Ocular Drug Distribution and Safety of a Noninvasive Ocular Drug Delivery System of Dexamethasone Sodium Phosphate in Rabbit

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Abstract

Purpose: To determine the ocular toxicity, systemic exposure, and amounts of dexamethasone sodium phosphate (DSP) in ocular tissues after administration of DSP with the Visulex system (DSP-Visulex).

Methods: DSP-Visulex was applied onto healthy rabbit eyes. DSP concentrations (4%, 8%, 15%, and 25%) and treatment durations (5, 10, and 20 min) were evaluated for the amounts of DSP in the ocular tissues and in plasma after single administrations of DSP-Visulex. The drug in eye tissues and plasma was analyzed by high-performance liquid chromatography-UV/VIS and by liquid chromatography-mass spectrometry, respectively. The safety and tolerability were ascertained based on clinical observations and histopathological examinations from repeat weekly DSP-Visulex treatments (4%, 8%, 15%, and 25% for 20 min) for 12 weeks.

Results: Significant amounts of DSP (ie, higher than 1 $\mu\text{g/g}$) were found in the anterior chamber, retina-choroid, cornea, vitreous, conjunctiva, and sclera after single applications of DSP-Visulex. The DSP concentrations in the ocular tissues and in plasma increased with increased DSP concentrations in the Visulex applicator and with increased application times. Systemic DSP was rapidly detected. The plasma half-life was 2–3 h. C_{max} was 148 and 1,844 ng/mL, and the area under the plasma drug concentration versus time curve (AUC) was 418 and 3,779 ng · h/mL for the low dose (4% DSP-Visulex for 5 min) and the high dose (15% DSP-Visulex for 20 min), respectively. Ocular findings over 12 weeks were mostly conjunctival injection and eye discharge. These were transient and mild. Histopathological examinations indicated the eyes to be normal.

Conclusions: DSP can be administered safely and effectively into the rabbit eye with the Visulex system. Treatment duration and DSP concentration are important factors in achieving therapeutic levels. Repeat applications of DSP-Visulex are safe and well tolerated for weekly administrations over 4–12 weeks. DSP-Visulex has clinical potential for the noninvasive treatment of ocular diseases.

Keywords: noninvasive, ocular drug delivery, ocular toxicity, dexamethasone, topical treatment, toxicokinetics

Introduction

DEXAMETHASONE SODIUM PHOSPHATE (DSP) is a highly water-soluble form of dexamethasone. DSP undergoes rapid hydrolysis to form dexamethasone (DEX) in plasma¹ and ocular tissues.² Both DEX and DSP have been used for the treatment of a wide variety of ocular inflammation conditions such as keratitis, blepharitis, iritis, conjunctivitis, uveitis, macular edema, and postoperative eye surgery.³ There are a number of dosage forms of DEX and DSP for ocular treatment, including eye drops, ointments, oral tablets, intraocular injections, and intravitreal implants. Current topical methods, however, cannot deliver corticosteroids to the posterior seg-

ment of the eye effectively, and their practice has been limited to treating anterior eye conditions.^{4–6} Eye drops often yield poor patient compliance due to the required adherence to frequent administration.⁷ The posterior segment of the eye can be treated through systemic routes, but significant systemic adverse effects are major concerns.⁵ Invasive methods, such as periocular injections, intravitreal (IVT) injections, or intravitreal implants (eg, Ozurdex[®], Retisert[®], and Iluvien[®]), are effective, but the cost of administration is high. They also involve a number of potential serious risks, including retinal detachment, endophthalmitis, increased intraocular pressure (IOP), and cataractogenesis.^{8–11} There is an unmet need for a new drug delivery system that can address such challenges.

