-201			6.0% OTO-201			Cetraxal® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	65.0	3.32	5	61.0	9.08	5	59.0	3.74	5	61.4	5.27	5	59.8	5.89	5	62.8	3.96	5
	Terminal	75.4	12.92	5	100.0	0.00	5	71.0	4.53	5	77.2	5.02	5	87.8	13.66	5	91.2	16.05	5
10 kHz	Pretest	46.2	1.79	5	45.8	4.21	5	45.4	2.51	5	43.2	3.35	5	42.8	4.44	5	44.8	3.03	5
	Terminal	50.6	5.18	5	97.0	4.24	5	56.2	11.61	5	55.4	5.03	5	71.8	16.68	5	77.8	20.61	5
20 kHz	Pretest	41.2	3.27	5	40.8	3.83	5	42.0	2.74	5	38.0	4.30	5	40.4	7.02	5	38.6	2.61	5
	Terminal	47.4	6.54	5	96.4	6.07	5	43.4	4.98	5	50.4	7.96	5	70.4	13.58	5	69.6	16.76	5

Summary of Auditory Brainstem Response Values, Left Ear, dB - FEMALE

		Saline			Gentamicin			Poloxamer 407			2.0% OTO-201			6.0% OTO-201			Cetraxal® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	63.6	3.51	5	64.8	4.50	4	67.6	4.51	5	66.6	2.07	5	64.0	2.83	5	61.0	3.24	5
	Terminal	67.6	1.14	5	99.4	1.34	5	77.2	2.59	5	72.2	10.03	5	76.8	8.70	5	91.8	4.97	5
10 kHz	Pretest	45.2	2.17	5	45.8	1.89	4	47.8	7.53	5	45.8	3.35	5	45.0	4.90	5	45.2	2.39	5
	Terminal	47.4	3.36	5	100.0	0.00	5	50.0	3.54	5	53.8	9.42	5	60.2	10.80	5	71.8	10.03	5
20 kHz	Pretest	38.8	2.28	5	39.0	3.16	4	38.8	3.19	5	37.8	4.02	5	39.0	5.79	5	36.8	1.30	5
	Terminal	41.4	1.82	5	98.6	3.13	5	47.6	1.14	5	54.0	6.63	5	57.4	12.72	5	63.6	17.27	5

Summary of Auditory Brainstem Response Values, Right Ear, dB - FEMALE

		Saline			Gentamicin			Poloxamer 407			2.0% OTO-201			6.0% OTO-201			Cetraxal® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	64.4	4.98	5	67.4	2.19	5	65.2	2.77	5	65.6	2.30	5	61.6	7.33	5	62.8	4.38	5
	Terminal	69.4	7.33	5	99.4	1.34	5	77.2	4.21	5	73.4	9.91	5	77.0	13.29	5	79.0	10.44	5
10 kHz	Pretest	46.4	4.16	5	52.0	8.86	5	45.8	2.28	5	48.6	3.13	5	45.0	1.22	5	43.2	2.59	5
	Terminal	49.8	6.38	5	98.6	3.13	5	51.6	4.28	5	56.6	5.90	5	61.6	14.76	5	57.0	7.78	5
20 kHz	Pretest	38.8	2.59	5	42.8	4.49	5	41.4	3.05	5	39.6	3.21	5	39.2	3.49	5	38.0	3.39	5
	Terminal	47.4	6.11	5	100.0	0.00	5	50.8	3.70	5	53.8	7.29	5	57.4	14.84	5	53.0	12.63	5

Feed Consumption, Ophthalmoscopy, ECG, Clinical Pathology: not assessed.

Gross Pathology

Middle ear gross pathology was performed on all ears and cytocochleogram analysis was performed on the left ears.

Gross Middle Ear Assessment

Ossicle mobility: Ossicle mobility was impaired in 3/20 ears in the gentamycin group, 3/20 ears in the Cetraxal® group, and 1/20 ears in the 6.0% OTO-201 group, but not in any ears in the saline, P407, or the 2.0% OTO-201 groups .

Tympanic membrane: Tympanic membrane appearance was abnormal in 1/20 ears in the saline group, 15/20 ears in the gentamycin group, 8/20 ears in the Cetraxal® group, and 14/20 ears in the 6.0% OTO-201 group, but not in any ears in the P407 and 2% OTO-201 groups. Tympanic membrane integrity was compromised in 2/20 ears in the saline group, 3/20 ears in the gentamycin group and in 7/20 ears in the Cetraxal® group but not in any ears in the P407, or the 2% and 6% OTO-201 groups.

Middle ear edema, fluid and fibrosis: The middle ear edema, fluid and fibrosis results are shown below in Table 10. Gentamycin produced the greatest incidence and severity for all three indices and OTO-201 at both concentrations produced mild-moderate middle ear edema and fluid at a somewhat greater incidence than the saline group and Cetraxal Otic®. P407 administration was associated with mild middle ear edema and fluid in only 1/20 ears. The pathology report indicated that the edema, fluid, and fibrosis findings were not corroborated by histological findings.

Table 10: The Incidence and Severity of Middle Ear Edema, Fluid and Fibrosis

Group	Incidence and Severity (ears/20 ears)			
		Edema	Fluid	Fibrosis
1: saline	Mild	6	6	0
	Moderate	0	0	0
	Severe	0	0	0
2: gentamycin	Mild	4	4	11
	Moderate	4	4	3
	Severe	9	9	2
3: P407	Mild	1	1	0
	Moderate	0	0	0
	Severe	0	0	0
4: 2% OTO-201	Mild	12	12	3
	Moderate	0	0	1
	Severe	0	0	0
5: 6% OTO-201	Mild	13	13	12
	Moderate	3	3	4
	Severe	0	0	1
6: Cetraxal®	Mild	5	5	5
	Moderate	1	1	1
	Severe	0	0	0

Reviewer Comment: *The pathology report included a sentence stating: "it should be noted that the gross necropsy findings of edema and fibrosis observed in a large fraction of ears across treatment groups could not be corroborated upon close examination of the tissues, as well as histologically."*

Cytocochleogram
The cochleae of all the animals in the saline, P407, 2.0 and 6.0% OTO-201 groups were within normal variability in terms of inner and outer hair patency. Gentamycin administration resulted in a large loss of inner and outer hair cells in all treated animals, and administration of Cetraxal Otic® produced mild to moderate hair cell loss.

Histopathology

Adequate Battery
Only auditory tissues in the right ears were examined
Peer Review
No
Histological Findings
All of the saline treated right ears exhibited minimal to mild subacute inflammation of the middle ear in conjunction with the presence of scattered aggregates of macrophages. The P407 and Cetraxal Otic® groups were indistinguishable from the saline group with the addition of foamy macrophages. Middle ear findings in the 2.0% OTO-201 group were similar to the saline group with the addition of minimal to mild granulomatous inflammation in 6/10 ears and the presence of foreign material. Similar findings occurred in the 6.0% OTO-201 group except with a higher incidence (10/10 ears) of minimal to mild granulomatous inflammation. The OTO-201 inflammatory findings were consistent with a dose-dependent foreign body reaction with the foreign body possibly consisting of ciprofloxacin particulates. Otherwise the OTO-201 effects were similar to those of P407 and Cetraxal Otic®. Gentamycin produced a marked chronic active inflammatory response in all right middle ears with evidence of fibroplasia and bone loss/remodeling.

Study Title: A One-Month GLP Acute Ototoxicity Study Evaluating the Sequential Administration of OTO-201 and Ciprodex in Guinea Pigs.

Study no.:	1740-014; OTO-201-RSP-037
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 3, 2012
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OTO-201, Lot # 059-30 (2%) and 059-50 (6%), 99.9% purity for ciprofloxacin. P407, Lot # 059-11 Ciprodex®, Lot # 196649F.

Reviewer Comment: *The P407 and OTO -201 lots that were used in this study were sterilized with the* (b) (4) *technique and shown to be comparable to the clinical batch intended for the initial clinical study including in terms of* (b) (4) *content.*

Key Study Findings

1. All treatment groups including the saline group experienced mild to moderate hearing loss suggesting the effects were caused by the Ciprodex Otic® treatment. OTO-201, 2 or 6% did not worsen the hearing loss.
2. The incidence of middle ear edema, fluid and fibrosis occurring in the OTO-201 groups was increased compared to the saline and P407 groups. However, the pathology report indicated that these effects were not corroborated by histopathology.
3. Cytocochleograms demonstrated similar incidences of mild to moderate cochlear hair cell loss in all treatment groups suggesting the cause was attributable to Ciprodex Otic® administration.
4. Middle ear histopathology for the saline group included minimal to mild subacute inflammation of the middle ear in conjunction with the presence of scattered aggregates of foamy macrophages and a low incidence of minimal granulomatous inflammation. P407 animals exhibited the same findings except a greater incidence of foamy macrophages. Both OTO-201 groups demonstrated a greater incidence of minimal to mild granulomatous inflammation and foreign material. The Sponsor indicated that the findings in the OTO-201 group were characteristic of a minimal to mild foreign body reaction with the foreign body hypothesized to be ciprofloxacin particulates.

Methods

Doses:	2.0, 6.0% OTO-201, Ciprodex Otic®. (0.3% ciprofloxacin plus 0.1% dexamethasone)
Frequency of dosing:	Bilateral single doses for vehicle and OTO-201. Bilateral twice daily for 7 days for Ciprodex®
Route of administration:	Intratympanic injection for OTO-201 and tympanostomy tube drops for Ciprodex Otic®.
Dose volume:	50 µl/ear
Formulation/Vehicle:	Poloxamer 407
Species/Strain:	Crl:HA (Albino Hartley) Guinea Pigs
Number/Sex/Group:	5/sex/group
Age:	Approximately 4-weeks old at receipt.
Weight:	Males: 348 to 426 grams and females: 309 to 375 grams at the time of randomization
Unique study design:	Six groups of male and female Albino Hartley guinea pigs (5/sex/group) were administered saline, P407 vehicle, and OTO-201 doses once on Day 0 by intratympanic injection to both ears. Ciprodex® was administered twice daily for 7 consecutive days (Days 3-9) via a tympanostomy tube in a fixed volume of 10 µl/ear/dose. All surviving animals were euthanized on Day 28.
Deviation from study protocol:	Multiple protocol deviations were noted for this study. However, the deviations were not considered to have altered the results or compromised the study integrity.

Table 11: Study Design for Study No.: OTO-201-RSP-037			
Group No.	**Dose Level [a]**	**Male**	**Female**
1	Saline	5	5
2	Vehicle (P407)	5	5
3	2.0% OTO-201	5	5
4	6.0% OTO-201	5	5
[a] Animals in Groups 1 and 5 were dosed once on Day 0 via an IT injection with saline, vehicle or OTO-201, followed by BID administration of Ciprodex Otic® from Days 3 to 9.			

Observations and Results

Mortality

All animals were examined for morbidity and mortality twice daily throughout the duration of the study.

All animals survived to scheduled termination.

Clinical Signs

All animals were examined for clinical signs twice per day. In addition, a detailed clinical examination of each animal was performed for all animals prior to dosing on Day 0, once following each dose, and weekly thereafter during the study. Observations included evaluation of skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactions to handling and bizarre behavior.

No clinical signs considered to be related to P407 or OTO-201 administration were observed.

Body Weights

Animal body weights were measured once prior to randomization and daily throughout the study.

No P407 or OTO-201-related changes in body weight were noted.

Physical Examinations

In addition to the physical signs assessment, a complete physical examination was conducted on all animals prior to termination except for Gentamicin animals. Otoscopic evaluations were performed during the examinations, and observations included any irritation and possible signs of infection.

No P407 or OTO-201-related changes in otoscopic evaluations were reported. Mild or moderate debris and irritation possibly related to Ciprodex® administration were observed in all groups except the P407 group.

Auditory Brainstem Response (ABR) Evaluations

ABR evaluations were performed on animals pretest and prior to necropsy. Examinations were conducted at each of three different frequencies (4, 10, and 20 kHz).

All treatment groups experienced mild to moderate hearing loss with no differences in gender (Table 12). Because the hearing loss was uniform between groups it appears to have been caused by the Ciprodex Otic® treatment. OTO-201, 2 or 6% did not worsen the hearing loss.

Table 12: Summary of Auditory BrainStem Responses

Summary of Auditory Brainstem Response Values, Left Ear - MALE

		Saline + CIPRODEX® Otic			Poloxamer 407 + CIPRODEX® Otic			2.0% OTO-201 + CIPRODEX® Otic			6.0% OTO-201 + CIPRODEX® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	63.6	5.77	5	62.6	6.02	5	65.2	1.79	5	62.0	4.85	5
	Terminal	83.4	7.20	5	71.6	6.15	5	76.0	10.10	5	84.8	12.87	5
10 kHz	Pretest	44.6	1.52	5	46.8	2.86	5	45.8	4.32	5	43.8	5.07	5
	Terminal	57.0	7.78	5	59.6	6.80	5	60.6	17.54	5	64.2	12.64	5
20 kHz	Pretest	41.2	1.30	5	43.2	4.66	5	40.2	2.77	5	36.8	2.59	5
	Terminal	54.4	10.26	5	56.0	8.57	5	59.6	22.74	5	69.0	22.10	5

Summary of Auditory Brainstem Response Values, Right Ear - MALE

		Saline+ CIPRODEX® Otic			Poloxamer 407 + CIPRODEX® Otic			2.0% OTO-201 + CIPRODEX® Otic			6.0% OTO-201 + CIPRODEX® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	64.4	6.11	5	60.6	5.13	5	66.2	3.90	5	65.6	4.72	5
	Terminal	87.8	12.09	5	78.8	16.51	5	77.6	15.40	5	77.8	11.34	5
10 kHz	Pretest	46.2	3.70	5	46.0	2.00	5	48.6	4.83	5	46.2	2.77	5
	Terminal	71.4	18.30	5	57.8	10.55	5	61.8	16.90	5	66.8	9.26	5
20 kHz	Pretest	40.4	2.61	5	39.8	2.49	5	41.6	4.77	5	40.2	4.15	5
	Terminal	69.0	23.37	5	54.4	15.47	5	56.0	11.29	5	64.6	9.13	5

Summary of Auditory Brainstem Response Values, Left Ear - FEMALE

		Saline + CIPRODEX® Otic			Poloxamer 407 + CIPRODEX® Otic			2.0% OTO-201 + CIPRODEX® Otic			6.0% OTO-201 + CIPRODEX® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	63.2	2.17	5	63.2	3.35	5	60.8	3.90	5	59.8	4.09	5
	Terminal	87.6	14.29	5	72.8	15.27	5	79.4	9.26	5	84.8	16.99	5
10 kHz	Pretest	43.8	2.59	5	44.2	1.92	5	45.4	4.56	5	44.0	4.00	5
	Terminal	63.2	18.86	5	60.6	18.51	5	55.6	9.61	5	69.8	14.62	5
20 kHz	Pretest	40.0	3.39	5	39.4	2.19	5	36.6	1.14	5	39.2	4.60	5
	Terminal	62.6	17.07	5	62.4	25.24	5	58.0	10.58	5	64.4	13.94	5

Summary of Auditory Brainstem Response Values, Right Ear - FEMALE

		Saline + CIPRODEX® Otic			Poloxamer 407 + CIPRODEX® Otic			2.0% OTO-201 + CIPRODEX® Otic			6.0% OTO-201 + CIPRODEX® Otic		
Frequency	Study Interval	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
4 kHz	Pretest	64.8	5.22	5	62.6	0.89	5	61.2	4.55	5	65.0	5.24	5
	Terminal	84.4	12.28	5	75.6	10.74	5	72.0	7.58	5	89.8	14.70	5
10 kHz	Pretest	45.8	2.68	5	46.4	1.34	5	46.2	6.22	5	50.8	8.50	5
	Terminal	63.0	12.79	5	61.0	15.98	5	68.6	19.67	5	67.0	20.63	5
20 kHz	Pretest	41.0	2.55	5	40.0	3.94	5	39.0	5.10	5	37.6	1.82	5
	Terminal	60.0	18.99	5	56.6	20.68	5	59.8	13.61	5	72.2	20.27	5

Feed Consumption, Ophthalmoscopy, ECG, Clinical Pathology: not assessed.

Gross Pathology

Animals received a gross middle ear assessment and a cytocochleogram.

Gross Middle Ear Assessment

Ossicle mobility: Ossicle mobility was impaired in 3/20 ears in the saline group, 2/19 ears in the P407 group, and 1/20 ears in the 2.0% OTO-201 group, but none of the animals in the 6.0% OTO-201 group demonstrated ossicle immobility.

Tympanic membrane: Tympanic membrane appearance and integrity abnormalities were as shown in Table 13. The highest concentration of OTO-201 produced the most tympanic membrane appearance and integrity abnormalities, but values were similar between groups with the least effects in the P407 group.

Table 13: Summary of Tympanic Membrane Appearance and Integrity Abnormalities		
Group	Tympanic Membrane Appearance Abnormalities	Tympanic Membrane-Integrity Abnormalities
1: saline	8 ears/20	4 ears/20
2. P407	3 ears/20	2 ears/20
3. 2% OTO-201	9 ears/20	7 ears/20
4. 6% OTO-201	13 ears/20	6 ears/20

Middle ear edema, fluid, and fibrosis: Results are shown below in Table 14. Values were similar between groups, with a slight increase in the incidence middle ear edema, fluid and fibrosis occurring in the OTO-201 groups compared to the saline and P407 groups. The pathology report indicated that these effects were not corroborated by histopathology findings.

Reviewer Comment: *The pathology report included a sentence stating: "it should be noted that the gross necropsy findings of edema and fibrosis observed in a large fraction of ears across treatment groups could not be corroborated upon close examination of the tissues, as well as histologically."*

Table 14: Summary of Middle Ear Edema, Fluid and Fibrosis.				
Group	Incidence and Severity (ears/20 ears)			
		Edema	Fluid	Fibrosis
1: saline	Mild	5	6	7
	Moderate	3	3	1
	Severe			
2: P407	Mild	5	5	6
	Moderate	4	4	
	Severe	1	1	1
3: 2% OTO-201	Mild	12	12	8
	Moderate	6	6	2
	Severe			
4: 6% OTO-201	Mild	13	13	14
	Moderate	5	5	2
	Severe			

Cytocochleogram: Cytocochleograms demonstrated the presence of mild to moderate cochlear hair cell loss (typically confined to the apical half of the cochlea) in all treatment groups. The incidence was comparable across the treatment groups suggesting the cause was attributable to Ciprodex® administration.

