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Pharmacokinetic Studies of Sustained-Release Depot of Dexamethasone in Beagle Dogs

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Abstract

Purpose: To examine the pharmacokinetic characteristics of sustained-release dexamethasone depots in two separate canine studies.
Methods: Dexamethasone depots loaded with a clinically representative (0.4 mg) dose (DEXTENZA™; Ocular Therapeutix) or an elevated (0.7 mg) dose were inserted into the canaliculi of beagle eyes ($n = 37$ and $n = 34$, respectively). Tear fluid was collected for pharmacokinetic analysis of dexamethasone in both studies at predetermined time points. Explanted 0.4 mg depots were collected weekly to measure remaining drug level. Clinical observations and ophthalmic examinations were performed in both studies at each visit.
Results: The 0.4 mg depots released a median 308 μg by day 15 and tapered to complete drug release by day 28. Median dexamethasone tear fluid concentrations in the 0.4 mg study group decreased from 2,805 ng/mL at day 7 to 0 ng/mL on day 28. Median dexamethasone tear fluid concentrations in the 0.7 mg study group decreased from 4,370 ng/mL at 6 h post insertion to 830 ng/mL on day 35. Mean ± standard deviation intraocular pressures in the 0.4 and 0.7 mg study groups were 20.7 ± 2.8 and 19.0 ± 4.1 mmHg at baseline, respectively, and demonstrated no meaningful change (20.5 ± 3.0 and 20.6 ± 2.9 mmHg, respectively) over the studies' durations. No ocular toxicities were attributed to the dexamethasone depot.
Conclusion: Sustained-release dexamethasone produced no identifiable ocular toxicity in this animal model, and pharmacokinetics demonstrated a sustained and tapered drug release over 28 days at a 0.4 mg dose and exceeded 35 days at a 0.7 mg dose.

Keywords: DEXTENZA, dexamethasone, hydrogel, sustained release, inflammation, pharmacokinetic

Introduction

Ophthalmic corticosteroids are a mainstay in the management of a number of ocular conditions, including uveitis, keratoconjunctivitis, ocular allergies, and postoperative inflammation.[1] However, corticosteroid dosing is challenging for many patients, because it typically requires frequent administration (up to 6 times/day), which is subsequently tapered over several weeks.

Adherence to any self-administered medication can be problematic,[2] and nonadherence to eye drops has been well documented.[3,4] Because adherence decreases as dosing rate increases,[5] adherence to the frequently administered ophthalmic corticosteroids is a particular concern. Moreover, improper administration has also been identified as a challenge with eye drops. A recent study of postoperative cataract patients demonstrated that over 90% of patients exhibited at least one of the following behaviors: missing the eye with the eye drop, instilling an incorrect amount of drops, contaminating the bottle tip, and failing to wash hands before instillation.[6] In addition, incorrect tapering of corticosteroids can lead to ocular rebound inflammation.[7] For these reasons, a superior technique for ophthalmic corticosteroid administration is an unmet need.

Punctum plugs loaded with drug have emerged as an attractive alternative to eye drops.[8] Initially developed for the treatment of dry eye,[9] punctum plugs have more recently demonstrated promise as intracanalicular depots, providing sustained drug delivery to the eye.[10] A dexamethasone-eluting depot called OTX-DP (DEXTENZA™; Ocular Therapeutix, Bedford, MA) has been developed and is currently under New Drug Application review with the US Food and Drug Administration (FDA) for the treatment of postoperative ocular pain, as well as in phase 3 clinical trials for the treatment of postoperative ocular inflammation and allergic conjunctivitis.