Some studies have reported that drugs administered topically can reach the back of the eye and the transscleral route can be a pathway for a drug to posterior eye tissues.^{12–19} To administer a drug through this topical pathway effectively, a high drug concentration at the front of the eye, particularly on the sclera, for a prolonged period of time is needed.⁴ However, the current topical ophthalmic products have failed to utilize this pathway effectively because of the short retention time at the site of application and the low drug concentration used in its formulation. Visulex is a passive diffusion-based technology developed by Aciont, Inc. It is a noninvasive drug delivery system that can be used to administer drug topically through the limbal sclera into the interior of the eye, utilizing the transscleral pathway.^{20,21} The Visulex applicator is designed to facilitate the drug molecule entering primarily through the conjunctiva-scleral surface and not the cornea. The applicator design also minimizes drug smearing over the eye and reduces the drug clearance from tearing and drainage into the nasolacrimal duct. These features enable an ophthalmic application of a high drug concentration, which may expedite the passive drug diffusion through the transscleral pathway without significant ocular toxicity.²¹ DSP is a suitable candidate for examining the potential of the Visulex drug delivery system because it is a potent corticosteroid that can be formulated to a high solution concentration. In this article, the combination of this high DSP concentration solution instilled into the Visulex applicator is referred to as DSP-Visulex.

The purpose of this investigation was to determine the potential ocular toxicity, systemic exposure, and amount of DSP delivered to the ocular tissues after administration of DSP with the Visulex system. We hypothesized that (1) a significant amount of DSP can be delivered into intraocular tissues of rabbit through a single ocular application of DSP-Visulex and (2) multiple ocular applications of DSP-Visulex are well tolerated and safe in healthy rabbit. There are 3 studies reported in this article: ocular drug distribution, ocular toxicity, and toxicokinetics. Three important parameters of the DSP-Visulex application were assessed: (1) DSP concentration, (2) treatment duration, and (3) the safety and tolerability of repeat treatments. In the ocular drug distribution study, DSP concentrations and treatment durations were evaluated for the amount of drug delivered into the ocular tissues. Systemic exposures were evaluated in the toxicokinetic study. The safety and tolerability were ascertained by repeat weekly DSP-Visulex applications over a 3-month period.

These data would be a basis for formulating dosing regimens of DSP-Visulex in clinical studies.

Methods

Materials and animals

DSP USP grade was supplied from Letco Medical (Decatur, AL). The concentrations of DSP solution were 4%, 8%, 15%, and 25% w/v. All DSP solutions containing 0.01% w/v of ethylenediaminetetraacetic acid (EDTA; Sigma-Aldrich, St. Louis, MO) with the pH adjusted to 7.0 using 1.0M hydrochloric acid (LabChem, Zelienople, PA) were freshly prepared in double deionized water on the day of dosing, using an aseptic technique. The Visulex applicator (Aciont, Salt Lake City, UT) for use in rabbit was fabricated from medical grade silicone rubber and a proprietary sponge material (Fig. 1). Ketamine hydrochloride injectable USP (100 mg/mL) and sodium chloride 0.9% USP were from Hospira, Inc. (Lake Forest, IL); proparacaine hydrochloride ophthalmic solution was from Bausch and Lomb (Tampa, FL); cyclopentolate hydrochloride ophthalmic solution was from Alcon Laboratories (Fort Worth, TX); and xylazine and potassium chloride (KCl) were from Sigma-Aldrich. Syringes and needles were from Becton, Dickinson and Company (Franklin Lakes, NJ). The binocular indirect ophthalmoscope used was the Keeler All Pupil II from Keeler Instruments (Broomall, PA) and it was complemented with the double aspheric lens 20D/50 mm for posterior chamber examination from Volk Optical, Inc. (Mentor, OH). Young adult New Zealand White rabbits each weighing 3–4 kg were obtained from Western Oregon Rabbit Co. (Philomath, OR). This study complied with the ARVO Statement for the use of Animals in Ophthalmic and Vision Research and was approved by The University of Utah Institutional Animal Care and Use Committee (Salt Lake City, UT). All animals were acclimated and observed for health issues for at least 2 weeks before being used in the study.

Study design

Sixty animals were randomly assigned into 20 groups of 3 ($n=3$) for 3 main studies: ocular drug distribution, ocular toxicity, and toxicokinetics.