Histopathology

Adequate Battery
Only auditory tissues from right ears were examined for histopathology
Peer Review
No
Histological Findings
The saline group exhibited minimal to mild subacute inflammation of the middle ear (10 out of 10 ears) in conjunction with the presence of scattered aggregates of foamy macrophages in 7 out of 10 ears and minimal granulomatous inflammation in 1/10 ears. P407 animals exhibited the same findings as the saline animals, except a greater incidence of foamy macrophages (10/10 ears). Both OTO-201 groups demonstrated a greater incidence of minimal to mild granulomatous inflammation (7/10 ears in the 2% OTO-201 group and 9/10 ears in the 6.0% OTO-201 group) and foreign material. The Sponsor indicated that the findings in the OTO-201 group were characteristic of a minimal to mild foreign body reaction consistent with the presence of foreign material which was hypothesized to be ciprofloxacin particulates.

SUMMARY EVALUATION

In response to previous nonclinical toxicology findings linking (b) (4) poloxamer 407 gel (P407) to cochlear damage in guinea pigs the Sponsor halted initiation of the first clinical trial and proposed a study plan intended to determine the cause(s) of ototoxicity. The Sponsor identified potential toxicant impurities ((b) (4) and (b) (4) in the (b) (4) P407 vehicle, and subsequently developed a new (b) (4) technique which was associated with much lower impurity levels.

Pharmacokinetic studies in guinea pigs indicated that (b) (4) OTO-201 injected by the IT-ANT route and (b) (4) OTO-201 preparations demonstrated similar pharmacokinetic profiles in the middle ear of guinea pigs. In addition, a pharmacokinetic study employing a 7-day course of Ciprodex Otic® administration beginning three days after a single intratympanic injection of (b) (4) P407 or (b) (4) OTO-201 (2 and 6%) indicated that ciprofloxacin did not accumulate and C_{max} levels were not elevated in guinea pig middle ears as a result of the combination treatment.

Non GLP-compliant toxicology studies indicated that the hearing loss as determined by auditory brainstem response (ABR) assessments following (b) (4) P407 injections were similar to that of saline and lower than the hearing loss produced by (b) (4) P407 formulations. The specific site of intratympanic injection was also shown to affect hearing loss with all injected substances, including saline producing more hearing loss when injected into the round window niche of the tympanum (IT-RWM) compared to injection into the anterior tympanum (IT-ANT). One limitation with the non-GLP studies was that the experimental P407 formulations (P407-SPC, P407-RTD, P407-GMP, P407-(b) (4) 580B, P407-(b) (4) 580C, P407-(b) (4) -580B, and P407-(b) (4) -580C) were not fully described beyond how they were sterilized and/or their route of administration, and it was not clear how differences in formulation might have influenced ototoxicity.

(b) (4) P407 and (b) (4) OTO-201 were further examined in two one-month GLP-compliant toxicology studies. In the first study, male and female guinea pigs were administered a single IT-ANT injection of saline, (b) (4) P407, (b) (4) OTO-201 (2 and 6%), gentamicin, or a 7-day course

of Cetraxal Otic®. The results of this study indicated that the [(b) (4)] 407 and [(b) (4)] OTO-201 groups exhibited the same normal pattern of inner and outer hair-cell patency as the saline group. Similarly the saline, [(b) (4)] P407, and 2% [(b) (4)] OTO-201 groups demonstrated a similar degree of minimal hearing loss as assessed with ABR while the 6% [(b) (4)] OTO-201 and Cetraxal Otic® produced similar moderate hearing loss. In contrast, gentamycin produced severe cochlear damage and hearing loss. These results indicate that the [(b) (4)] preparations when injected into the anterior tympanum produced less ototoxicity than what was previously demonstrated for [(b) (4)] formulations injected into the round window niche of the tympanum. In particular, the [(b) (4)] treatment produced comparable effects to the saline treatment across all of the measured toxicological indices including middle ear histopathology.

In the second GLP-compliant, one-month toxicology study, a 7-day treatment course of Ciprodex Otic® was administered 3 days after a single IT-ANT injection of saline, [(b) (4)] P407, or [(b) (4)] (2 and 6%). In this study, a similar moderate degree of hearing loss and cochlear damage was exhibited across all of the study groups including the saline group, suggesting the effects were mediated by the treatment course of Ciprodex Otic® administered to all the groups. As in the first experiment, with reference to all the toxicological evaluations including middle ear histopathology, [(b) (4)] P407 produced effects comparable to those of the saline group.

In both experiments the significance of a moderate incidence of middle ear edema, fluid, and fibrosis reported to occur in saline and [(b) (4)] P407 groups, but at a higher incidence and severity in the [(b) (4)] OTO-201 groups was difficult to interpret because of a lack of corroboration by the histopathology evaluation. The pathology reports for both of the studies included the statement: "it should be noted that the gross necropsy findings of edema and fibrosis observed in a large fraction of ears across treatment groups could not be corroborated upon close examination of the tissues, as well as histologically," suggesting the middle ear edema, fluid and fibrosis observations may have been in error. While troubling, this explanation is consistent with the middle ear pathology results from two previous one-month toxicology studies submitted in conjunction with IND 110244 (OTO-201-RSP-008 and OTO-201-RSP-010) where a much lower incidence of middle ear edema, fluid and/or fibrosis was reported.

The histopathology findings for both studies included the following. The saline group exhibited minimal to mild subacute inflammation of the middle ear in conjunction with the presence of scattered aggregates of macrophages. [(b) (4)] P407-treated animals exhibited the same findings, except a greater incidence of foamy macrophages. Both [(b) (4)] OTO-201 groups additionally demonstrated minimal to mild granulomatous inflammation and the presence of foreign material. The Sponsor indicated that the findings in the OTO-201 groups were characteristic of a minimal to mild foreign body reaction consistent with the presence of foreign material which was hypothesized to be ciprofloxacin particulates. In any case, the [(b) (4)] P407 treatment produced histopathology approximating that of saline consistent with minimal or no ototoxicity associated with the [(b) (4)] sterilized vehicle.

The nonclinical results as a whole support the safety of the P407 and OTO-201 formulations sterilized by the new [(b) (4)] method and injected into the anterior tympanum. As indicated in the revised clinical protocol (Protocol # 201-201201) the initial clinical trial will utilize [(b) (4)] preparations and injections into the anterior tympanum as opposed to the round window niche.

Based on the nonclinical results and the changes to the clinical protocol, there are no pharmacology/toxicology objections to removal of the full clinical hold and re-initiation of the proposed clinical trial.

APPENDIX 1

Batch analyses for the (b) (4) nonclinical and clinical drug product and diluent batches (section 3.2.P.5.4 in the 11/2/2012 IND 110244 (b) (4)).

Table 1: Batch Analysis for OTO-201 Drug Product

	Formula	OTO-201 (b) (4)	OTO-201 (b) (4)	OTO-201 (b) (4)	OTO-201 (b) (4)
	Lot Number	FG-10-0016	059-030	059-050	W0006476
	Dosage Strength	120 mg/mL	20 mg/mL	60 mg/mL	60 mg/mL
	Manufacturer	(b) (4)	Otonomy, Inc.	Otonomy, Inc.	(b) (4)
	Date of Manufacture	December 13, 2010	June 28, 2012	June 28, 2012	September 01, 2012
	Batch Size				(b) (4)
	Drug Substance Lot	CHI1110002	CBI1412001	CBI1412001	CBI1211009
	Use	Pharmaceutical Development and Toxicology Studies	Pharmaceutical Development and Toxicology Studies	Pharmaceutical Development and Toxicology Studies	Clinical Studies
Tests	Current Specifications[a]	Results			
Appearance	Description[b]	Conforms	Conforms	Conforms	Conforms
pH	7.0-8.0	7.6	7.9	7.9	7.7
Identification	Retention time of sample conforms to retention time of standard within (b) (4)%	Conforms to reference standard	Conforms to reference standard	Conforms to reference standard	Conforms to reference standard
Assay	85% - 115% Label Claim				(b) (4)
Total Product Purity	NLT (b) (4)%				
Individual Related Substances	Report any related substance (b) (4)% Individual related substances NMT (b) (4)%				
Total Product Related Impurities	NMT (b) (4)%				

Table 1: Batch Analysis for OTO-201 Drug Product (Continued)

Tests	Current Specifications[a]	Results			
Uniformity of Dosage Units	Report Results	Conforms (b) (4)	Conforms (b) (4)	Conforms (b) (4)	Conforms (b) (4)
Endotoxin	NMT	(b) (4)			
Sterility	Sterile per USP <71>	No Growth[c]	Sterile[d]	Sterile[d]	Sterile[d]
Temperature of Gelation	Report Results (°C)	N/A	(b) (4)		
Gelation	Complete Gelation (b) (4)C	Conforms	N/A	N/A	N/A
(b) (4) Assay	Report Results (%w/w)	N/A	(b) (4)		
(b) (4) Assay	Report Results (µg/mL)	N/A	(b) (4)		
(b) (4) Assay	Report Results (µg/mL)	N/A			
(b) (4) Assay	Report Results (µg/mL)	N/A			
Osmolality	Report Results (mOsm/kg)	285	295	309	307
Viscosity at 20°C	Report Results (cP)	N/A	(b) (4)		
Particle Size Distribution	Report Results (d_{10}, d_{50}, d_{90}; µm)	(b) (4)			
Release (Dissolution)	Report Results MDT (min)	(b) (4)			

Table 2: Batch Analysis for OTO-201 Diluent

	Formula	OTO-201- (b) (4)	OTO-201- (b)	OTO-201- (b)
	Lot Number	FG-10-0016	059-011	W0006474
	Dosage Strength	N/A	N/A	N/A
	Manufacturer	(b) (4)		
	Date of Manufacture	December 15, 2010	June 28, 2012	August 29, 2012
	Batch Size	(b) (4)		
	Use	Pharmaceutical Development and Toxicology Studies	Pharmaceutical Development and Toxicology Studies	Clinical Studies
Tests	Current Specifications[a]	Results		
Appearance	Description[b]	Conforms	Conforms	Conforms
Gelation	Complete gelation (b) (4)C	Complete gelation (b) (4)C	N/A	N/A
pH	7.0-8.0	7.6	7.7	7.8
Endotoxin	NMT	(b) (4)		
Sterility	Sterile Per USP <71>	No growth (sterile)	Sterile	Sterile
Temperature of Gelation	Report Results (°C)	N/A	(b) (4)	
Osmolality	Report Results (mOsm/kg)	325	276	301
Viscosity at 20°C	Report Results (cP)	N/A	(b) (4)	
Polymer Assay	Report Results (%w/w/)	N/A		
(b) (4) Assay	Report Results (µg/mL)	N/A		
(b) (4) Assay	Report Results (µg/mL)	N/A		
(b) (4) Assay	Report Results (µg/mL)	N/A		

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/s/

JAMES S WILD
12/04/2012

WENDELYN J SCHMIDT
12/05/2012

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	IND 110244
Supporting document/s:	Support Document No.: 5
Sponsor's letter date:	8/26/2011
CDER stamp date:	8/26/2011
Product:	OTO-201
Indication:	Intra-operative treatment for middle ear effusion in pediatric subjects requiring tympanostomy tube placement
Sponsor:	Otonomy
Review Division:	Division of Anti-infective Products
Reviewer:	James Wild, Ph.D.
Supervisor/Team Leader:	Wendelyn Schmidt, Ph.D.
Division Director:	John Farley, M.D.
Project Manager:	Jane Dean, RN, MSN

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

OTO-201 is a suspension of ciprofloxacin hydrate in buffered solution (pH 7-8) containing (b) (4)% poloxamer 407, a glycol polymer. OTO-201 exists as a liquid at room temperature and gels immediately upon transitioning to body temperature. Two dose levels of OTO-201 will be investigated in the Phase 1b clinical study: 4 mg ciprofloxacin and 12 mg ciprofloxacin. Inactive ingredients include tromethamine, poloxamer 407, sodium chloride, hydrochloric acid (b) (4), and water. The drug product is packaged in a sterile vial for injection.

1.2 Brief Discussion of Nonclinical Findings

- Two ciprofloxacin-containing marketed products Cetraxal® (0.2% ciprofloxacin solution) and Ciprodex® (0.3% ciprofloxacin plus 0.1% dexamethasone) are currently the standard of care for the treatment of acute otitis externa. In addition Ciprodex® is used to treat acute otitis media in patients with tympanostomy tube placement. Both products are administered as ear drops BID for 7 days. Compliance may be a problem for both products and for Ciprodex® in patients with tympanostomy tube placement, delivery of the product to the middle ear is potentially inconsistent. OTO-201 is a sustained release preparation by virtue of the suspension of ciprofloxacin in (b) (4)% poloxamer 407. It is hoped that a single intratympanic injection of OTO-201 will provide more consistent ciprofloxacin coverage in the middle ear than a treatment course of Ciprodex® over 7 days.
- Inactive ingredients in the OTO-201 preparations include tromethamine (b) (4)% poloxamer 407, sodium chloride, hydrochloric acid (b) (4), and water. The drug product is packaged in a sterile vial for injection. Poloxamer 407 and tromethamine have not been used in any marketed otic products. Poloxamer 407 has been used in oral, topical, ophthalmic and periodontal products at lower concentrations. The periodontal product contains (b) (4)% poloxamer 407. Tromethamine has been used in many different kinds of approved products including IV, IM, intrathecal, oral and topical products and at higher percent concentrations than the present product ((b) (4)%) in IV, and ophthalmic products.
- Several patterns were apparent in multiple pharmacokinetic studies. The middle ear C_{max} levels of ciprofloxacin did not vary greatly with increasing concentrations of ciprofloxacin (0.6, 2.0, 6.0, and 12.0%) in OTO-201 preparations. However AUC and mean residence time increased with dose although in a less than dose-proportional manner. Inner ear concentrations were much less than the middle ear values. The results indicate that maximum middle ear concentration of ciprofloxacin cannot be increased by increasing the dose of OTO-201, but higher ciprofloxacin concentrations are maintained longer with higher doses of OTO-201. Even moderate dosage concentrations of ciprofloxacin In OTO-201 can result in substantial ciprofloxacin concentrations remaining in the middle ear longer than a therapy period of one to two weeks. Results from additional pharmacokinetic studies indicated ciprofloxacin was retained longer at the site of action, middle ear epithelium, compared to the middle ear lumen, and also in

"wet" guinea pig ears which model the frequent pattern of otorrhea occurring in young patients with ear infections.

- In the toxicology study where guinea pigs received intratympanic OTO-201 or vehicle or separate treatment courses of Cetraxal® or Ciprodex®, limited otic toxicity was noted in the high-dose OTO-201 group with more pronounced toxicity in the Cetraxal® and Ciprodex® groups. Notably auditory brain responses and cochlear hair cell loss were not altered for the vehicle or OTO-201-treated animals at any dose. In contrast, males receiving Cetraxal® demonstrated a mild threshold elevation in both their left and right ears, and 80% of the animals in the Ciprodex® group experienced cochlear hair cell loss. The incidence of middle ear foreign material was similar in the OTO-201 and vehicle control groups. Other auditory system findings included a small potential of minimal-moderate tissue reaction in the middle ear at times associated with minimal fibrosis and ossicle immobility in the high-dose OTO-201 group which was comparable to Cetraxal®. The highest incidence of ossicle immobility was 4 ears out of 20. Foamy macrophages were present in a small percentage of Eustachian tubes from all of the groups receiving poloxamer 407 suggesting foamy macrophages may represent uptake of the vehicle.
- Patients that do not respond to OTO-201 treatment in the first clinical trial are scheduled to receive rescue treatment with a 7-day treatment course of BID Ciprodex®. A possible concern is that the combination treatment of OTO-201 and Ciprodex® may lead to prolonged retention and/or higher middle ear ciprofloxacin levels with possible toxicological consequences. In a guinea pig toxicology study using combinations of Ciprodex® plus (b) (4)% poloxamer 407 vehicle or OTO-201, auditory brain response thresholds were elevated to about the same extent in all groups suggesting the effect was mediated by Ciprodex® treatment and not OTO-201 dose. The remaining otic toxicity, also occurred at a similar incidence in the vehicle control and OTO-201 treatment groups suggesting a controlling influence by the co-administration of Ciprodex® and not the dose of OTO-201. However, middle ear foreign material occurred only in the OTO-201 plus Ciprodex® treatment groups in a OTO-201 dose-responsive manner suggesting the foreign material may have been ciprofloxacin and that combination treatment with OTO-201 and Ciprodex® may increase middle-ear ciprofloxacin retention.
- The NOAEL can be considered to be the high OTO-201 dose (50 μl of 6.0%/ear) as it caused only marginal systemic toxicity and generally equivocal otic toxicity. This dose corresponds to a per ear dose of 3 mg/500 grams body weight or 6 mg/kg. The initial clinical dose planned for the proposed clinical study is 4 mg OTO-201/ear (200 μl of 2% OTO-201) which is equivalent to a total dose of 8 mg per average 10 kg subject or 800 μg/kg and higher for small patients as young as 6-months old. The safety margin based on human equivalent dose conversion based on relative body surface area is 1.65 fold. The 1.65 fold safety margin is much lower than the preferred 10-fold safety margin, but other findings and comparisons suggest relative safety for OTO-201 systemic toxicity. The primary OTO-201-related systemic effects noted in the guinea pig toxicology studies were reduced platelets and eosinophils in male or female guinea pigs receiving the

highest OTO-201 dose. Also relatively low plasma concentrations occurred in the guinea pig toxicology studies following intratympanic injection of OTO-201 or co-administration of OTO-201 and Ciprodex®. In guinea pigs, the maximal plasma ciprofloxacin concentrations associated with administration of OTO-201 with and without Ciprodex® were on the order of 100 to 500 fold lower than the peak serum concentrations of ciprofloxacin associated with clinical administration of 250 mg oral ciprofloxacin in humans. The comparatively low plasma ciprofloxacin concentrations following intratympanic administration of OTO-201 in guinea pigs suggest a low potential for OTO-201-related systemic toxicity in humans.