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OTX-DP is composed of micronized dexamethasone particles suspended within a polyethylene glycol (PEG) hydrogel matrix. Hydrogels are already used in several FDA-approved applications as drug delivery agents, and PEG, a hydrophilic polymer, is found in a number of pharmaceuticals, from laxatives to lubricant eye drops. PEG hydrogels have been established as safe biocompatible substances.[11,12]

OTX-DP is a noninvasive single-application depot that provides sustained release of dexamethasone. Upon insertion into the vertical canaliculus and contact with tear fluid, the OTX-DP hydrogel swells to conform to the canalicular anatomy to ensure depot retention and dexamethasone release over a 30-day period. The OTX-DP hydrogel is conjugated with fluorescein so it can be visualized in the canaliculus through the tissue using a slit lamp with a blue light and yellow optical filter. The PEG hydrogel degrades and liquefies through bulk hydrolysis over time[13] and is cleared through the nasolacrimal system (data not shown), obviating the need for removal by the physician when treatment is complete. Furthermore, this depot has demonstrated stability at high temperatures, with depots stored at 40°C and refrigerated depots showing no meaningful difference in drug release over time.[14]

This article describes 2 preclinical studies performed in beagle dogs to examine the pharmacokinetic characteristics of (1) the OTX-DP sustained-release dexamethasone depot at a clinically representative dose and (2) a sustained-release dexamethasone depot at an elevated dose.

Methods

The nonclinical laboratory studies were approved by the Institutional Animal Care and Use Committee. All institutional and national guidelines for the care and use of laboratory animals were followed, including the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. These were preclinical studies designed, in part, to determine dexamethasone levels in tear fluid over time after dexamethasone depot insertion into the canaliculi of canines. Beagle dogs at least 6 months of age and weighing ~5 to 10 kg were used in these studies; female dogs were used for the 0.4 mg depot study and both sexes were used for the 0.7 mg depot study. The beagle model was chosen because the ocular and nasolacrimal systems are similar in anatomy to humans, thus enabling the same clinical route of exposure.

Dexamethasone intracanalicular depots

OTX-DP is a punctum plug containing dexamethasone within a biodegradable PEG hydrogel matrix that is conjugated with fluorescein to aid plug visualization. Dexamethasone incorporated into the hydrogel is USP/EP micronized grade having a particle size of not less than (NLT) 98% <10 μm and NLT 90% <5 μm. Dexamethasone was suspended in a multiarm PEG precursor solution and injected into small-bore tubing before cross-linking. The drug-loaded hydrogel matrix was dried and cut into punctum plugs containing either 0.4 mg (DEXTENZA) or 0.7 mg dexamethasone per plug (both plug types were manufactured by Ocular Therapeutix, Inc.). All depots were designed to degrade through bulk hydrolysis, soften, and liquefy over time following insertion.

Depot insertion and removal

Animals were fasted overnight before sedation, which was performed with an intramuscular injection of dexmedetomidine (Dexdomitor®; Zoetis, Florham Park, NJ). If the eyes were not stable enough to perform the procedure, the animal was placed on isoflurane through inhalation. One to 2 drops of 0.5% proparacaine hydrochloride was administered to each eye before the procedure.

An intracanalicular depot was inserted into either the superior or inferior canaliculus of both eyes using forceps, after which the punctal areas were hydrated with BSS (balanced saline solution). The insertion location (superior or inferior canaliculus) was determined based on punctum diameter and anatomical morphology, which were assessed using a punctal gauge dilator under an operating microscope. Atipamezole (Antisedan®; Zoetis) was intramuscularly administered to reverse the effects of the sedative. Following insertion, animals were first recovered from anesthesia and then food was returned.

For the 0.4 mg depot study, 3 animals each (6 eyes) were chosen on days 7, 15, 21, and 28 for tear fluid collection, bilateral depot removal, and study discontinuation. At each time point, depots were removed using manual extraction after tear fluid collection to determine remaining drug content. First, the animals were anesthetized and eyes were administered proparacaine, as previously described. The punctum was gently dilated and the softened depot was manually expressed through the punctal opening by applying pressure to the tissue just behind the distal portion of the depot. The recovered depots were collected for photography and drug content analysis. Animals whose depots were explanted were removed from the study; all other animals remained on study.