For the ocular drug distribution study, there were a total of 12 groups. The test parameters were 4 DSP concentrations (ie, 4%, 8%, 15%, and 25% w/v) and 3 application times (ie, 5, 10, and 20 min). Each group received a single

FIG. 1. (A) Visulex applicator with an annular white sponge. (B) DSP-Visulex in a rabbit eye with the sponge in contact with the eye on the sclera and the surface area of sponge is $\sim 200 \text{ mm}^2$. Note: DSP (dexamethasone sodium phosphate) solution is loaded into the sponge using a pipettor. In (B), the sponge is underneath the eyelid and cannot be seen.

DSP-Visulex treatment at a prespecified concentration and application time in both eyes concurrently (within 10–20 s apart). The rabbits were sacrificed immediately after dosing (generally within 5 min). The eyes were then enucleated and analyzed for DSP and DEX using high-performance liquid chromatography (HPLC). The total of 6 eyes was used for averaging the amount of drug in each group. The rationale for this study was to answer whether or not a single application of Visulex can deliver a meaningful amount of DSP into the deeper eye tissues. Since there is no established minimum effective concentration of DEX or DSP in ocular tissues, the target concentration of DSP in each eye tissue (immediately after the application) that is considered meaningful was arbitrarily set at 1 µg/g. This was based on the fact that 1 µg/mL DEX showed a marked reduction of lung phospholipase activity in guinea pig,²² and 1 µg/mL was also the quantification limit of HPLC assay in this study. This number can very well be on the high side as even a concentration of DEX at 10^{-7} M (~40 ng/mL) has been reported to inhibit prostaglandin release from rabbit coronary microvessel endothelium.²³

For the ocular toxicity study, there were 4 groups. The longest application time of interest, 20 min, was selected for testing safety and tolerability of the 4 DSP concentrations. Each rabbit received a weekly DSP-Visulex application (ie, 4%, 8%, 15%, or 25% DSP concentrations) for 20 min in 1 eye (right eye) leaving the other (left eye) as an untreated control. The total exposure was 12 doses over the period of 12 weeks. Clinical observations were performed before and after each dosing on weekdays. Following the final observations (ie, 1 week after the last dose), the rabbits were sacrificed and the eyes were processed for histological evaluation.

For the toxicokinetic study, there were 4 groups of rabbits. Each group received a single dose of 5- or 20-min application of 4% or 15% of DSP-Visulex in 1 eye. Blood was collected and processed for plasma at predose, 5, 30, 60, 120, 240, and 360 min, and 24, 48, 72, 96, and 168 h after administration. Plasma concentration analysis for DEX and DSP was performed using liquid chromatography–mass spectrometry (LC-MS).

At the termination point in all 3 studies, the animals received a 2.5 mL intramuscular injection containing 5 mg ketamine and 30 mg xylazine per mL as general anesthetic. For each animal, the depth of anesthesia was confirmed by the absence of corneal blink reflex or toe pinch response to ensure humane euthanasia. The animal was then sacrificed by an intracardiac injection of 2 mL of saturated KCl with a 3 mL syringe and 18GA × 1" needle. The eyes were collected and processed for drug analysis or histological evaluation.

DSP-Visulex administration

Each rabbit was placed in a rabbit restrainer to limit movement during the DSP-Visulex administration. One drop of sterile proparacaine hydrochloride (a local anesthetic) was put in the eye (to be treated) 5 min before dose administration. DSP solution (250 µL) was loaded onto the annular white sponge of the Visulex applicator using an Eppendorf pipettor. Then, Visulex containing the DSP solution (DSP-Visulex) was gently applied to the scleral surface of the eye of each rabbit. The position of the Visulex system was checked to ensure that the drug sponge was in immediate contact with the white part of the eye, but not the

cornea. Digital timers were used for accurate application times (ie, 5, 10, or 20 min). After the given application duration, the applicator was carefully removed from the eye.