- The whole body surface area comparison is not appropriate for determining otic toxicity safety margins. Given the primary site of action for OTO-201 is the middle ear and specifically middle ear epithelium, a better safety margin variable for predicting safe human doses based on the guinea pig results is middle ear volume which, unfortunately, are not well described in the literature. The highest dose of OTO-201 did not produced pronounced otic toxicity as measured by several indices. However, it is uncertain how the pharmacokinetics and safety of intratympanic injection into healthy “dry’ guinea pig ears compares to similar administration into the infected ears of young patients with accompanying otorrhea.
- Some concerns regarding administration of OTO-201 to infants and young children have not been sufficiently addressed by the submitted nonclinical studies. These include the unknown toxicity of poloxamer 407 in human juvenile ears and the uncertain toxicological consequences of potentially high ciprofloxacin middle ear concentrations after co-administration of OTO-201 and Ciprodex®. In order to partially address the second concern, a pharmacokinetic study in guinea pigs examining middle ear ciprofloxacin concentrations following co-administration of OTO-201 and a treatment course of Ciprodex® is recommended.
- Juvenile animal studies are not expected to shed light on the effects that might be expected of ciprofloxacin and polaxamer 407 in human juvenile ears, mainly because ear development in the most appropriate experimental species (guinea pigs, sheep) has not been adequately studied.
- In order to achieve a ciprofloxacin therapeutic effect over a therapy period of one to two weeks, but minimize the potential for ciprofloxacin-related toxicity, an optimal dose of OTO-201 must be determined. The pharmacokinetic study results suggest that OTO-201 administration particularly at the higher ciprofloxacin concentrations can lead to prolonged middle ear retention of ciprofloxacin with possible toxicological consequences. In theory at least, this problem may be compounded when OTO-201 administration is closely followed by a treatment course of Ciprodex®. The safest course for the initial study will be to administer the lowest efficacious concentration of ciprofloxacin in OTO-201 and closely monitor juvenile patients for auditory responses during the treatment period and for a suitable recovery period following dosing.
- The genetic toxicology, carcinogenicity and reproductive toxicology status of ciprofloxacin is as noted on the product labels for approved products containing ciprofloxacin and no further studies in these categories are planned.

- OTO-201 was shown to produce negative results in an acute dermal toxicity assay and a skin sensitization assay.

1.3 Recommendations

1.3.1 Clinical Study (ies) Safe to Proceed: Yes with dosing beginning at the lowest efficacious dose and rigorous auditory response measurements instituted for patients.

1.3.2 If Not Safe to Proceed

Nonclinical deficiencies

Nonclinical information needed to resolve deficiencies

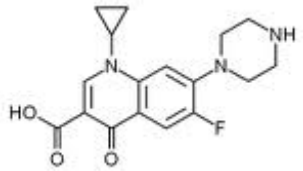
1.3.3 Additional Recommendation(s) (Non-hold comments/advice to sponsor) *if any.*

1. Before proceeding to phase 2 trials, a pharmacokinetic study in guinea pigs assessing middle ear ciprofloxacin concentrations following co-administration of poloxamer 407 vehicle, or OTO-201 (encompassing a range of ciprofloxacin concentrations) plus a treatment course of Ciprodex® is recommended.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)
85721-33-1
Generic Name
Ciprofloxacin
Code Name
OTO-201
Chemical Name
1-cyclopropyl- 6-fluoro-1,4-dihydro- 4-oxo- 7-(1-piperazinyl)-3- quinolinecarboxylic acid
Molecular Formula/Molecular Weight
$C_{17}H_{18}FN_3O_3$ (anhydrate)/331.34
Structure or Biochemical Description



Pharmacologic Class
Antibiotic

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND [redacted]

2.3 Drug Formulation

The OTO-201 drug product is a suspension formulation of ciprofloxaxin [redacted] in buffered [redacted]% poloxamer 407 solution. The additional excipients included in the formulation are shown below in Table 1

2.4 Comments on Novel Excipients

The complete list of the product excipients is shown in Table 1 below.

Table 1: OTO-201 Excipients

Excipients	MW	CAS#	% w/w
Poloxamer 407, NF	[redacted]	9003-11-6	[redacted]
NaCl		7647-14-5	
HCl, NF ([redacted]% w/w)		7647-01-0	
Tromethamine, USP		77-86-1	
Water		7732-18-5	

Poloxamer 407 and tromethamine have not been used in any marketed otic products. Poloxamer 407 has been used in oral, topical, ophthalmic and periodontal products at lower concentrations. The periodontal products contained [redacted]% poloxamer 407. Tromethamine has been used in many different kinds of approved products including IV, IM, intrathecal, oral and topical products and at higher percent concentrations than the present product ([redacted]%) in IV, and ophthalmic products.

2.5 Comments on Impurities/Degradants of Concern

Impurities and degradants have yet to be identified.

2.6 Proposed Clinical Protocol

Study Title: A Prospective, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Phase 1B Study of OTO-201 Given as a Single Intratympanic Injection for Intra-Operative Treatment of Middle Ear Effusion in Pediatric Subjects Requiring Tympanostomy Tube Placement. Protocol No.: 201-201101

Study Population: The study population will be composed of male and female patients of ages 6 months to 12 years with a clinical diagnosis of bilateral middle ear effusion requiring tympanostomy tube placement.

Dosing regimen: Eligible subjects will be randomized to either OTO-201 or placebo in a 2:1 ratio. Two dose levels of OTO-201 will be evaluated and the cohorts will be stratified by age with cohort 1 composed of subjects 6 months to 2 years old and cohort 2 composed of subjects older than 2 years.

Cohort 1:

1. OTO-201 4 mg; single 200 µL intratympanic injection to each ear of 2% OTO-201.
2. Placebo (Vehicle) for OTO-201; single 200 µl intratympanic injection to each ear.

Cohort 2:

1. OTO-201 12 mg; single 200 µl intratympanic injection to each ear 0f 6% OTO-201.
2. Placebo (Vehicle) for OTO-201; single 200 µl intratympanic injection to each ear.

Subjects with visible otorrhea in the auditory canal on external examination by the blinded investigator will be considered treatment failures if otorrhea is observed on or after 3 days post-surgery (Day 4) through Day 15. Treatment failures will be eligible for rescue medication and will be treatment failures for the remainder of the study. The rescue treatment for subjects who fail study drug due to ottorhea is Ciprodex®. Enrollment for the second cohort is scheduled to begin when at least 12 subjects in the 4 mg dose cohort have completed the Day 8 visit and no safety issues have been identified.

2.7 Previous Clinical Experience

To date no clinical efficacy studies have been conducted with OTO-201. However, the active pharmaceutical ingredient in OTO-201, ciprofloxacin, is currently used in ear drops in FDA approved products, Cetraxal® and Ciprodex®, and administered orally (Cipro®, Proquin XR®) and as eye drops (Ciloxan®) in FDA approved products.

The vehicle for OTO-201 (b) (4) % poloxamer 407) was recently tested in a clinical trial for a different investigational product (b) (4), (IND (b) (4)), which is also administered by intratympanic injection into the middle ear. In this study, subjects received a single intratympanic injection of either vehicle (b) (4) % poloxamer 407) or one of two doses of (b) (4) in the affected ear. Subjects were followed for 3 months post injection. A total of 14 patients were administered vehicle. All enrolled subjects completed the study. There were no deaths on study, no serious adverse events and no adverse events of interest associated with a single intratympanic injection of the poloxamer 407 vehicle. Moreover, there were no adverse findings based on laboratory measurements, physical examination, vital signs, or ECG.

Reviewer Comment: *The clinical effects of intratympanic administration of (b) (4) % poloxamer 407 on balance and hearing tests were not described in the study synopsis provided with the IND 110244 submission.*

2.8 Regulatory Background

This is the original IND for OTO-201. FDA answers to PIND Sponsor's questions were submitted to the Sponsor on 11/17/2010.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacokinetics

1. Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-002-01.
2. Middle Ear Pharmacokinetics of OTO-201 in "Wet Ear" Conditions in Guinea Pigs. Study No.: OTO-201-RSP-005-01.
3. Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs (Middle Ear Epithelium). Study No.: OTO-201-RSP-019-01.
4. Volume Range Pharmacokinetics of 2.0% OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-004-01.
5. Pharmacokinetics of Ciprodex Otic® in Guinea Pigs. Study No.: (b) (4) .
6. Pharmacokinetics of Co-Administration of OTO-201 Vehicle and Ciprodex Otic® in Guinea Pigs. Study No.: (b) (4).

Toxicology

Repeated-Dose Studies

1. A One-Month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-008.
2. Co-Administration of OTO-201 and Ciprodex® Otic: A One-Month GLP Ototoxicity Study in Guinea Pigs. Study No.: OTO-201-RSP-010.

Special Toxicology

1. An Acute Dermal Toxicity Study of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-009.
2. Skin Sensitization (Buehler Method) Study of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-007.
3. Ventilation Tube Patency Following Intratympanic Administration of (b) (4)% Poloxamer 407 (b) (4) in Guinea Pigs. Study No.: OTO-200-RSP-022-01.

3.2 Studies Not Reviewed

1. Validation of an LC-MS/MS Method For the Analysis of Ciprofloxacin in Guinea Pig Sodium Heparin Plasma. Study No.: ET-0011-RB-BV.
2. Low Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-014-01.
3. Pharmacokinetics of Cetraxal Otic® in Guinea Pigs. Study No.: OTO-201-RSP-011-01.
4. Pharmacokinetics of Cetraxal Otic® in Guinea Pigs (Middle Ear Epithelium). Study No.: OTO-201-RSP-025-01.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

The primary pharmacology studies for this IND were reviewed by the Microbiology reviewer.

4.2 Secondary Pharmacology

No secondary pharmacology studies with OTO-201 were conducted.

4.3 Safety Pharmacology

No safety pharmacology studies with OTO-201 were conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Analytical Methods

The Sponsor submitted a single study report describing the validation of an LC-MS/MS method for the analysis of ciprofloxacin in guinea pig plasma treated with sodium heparin (Study No.: FRP-ET-011-RB-BV-01-00). This study was not reviewed.

Absorption

Multiple studies examining the pharmacokinetics of OTO-210 in the middle ear and middle ear epithelium following a single intratympanic injection in guinea pigs were reviewed (Study Nos.: OTO-201-RSP-002, OTO-201-RSP-014, OTO-201-RSP-019, OTO-201-RSP-004, and OTO-201-RSP-005). These studies examined variations associated with different ciprofloxaxin concentrations, variable injection volumes, and injections in wet middle ear conditions. In addition two studies examining the pharmacokinetics of Ciprodex® alone and in the presence of (b)(4)% poloxamer 407 were reviewed (Study Nos.: (b)(4)).

Study Title: Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs. Study No.: OTO-201-RSP-002-01.

Methods

Guinea pigs received a single bilateral intratympanic injection of either 0.6, 2.0, 6.0, or 12.0% ciprofloxacin suspended in (b)(4)% poloxamer 407 (b)(4) (OTO-201) in the inferior anterior quadrant of the tympanum on Study Day 0. Animals were followed for 28 days and middle and inner ear samples were collected on Days 1, 3, 7, 14, 21, and 28. Ciprofloxacin concentrations in both middle and inner ear cavities were assessed with a LC-MS/MS technique.

The middle ear was sampled according to the following procedure. The lower anterior and posterior portion of the tympanic membrane was removed while attempting to leave the upper anterior and upper posterior tympanic membrane undisturbed. Using 22 and 27 gauge blunt needles, the middle ear cavity was lavaged twice with 100 µl of sterile water. The lavaged volumes were collected and ciprofloxacin concentrations were assessed using an LC-MS/MS technique.

The inner ear perilymph was sampled as follows. An incision was made behind the ear and a hole was drilled through the bulla using a dental burr so that the middle ear was exposed and accessed. The cochlea and the round window membrane were visualized under a stereo surgical microscope. A unique microhole was hand drilled through the cochlear capsule adjacent to the round window. Perilymph in a volume of 5 ml was then collected using a microcapillary inserted into the cochlear scala tympani.

Results

Substantial levels of ciprofloxacin were observed in the middle ear for all groups (Figure 1, Table 2). C_{max} levels did not significantly differ between the 2.0, 6.0, and 12.0% OTO-201 groups and these values were not much higher than the C_{max} for the low dose (0.6%). The AUC values increased in a less than dose proportional manner by more than four fold between the 0.6% dose and the 12% dose. The mean residence time also increased with dose but in a less than dose-proportional manner ranging from 6 days to 12.2 days.

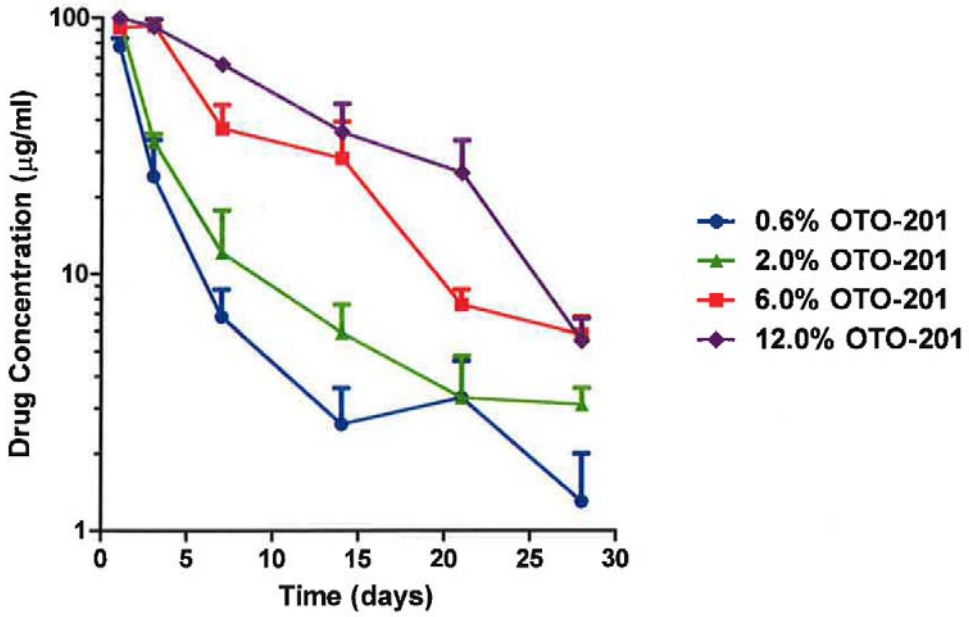


Figure 1: Middle Ear Ciprofloxacin Levels Following a Single Intratympanic Injection. (Sponsor's Figure).

Table 2: Summary of Ciprofloxacin Pharmacokinetic Parameters. (Sponsor's Table)

	Cmax µg/ml	AUC µg.h/ml	AUC_{0-24} µg.h/ml	MRT h	T>MIC h	Cmax/MIC ratio	AUC_{0-24}/MIC h
OTO-201							
0.6	77.4	7663	1858	143	322	39	929
2.0	96.9	11025	2326	200	413	48	1163
6.0	91.7	23921	2200	246	715	46	1100
12.0	99.9	32026	2398	293	721	50	1199

Concentrations of OTO-201 were 5 to 25 fold lower in the inner ear compared to the middle ear (Figure 2).

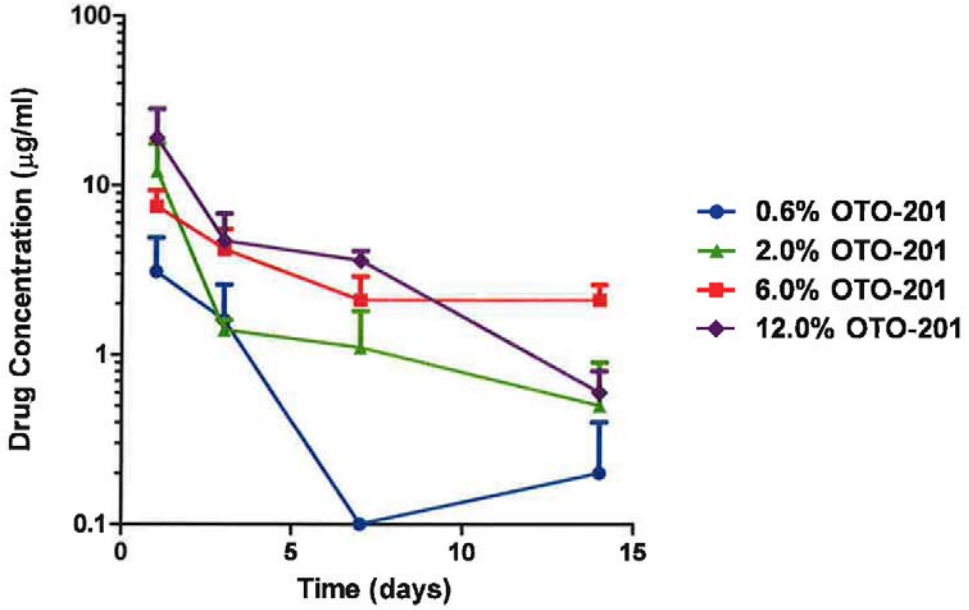


Figure 2: Inner Ear Ciprofloxacin Levels Following a Single Intratympanic Injection. (Sponsor's Figure)

Study Title: Middle Ear Pharmacokinetics of OTO-201 in "Wet Ear" Conditions in Guinea Pigs (Study No.: OTO-201-RSP-005-01).

Methods

On study Day 0, female guinea pigs received a single bilateral trans-bulla injection of either 0.6% or 2.0% OTO-201 in the inferior anterior quadrant of the tympanum. The guinea pigs were followed for 21 days after dosing and samples from the middle ear were obtained on Days 1, 3, 7, 14, and 21.

The trans-bulla injection in guinea pigs triggers a transient trauma of the middle ear associated with an inflammatory reaction and fluid exudation typically lasting for 3-4 days (wet ear environment).

The middle ear was sampled according to the following procedure. The lower anterior and posterior portion of the tympanic membrane was removed while attempting to leave the upper anterior and upper posterior tympanic membrane undisturbed. Using 22 and 27 gauge blunt needles, the middle ear cavity was lavaged twice with 100 µl of sterile water. The lavages were collected and ciprofloxacin was measured using a LC-MS/MS technigue.

Results

The middle ear C_{max} values for the 0.6 and 2.0% OTO-201 injections were both approximately 70 µg/ml (Figure 3). However, the middle ear concentrations decreased more rapidly for the low dose group to below 1 µg/ml by Day 7 as compared to over 6 µg/ml for the high dose group on Day 7. While the middle ear ciprofloxacin concentrations continued to decrease to approximately 0.1 µg/ml by Day 21 for the low dose group, the Day 21 middle ear ciprofloxacin concentrations were still about 6 µg/ml for the high-dose group.