Ocular assessments

To ensure continuous drug dosing, the punctal area of each study eye was evaluated for visualization of the depot (the hydrogel conjugated with fluorescein allows visualization using a slit lamp with a blue light and yellow optical filter) weekly for the 0.4 mg depot study and twice weekly for the 0.7 mg depot study. If a depot could not be visualized, the tear fluid samples and other measurements for that eye were removed from the study and no additional data were collected.

Ophthalmic examinations were performed on the 0.7 mg depot study animals predose (day 0) and on days 1, 14, and 35; anterior segment structures (conjunctiva, cornea, iris, corneal pannus, and lens) and posterior segment structures (vitreous body, optic disc/nerve, choroid, retina, and retinal blood vessels). A gross ocular examination, in which eyes were monitored for signs of irritation (e.g., discharge and redness) and overall health, was performed weekly on all 0.4 mg depot study animals beginning on day 0.

Intraocular pressure (IOP) measurements using a TonoVet tonometer (Jorgensen Laboratories, Loveland, CO) were performed on both eyes of each study animal on days 0, 15, and 28 in the 0.4 mg depot study and predose and on days 1, 14, and 35 in the 0.7 mg depot study. The TonoVet tonometer collects 6 IOP measurements per eye and provides the average IOP value of those 6 readings.

In the 0.4 mg depot study, tear fluid samples were collected from 3 animals (6 eyes) using a Schirmer Tear Test

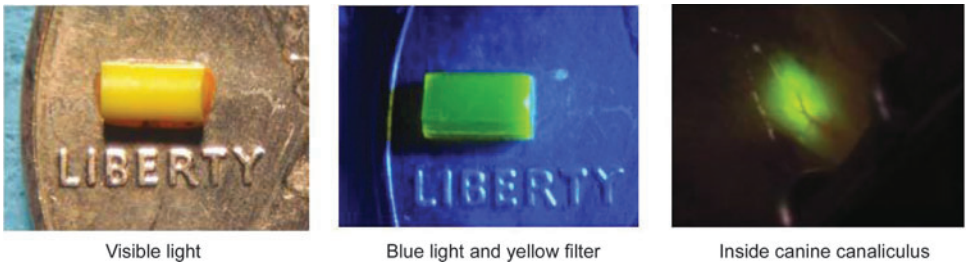

FIG. 1. Visualization of OTX-DP depot *ex vivo* and *in vivo.*

Strip (Alcon Laboratories, Inc., Fort Worth, TX) on days 7, 15, 21, and 28 (each animal underwent tear fluid collection only once, after which the animal was removed from the study). In the 0.7 mg study, tear fluid samples were collected from both eyes of all on-study animals using a Schirmer Tear Test Strip on days 0 (6 h postinsertion), 7, 14, 21, 28, and 35. The tear fluid containing test strips was immediately placed in microfuge tubes on dry ice and stored at −20°C. Before analysis, the samples were thawed and, subsequently, extracted using centrifugation and water dilution.

Concentrations of dexamethasone from the tear fluid samples in both studies were analyzed using a validated method consisting of high-performance liquid chromatography (HPLC) combined with a triple quadrupole mass spectrometer. The HPLC-MS/MS system consisted of Shimadzu AD10vp pumps, CTC autosampler, and an ABI 2000 tandem mass spectrometer controlled by Analyst 1.4.2 software. The lower limit of quantitation was 1.0 ng/mL. The method was linear ($R = 0.998$) and accuracy (recovery) over the range of the curve was 91% to 104% with a method precision of 5% to 7% coefficient of variation. Dexamethasone content in the 0.4 mg depot study from preinsertion (time zero) and explanted depots (days 7, 15, 21, and 28) was analyzed using solvent extraction and HPLC analysis and concentration determined relative to a standard curve.

Statistical analyses

The median and standard deviation (SD) of dexamethasone concentrations in tear fluid in both studies and in explanted drug depots in the 0.4 mg study were assessed at each time point. The mean and SD of IOP in animals from both studies were assessed predose, at 2 weeks, and at study completion. All other results were descriptive measures. Statistical analyses were performed using Microsoft Excel 2010 (version 14).