Eye tissue collection and drug extraction

For drug analysis,²⁰ the eyes were immediately dissected after enucleation into 7 tissue sections: anterior chamber, lens, retina-choroid, cornea, vitreous, conjunctiva, and sclera. The anterior chamber consists of iris, ciliary muscles, and aqueous humor. After dissection, the drug was extracted from each tissue overnight with 5 mL of the extraction solvent (60% chloroform–40% methanol). Each tissue section was then separated from the extraction solution by centrifuge at 3,400 rpm for 10 min. The extraction solutions were concentrated by evaporation of the solvent in a water bath at 50°C, using nitrogen gas, and then reconstituted in 1 mL of the reconstitution solvent (95% methanol/5% 1 M HCl). The amounts of total DSP and DEX in the eye tissues were then determined by HPLC analysis. It is to be noted that each entire tissue section of sclera, anterior chamber, retina-choroid, cornea, vitreous, and lens was used for the drug analysis. Although the entire conjunctiva tissue was not used for the drug analysis, careful and consistent trim of the conjunctiva was made during the tissue dissection.

For histopathology, the enucleated eyes were stored in Davidson's solution (ie, 34.7% deionized water, 11.1% glacial acetic acid, 32.0% ethanol, and 22.2% formalin) for 24 h and then transferred to plastic conical tubes containing 20 mL of 70% ethanol in water. The eyes were sent for histopathological processing and evaluation at Colorado Histo-Prep.

Blood collection

Blood was collected at predose (–20 min), 5, 30, 60, 120, 240, and 360 min, and 24, 48, 72, and 168 h after DSP-Visulex application. Approximately 1 mL of blood was collected by direct venipuncture of the jugular vein with a 3 mL syringe and 21GA × 1" needle. Blood was immediately transferred into anticoagulant (potassium EDTA)-coated microcentrifuge tubes. Blood was then centrifuged for 5 min at 3,000 g at 4°C. Plasma was immediately separated into another microcentrifuge tube and kept in –20°C freezer for LC-MS analysis.

HPLC analysis

The amounts of DSP and DEX in the eye tissues were determined by HPLC analysis, which was slightly modified from Miller et al.²⁰ The HPLC system used was Waters 2695 separation module equipped with Waters 2487 dual wavelength detector (Waters Corporation, Milford, MA) and Kinetex C18 column 2.6 µm 100 × 4.6 mm (Phenomenex, Torrance, CA). All chemical reagents for making HPLC mobile phases were HPLC grade from Sigma-Aldrich. The mobile phase was 30% by volume of acetonitrile and 0.1% by volume of trifluoroacetic acid (99%) in double-deionized water. The HPLC method was isocratic with a 1.2 mL/min flow rate and column temperature was 30°C. The injection volume was 10 µL. A single UV wavelength mode was set at $\lambda = 240$ nm. Retention times for DSP and DEX were 4.2 and 6.9 min, respectively. The DSP and Dex standard curves of 0.0005–0.5 mg/mL (ie, concentration vs. absorbance) were generated. The lower limit of quantification of this method was 0.001 mg/mL.

LC-MS analysis

All plasma analyses for DSP and DEX were performed at Tandem Labs (Salt Lake City, UT) using LC-MS. Briefly, the samples were assayed by Shimadzu SCL-10A controller with LC-10AD pump. The mobile phase was 50% by volume of 10 mM ammonium acetate and 50% by volume of methanol. The HPLC column was an XBridge Phenyl column, 5 μ m, 50 $\times$ 2.0 mm. An isocratic elution was applied at 0.500 mL/min flow rate and column temperature was 30°C. An API 5000 (Applied Biosystem/Sciex) mass detector with an electrospray interface in positive mode (source temperature set at 400°C) was used to detect the MS/MS transition m/z 393 to m/z 373.4 for DEX and m/z 473 to m/z 435 for DSP. The injection volume was 10 μ L. The retention times for DSP and DEX were 1.2 and 2.5 min, respectively. DSP and DEX standard curves of 0.2–200 ng/mL were generated. The limit of quantitation of this method was 1 ng/mL.