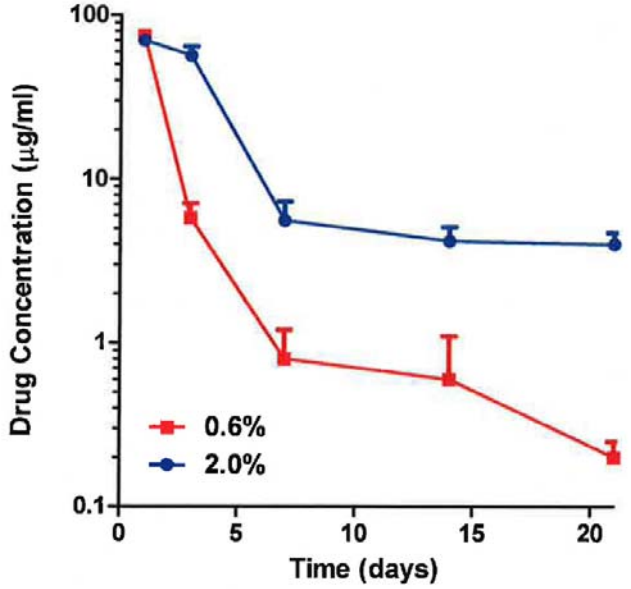


Figure 3: Middle Ear Ciprofloxacin Levels Following a Single Trans-Bulla Injection of Either 0.6% or 2.0% OTO-201. (Sponsor's Figure)

Study Title: Dose Range Pharmacokinetics of OTO-201 in Guinea Pigs (Middle Ear Epithelium). Study No.: OTO-201-RSP-019-01

Methods

Female guinea pigs (4/group) received a single bilateral intratympanic injection of 0.6%, 2.0%, 6.0%, or 12.0% of the (b) (4) suspension (OTO-201) in the inferior anterior quadrant of the tympanum (directed away from the round window niche). Animals were followed for up to 14 days. Middle ear epithelium samples were obtained at Days 1, 3, 7, and 14.

Results

As shown in Figure 4, The C_{max} levels (Mean ± SEM) of ciprofloxacin in the middle ear epithelium following intratympanic doses of 0.6% and 2% OTO-201 were similar (37.0 ± 10.2 and 62.5 ± 12.5 μg/ml respectively) and approximately 10 fold lower than the C_{max} values for the 6.0% and 12.0% doses of OTO-201 (393.3 ± 82.0, and 585.9 ± 309.2 μg/ml respectively). Over the 14 day sampling period the middle ear epithelium concentrations of ciprofloxacin normalized to similar levels (55.5 ± 5.4, 27.3 ± 17.1, and 103.8 ± 72.4 μg/ml respectively) for the 2, 6, and 12% doses of OTO-201, while the concentration for the 0.6% dose was substantially lower (7.0 ± 2.1 μg/ml).

Reviewer Comment: *These data suggest that middle ear epithelial cell ciprofloxacin concentrations are approximately 10-fold higher than middle ear lumen concentrations for the same doses of OTO-201. Also retention times appear to be similar or somewhat longer in epithelial cells.*

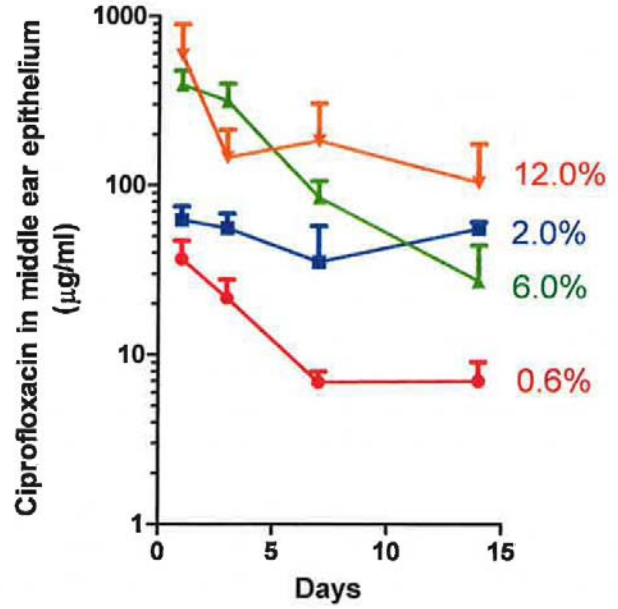


Figure 4: Middle Ear Epithelium Ciprofloxacin Levels Following a Single Intratympanic Injection of OTO-201. (Sponsor's Figure)

Study Title: Volume Range Pharmacokinetics of 2.0% OTO-201 in Guinea Pigs.
Study No.: OTO-201-RSP-004-01.

Methods

Female guinea pigs (4/group) received a single unilateral intratympanic injection of either 25 μl, 50 μl, or 75 μl of 2.0% OTO-201 (b) (4) suspension into the inferior anterior quadrant of the tympanum, and animals were followed for up to 14 days. Middle ear samples were collected on Days 1, 3, 7, and 14. Using 22 and 27 gauge blunt needles, the middle ear cavity was lavaged twice with 100 μl of sterile water. The lavages were collected and ciprofloxacin was measured using an HPLC method.

Results
Intratympanic injection of all of different volumes of OTO-201 resulted in substantial levels of middle ear ciprofloxacin (Figure 5). The middle ear C_{max} values followed a general trend of dose-dependent increase with values of 56.0 ± 3.5, 70.8 ± 7.1, and 94.3 ± 5.7 μg/ml for the 25, 50, and 75 μl dosage volumes respectively. Over time the differences in the dosage curves for the three volumes merged to some degree suggesting dosage volume had less effect on the overall drug elimination profile.

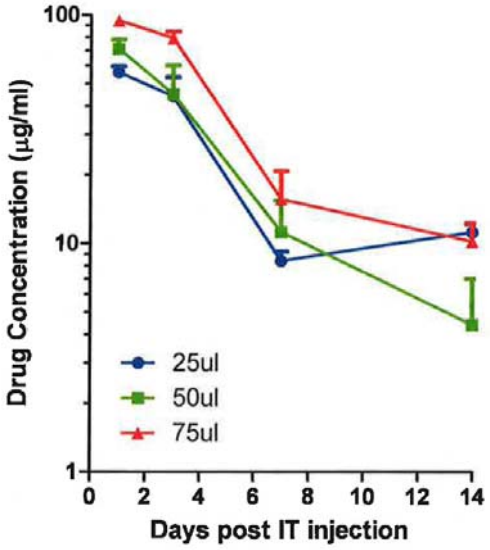


Figure 5: Middle Ear Ciprofloxacin Levels Following a Single Intratympanic Injection. (Sponsor's Figure)

Study Title: Pharmacokinetics of Ciprodex Otic® in Guinea Pigs (Study Number: (b) (4)).

Methods
Female guinea pigs (N = 4/group) were fitted with tympanostomy tube placement, then dosed with Ciprodex® in 10 μl volumes into the tubes in a single dose or twice daily for 7 days.

Middle ear lavage samples were obtained at 1, 3, or 6 hours after the single dose administration of Ciprodex® and on Days 1, 3, 5, 7, 14, 21, and 28 in conjunction with the 7 day administration course. The middle ears were sampled as follows. The lower anterior and posterior portion of the tympanic membrane was removed while attempting to leave the upper anterior and upper posterior tympanic membrane undisturbed. Using 22 and 27 gauge blunt needles, the middle ear cavity was lavaged twice with 100 μl of sterile water. The lavages were collected and ciprofloxacin was measured using a LC-MS/MS method.

Results
Ciprofloxacin middle ear concentrations were highest at the first and second sampling timepoints (1 and 3 hours) after a single dose of Ciprodex® then decreased

approximately 3 fold by 6 hours after dosing (Figure 6; Table 3). During the 7-Day course of BID Ciprodex®, ciprofloxacin middle ear concentrations (trough levels) peaked at approximately 5-9 μg/ml by Day 3 and remained at this level until Day 14 before falling to 1.3 μg/ml by Day 28 (Figure 7; Table 3).

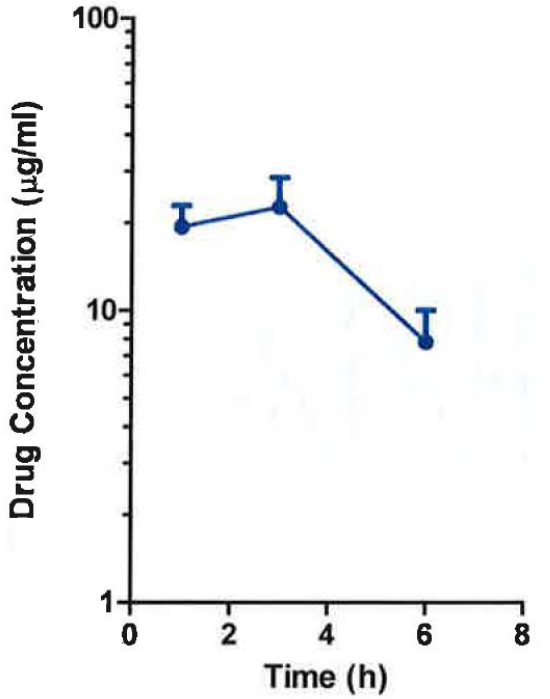


Figure 6: Middle Ear Ciprofloxacin (Peak Levels) Following a Single Administration of Ciprodex® Otic Drops. (Sponsor's Figure)

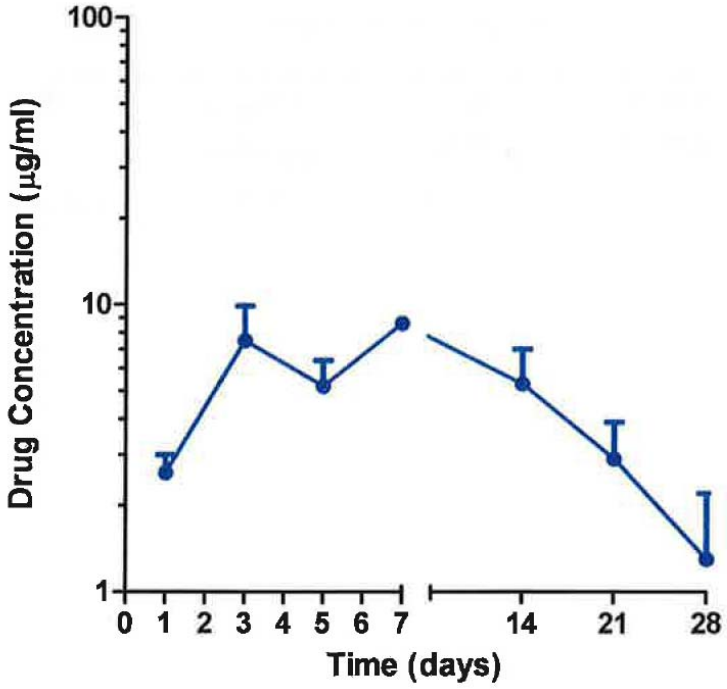


Figure 7: Middle Ear Ciprofloxacin (Trough Levels) Following a 7-day (BID) Treatment Course of Ciprodex® Otic Drops. (Sponsor's Figure)

Table 3: Middle Ear Ciprofloxacin Levels Following a Single Administration or a 7-Day BID Treatment Course of Ciprodex®. (Sponsor's Table)

	Middle Ear Ciprofloxacin					
	#1	#2	#3	#4	Mean	SEM
Peak levels						
1 Hour	20.1	11.3	17.6	28.2	19.3	3.5
3 Hour	37.7	17.4	9.9	25.5	22.6	5.9
6 Hour	12.9	2.3	8.9	7.1	7.8	2.2
Trough levels						
Day 1	1.6	3.5	2.7	2.7	2.6	0.4
Day 3	5.2	3.4	14.4	7.1	7.5	2.4
Day 5	2.4	7.1	7.3	3.9	5.2	1.2
Day 7	7.7	9.3	7.9	9.3	8.6	0.4
Day 14	7.7	8.8	2.8	1.8	5.3	1.7
Day 21	5.9	1.3	2.4	2.1	2.9	1.0
Day 28	3.9	0.5	0.4	0.6	1.3	0.9

Guinea pigs (n=4 per group) were sampled at the indicated times in the middle ear. Values (mean ± SEM) are expressed as µg/ml.

Reviewer Comment: *The measured C_{max} concentrations of middle ear ciprofloxacin after a single administration of Ciprodex® were lower than those obtained with all doses of OTO-201 in other studies.*

Study Title: Pharmacokinetics of Co-Administration of OTO-201 Vehicle and Ciprodex® in Guinea Pigs. (Study No.: (b) (4))

Methods

Guinea pigs (4 per group) received a single intratympanic injection of OTO-201 vehicle followed 3 days later by a either a single dose of Ciprodex® or a treatment course of Ciprodex® (BID dosing for 7 days). Ciprodex® was administered through a tympanostomy tube. The animals receiving a single dose of Ciprodex® were monitored for 6 hours and the animals receiving the combination treatment were monitored for up to 28 days.

The middle ear was sampled at 1, 3, and 6 hours following the single Ciprodex® administration, or on Days 4, 10, 14, 21, and 28 for the 7 day treatment of Ciprodex® where the OTO-201 vehicle was administered on Day 0 and Ciprodex® was administered BID for 7 days from Day 3 to Day 10. The middle ears were sampled as follows. The lower anterior and posterior portion of the tympanic membrane was removed while attempting to leave the upper anterior and upper posterior tympanic membrane undisturbed. Using 22 and 27 gauge blunt needles, the middle ear cavity was lavaged twice with 100 μl of sterile water. Middle ear ciprofloxacin concentrations were assessed using a LC-MS/MS method.

Results

Three days after guinea pigs received intratympanic injections of OTO-201 vehicle, middle ear concentrations of ciprofloxacin were highest at the first and second sampling time-points (1 and 3 hours) after a single dose of Ciprodex® then decreased approximately 4 fold by 6 hours after dosing (Table 4). During the 7-Day course of BID Ciprodex®, ciprofloxacin middle ear concentrations (trough levels) peaked at approximately 4-8 μg/ml by Day 3 and remained at this level until Day 14 before falling to 1.0 μg/ml by Day 28 (Figure 8; Table 4). The trough levels and the rate of elimination of ciprofloxacin from guinea pig middle ears was very similar to what occurred in a similar study ((b) (4)) that did not include the co-administration of OTO-201 vehicle. In contrast, C_{max} levels were increased approximately 3-fold relative to Ciprodex® administered in the absence of (b) (4)% poloxamer 407. These results suggest the presence of (b) (4)% poloxamer 407 in the middle ear does not increase the persistence of ciprofloxacin derived from a 7-day BID treatment course of Ciprodex®, but peak middle ear ciprofloxacin concentrations are increased.

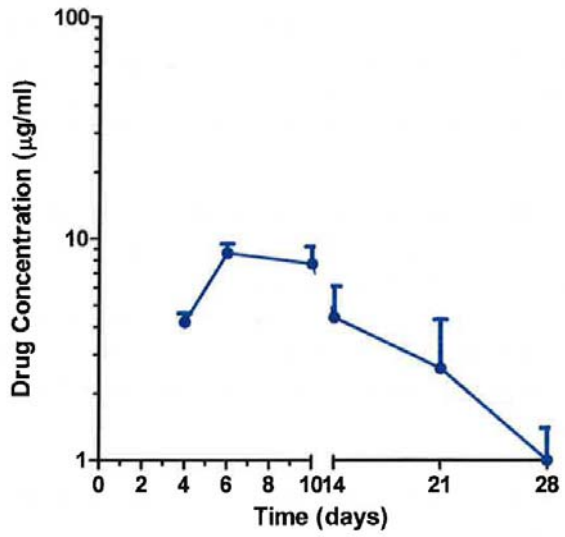


Figure 8: Middle Ear Ciprofloxacin (Trough Levels) Following a 7 Day BID Treatment Course of Ciprodex® Initiated 3 Days Post IT Injection of 0% OTO-201. (Sponsor's Figure)

Table 4: Summary of Middle Ear Ciprofloxacin Levels. (Sponsor's Table)

	Middle Ear Ciprofloxacin					
	#1	#2	#3	#4	Mean	SEM
Peak levels						
1 Hour	65.1	89.2	69.6	46.6	67.6	8.7
3 Hour	38.6	49.8	83.4	16.4	47.1	14.0
6 Hour	14.4	9.5	14.8	9.0	11.9	1.5
Trough levels						
Day 4	4.8	6.8	5.4	5.8	5.7	0.4
Day 10	5.8	5.5	12.0	7.3	7.7	1.5
Day 14	3.0	6.5	0.5	7.8	4.4	1.7
Day 21	1.0	1.5	0.1	7.7	2.6	1.7
Day 28	0.1	0.7	2.2	0.9	1.0	0.4

Guinea Pigs (N =4) were sampled at the indicated times in the middle ear. Values (mean ± SEM) are expressed as µg/ml.

6 General Toxicology

6.1 Single-Dose Toxicity

Two general toxicology studies examining primarily the effects of a single dose of OTO-201 on the auditory system and systemically with and without the combination

administration of a treatment course of Ciprodex® (Study Nos.: OTO-201-RSP-008 and OTO-201-RSP-010 respectively) are reviewed below.

Study title: A One-Month GLP Acute Ototoxicity Study of OTO-201 in Guinea Pigs.

Study no.:	1740-008; OTO-201-RSP-008
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Dec. 29, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OTO-201, Lot # FG-10-0016, 97% purity for ciprofloxacin. Cetraxal®, Lot # E09117. Ciprodex®, Lot # 180695F

Key Study Findings

1. The NOAEL for OTO-201 was considered to be the highest administered dose of 0.6% OTO-201.
2. No effect of treatment on the ABR was noted for either gender in the vehicle control group (Group 1) or any of the OTO-201 treatment groups (Groups 2-4). Males, but not females in Group 5 receiving Cetraxal® demonstrated a mild threshold elevation in both their left and right ears suggesting mild hearing loss.
3. In the 6.0% OTO-201 group, the presence of mild focus was noted in one ear (out of 20), as well as a small potential for minimal-moderate tissue reaction in the middle ear, at times associated with minimal fibrosis and ossicle immobility which was comparable to Cetraxal®. The highest incidence of ossicle immobility was 4 ears out of 20. In the Cetraxal® group, the presence of deformation/ perforation/white foci were noted in 3 ears, and in 7 ears in the Ciprodex® group.
4. Administration of OTO-201 and Cetraxal® was not associated with pronounced inner ear hair loss. In contrast, 60% of the animals were deleteriously affected in the Ciprodex® group.
5. In the middle ear, the presence of foreign material was noted at about the same incidence in all the groups including the vehicle control group.
6. Foamy macrophages were present in a small percentage of eustacian tubes from all of the groups receiving poloxamer 407 suggesting foamy macrophages may represent uptake of the vehicle.
7. Significant OTO-201-related reductions in total blood platelets, eosinophils, basophils, and MCHC were not thought to be toxicologically relevant due to a lack of dose response, similar effects in the Cetraxal® group, and/or inconsistent effects between the genders.

Methods

Doses:	0.6, 2.0, 6.0% OTO-201, Cetraxal® (0.2% ciprofloxaxin solution), or Ciprodex® (0.3% ciprofloxacin plus 0.1% dexamethasone).