Results

The intracanalicular depots in the 0.4 mg depot study were loaded with an average of 386 µg dexamethasone (median 383 µg), representing the 0.4 mg clinical ophthalmic dose of dexamethasone in humans; the depots in the 0.7 mg depot study were loaded with an average of 729 µg dexamethasone (median 731 µg), representing an elevated dose in humans.

A total of 19 animals from the 0.4 mg depot study had an OTX-DP depot inserted into the canaliculi of 37 eyes. A total of 17 animals from the 0.7 mg depot study had a dexamethasone depot inserted into the canaliculi of 34 eyes. Figure 1 demonstrates the size of the depot, as well as its

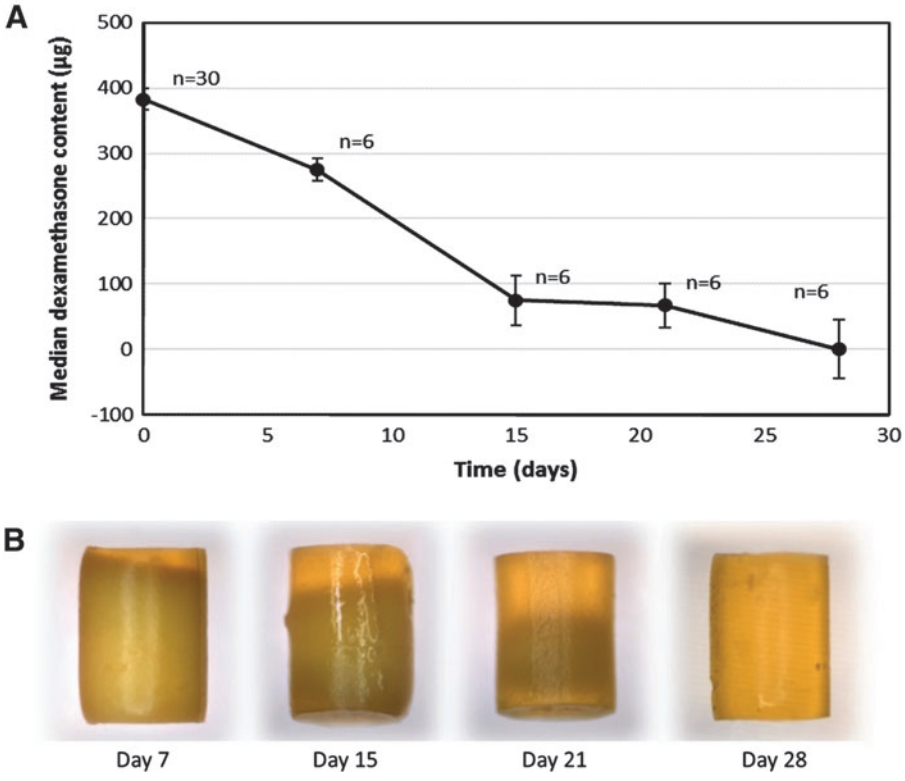

FIG. 2. Drug content in intracanalicular OTX-DP (0.4 mg) depots. **(A)** Median dexamethasone content in predosed depots (day 0) and explanted depots across the study time points. **(B)** Explanted depots at various time points demonstrate the directional drug release into the tear fluid and the complete release by day 28.