Toxicokinetic data analysis

Toxicokinetic data analysis was based on standard non-compartmental pharmacokinetic methods. Plasma concentration of DSP equivalent was used in the analysis to express systemic exposure of DSP and DEX as a single entity. The DSP equivalent was calculated by converting DEX to DSP using 392.5 g of DEX equivalent to 516.4 g of DSP. The maximum observed plasma concentration (C_{max}) was determined by visual estimation from the data plot. Area under the plasma drug concentration versus time curve (AUC) from 0 to the time of last measurable concentration was calculated by the linear trapezoidal method. Elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/k_e$, where k_e is the elimination rate constant determined by linear regression of the last 3 analytically measured points on the plasma concentration versus time curve.

Clinical observations

Body weights of the animals were taken upon arrival, and then monthly. All animals (both left and right eyes) were examined by indirect ophthalmoscopy of the cornea, conjunctiva, anterior chamber, vitreous, posterior chamber, and sclera. One to two drops each of phenylephrine hydrochloride and cyclopentolate hydrochloride were used as mydriatics. Observations on the anterior and posterior segments of the eye were made, graded, and recorded. A modified McDonald-Shadduck scale²⁴ was used for grading eye irritation and ocular toxicity.

Histopathology

The histopathological processing and evaluation were conducted at Colorado Histo-Prep (Fort Collins, CO). Briefly, a central cut of the eye globe was taken, as well as 2 cuts on either side of the central cut (calottes) at trim. For each eye, the central cut was placed into 1 cassette, and the 2 calottes were placed together into a separate cassette. The tissues were processed, embedded in paraffin wax, sectioned by microtome, and stained. Histopathology of the tissues was conducted on slides stained with hematoxylin and eosin. A pathologist who evaluated the tissues had no knowledge of the specific pharmacologic activity or formulation of the test articles. Standardized toxicological pathology criteria

and nomenclature for the rabbit were used to categorize microscopic tissue changes.^{25,26}

Statistical analysis

The data are reported as mean $\pm$ standard deviation (unless otherwise indicated). GraphPad InStat 3.10 software (La Jolla, CA) was used for statistical analysis. The differences in mean between the groups were evaluated by one-way analysis of variance with Tukey-Kramer multiple comparisons test. This included total amount of the drug in the eye and the drug concentration in each tissue (ie, anterior chamber, lens, retina-choroid, cornea, vitreous, conjunctiva, sclera, and plasma). The differences in mean body weight at baseline and the end of study were tested by paired t -test. For all statistical analyses, differences were considered significant at $P < 0.05$.

Results

Ocular drug distribution study

After single applications of DSP-Visulex for 5, 10, or 20 min and for all DSP concentrations, significant amounts of DSP and some DEX were found in all the tissues. A typical rank order of DSP amounts in the eye tissue is sclera, conjunctiva, cornea, retina-choroid, anterior chamber, vitreous, and lens. The total amount of drugs in each tissue except vitreous and lens appears to correlate well with the DSP concentration and application time of DSP-Visulex. In Fig. 2, the total amount of DSP delivered by Visulex was calculated by the sum of DSP and DEX in microgram equivalent for the purpose of drug delivery analysis. Generally, at a given application duration (ie, 5, 10, or 20 min), a higher DSP formulation concentration yielded a higher amount of DSP in the eye. Similarly, at a given concentration, a longer application duration of DSP-Visulex yielded a higher amount of DSP in the eye.

The concentration of DSP in each tissue was also calculated in μ g/g and summarized in Table 1 for potential efficacy evaluation of DSP-Visulex. As discussed earlier, the concentration of 1 μ g/g or higher in the tissue is considered the potential therapeutic level. After a single administration of DSP-Visulex, with exception of the lens, DSP found in

FIG. 2. A plot (mean $\pm$ SD, $n = 6$ eyes) between amount of drug in the eye, application time, and DSP concentration after single administration of DSP-Visulex (4%, 8%, 15%, and 25% DSP for 5, 10, or 20 min). SD, standard deviation.