Frequency of dosing:	Bilateral single doses for vehicle and OTO-201. Bilateral twice daily for 7 days for Cetraxal® and Ciprodex®
Route of administration:	Intratympanic injection for OTO-201 and tympanostomy tube drops for Cetraxal® and Ciprodex®
Dose volume:	50 µl/ear
Formulation/Vehicle:	Poloxamer 407
Species/Strain:	Crl:HA (Albino Hartley) Guinea Pigs
Number/Sex/Group:	7/sex/group
Age:	5-8 weeks old at dosing initiation
Weight:	Males: 220 to 396 grams and females: 265 to 349 grams at the time of randomization
Satellite groups:	Two animals per group were designated for eustachian tube removal.
Unique study design:	See Table 5. Six groups of male and female Albino Hartley guinea pigs (7/sex/group) were administered the poloxamer 407 vehicle, 0.6, 2.0, and 6.0% OTO-201, or the positive control articles, Cetraxal®, and Ciprodex®. The vehicle and OTO-201 doses were administered once on Day 1 by intratympanic injection to both ears. The positive control drugs were administered twice daily for 7 consecutive days via a tympanostomy tube. All of the surviving animals except those designated for Eustachian tube removal (2/sex/group) were euthanized on Day 30.
Deviation from study protocol:	Multiple protocol deviations were noted for this study. However, the deviations were not considered to have altered the results or compromised the study integrity.

Table 5: Study Design for the One-Month OTO-201 Toxicology Study in Guinea Pigs. (Sponsor's Table)

Group Assignments			
Group Number	Dose Level[a]	Number of Animals[b] Male	Female
1	Poloxamer 407	7	7
2	0.6% OTO-201	7	7
3	2.0% OTO-201	7	7
4	6.0% OTO-201	7	7
5	CETRAXAL® Otic	7	7
6	CIPRODEX® Otic	7	7

[a]Groups 1 through 4 received the vehicle or test article once on Day 1. Groups 5 and 6 were dosed twice daily for 7 continuous days starting on Day 1.
[b]Two animals/sex/group were designated for Eustachian tube removal only.

Observations and Results

Mortality

All animals were examined for morbidity and mortality twice daily throughout the duration of the study.

No animals were determined to have died due to test article-related causes.

Clinical Signs

All animals were examined for clinical signs twice per day. In addition, a detailed clinical examination of each animal was performed for Groups 1 through 4 twice on Day 1 (before and after dosing), and daily thereafter during the study. A detailed clinical examination of each animal in Groups 5 and 6 was performed before and after each dose on Day 1, after each dose on Days 2 through 7 and once daily thereafter during the study. Observations included evaluation of skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactions to handling and bizarre behavior. Animals were also evaluated for acute dosing effects including nystagmus, head tilting, circling, loss of balance, and/or ataxia.

No clinical signs considered to be related to test-article administration were observed. The primary clinical signs were discolored eyes and discolored material around the eyes and these findings were attributed to the orbital sinus blood collection procedure.

Body Weights

Animal body weights were measured once prior to randomization and daily throughout the study.

Male body weights did not vary between groups. Body weights for females in the high-dose OTO-201 group showed a trend toward reduction over the course of the study beginning on Day 5. Body weights for the females in the high-dose group were significantly lower than those of control females on Days 15 and 16. Subsequently the differences were not significant, but the overall trend continued for the remainder of the study.

Physical Examinations

In addition to the physical signs assessment, a complete physical examination was conducted on all animals on Day 8 and on designated animals on Day 28. Otoscopic evaluations were performed during the examinations, and observations included any irritation and possible signs of infection.

No OTO-201-related changes in physical examination results were noted. At least one animal from all groups demonstrated erythema in the ear canal during the pretest, Day 8, and/or Day 28 observations. These findings ranged from mild to severe, were similar across all groups at all time points, and did not appear to occur in a dose- or gender-dependent manner. Also at times the erythema was associated with mild to moderate debris in the ear canal. The Sponsor indicated that a low incidence of erythema and ear canal debris is considered normal in guinea pigs.

Auditory Brainstem Response (ABR) Evaluations

Excluding the animals designated for Eustachian tube removal, ABR evaluations were performed on animals pretest and animals in Groups 1-5 on Day 29. Examinations were conducted at each of three different frequencies (4, 10, and 20 kHz). The animals in group 6 treated with Ciprodex® did not receive ABR evaluations.

No effect of treatment on the ABR was noted for either gender in the vehicle control group (Group 1) or any of the OTO-201 treatment groups (Groups 2-4). Males, but not females in Group 5 receiving Cetraxal® demonstrated a mild threshold elevation in both their left and right ears suggesting mild hearing loss.

Table 6: Pre-dose and Day 29 Auditory Brain Stem Response Measurements in Male and Female Guinea Pigs. (Sponsor's Table)

Table 1. Pre and Post Group Averages (with Standard Deviation) for the Left and Right Ears at Each Test Frequency for Males

	Pre 4 khZ Average	Post 4 khZ Average	Pre 10 khZ Average	Post 10 khZ Average	Pre 20 khZ Average	Post 20 khZ Average
Group 1						
Left	42.2 (1.64)	39.0 (7.48)	27.0 (3.94)	28.4 (9.53)	16.6 (2.3)	20.4 (10.45)
Right	42.4 (2.3)	47.4 (12.1)	28.6 (4.98)	35.4 (14.08)	17.4 (1.14)	23.6 (20.14)
Group 2						
Left	38 (5.89)	38.8 (4.02)	23.4 (3.21)	26.4 (6.95)	16.4 (3.21)	15.4 (6.02)
Right	39.4 (3.43)	36.8 (5.97)	26 (4)	25.2 (5.02)	15.4 (3.13)	9.2 (6.42)
Group 3						
Left	42.6 (6.35)	36.2 (6.83)	35.2 (3.56)	24.2 (3.9)	18 (1.22)	20.2 (5.45)
Right	46 (5.15)	37.6 (5.37)	30.6 (4.72)	24.6 (3.78)	17.8 (5.54)	13.6 (4.51)
Group 4						
Left	42 (5.66)	34.6 (6.95)	32.4 (5.68)	26.8 (9.31)	21 (5.15)	21.8 (5.93)
Right	44.6 (3.36)	39.4 (10.81)	27.6 (5.37)	26.2 (2.05)	15.6 (1.67)	19.8 (10.43)
Group 5						
Left	45.2 (5.93)	57.6 (19.88)	31 (5.15)	44.2 (19.02)	18 (3.39)	40.2 (18.65)
Right	43.6 (6.19)	57.4 (22.42)	27.6 (2.88)	41 (24.37)	13.6 (4.16)	38.2 (25.2)

Table 2. Pre and Post Group Averages (with Standard Deviation) for the Left and Right Ears at Each Test Frequency for Females

	Pre 4 khZ Average	Post 4 khZ Average	Pre 10 khZ Average	Post 10 khZ Average	Pre 20 khZ Average	Post 20 khZ Average
Group 1						
Left	38.8 (8.64)	38.6 (11.22)	27.4 (6.27)	30.2 (13.52)	15.6 (7.57)	12.2 (13.94)
Right	40 (7.03)	28.8 (6.46)	30.6 (4.98)	21 (5.15)	15 (3.94)	4.4 (1.14)
Group 2						
Left	45.2 (4.44)	32.2 (1.64)	32.8 (4.32)	23 (3.81)	20 (3)	6.6 (1.82)
Right	47.6 (5.81)	32.5 (12.87)	33.8 (7.46)	26 (3.16)	16.6 (1.67)	4 (0.71)
Group 3						
Left	41.4 (5.18)	37 (14.37)	27.4 (4.1)	33 (19.62)	17.8 (1.48)	21.2 (18.63)
Right	39.6 (3.58)	37.4 (20.23)	26.4 (4.1)	31.8 (21.79)	17.2 (2.86)	16 (18.21)
Group 4						
Left	41.2 (5.63)	45 (15.79)	31.6 (4.51)	36.6 (17.18)	17.8 (4.27)	16.6 (10.29)
Right	41.2 (4.55)	34.2 (10.38)	28.6 (5.13)	25.2 (2.77)	15 (2.35)	10.2 (6.76)
Group 5						
Left	43.8 (5.63)	44 (18.48)	34.2 (4.76)	31.2 (13.03)	22.8 (4.76)	17.6 (10.97)
Right	44.8 (6.91)	36.6 (11.86)	31.2 (2.59)	34.4 (12.68)	18 (4.47)	28.4 (16.91)

Feed Consumption, Ophthalmoscopy, ECG: were not assessed.

Hematology

Blood samples were collected for hematology and clinical chemistry analysis from all animals except those designated for Eustachian tube removal in Groups 1-5 on Day 29

(the day prior to terminal necropsy). Coagulation parameters were not assessed and samples from Group 6 animals were not collected.

No changes that were considered to be toxicologically serious were noted in any of the hematology or coagulation parameters in response to OTO-201 or Cetraxal®. Total blood platelets were significantly reduced by about the same amount (≈ 19-26%) in male guinea pigs in all three OTO-201 dose groups. Group 5 males receiving Cetraxal® were not similarly affected. Total blood eosinophils were also significantly reduced in male guinea pigs in all three OTO-201 treatment groups by approximately 50-65% without a dose effect and in males in the Cetraxal® treatment group by 50%. Basophils were significantly reduced only in males receiving 0.6% OTO-201. Females were not similarly affected for either platelets, eosinophils, or basophils. In females, MCHC was slightly (≤ 3%) but significantly reduced in all three OTO-201 treatment groups without a dose effect. Females in the Cetraxal® treatment group were similarly affected.

Clinical Chemistry

Clinical chemistry samples were collected from the same animals and at the same time as the hematology samples.

No toxicologically relevant changes in any of the clinical chemistry parameters were noted in response to treatment with any of the test articles. Relative to the values for the vehicle control group, GGT was significantly decreased in males in the 0.6% and 6% OTO-201 groups to a similar degree of as much as 46% but it was not decreased in the 2% OTO-201 males or in any of the females.

Urinalysis: Not performed

Gross Pathology

Animals were examined for gross pathology internally and externally following euthanasia. In addition to the overall gross pathology assessment, animals received a gross middle ear assessment.

General Gross Pathology Results: One male in the high-dose (6.0%) OTO-201 group exhibited a tan discoloration of one testis. This was considered to be an incidental finding.

Auditory System Gross Pathology: There were minimal and infrequent OTO-201-related effects in the tympanic membrane or middle ear observed macroscopically. In the tympanic membrane the effects of OTO-201 were less severe than Cetraxal® or Ciprodex®. No findings were observed for the vehicle, 0.6% and 2.0% OTO-201 groups. In the 6.0% OTO-201 group, the presence of mild focus was noted in one ear (out of 20). In the Cetraxal® group, the presence of deformation/perforation/white foci were noted in 3 ears, and in 7 ears in the Ciprodex® group.

In the middle ear, the presence of foreign material was noted in the vehicle group (4 ears), 0.6% OTO-201 (1 ear), 2.0% OTO-201 (6 ears), 6.0% OTO-201 (5 ears) and in the comparator groups Cetraxal® (5 ears) and Ciprodex® (4 ears). The presence of

fluid was observed in some animals receiving 2.0% OTO-201 (7 ears), 6.0% OTO-201 (4 ears), Cetraxal® (2 ears) and Ciprodex® (1 ear). Mild discoloration of the middle ear was present in 1 ear of the vehicle group, and in the Cetraxal® group (4 ears).

Gross Middle Ear Assessment Evaluations

It should be noted that the gross middle ear assessment was conducted following the removal of the temporal bones. The Sponsor noted that in the opinion of Dr. (b) (4) , and an expert in middle ear toxicology, the procedure is sound for the determination of erythema, edema, or fibrosis. However, Dr. (b) (4) advised the Sponsor that based on his years of experience with this animal model that the surgical procedure alone could stimulate fluid in the middle ear with this finding thus occurring as an artifact.

The intratympanic administration of OTO-201 at the high dose of 6.0% was associated with a small potential of minimal-moderate tissue reaction in the middle ear, which was comparable to Cetraxal®. One female animal in the vehicle group was found with moderate fibrosis and impaired ossicle mobility in the left ear. The right ear of this animal and all the remaining middle ear assessment evaluations including ossicle mobility were normal in vehicle control animals. Middle ear assessment evaluations identified also that all middle ears and ossicle mobility were normal in the 0.6% OTO-201 group.

Middle ear assessment evaluations in the 2.0% OTO-201 group identified minimal to moderate fluid in the right ears of 5 males and 2 females. One female also had moderate fibrosis in the right ear. The left ears of two of the male animals also had fluid. None of these findings were associated with impaired ossicle mobility. Middle ear assessment evaluations in the 6.0% OTO-201 group identified the presence of minimal fibrosis in one female associated with fluid in the right ear. One male was also observed with fluid in the right ear and a second male was observed with moderate fibrosis in the right ear. The left ears of two males were observed with minimal or moderate fibrosis. Ossicle mobility was impaired in the right ear of three females and in the left ear of one male animal.

Middle ear assessment evaluations in the Cetralex® Otic group identified the presence of moderate fibrosis in one male associated with ossicle immobility in the right ear. One female was also observed with minimal fibrosis in the right ear. Ossicle mobility was present in the middle ears of all of the remaining animals.

Middle ear assessment evaluations in Ciprodex® group were normal. Ossicle mobility was impaired in two males and one female in this group.

Organ Weights

Following euthasia, the organs listed in Table 16 were weighed from all the animals not designated for Eustacian tube removal and absolute organ weights as well as organ weights relative to body weights and brain weights were recorded. Paired organs were weighed together. The thyroid gland and the bilateral parathyroid glands were weighed together, and the right mandibular/sublingual salivary glands were weighed together.

No OTO-201-related changes in organ weights were noted. Statistically significant decreases in mean absolute epididymides weight, and epididymides weight relative to body weight and brain weight were observed in 2 males receiving 0.6% OTO-201, 5 males receiving 2.0% OTO-201, 3 males receiving 6.0% OTO-201, and 2 males treated with Ciprodex®. However, the changes in epididymides weight did not correlate with decreased testes weights and the Sponsor thought the changes might have occurred due to irregular collection of the epididymides.

Reviewer Comment: *Neither the testes nor the epididymides were examined for histopathology, thus the relationship to OTO-201 administration remains uncertain.*

Histopathology

Adequate Battery
Yes
Peer Review
No
Histological Findings
Minimal numbers of foamy macrophages were observed within the lumen of the eustachian tube of two males and one female at 6.0% OTO-201, one female at 2.0% OTO-201, one male at 0.6% OTO-201, and one female of the vehicle control group. The macrophages were pale, plump, and filled with a foamy cytoplasm. The foamy macrophages may represent uptake of the vehicle by macrophages.

Minimal foreign material was observed in the lumen of the eustachian tube of one male at 6.0% OTO-201. The foreign material was granular and basophilic in appearance and was surrounded by a minimal number of foamy macrophages. The Sponsor indicated that the foreign material may have represented either the test article or vehicle.

The lining epithelium of the Eustachian tube of one male of the comparator Cetraxal® group was focally infiltrated by minimal numbers of heterophils with no evidence of epithelial degeneration. The toxicological significance of this finding is unknown but likely represents an incidental finding.

Mild, multifocal, ulceration of the gastric mucosa with associated minimal acute inflammation was observed in only one male of the comparator Cetraxal® group. This finding in the stomach may be secondary to stress; however, a test-article relation cannot be excluded.

Auditory System Histopathology Report.
Cytococleograms: The appearance of hair cell loss in the poloxamer 407 vehicle group and in the 6.0% OTO-201 group was within the normal range of variability across groups of animals. Neither the vehicle alone nor OTO-201 at 6.0% produced otopathology. In the Cetraxal® group, one animal stood out with increased otopathology, while the other nine were within normal range suggesting an equivocal finding. In contrast, the Ciprodex® group, only four of ten animals that fell completely within the normal range of variability, two animals with large regions of moderate hair

cell loss and two animals with spikes that included pronounced inner hair cell loss. This strongly suggests there could be a treatment effect of Ciprodex®.

Toxicokinetics

Toxicokinetic samples were collected from all animals except those designated for eustacian tube removal on Days 2 (24 hours after dosing) 7, 14, and 29. Plasma concentrations of ciprofloxacin were determined with a validated LC-MS/MS analysis method.

Systemic exposures to ciprofloxacin following administration of OTO-201, Cetraxal® or Ciprodex® are shown in Table 7. The plasma T_{max} for OTO-201 administration was Day 2 as opposed to later Day 7 T_{max} values for Cetraxal® and Ciprodex®. OTO-201 demonstrated a dose-dependent increase in plasma concentrations on all sampling days. The highest dose of OTO-201 (6.0%; Group 6) produced mean plasma ciprofloxacin concentrations on Day 2 that were approximately 4-fold higher than the peak ciprofloxacin concentrations produced by Cetraxal® and Ciprodex® on Day 7. While plasma ciprofloxacin levels were below the level of quantification (< 1.0 ng/ml) or substantially lower than peak levels on Days 14 and 29 for the low and mid-dose OTO-201 groups and the Cetraxal® and Ciprodex® dose groups, >10 ng/ml plasma levels persisted on Day 29 for the highest OTO-201 dose group. However, for all groups, plasma ciprofloxacin concentrations were much lower than the approximately 1 to 5 μg/ml peak serum ciprofloxacin concentrations reported to occur with clinical oral administration of 250 to 1000 mg ciprofloxacin in humans.

Table 7: Plasma Ciprofloxacin Levels Following Intratympanic OTO-201 Injection or treatment Courses of Cetraxal® or Ciprodex®.

Sampling Time	Plasma Concentrations (ng/ml)					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	BQL	10.1 ± 3.0	17.1 ± 8.9	22.9 ± 7.4	BQL – 2.3	BQL – 13.9
Day 7	BQL	BQL – 2.2	4.1 ± 1.5	16.2 ± 4.9	5.8 ± 2.2	6.0 ± 1.6
Day 14	BQL	BQL – 1.2	BQL – 8.0	12.1 ± 2.7	BQL	BQL
Day 29	BQL	BQL	2.3 ± 1.3	10.5 ± 4.0	BQL	BQL
BQL = below quantification level (< 1.0 ng/ml)						

Dosing Solution Analysis

In support of both the OTO-201 alone and OTO-201 + Ciprodex® toxicology studies (Study Nos.: OTO-201-RSP-008 and OTO-201-RSP-010) six OTO-201 samples representing three dose levels (0.6%, 2.0%, and 6.0% OTO-201) were analyzed on May 10-11, 2011. These samples were prepared from the OTO-201 batches used in the toxicology experiments but the samples were not obtained from the actual dosing solutions used in the experiments. Ciprofloxacin concentrations were determined using HPLC and ciprofloxacin reference standards.