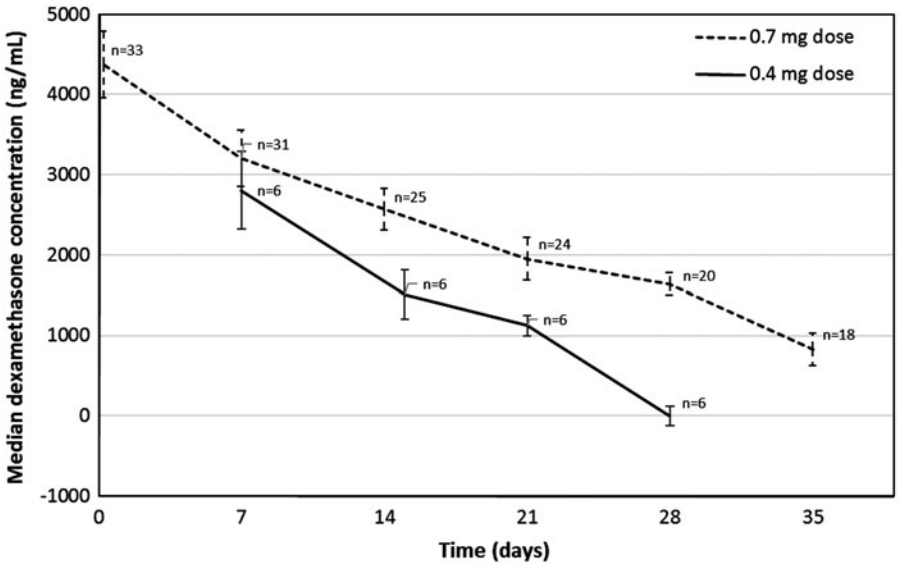

FIG. 3. Median dexamethasone concentration in tear fluid across study time points for the 0.4 and 0.7 mg doses.

fluorescence under blue light with use of a yellow filter both *ex vivo* and *in vivo*, with the latter showing the ease of depot visibility inside a canine canaliculus.

At each time point, none of the findings from the ophthalmic examination of all eyes in both studies were considered adverse; thus, no ocular toxicity was observed (data not shown).

Pharmacokinetic results

In the 0.4 mg depots (which were loaded with a median 383 μg dexamethasone), the median dexamethasone content was 267 μg on day 7 tapering to 0 μg on day 28 (Fig. 2A). Figure 2B illustrates the directional release of drug from the depot into the tear fluid, with complete release by day 28.

The tear fluid of the beagle dogs was sampled across the studies and analyzed for drug content. In the 0.4 mg depot study, the median dexamethasone from the depots was 2,805 ng/mL on day 7 tapering to 0 ng/mL on day 28 (Fig. 3). In the 0.7 mg depot study, the median dexamethasone from the depots was 4,370 ng/mL 6 h after insertion tapering to 830 ng/mL on day 35.

IOP results

In the 0.4 mg depot study, baseline IOP was measured in 38 eyes (incapable of depot insertion in one eye due to a preexisting punctal anatomy, and thus, that eye was removed from the study). Mean ± SD baseline IOP was 20.7 ± 2.8 mmHg, which increased to 24.4 ± 3.5 mmHg at day 15 ($n = 28$; 9 plugs were removed or lost after baseline) and decreased to baseline level (20.5 ± 3.0 mmHg) at day 28 ($n = 8$; 20 additional plugs were removed or lost after day 15) (Table 1). To provide the most judicious IOP comparison over the study duration, only the 18 study eyes in the 0.7 mg depot study that retained plugs were compared; mean ± SD IOP was 19.0 ± 4.1 mmHg at baseline, 21.0 ± 4.6 mmHg at day 14, and 20.6 ± 2.9 mmHg at day 35.

Discussion

In these 2 preclinical studies of beagle dogs with bilateral intracanalicular depots, pharmacokinetic analysis of the dexamethasone depot demonstrated a tapered dexamethasone release profile over the course of the studies (28 days for the 0.4 mg depot study and 35 days for the 0.7 mg depot study). Moreover, no detectable ocular toxicity or IOP increase was observed with depot use. Previous preclinical examination of OTX-DP (the 0.4 mg depot) in this animal model has shown a lack of any systemic or ocular adverse effects with this depot,[10] providing further evidence that this dexamethasone depot is well tolerated in a preclinical model.

Topical corticosteroids, such as dexamethasone, have widespread use in the field of ophthalmology, due to their ability to suppress inflammation.[15,16] As such, they are used to treat a wide variety of ocular conditions, including uveitis, keratitis, blepharitis, macular edema, allergic conjunctivitis, and dry eye, as well as to reduce inflammation following numerous ocular surgeries.