The two mid dose (2.0%) and high-dose (6.0%) OTO-201 samples were determined to contain 95.6 to 97.75% of the label claim for ciprofloxacin. The two low-dose samples were initially shown to contain between 74.43 to 77.85% of the label claim for

ciprofloxacin. Reanalysis of the low-dose ciprofloxacin concentration on May 26-27, 2011 determined that the low-dose solution contained between 97.45% and 100.34% of the label claim.

Study title: Co-Administration of OTO-201 and Ciprodex®: A One-Month GLP Ototoxicity Study in Guinea Pigs.

Study no.:	OTO-201-RSP-010
Study report location:	Electronic transmission
Conducting laboratory and location:	Otonomy Inc., 5626 Oberlin Drive, San Diego, California 92121
Date of study initiation:	December 29, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OTO-201, Lot # FG-10-0016, 97% purity Ciprodex®, Lot No.: 180695F

Key Study Findings

1. Co-administration of OTO-201 and Ciprodex® did not produced systemic toxicity (mortality, clinical signs, changes in body weights, hematology and clinical chemistry parameters, or organ weights) as noted 29 days after OTO-201 dosing.
2. Auditory brain response thresholds were elevated in all groups suggesting the effect was mediated by Ciprodex® treatment.
3. Most of the auditory-system gross pathology including mild deformation/white foci/perforation of the tympanum and middle ear ossicle immobility occurred at a similar incidence in the vehicle control group and the OTO-201 treatment groups.
4. Foreign material associated with the tympanum and in the middle ear occurred only in the OTO-201-treatment groups in a ciprofloxacin dose-responsive manner suggesting the foreign material may have been ciprofloxacin.
5. The histological determination of cochlear hair loss occurred at a similar low incidence in the OTO-201 treatment and vehicle control groups.

Methods

Doses:	0, 0.6, or 6.0% OTO-201 followed by Ciprodex®.
Frequency of dosing:	Single bilateral dose of vehicle or OTO-201 followed by 7 BID bilateral doses of Ciprodex®
Route of administration:	OTO-201: intratympanic; Ciprodex®: via tympanostomy tubes.
Dose volume:	OTO-201: 50 μl; Ciprodex®: 10 μl.
Formulation/Vehicle:	Poloxamer 407
Species/Strain:	Crl:HA (Albino Hartley) Guinea pigs
Number/Sex/Group:	7/gender/group
Age:	Approximately 3.5 to 4 weeks of age at receipt. A two-week acclimation period followed.
Weight:	Males: 263 to 341 grams. Females: 274 to 317 grams.
Satellite groups:	Two animals/sex/group were designated for

	Eustachian tube (ET) removal.
Unique study design:	See Table 8 below. Vehicle (poloxamer 407) or OTO-201 (0.6 and 6.0%) were administered once during the study as a single intratympanic (bilateral) injection (50 µl) immediately prior to undergoing tympanostomy tube placement on study Day 1. The co-article (Ciprodex®) was administered topically BID for 7 consecutive days beginning on Day 4 at a dose of 10 µl per ear. In this dosing procedure, animals were immobilized, the tympanostomy tubes were visualized and the doses were administered bilaterally via pipette. Delivery to the middle ear was facilitated by pumping the tragus.
Deviation from study protocol:	Multiple deviations in the study protocol were noted. However, none was considered sufficient to negate the results or compromise the integrity of the findings.

Table 8: Study Design for Study No.: OTO-201-RSP-010. (Sponsor's Table)

Group Assignments			
Group Number	Dose Level[a] (% OTO-201)	Number of Animals[b]	
		Male	Female
1	0 (Poloxamer 407)	7	7
2	0.6	7	7
3	6.0	7	7

[a]Animals were dosed with the appropriate amount of OTO-201 on Day 1, followed by twice daily administration of CIPRODEX® Otic from Days 4 to 10.
[b]Two animals/sex/group designated for Eustachian tube (ET) removal only.

Observations and Results

Mortality

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily throughout the duration of the study.

There were no unscheduled deaths.

Clinical Signs

A detailed clinical examination of each animal was performed prior to dosing on Day 1, prior to each Ciprodex® administration on Days 4 through 10, and weekly thereafter

during the study. Observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior. Also animals were observed for acute effects of vehicle or test article treatment following dosing, including nystagmus, head tilting, circling, loss of balance, and/or ataxia.

No clinical signs appeared to be related to treatment with OTO-201 and Ciprodex®. Some clinical signs, red discharge in the left ears of some males and eye material and lacrimation were thought to be related to the dosing procedure for Ciprodex® or the orbital blood collection procedure respectively.

Body Weights

Body weights for all animals were measured and recorded within one day of receipt, twice prior to randomization, and approximately twice weekly during the study.

Body weights and body weight gains did not substantially differ between groups over the course of the experiment.

Feed Consumption: Not performed

Physical Examinations

A complete physical examination was conducted on all animals pretest and on all animals not designated for ET removal prior to the terminal necropsy (Day 29). Otoscopic evaluations were performed on all animals during the physical examinations. Observations included any irritation and possible signs of infection.

Erythema was observed pretest (prior to dosing) in the left or right ear canal of two animals in the poloxamer 407 vehicle group and in one animal in the 0.6% OTO-201 group. On Day 29, erythema was observed one or both ear canals of two 0.6% OTO-201 animals and one animal in the 6.0% OTO-201 group. At times, these findings were associated with mild or moderate debris in the ear canal, however not in every case. The findings were not considered treatment-related because both ear erythema and debris are relatively common in guinea pigs, and the findings sometimes occurred pre-test, were similar across all groups and time points, and did not appear to have a dose-response or sex-dependence relationship.

Auditory Brainstem Response (ABR) Evaluations

ABR evaluations were performed on all animals pretest and on all animals not designated for Eustacian Tube removal on Day 29 by trained MPI Research personnel. These examinations were conducted at each of three different frequencies (4, 10, and 20 kHz),

The mean hearing thresholds were elevated at variable frequencies for both males and females in all groups including Group 1 (vehicle plus Ciprodex®) on Day 29 compared to pre-test values (Table 9). These results were similar for all groups suggesting that

increasing concentrations of OTO-201 did not affect auditory function as measured by ABR and that the hearing threshold shift was due to the Ciprodex® treatment.

Table 9: Pretest and Day 29 Auditory Brainstem Response (ABR) Measurements for Male and Female Guinea Pigs. (Sponsor's Table)

Table 1. Pre and Post Group Averages (with Standard Deviation) for the Left and Right Ears at Each Test Frequency for Males

	Pre 4 khZ Average	Post 4 khZ Average	Pre 10 khZ Average	Post 10 khZ Average	Pre 20 khZ Average	Post 20 khZ Average
Group 1						
Left	40.4 (6.62)	62.0 (13.64)	29.8 (8.01)	56.8 (15.45)	17.8 (2.77)	64.0 (7.97)
Right	36.8 (4.6)	62.2 (20.54)	29.2 (5.63)	61.0 (17.94)	15.8 (5.22)	45.0 (16.94)
Group 2						
Left	40.0 (4.3)	63.2 (14.24)	28.0 (4.3)	54.6 (15.6)	13.6 (3.78)	49.4 (17.8)
Right	39.8 (2.28)	49.8 (19.41)	29.2 (4.66)	43.2 (21.19)	12.2 (4.44)	31.2 (19.94)
Group 3						
Left	40.2 (5.12)	54.0 (13.29)	31.0 (3.67)	44.8 (8.23)	18.6 (4.04)	34.2 (9.01)
Right	40.6 (4.28)	58.0 (20.77)	30.4 (8.68)	47.6 (12.64)	19.4 (4.98)	46.6 (10.26)

Table 2. Pre and Post Group Averages (with Standard Deviation) for the Left and Right Ears at Each Test Frequency for Females

	Pre 4 khZ Average	Post 4 khZ Average	Pre 10 khZ Average	Post 10 khZ Average	Pre 20 khZ Average	Post 20 khZ Average
Group 1						
Left	35.2 (3.96)	49.0 (14.34)	30.0 (4.85)	50.0 (15.12)	11.4 (5.77)	35.6 (11.15)
Right	36.8 (4.32)	58.6 (15.37)	27.0 (6.2)	52.4 (12.18)	12.0 (3.39)	38.8 (18.07)
Group 2						
Left	39.8 (2.77)	59.6 (12.12)	31.2 (8.7)	47.4 (16.7)	13.0 (5.43)	44.0 (19.57)
Right	36.0 (3.0)	57.6 (11.7)	29.6 (5.46)	49.8 (12.76)	13.8 (3.83)	35.0 (12.02)
Group 3						
Left	35.4 (0.89)	54.0 (11.6)	28.6 (5.13)	47.4 (12.9)	12.6 (5.37)	45.6 (17.9)
Right	35.4 (6.35)	56.2 (11.78)	26.6 (6.31)	57.4 (16.35)	12.4 (3.85)	40.0 (8.19)

Ophthalmoscopy and ECG: not performed

Hematology

Clinical pathology evaluations (hematology coagulation parameters and clinical chemistry) were conducted on all animals not designated for ET removal on the day prior to the terminal necropsy (Day 29).

There were no clear OTO-201-related effects on hematology parameters. Total platelets were reduced in females in both the 0.6% and 6.0% OTO-201 groups by ≈ 22% compared to vehicle control group females. Platelets were not reduced in males, however, and the total platelets in the male vehicle control group were similar to the

values for the female 0.6% and 6.0% groups. In addition, there were statistical differences for male MCHC, total eosinophils, and total basophils, and female MCV in the 0.6% OTO-201 group compared to the gender-specific vehicle control groups. These changes were not considered toxicologically meaningful due to the small magnitude or direction of change, inconsistency between genders, and/or a lack of dose response.

Clinical Chemistry

Blood samples were collected for serum chemistry analysis according to the same schedule and from the same animals as for the hematology samples.

Aspartate aminotransferase (AST; 1.5-fold) and alanine aminotransferase (ALT; 1.4-fold) were moderately increased in multiple males receiving 0.6% OTO-201, but the changes were not statistically significant relative to the vehicle group, and changes were not noted in the high-dose OTO-201 group. The mean sorbitol dehydrogenase (SDH; 2.3-fold) was moderately increased (statistically significant) in males receiving 0.6% OTO-201. However, no increase was demonstrated in the high-dose OTO-201 group for either gender. Creatinine was also statistically lower in males and females receiving 0.6% OTO-201, but values remained within expected ranges and these findings were not observed at the higher dose of 6.0% OTO-201. The small magnitude of change and the lack of dose-dependency for creatinine suggest that these findings are not toxicologically relevant.

Urinalysis: Not performed.

Gross Pathology

General Gross Pathology: The animals were examined carefully for external abnormalities including masses following perfusion and the removal of the middle ear tissues. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and, where required, placed in fixative.

The Sponsor reported that macroscopic observations were limited to the middle ear and tympanic membrane.

Auditory System Gross Pathology: There were no test-article related findings as the degree and severity of observations were comparable across the treatment groups. In the tympanic membrane, the presence of mild deformation/white foci/perforation was noted in (out of 20 ears) 2 ears in the poloxamer 407 + Ciprodex® group, 6 ears in the 0.6% OTO-201 + Ciprodex® group and in 2 ears in the 6.0% OTO-201 + Ciprodex®-treated animals. In the middle ear, a mild edema was noted in 1 ear in the 0.6% OTO-201 + Ciprodex® group. Fluid was observed in 1-2 ears across all groups. No other findings were reported, with the exception of the presence of foreign material in one ear of the 0.6% OTO-201 + Ciprodex® group, and in 7 ears in the 6.0% OTO-201 + Ciprodex® group. The incidence of middle ear foreign material appeared to increase in an OTO-201 dose-proportional manner, suggesting that the foreign material may have been ciprofloxaxcin.

Gross Middle Ear Assessment Evaluations: Animals not designated for Eustachian tube removal had both temporal bones removed and a gross assessment of each middle ear was performed using a dissecting microscope. During this assessment, the middle ear was opened by removing regions of the temporal bone in order to reveal the ossicles. The presence of fibrous tissue, edema/swelling and fluid was noted and rated. A forceps was used to gently apply pressure to the ossicles and mobility was noted as present or not present.

Edema was observed in the right ear of one animal in the poloxamer 407 vehicle group, and two animals in the 0.6% OTO-201 group. Fibrosis was observed in one animal in the poloxamer 407 group. Ossicle mobility was not present in one ear in each of four animals in the poloxamer 407 group, two animals (two ears) in the 0.6% OTO-201, and three animals (four ears) in the 6.0% OTO-201 group. As ossicle immobility was observed in the poloxamer 407 group and occurred at a similar incidence in the OTO-201 treated groups, ossicle immobility may have resulted from the co-administration of the Ciprodex®, possibly due to poloxamer 407, or as artifact.

Organ Weights

Body weights and organ weights for the organs listed in Table 16 were recorded for all surviving animals not designated for ET removal at the scheduled necropsy and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together. A combined weight of the thyroid gland with bilateral parathyroid glands was obtained, and the right mandibular/sublingual salivary glands were weighed together. The thyroid/parathyroid gland and pituitary gland were weighed following fixation.

There were no OTO-201-related changes in organ weights or statistically significant organ weight changes.

Histopathology

Adequate Battery
General Histopathology: The tissues examined for histopathology other than the auditory organs were limited to the Eustachian tube, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum.
Auditory System Histopathology: Following gross assessment, cochleas (left ear) were dissected and stained, and a cytocochleogram analysis was performed at the Kresge Hearing Research Institute (KHRI). The right ear tissues were stored in paraformaldehyde and processed for histology and light microscopic evaluations at KHRI.
Peer Review
No
Histological Findings
General Histopathology: There were no test article-related microscopic findings in the tissues examined. The few remaining microscopic observations were either, common background findings in guinea pigs, or incidental, and were therefore considered unrelated to treatment.

Auditory System Histopathology: The middle ear histology was normal in the majority of animals in all groups. Two of ten animals in the 0.6% OTO-201 + Ciprodex® treatment group had foreign material in the middle ear and some foamy macrophages. However, the remaining animals in this group did not display the same findings. This response also was not found in the vehicle group or in the higher dosage treatment group. Overall, there was no treatment effect on middle ear histology of the administration of vehicle, 0.6% or 6.0% OTO-201 in combination with Ciprodex®.
Cytocochleograms: The overall large majority of animals in all groups had normal appearing cochlear histology. The appearance of hair cell loss in the OTO-201 vehicle (poloxamer 407) + Ciprodex® group and in the 6.0 % OTO 201 + Ciprodex® group was within the normal range of variability across groups of animals, with 2 of 10 showing only minimal hair cell loss in both groups and 1 of 10 showing minimal-moderate loss in the vehicle group. The majority of animals in the 0.6% OTO 201 + Ciprodex® group were within the normal range, with 2 of 10 showing only minimal hair cell loss and 2 of 10 having greater hair cell loss (minimal to moderate for one animal and moderate to large for the other). Overall, because of the lack of differences between the treatment and vehicle control groups, the observed incidence of minimal hair cell loss may be attributable to the co-administration of Ciprodex®.

Toxicokinetics

Blood samples (approximately 1 ml) were collected from all animals not designated for ET removal via the orbital sinus after anesthesia with isoflurane administration for determination of the plasma concentrations of the test article[9]. Samples were collected on Day 1 (24 hours postdose), Day 3 (prior to Ciprodex® dosing), Day 11 (following completion of Ciprodex® dosing), Day 14, and Day 29.

Plasma exposure to ciprofloxacin following co-administration of OTO-201 and Ciprodex® appeared minimal at all doses and at all time points. In the vehicle control group (poloxamer 407 + Ciprodex®) levels peaked at ≈ 1.7 ng/ml on Day 11 following completion of the Ciprodex® treatment course. In the OTO-201 plus Ciprodex® treatment groups plasma levels peaked on Day 1 (the day following intratympanic injection of OTO-201) reaching 9.8 to 19.2 ng/ml and 9.7 to 33.3 ng/ml for the 0.6 and 6.0% OTO-201 doses respectively.

Dosing Solution Analysis

In support of both the OTO-201 alone and OTO-201 + Ciprodex® toxicology studies (Study Nos.: OTO-201-RSP-008 and OTO-201-RSP-010) six OTO-201 samples representing three dose levels (0.6%, 2.0%, and 6.0% OTO-201) were analyzed on May 10-11, 2011. These samples were prepared from the OTO-201 batches used in the toxicology experiments but the samples were not obtained from the actual dosing solutions used in the experiments. Ciprofloxacin concentrations were determined using HPLC and ciprofloxacin reference standards.

The two mid dose (2.0%) and high-dose (6.0%) OTO-201 samples were determined to contain 95.6 to 97.75% of the label claim for ciprofloxacin. The two low-dose samples were initially shown to contain between 74.43 to 77.85% of the label claim for ciprofloxacin. Reanalysis of the low-dose ciprofloxacin concentration on May 26-27,

2011 determined that the low-dose solution contained between 97.45% and 100.34% of the label claim.

7 Genetic Toxicology

Genetic Toxicology Studies were not performed with OTO-201.

The Ciprodex® and Cetraxal® product labels include the following information.
Eight *in vitro* mutagenicity tests have been conducted with ciprofloxacin and the test results are listed below:

1. *Salmonella*/Microsome Test (Negative)
2. *Escherichia coli* DNA Repair Assay (Negative)
3. Mouse Lymphoma Cell Forward Mutation Assay (Positive)
4. Chinese Hamster V79 Cell HGPRT Test (negative).
5. Syrian Hamster Embryo Cell Transformation Assay (Negative).
6. *Saccharomyces cerevisiae* Point Mutation Assay (Negative)
7. *Saccharomyces cerevisiae* Mitotic Crossover and Gene Conversion Assay (Negative).
8. Rat Hepatocyte DNA Repair Assay (Positive).

8 Carcinogenicity

Carcinogenicity studies were not performed with OTO-201

The information included on the Ciprodex® and Cetraxal® product labels indicates that "Long-term carcinogenicity studies in mice and rats have been completed for ciprofloxacin. After daily oral doses of 750 mg/kg (mice) and 250 mg/kg (rats) were administered for up to 2 years, there was no evidence that ciprofloxacin had any carcinogenic or tumorigenic effects in these species."

9 Reproductive and Developmental Toxicology

Reproductive and Developmental Toxicology Studies were not performed with OTO-201.

Regarding pregnancy and teratogenic effects, the Ciprodex® and Cetraxal® product labels indicate: "Pregnancy Category C: Reproduction studies have been performed in rats and mice using oral doses of up to 100 mg/kg and intravenous (IV) doses up to 30 mg/kg and have revealed no evidence of harm to the fetus as a result of ciprofloxacin. In rabbits, ciprofloxacin (30 and 100 mg/kg orally) produced gastrointestinal disturbances resulting in maternal weight loss and an increased incidence of abortion, but no teratogenicity was observed at either dose. After intravenous administration of doses up to 20 mg/kg, no maternal toxicity was produced in the rabbit, and no embryotoxicity or teratogenicity was observed."

Regarding fertility effects, the Ciprodex® and Cetraxal® product labels indicate: "Fertility studies performed in rats at oral dose of ciprofloxacin up to 100 mg/kg/day revealed no evidence of impairment." Paraphrased information from both labels indicates that an

oral dose of 100 mg/kg/day would be over 100 times the maximum recommended clinical dose of ototopical ciprofloxacin based upon body surface, assuming total absorption of ciprofloxacin from the ear of patients treated with Ciprodex® or Cetraxal® twice per day.

10 Special Toxicology Studies

Study title: Ventilation Tube Patency Following Intratympanic Administration of (b) (4)% Poloxamer 407 (b) (4) in Guinea Pigs

Study no.:	OTO-200-RSP-022-01
Study report location:	Electronic transmission
Conducting laboratory and location:	Otonomy Inc.
Date of study initiation:	The initiation date was not identified
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	(b) (4)% poloxamer 407 dyed with Evans Blue, Lot # and purity were not identified.

Methods

Doses:	Only the vehicle, (b) (4)% poloxamer 407 dyed with Evans Blue.
Frequency of dosing:	Single dose
Route of administration:	Intratympanic
Dose volume:	50 µl
Formulation/Vehicle:	(b) (4)% poloxamer 407
Species/Strain:	Guinea pigs2
Number/Sex/Group:	4 females per group
Age:	6-8 weeks of age
Weight:	200-300 grams
Satellite groups:	none
Unique study design:	Four female guinea pigs received a single intratympanic injection (50 µl) of (b) (4)% poloxamer 407 while anesthetized. Subsequently, while still anesthetized, a ventilation tube was inserted through the tympanic membrane into the middle ear. The opening of the ventilation tube was visually assessed for clogging using a surgical microscope on Days 1 and Days 3.
Deviation from study protocol:	Deviations were not noted.

Observations and Results

The study report included two photographs taken with a surgical microscope of the ear drum and the opening of the ventilation tube. At least for the Day 3 photograph, the opening to the ventilation tube does not appear to be occluded by the poloxamer 407 (dyed with Evan's Blue dye).

Reviewer Comment: *It is difficult to assess the patency and occlusion status of the ventilation tubes from the photographs that were provided. More comprehensive information could have been provided by assessing and photographing the entire length of the tubes following termination of the study animals and tube removal.*

Study title: An Acute Dermal Toxicity Study of OTO-201 in Guinea Pigs.

Study no.:	OTO-201-RSP-009
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OTO-201, FG-10-0016

Methods

Doses:	0.2% ciprofloxacin in Cetraxal®, and 0.6% ciprofloxacin in OTO-201.
Frequency of dosing:	Single dose held onto the skin for 24 hours
Route of administration:	Topical dermal
Dose volume:	1 ml
Formulation/Vehicle:	Buffered (b) (4)% poloxamer 407 solution (with 50 mM TRIS).
Species/Strain:	Guinea pig/Crl:HA (Albino Hartley)
Number/Sex/Group:	5 animals/sex/group
Age:	Approximately 5 weeks
Weight:	385-436 grams (male) and 319-363 grams (female).
Satellite groups:	none
Unique study design:	See Table Below. The comparator (Cetraxal®; 0.2% ciprofloxacin) and test article (OTO-201; 6.0% ciprofloxacin) were administered once to each animal in 1 ml dose volumes by topical dermal application. The comparator and test articles were administered on Day 1 and held in contact with the skin for 24 hours.
Deviation from study protocol:	Study protocol deviations were not considered to have influenced the study results or study integrity.

Table 10: Study Design for Study No.: OTO-201-RSP-009 (Sponsor's Table)

Group Assignments					
Group Number	Treatment	Ciprofloxacin Dose Level	Dose Volume	Number of Animals	
				Male	Female
1	Cetraxal Otic	0.2% (2 mg/mL)	1.0 mL	5	5
2	OTO-201	6.0% (60 mg/mL)	1.0 mL	5	5

Observations and Results

No mortality occurred, and no dermal toxicity was observed in either the Cetraxal® or OTO-201 groups.

Study title: Skin Sensitization (Buehler Method) Study of OTO-201 in Guinea Pigs.

Study no.:	OTO-201-RSP-007
Study report location:	Electronic transmission
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 3, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	OTO-201, FG-10-0016 Cetraxal®, Lot # E09117

Methods

Doses:	0, 0.6, 2, and 6% OTO-201, and one 0.4 ml dose of Cetraxal®.
Frequency of dosing:	Three induction doses and one challenge dose.
Route of administration:	Topical dermal
Dose volume:	0.4 ml for all
Formulation/Vehicle:	For OTO-201: (b) (4) % Poloxamer 407 Solution (with 50 mM Tris).
Species/Strain:	Guinea pigs/Crl:HA Albino Hartley
Number/Sex/Group:	45/sex
Age:	3.5 to 5 weeks (males) and 4-5 weeks (females)
Weight:	214 to 438 grams (males) and 191 to 373 grams (females).
Satellite groups:	None
Unique study design:	During the Induction Phase, vehicle, comparator, and test article formulations were administered to the left scapular region on Days 1, 8, and 15. Animals were not treated for 2 weeks prior to the Challenge Phase. During the Challenge Phase (Day 29), comparator or test article formulations were administered once to the left flank of the

	same animals used in the Induction Phase (including the designated control animals). On the day after the Challenge Phase dose (at least 6 hours before the first scoring), the animals were depilated with a short application of lotion hair remover. Dermal observations and scores for erythema and edema were recorded at approximately 24 and 48 hours post-challenge.
Deviation from study protocol:	No significant study deviations were noted for this study.

Table 11: Study Design for Study No.: OTO-201-RSP-007. (Sponsor's Table)

Group Assignments			
Group Number	Targeted Dose Level (0.4 mL)	Number of Animals[a]	
		Male	Female
	Induction Phase		
1	Vehicle	5	5
2	Cetraxal Otic	10	10
3	0.6% OTO-201	10	10
4	2.0% OTO-201	10	10
5	6.0% OTO-201	10	10
	Challenge Phase		
1	6.0% OTO-201	5	5
2	Cetraxal Otic	10	10
3	0.6% OTO-201	10	10
4	2.0% OTO-201	10	10
5	6.0% OTO-201	10	10

[a]The same animals were treated during the Induction and Challenge Phases. The respective material was administered to the left scapular region during the Induction Phase and to the left flank during the Challenge Phase.

Observations and Results

No dermal irritation was observed in any animal dosed with the vehicle, Cetraxal®, or OTO-201 at any strength. These results were compared to a historical positive control, hexyl cinnamic aldehyde (HCA) which induced slight to moderate erythema in 95% of treated animals from a previous study.

Table 12: Dermal Irritation Scores. (Sponsor's Table)

Summary of Dermal Irritation Scores – Challenge Phase							
Group	Number of Animals	Sum of 24 Hour Scores	Sum of 48 Hour Scores	Number of Animals with Scores of ≥1 at either 24 and/or 48 hours[a]	Severity Index (SI) 24 Hour Score	Severity Index (SI) 48 Hour Score	Sensitization Incidence Index (SII)
Vehicle	10	0	0	0	0	0	0
Cetraxal Otic	20	0	0	0	0	0	0
0.6% OTO-201	20	0	0	0	0	0	0
2.0% OTO-201	20	0	0	0	0	0	0
6.0% OTO-201	20	0	0	0	0	0	0
HCA 50%[b]	20	16.5	16	13	0.825	0.8	65

[a]Animals showing a positive response at both observations are counted as 1 incidence.
[b]Historical positive control data established on (b) (4) Study Number 999-742.

11 Integrated Summary and Safety Evaluation

OTO-201 is a suspension of ciprofloxacin hydrate in buffered solution (pH 7-8) containing (b) (4)% poloxamer 407, a glycol polymer. OTO-201 exists as a liquid at room temperature and gels immediately upon transitioning to body temperature.

Two ciprofloxacin-containing marketed products Cetraxal® (0.2% ciprofloxacin solution) and Ciprodex® (0.3% ciprofloxacin plus 0.1% dexamethasone) are currently the standard of care for the treatment of acute otitis externa. In addition, Ciprodex® is used to treat acute otitis media in patients with tympanostomy tube placement. Both products are administered BID as ear drops for 7 days. Compliance may be a problem for both products and for Ciprodex® in patients with tympanostomy tube placement, delivery of the product to the middle ear is potentially inconsistent. OTO-201 is a sustained release preparation by virtue of the suspension of ciprofloxacin in (b) (4)% poloxamer 407, and it is hoped that a single intratympanic injection of OTO-201 will provide more consistent ciprofloxacin coverage in the middle ear than a treatment course of BID doses of Ciprodex® over 7 days.

Inactive ingredients in the OTO-201 preparations include tromethamine, poloxamer 407, sodium chloride, hydrochloric acid (b) (4), and water. The drug product is packaged in a sterile vial for injection. Poloxamer 407 and tromethamine have not been used in any marketed otic products. Poloxamer 407 has been used in oral, topical, ophthalmic and periodontal products at lower concentrations. The periodontal products contained (b) (4)% poloxamer 407. Tromethamine has been used in many different kinds of approved products including IV, IM, intrathecal, oral and topical products and at higher percent concentrations than the present product ((b) (4)%) in IV, and ophthalmic products.

The Sponsor conducted multiple pharmacokinetic studies in guinea pigs in an effort to characterize middle and inner ear concentrations of ciprofloxacin under varying conditions. These studies examined variations associated with different ciprofloxacin concentrations in OTO-201 preparations, variable OTO-201 injection volumes, and OTO-201 injections in wet middle ear conditions. In addition, the middle ear pharmacokinetics of Ciprodex® alone and in the presence of (b) (4)% poloxamer 407 was

examined. Several patterns were apparent in the findings. The middle ear C_{max} levels of ciprofloxacin did not vary greatly with increasing concentrations of ciprofloxacin (0.6, 2.0, 6.0 and 12.0%) in OTO-201 preparations. However AUC and mean residence time increased with dose although in a less than dose-proportional manner. Inner ear concentrations followed a similar pattern in terms of retention time and the dose-relationship of the AUC values, but C_{max} and AUC were much less than the middle ear values. Thus the highest concentrations of ciprofloxacin occurred in the middle ear following intratympanic injection of OTO-201. The results indicate that maximum middle ear concentration of ciprofloxacin cannot be increased by increasing the dose of OTO-201, but higher ciprofloxacin concentrations are maintained longer with higher middle ear concentrations depending on the dose of OTO-201. In order to achieve a ciprofloxacin therapeutic effect over a therapy period of one to two weeks, but minimize the potential for ciprofloxacin-related toxicity associated with prolonged middle ear retention of high ciprofloxacin concentrations, an optimal dose of OTO-201 must be determined. The guinea pig studies of OTO-201 in the middle ear suggest even moderate concentrations of ciprofloxacin in OTO-201 can remain in the middle ear longer than is optimal.

The site of action for ciprofloxacin is bacteria colonizing the middle ear epithelium. In contrast to the middle ear lumen, ciprofloxacin C_{max} values for the middle ear epithelium followed a dose-responsive pattern as did the AUC values. However, retention of ciprofloxacin in the middle ear epithelium appeared slightly increased compared to the middle ear lumen even at the lowest ciprofloxacin dose concentration of 0.6% suggesting that prolonged retention and the potential for ciprofloxacin toxicity is accentuated in middle ear epithelial tissue. A similar pattern was evident in guinea pig “wet ears” with increased AUC and retention time for the 2.0% ciprofloxacin concentration in OTO-201 compared to the same dose in dry guinea pig ears. Because post-surgical otorrhea occurs at a high frequency in the ears of patients receiving tympanostomies, the guinea pig “wet ear” model probably simulates the actual disease conditions best.

According to the Ciprodex® product label, “the safety and efficacy of Ciprodex® Otic have been established in pediatric patients 6 months and older (937 patients) in adequate and well controlled clinical trials.” This information suggests that in guinea pig toxicology studies, safe doses of OTO-201 are those that produce equivalent or less toxicity than Ciprodex®. In the toxicology study where guinea pigs received intratympanic OTO-201 or vehicle or separate treatment courses of Cetraxal® or Ciprodex® (Study No.: OTO-201-RSP-008), limited otic toxicity was noted in the high-dose OTO-201 group with more pronounced toxicity in the Cetraxal® and Ciprodex® groups. Notably auditory brain responses were not altered for the vehicle or OTO-201-treated animals at any dose. Also the appearance of hair cell loss in the inner ear in the poloxamer 407 vehicle group and the high-dose OTO-201 group were within the normal range of variability. In contrast, males but not females receiving Cetraxal® demonstrated a mild threshold elevation in both their left and right ears suggesting mild hearing loss, and in the Ciprodex® group, only four of ten animals had hair cell loss that fell completely within the normal range of variability, with two animals with large regions

of moderate hair cell loss and two animals with spikes that included pronounced inner hair cell loss.

Other abnormal otic findings included the presence of mild focus in one ear (out of 20) in the 6.0% OTO-201 group. In the Cetraxal® group, the presence of deformation/ perforation/white foci were noted in 3 ears, and in 7 ears in the Ciprodex® group. In the middle ear, the presence of foreign material was noted at a similar incidence in all groups, in the vehicle group (4 ears), 0.6% OTO-201 (1 ear), 2.0% OTO-201 (6 ears), 6.0% OTO-201 (5 ears) and in the comparator groups Cetraxal® (5 ears) and Ciprodex® (4 ears). The intratympanic administration of OTO-201 at the high dose of 6.0% was associated with a small potential of minimal-moderate tissue reaction in the middle ear, at times associated with minimal fibrosis and ossicle immobility which was comparable to Cetraxal®. The highest incidence of ossicle immobility was 4 ears out of 20. Foamy macrophages were present in a small percentage of eustacian tubes from all of the groups receiving poloxamer 407 suggesting foamy macrophages may represent uptake of the vehicle.

Patients that do not respond to OTO-201 treatment in the first clinical trial are scheduled to receive rescue treatment with a 7-day treatment course of BID Ciprodex®. For this reason it is important to understand what middle ear ciprofloxacin concentrations and possible otic toxicity might result from combination treatment with OTO-201 and Ciprodex®. The combination of intratympanic (b)(4)% poloxamer 407 followed by a treatment course of Ciprodex® was examined in one study. In this study, C_{max} levels of middle ear ciprofloxacin increased about 3 fold with a single Ciprodex® administration following intratympanic (b)(4)% poloxamer 407 compared to the middle ear C_{max} levels produced by a single Ciprodex® administration in the absence of (b)(4)% poloxamer 407 in another study. In contrast, the daily trough middle ear ciprofloxacin concentrations were very similar in both studies. Ciprofloxacin levels were substantially reduced to 1-2 μg/ml 28 days after the first dose compared to previous trough levels of approximately 10 μg/ml for the first 7 days. This data suggests that combining Ciprodex® with poloxamer 407 *in vivo* does not result in prolonged retention of ciprofloxacin in the middle ear, but peak middle ear ciprofloxacin concentrations may increase. A concern is that the combination treatment of OTO-201 and Ciprodex® may lead to higher levels and prolonged retention of middle ear ciprofloxacin with possible toxicological consequences. Unfortunately middle ear ciprofloxacin levels resulting from combination treatment with OTO-201 and Ciprodex® were not elucidated in the guinea pig toxicology study where poloxamer 407 vehicle or OTO-201 were co-administered with Ciprodex®. One finding in this study which is consistent with prolonged retention of middle ear ciprofloxacin was the OTO-201 dose-proportional increase in the incidence of foreign material associated with the tympanum and middle ear. Middle-ear foreign material was not observed in the vehicle + Ciprodex® group suggesting the foreign material was retained ciprofloxacin occurring as a result of OTO-201 and Ciprodex® co-administration.

However, an OTO-201 dose-dependent increase in otic toxicity was not observed in this study. ABR thresholds were similarly elevated in all groups suggesting the effect was

mediated by Ciprodex® treatment but not by OTO-201 dose. The remaining otic toxicity, including mild deformation/white foci/perforation of the tympanum and middle ear ossicle immobility occurred at a similar incidence in the vehicle control and OTO-201 treatment groups also suggesting a controlling influence by the co-administration of Ciprodex®, but not by OTO-201. Minimal cochlear hair loss also occurred at a similar incidence in the OTO-201 and vehicle control groups. While OTO-201 related toxicity was not detected in the OTO-201-Ciprodex® co-administration study, it will still be useful to determine if middle ear ciprofloxacin concentrations and/or retention are expected to increase with co-administration to better assess the potential for otic toxicity in juvenile patients. In order to obtain this information, a pharmacokinetic study examining middle ear ciprofloxacin concentrations in conjunction with co-administration of OTO-201 and a treatment course of Ciprodex® in guinea pigs is recommended.

In the absence of co-administration with Ciprodex®, the high OTO-201 dose (50 μl of 6.0%/ear) can be considered to be the NOAEL dose as it caused only marginal systemic toxicity and generally equivocal otic toxicity. This dose corresponds to a per ear dose of 3 mg/500 grams body weight or 6 mg/kg. The initial clinical dose planned for the proposed clinical study is 4 mg OTO-201/ear (200 μl of 2% OTO-201) which corresponds to a total dose of 8 mg per average 10 kg subject or 800 μg/kg and higher for small patients as young as 6-months old. The safety margin for toxicity based on body surface area comparison and determination of the human dose equivalent for the NOAEL dose is 1.65 fold (Table 13). The 1.65 fold safety margin is much lower than the preferred 10-fold safety margin, but other findings and comparisons suggest relative safety for OTO-201 with regard to systemic toxicity. The primary systemic effects noted in the guinea pig toxicology studies were reduced platelets and eosinophils in guinea pigs. Also, relatively low plasma ciprofloxacin concentrations occurred in the guinea pig toxicology studies following intratympanic injection of OTO-201 or co-administration of OTO-201 and Ciprodex®. In guinea pigs, the maximal plasma ciprofloxacin concentrations associated with administration of the OTO-201 with and without co-administration of Ciprodex® were on the order of 100 to 500 fold lower than the peak serum concentrations of ciprofloxacin associated with clinical administration of 250 mg oral ciprofloxacin in humans. The comparatively low plasma ciprofloxacin concentrations following intratympanic administration of OTO-201 in guinea pigs suggest a low potential for OTO-201-related systemic toxicity in humans.