The challenges with topical ophthalmic corticosteroids are 2-fold: adherence and safety. As already discussed, adherence to eye drops is poor,[3,4] and this can be exacerbated by improper administration[6] or the need for frequent administration,[5] as with ophthalmic corticosteroids. Furthermore, corticosteroids are associated with certain ocular complications, including glaucoma, posterior subcapsular cataract, and corneal thinning.[17] Another safety issue—ocular rebound inflammation—can be caused if corticosteroids are not gradually tapered.[7]

TABLE 1. IOP RESULTS FROM ANIMALS IN BOTH STUDIES

	0.4 mg depot study			*0.7 mg depot study*		
Time point	n	*Mean (mmHg)*	*SD (mmHg)*	N	*Mean (mmHg)*	*SD (mmHg)*
Day 0	38	20.7	2.8	18	19.0	4.1
Day 14/15[a]	28	24.4	3.5	18	21.0	4.6
Day 28[b]/35[a]	8	20.5	3.0	18	20.6	2.9

[a]Animals from the 0.4 mg depot study had IOP measured on days 15 and 28; animals from the 0.7 mg depot study had IOP measured on days 14 and 35.
[b]Two animals were tested on day 26 instead of day 28.
SD, standard deviation; IOP, intraocular pressure.

A dexamethasone-eluting depot addresses both adherence and safety concerns. Because the depot is inserted in the eye care clinician's office by a clinician, adherence and improper administration concerns are minimized and postoperative management is simplified. In addition, the pharmacokinetic properties of the drug-eluting depot demonstrate, as shown by the results of this preclinical study, a sustained but tapered drug release into the tear film, minimizing the chance of ocular rebound inflammation. Previous studies assessing drug levels in the posterior segment from topical eye drops have demonstrated that delivery to the back of the eye is limited and below therapeutic target levels.[18–20]

Although numerous *in vitro* drug release studies have been performed demonstrating sustained release of OTX-DP (data not shown), these results are not an accurate representation of drug release within the confines of the canaliculus, because drug release from the hydrogel is driven by aqueous solubility rather than by degradation,[21] and therefore, drug release must be determined empirically in this animal model. Sustained drug release in the current study was demonstrated by sustained drug levels present in the tear fluid, by drug content measured in explanted depots, and by images of the drug remaining in the explanted depots over time.

As shown in Fig. 2B, the depot maintained structural integrity during the 28-day therapeutic period to afford drug delivery. The depot degrades through hydrolysis using a similar mechanism as either dissolvable sutures or biodegradable microparticles/implants that have been fabricated with poly lactic-co-glycolic acid (PLGA) or polylactic acid (PLA). The hydrogel depot starts as a gel and softens over time during hydrolysis to its final liquid form. *In vitro* testing in simulated physiological conditions demonstrates that the hydrogel component of the depot persists for >1 month (data not shown).

The current studies, which examined both a clinically relevant dexamethasone dose (0.4 mg) and an elevated dose (0.7 mg), do have limitations. Using both eyes of the study animals includes a risk of contralateral eye drug diffusion. However, previous OTX-DP safety studies performed in beagles measuring systemic dexamethasone levels have observed no drug above the limit of quantitation (LOQ) in plasma (data not shown), making it unlikely that drug would be present above the LOQ in the tear film of the contralateral eye in this study. As with all animal studies, these studies can only provide surrogate information for human studies. Although the relevant anatomy and physiology of canines are similar to that of humans, there are important differences. For instance, canines have a straighter canaliculus than humans and they have higher tear production and blink rates, both of which may negatively impact drug concentration in the tear film. Therefore, the risks and benefits of dexamethasone depot use in canines, as demonstrated in these studies, may not accurately correlate with the risks and benefits experienced by patients. Moreover, the short durations of the studies (i.e., 28 and 35 days) preclude the identification of any long-term adverse effects.

Conclusion

OTX-DP demonstrated a sustained and tapered drug release over the course of these 2 preclinical studies, while producing no identifiable ocular toxicity or IOP increase, thereby justifying additional clinical development of this ocular product.

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Author Disclosure Statement

All authors are all employees and equity holders of Ocular Therapeutix.

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