Table 13: Safety Margin Determination for Systemic Toxicity

Study Number	Species	NOAEL	HED	Safety Margin[c]
OTO-201-RSP-008	Guinea Pig	6.0% (50 μl/ear) = 6 mg/kg[a]	1.32 mg/kg[b]	1.65 fold

[a] Based on an average guinea pig body weight of 500g.
[b] The conversion factor for guinea pig doses to human equivalent doses (HEDs) is 0.22.
[c] The initial clinical dose is 4 mg/ear for a total of 8 mg per an average 10 kg subject (patients 6 months to 2 years of age) = 800 μg/kg.

The whole-body surface area comparison is not appropriate for determining otic toxicity. Given the primary site of action for OTO-201 is the middle ear and specifically middle ear epithelium, a better safety margin variable for predicting safe human doses based on the guinea pig results would seem to be middle ear volume. Unfortunately, middle ear volumes for different species, unlike inner ear perilymph volumes, are not well described in the literature. The highest dose of OTO-201 did not produce pronounced otic toxicity in guinea pigs as measured by several indices. However, it is uncertain how intratympanic injection into healthy "dry" guinea pig ears compares to similar administration into the infected ears of young patients with accompanying otorrhea.

The previous safety results for the clinical intratympanic administration of (b)(4)% poloxamer 407 in 14 adult subjects (Study (b)(4)) suggests the vehicle may be safe for administration to adults, but its retention pattern and safety in juvenile ears with accompanying otorrhea has not been characterized. The middle ear biodegradation pattern of poloxamer 407 generally remains unclear. A single study in the literature ((b)(4) reports that (b)(4)% poloxamer 407 largely disappeared from the middle ears of guinea pigs 49 days after ear perfusion. The guinea pig toxicology studies in the present IND submission did not comprehensively evaluate poloxamer degradation or elimination from the middle ear. However, histological evaluation did not reveal substantial or frequent evidence of debris remaining in the middle ears of guinea pigs treated with OTO-201 or vehicle. As noted, foamy macrophages were noted in several Eustachian tubes suggesting macrophage phagocytosis may be one mechanism for poloxamer 407 elimination.

Juvenile animal studies are not expected to shed light on the effects that might be expected of ciprofloxacin and/or poloxamer 407 in human juvenile ears, mainly because ear development in the experimental species with ear anatomy most similar to that of humans (guinea pigs, sheep) has not been adequately studied. It is not known at what age guinea pigs or sheep demonstrate the auditory-system developmental patterns simulating those of young patients of ages as low as six months.

In order to achieve a ciprofloxacin therapeutic effect over a therapy period of one to two weeks, but minimize the potential for ciprofloxacin-related toxicity, an optimal dose of OTO-201 must be determined. The guinea pig pharmacokinetic study results suggest that OTO-201 administration particularly at the higher ciprofloxacin concentrations can lead to prolonged middle ear retention of ciprofloxacin, thus increasing the potential for toxicological consequences. In theory at least, this problem may be compounded when OTO-201 administration is closely followed by a treatment course of Ciprodex®. In the absence of conclusive data, the safest course for the initial study will be to administer the lowest efficacious concentration of ciprofloxacin in OTO-201 and closely monitor juvenile patients for auditory responses during the treatment period and for a suitable recovery period following dosing.

OTO-201 was shown to produce negative results in an acute dermal toxicity assay and skin sensitization assay. The genetic toxicology, carcinogenicity, and reproductive

toxicology status of ciprofloxacin is as noted on the product labels for Cetraxal® and Ciprodex® and no further nonclinical studies in these categories are currently planned.

12 Appendix/Attachments

Table 14: Hematology Parameters

Study No.	OTO-201-RSP-008	OTO-201-RSP-010
Species	**Guinea Pig**	**Guinea Pig**
Hemoglobin concentration	X	X
Hematocrit	X	X
Erythrocyte count	X	X
Platelet count	X	X
Mean platelet volume		
Mean corpuscular volume	X	X
Mean corpuscular hemoglobin	X	X
Mean corpuscular hemoglobin concentration	X	X
Red cell distribution width		
Total leukocyte count	X	X
Reticulocyte count	X	X
Reticulocyte hemoglobin content		
Differential leukocyte count (Absolute neutrophil, lymphocyte, monocyte, eosinophil, basophil counts)	X	X
Blood smear for cell morphology (if necessary for interpretation)		

Table 15: Serum Chemistry Parameters

Study No.	OTO-201-RSP-008	OTO-201-RSP-010
Species	**Guinea Pig**	**Guinea Pig**
Aspartate aminotransferase	X	X
Alanine aminotransferase	X	X
Alkaline phosphatase	X	X
Blood urea nitrogen	X	X
Creatinine	X	X
Glucose	X	X
Cholesterol	X	X
Triglycerides	X	X
Total protein	X	X
Albumin	X	X
Total bilirubin	X	X

Sodium	X	X
Sorbitol dehydrogenase	X	X
Potassium	X	X
Chloride	X	X
Calcium	X	X
Inorganic phosphorus	X	X
Gamma-glutamyl transferase	X	X
Glutamate dehydrogenase		
Globulin	X	X
Albumin/globulin ratio	X	X

Table 16: Organ Weight Inventory Table

Study #	**OTO-201-RSP-008**	**OTO-201-RSP-010**
Species	**Guinea Pig**	**Guinea Pig**
Adrenals	X	X
Aorta		
Bone Marrow smear		
Bone (sternum, and/or femur and/or rib)		
Brain	X	X
Cecum		
Cervix		
Colon		
Conjunctiva		
Duodenum		
Epididymis	X	X
Esophagus		
Eye		
External ear		
Fallopian tube		
Gall bladder		
Gross lesions		
Harderian gland		
Heart	X	X
Hypophysis		
Ileum		
Infusion site		
Jejunum		
Joint, tibiofemoral		
Kidneys	X	X

Lachrimal gland		
Larynx		
Liver	X	X
Lungs	X	X
Lymph nodes, inguinal		
Lymph nodes, mediastinal		
Lymph nodes mandibular		
Lymph nodes, mesenteric,		
Mammary Gland		
Muscle (biceps, femoris)		
Nasal cavity		
Nasal turbinates		
Optic nerves		
Ovaries	X	X
Oviduct		
Pancreas		
Parathyroid	X	X
Peripheral nerve		
Peyer's patches		
Pharynx		
Pituitary	X	X
Prostate		
Rectum		
Salivary gland	X	X
Sciatic nerve		
Seminal vesicles		
Skeletal muscle		
Skin		
Spinal cord		
Spleen	X	X
Sternum		
Stomach		
Testes	X	X
Thymus	X	X
Thyroid	X	X
Tongue		
Tonsils		
Trachea		
Ureter		

Urinary bladder		
Uterus		
Vagina		
Vertebra, Lumbar		
Zymbal gland		

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/s/

JAMES S WILD
12/20/2011

WENDELYN J SCHMIDT
12/20/2011

MEMO: IND 110244: OTO-201 for the treatment of middle ear effusion at the time of tympanostomy tube placement. Sponsor: Otonomy, Inc.

SUBMISSION DATE:	November 23, 2011
TO:	Jane Dean, Project Manager, DAIP
FROM:	James Wild, Ph.D., Pharmacologist, DAIP
THROUGH:	Wendelyn Schmidt, Ph.D., Supervisory Pharmacologist, DAIP
RE:	New Nonclinical Data

BACKGROUND

The original IND 110244 for OTO-201 (ciprofloxacin in (b)(4)% poloxamer 407) was submitted on 8/26/2011. Based on the data submitted, no holds were placed on the initial clinical study but design modifications were requested and the clinical study design was modified. Another IND ((b)(4)) submitted by the same Sponsor for a related product ((b)(4)

On November 23, 2011, in conjunction with IND (b)(4), but also referenced for IND 110244, the Sponsor submitted new nonclinical data that included description of cochlear toxicity associated with the (b)(4) form of the (b)(4)% poloxamer 407 (b)(4) in two guinea pig studies. This toxicity was not noted for the original (b)(4) However the Sponsor had intends to use the new (b)(4) formulation in their planned clinical studies (b)(4) for the OTO-201 (b)(4)

Based on the data from the two guinea pig studies, the Sponsor in an 11/4/2011 letter submitted to IND (b)(4) and IND 110244, indicated the following:

"Based on these findings, we have made the decision to suspend screening in the proposed Clinical Study … as well as the proposed Clinical Study 201-201101 (IND 110,244) entitled 'A Prospective, Randomized, Double-blind, Placebo-controlled, Multicenter, Phase 1B Study of OTO-201 Given as a Single Intratympanic Injection for Intra-Operative Treatment of Middle Ear Effusion in Pediatric Subjects Requiring Tympanostomy Tube Placement.' Otonomy is thoroughly investigating the findings from this GLP toxicology study and planning additional investigations to further elucidate the observations. Otonomy will provide a complete analysis of these data to the Agency when available. No sites have been initiated for Study 201-201101, and no sites have received study drug. Therefore no subjects have been exposed to study drug (Ready to Dilute OTO-201). Until the findings from the toxicology study are clarified with the Agency, no subjects will be enrolled in any clinical studies of OTO-201 in the US."

The Sponsor has submitted preliminary data from two guinea pig toxicology studies where the cochlear toxicity was noted and described plans to further characterize the toxicity potential of several differently processed poloxamer 407 vehicle formulations.

The findings and plans are summarized below as well the Pharm/Tox perspective regarding the plans.

NONCLINICAL DATA

The studies shown in Table 1 utilized P407-[redacted] for single- and repeated-intratympanic dose administrations in guinea pigs. These studies are summarized below.

Table 1: New Guinea Pig Studies Using P407-[redacted].

Study	Study description	Study duration	Species	Route	Study number
1	Acute ototoxicity of [redacted]- GLP	4 week	GP	IT	OTO-104-RSP-025
2	20-week repeat dose ototoxicity study of [redacted] followed by a 6-week recovery period - GLP	26 week	GP	IT	OTO-104-RSP-024

GP: guinea pig; IT: intratympanic injection

1. **26-Week Repeat-Dose Ototoxicity of [redacted]**. (Study No.: OTO-104-RSP-024)

Methods

The ototoxicity potential of [redacted] administered bilaterally by intratympanic injection every four weeks to male and female guinea pigs (5/gender/group) for a total of 6 doses was evaluated in this study. The dose groups were P407[redacted] control group), and [redacted].
Following the last dose, animals were monitored for a 6-week recovery period. Otic examinations included auditory brainstem responses (ABR) at frequencies of 4, 10, and 40 kHz, histology of the middle ear (tissue reaction, inflammation at the tympanic membrane, malleus, and tensor tympani muscles, and ossicle profiles), and for the P407-[redacted] groups, cytocochleograms measuring cochlear hair cell patency.

Results

The average ABR-threshold levels observed for the pre-and post-treatment measurements suggested an effect of treatment (including the P407[redacted] group), in each ear, for each gender (Table 2). Overall, there was a significant hearing threshold shift (>40 dB SPL) observed in the majority of animals (75 90%), across treatment groups and frequencies. There were no differences observed between the treatment groups including the P407[redacted] group.

Middle ear histological assessments indicated that the majority of animals in the P407[redacted] group had normal middle ear histology.

Cytocochleograms indicated significant cochlear hair cell loss in the majority of animals from both the P407[redacted] (90% of animals) and 12%[redacted] groups (75% of

animals). There was no difference in the degree or extent of hair cell loss between the P407 (b) (4) groups suggesting the toxicity was associated with exposure to P407 (b) (4).

Table 2: ABR Hearing Thresholds of Guinea Pigs Following Repeated-Intratympanic Administration of (b) (4) P407 (b) (4) Vehicle Control. (Sponsor's Table)

Male	Baseline 4kHz	Termination 4kHz	Baseline 10kHz	Termination 10kHz	Baseline 20kHz	Termination 20kHz
0% (b) (4)	41.2 (1.5)	75.2 (24.2)	28.8 (2.9)	81.2 (27.1)	11.0 (3.3)	86.6 (18.3)
	39.4 (6.0)	82.4 (23.5)	28.6 (2.6)	81.6 (28.4)	15.8 (7.2)	59.6 (41.8)
2%	41.6 (4.16)	81.6 (20.4)	29.2 (6.3)	71.0 (26.9)	8.8 (4.9)	55.4 (35.3)
	43.2 (4.5)	66.6 (19.9)	33.0 (6.0)	58.4 (26.5)	11.2 (5.0)	40.0 (28.5)
6%	45.2 (5.3)	74.0 (16.4)	29.4 (1.5)	66.4 (21.3)	15.0 (1.8)	55.4 (27.0)
	38.2 (5.5)	56.0 (21.5)	27.4 (5.5)	50.8 (22.9)	10.4 (2.5)	44.8 (29.4)
12%	37.0 (9.7)	86.6 (27.7)	28.8 (6.7)	88.4 (25.9)	10.8 (2.1)	85.2 (33.0)
	36.4 (3.5)	94.8 (11.6)	30.0 (4.8)	80.8 (18.5)	11.8 (3.7)	79.8 (18.8)

Female	Baseline 4kHz	Termination 4kHz	Baseline 10kHz	Termination 10kHz	Baseline 20kHz	Termination 20kHz
0% (b) (4)	38.4 (6.5)	84.0 (25.9)	29.0 (3.3)	81.4 (29.6)	13.8 (5.2)	77.4 (25.5)
	41.8 (4.1)	74.6 (23.3)	29.8 (3.9)	63.6 (33.8)	11.6 (3.3)	44.2 (35.9)
2%	45.4 (5.8)	80.8 (19.6)	30.4 (6.2)	71.0 (18.4)	16.0 (4.1)	73.8 (26.3)
	41.4 (5.0)	79.6 (28.5)	29.0 (4.0)	77.6 (31.1)	11.2 (4.7)	66.6 (38.6)
6%	40.6 (4.1)	83.8 (23.5)	28.4 (4.7)	85.0 (20.7)	10.8 (3.8)	90.0 (10.7)
	40.8 (3.9)	69.8 (23.4)	30.4 (6.7)	64.2 (29.5)	10.6 (3.4)	75.4 (26.2)
12%	40.8 (5.7)	69.6 (28.4)	29.4 (5.5)	73.2 (29.4)	11.4 (5.9)	73.2 (39.0)
	37.2 (7.3)	66.0 (11.3)	31.6 (5.6)	73.2 (29.0)	10.2 (3.7)	62.0 (35.9)

Data are presented as mean (standard deviation) for the left (top in each cell) and right (bottom in each cell) ears at each frequency.

2. **4-Week Acute Single Dose Ototoxicity of (b) (4)** (Study No: OTO-104-RSP-025).

Reviewer Comments: *A final report for this study was submitted to IND (b) (4) on June 11, 2011. The following is a summary of the data provided in summary by the Sponsor. A more complete Pharm/Tox evaluation will be undertaken once the final study report for Study No.: OTO-104-RSP-024 has been submitted.*

Methods

Male and female guinea pigs (5/gender/group) were administered a single bilateral intratympanic injection (50 µl) of P407 [redacted] concentrations. The acute ototoxicity potential of the different treatments was assessed by auditory brainstem responses (ABR) at frequencies of 4, 10, and 40 kHz, histology of the middle ear (tissue reaction, inflammation at the tympanic membrane, malleus, and tensor tympani muscles, and ossicle profiles), and for the P407 [redacted] groups, cytocochleograms measuring cochlear hair cell patency.

Results

Mean ABR thresholds in the P407 [redacted] group and the [redacted] groups did not differ significantly from baseline upon study completion. However, in the P407 [redacted] group, hearing threshold shifts in excess of 30 dB SPL to at least one frequency were noted in 4 out of 20 ears (20%). In the [redacted] treatment groups a similar small percentage (10-25%) of ears were affected.

Middle ear histology was normal in all animals of the P407 [redacted] vehicle group.

Three animals (out of ten) treated with P407 [redacted] and four animals (out of ten) treated with [redacted] had substantial loss of both outer and inner hair cells across the length of the cochlea. The findings observed in the [redacted] group were indistinguishable from those of the P407 [redacted] control group suggesting the toxicity was associated with exposure to P407 [redacted].

OVERALL CONCLUSIONS

The data indicate that a single intratympanic injection of P407 [redacted] was associated with substantial hearing impairment and related outer and inner cochlear hair cell loss in approximately 10-30% of the treated animals (Table 3). The effects were accentuated with repeated intratympanic administration of P407 [redacted] (six injections at a four-week dosing interval) where significant hearing loss and associated cochlear hair cell loss occurred in the majority of treated guinea pigs. These results are in contrast to an earlier single-intratympanic-dose study (OTO-104-RSP-002) in guinea pigs using the P407 [redacted] vehicle formulation where 20% of the treated ears demonstrated minimal hearing loss, and significant outer and inner hair cell loss was not noted in any treated ears. These preliminary results suggest that some chemical or other factor of P407 [redacted] which is presumably absent or reduced in P407 [redacted] is responsible for the ototoxicity.

Table 3: Ototoxicity Profiles of P407 (b) (4) and P407 (b) (4) Following Single- and Repeated-Intratympanic Administration. (Sponsor's Table)

Formulation	Dosing	ABR	Cytocochleogram
P407 (b) (4)	Single IT	20% ears with minimal hearing loss (20 dB SPL) at one frequency only	0% ears with significant outer and inner hair cell loss
P407	Single IT	20% ears with significant hearing loss (30 to >40 dB SPL) across frequencies	30% ears with significant outer and inner hair cell loss
P407	6 injections 4-week interval	90% ears with significant hearing loss (>40 dB SPL) across frequencies	90% ears with significant outer and inner hair cell loss

PLANNED STUDIES

In their 11/23/2011 submission, the Sponsor summarized their plan of action to better understand and characterize the toxicity potential of different preparations of P407 as follows:

"Otonomy is currently conducting an extensive cross-functional investigation into the toxicology findings reported above. First, P407 (b) (4) and P407 (b) (4), as well as several other differently processed P407 formulations, are being subjected to extensive analytical characterization to discern chemical differences. Second, we have developed *in vitro* and *in vivo* screening systems that may provide information as to the topical toxicity potential of differently processed P407 formulations. Finally, a large non-GLP study in guinea pigs given repeated intratympanic injections of P407 (b) (4), P407 (b) (4) or several other differently processed P407 formulations at different dosing intervals is also underway to confirm these findings and better understand the in vivo toxicity profiles."

Pharm/Tox Comments: *We agree that your plan appears to be appropriate for characterizing the chemical entities underlying the P407-related ototoxicity and for providing information useful in determining which types of P407 processing are associated with ototoxicity. Please include negative (saline) and positive (chemicals or drugs known to produce cochlear hair cell loss) controls in the in vitro and in vivo studies where appropriate.*

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

JAMES S WILD
12/13/2011

WENDELYN J SCHMIDT
12/15/2011