TABLE 1. DEXAMETHASONE SODIUM PHOSPHATE-EQUIVALENT CONCENTRATIONS IN OCULAR TISSUES

Dose	Tissue (mean $\pm$ SD, μ g/g)						
	Cornea	Anterior chamber	Lens	Vitreous	Retina-choroid	Sclera	Conjunctiva
4% DSP, 5 min	108 $\pm$ 74	14 $\pm$ 4	0 $\pm$ 0	0 $\pm$ 1	18 $\pm$ 16	59 $\pm$ 25	33 $\pm$ 29
4% DSP, 10 min	216 $\pm$ 86	11 $\pm$ 8	2 $\pm$ 0	2 $\pm$ 1	59 $\pm$ 86	131 $\pm$ 52	56 $\pm$ 14
4% DSP, 20 min	147 $\pm$ 75	12 $\pm$ 4	3 $\pm$ 1	1 $\pm$ 1	24 $\pm$ 7	154 $\pm$ 49	49 $\pm$ 26
8% DSP, 5 min	288 $\pm$ 73	23 $\pm$ 3	13 $\pm$ 0	5 $\pm$ 1	74 $\pm$ 23	233 $\pm$ 53	84 $\pm$ 36
8% DSP, 10 min	459 $\pm$ 148	23 $\pm$ 9	0 $\pm$ 0	2 $\pm$ 1	63 $\pm$ 61	314 $\pm$ 74	104 $\pm$ 33
8% DSP, 20 min	567 $\pm$ 397	56 $\pm$ 54	14 $\pm$ 24	6 $\pm$ 8	54 $\pm$ 38	306 $\pm$ 207	113 $\pm$ 58
15% DSP, 5 min	367 $\pm$ 118	18 $\pm$ 3	5 $\pm$ 0	5 $\pm$ 3	182 $\pm$ 176	328 $\pm$ 60	150 $\pm$ 26
15% DSP, 10 min	467 $\pm$ 173	43 $\pm$ 11	17 $\pm$ 1	7 $\pm$ 1	113 $\pm$ 32	512 $\pm$ 54	222 $\pm$ 45
15% DSP, 20 min	1128 $\pm$ 521	89 $\pm$ 42	13 $\pm$ 3	12 $\pm$ 3	351 $\pm$ 275	615 $\pm$ 336	287 $\pm$ 94
25% DSP, 5 min	714 $\pm$ 252	39 $\pm$ 17	6 $\pm$ 1	6 $\pm$ 3	114 $\pm$ 82	452 $\pm$ 214	221 $\pm$ 126
25% DSP, 10 min	512 $\pm$ 327	35 $\pm$ 32	1 $\pm$ 1	4 $\pm$ 4	60 $\pm$ 77	429 $\pm$ 231	184 $\pm$ 109
25% DSP, 20 min	2,225 $\pm$ 886	169 $\pm$ 67	13 $\pm$ 4	9 $\pm$ 4	207 $\pm$ 101	731 $\pm$ 189	347 $\pm$ 107

DSP, dexamethasone sodium phosphate; SD, standard deviation.

most of the ocular tissues, including cornea, sclera, conjunctiva, retina-choroid, and anterior chamber, was significantly higher than the target level of 1 μ g/g in all the DSP-Visulex regimens tested in this study. DSP concentrations in the vitreous were around or slightly above 1 μ g/g in most cases except for the 5-min 4% DSP application. The typical order of concentration of DSP in ocular tissues from high to low was cornea > sclera > conjunctiva > retina-choroid > anterior chamber > lens > vitreous. The drug concentration in the ocular tissues (except lens and vitreous) correlated well with both increasing DSP concentration in the Visulex system and treatment duration. It may be noted that the data from 25% DSP for 10 min are significantly lower from this trend (both Fig. 2 and Table 1). It is speculated that the contact of DSP-Visulex on the eye may have been compromised (in 2 eyes) and therefore the averaged values of DSP in this case are minimized. However, as there was no apparent reason to exclude these data from the study, the much low values were included in the mean calculation.

Ocular toxicity study

Over the course of the 12-week toxicity study entailing 12 weekly doses of DSP-Visulex applications, ocular findings noted with the treated eyes (right eye) were conjunctival injection, discharge, and corneal haze. These ocular findings were transient and mild in nature. No abnormalities or signs of ocular toxicity were observed in untreated eyes (left eye). Details of the ocular findings are given below and a summary of clinical observations over the 12 weeks, including

the conjunctival injection scores, histopathological results, and body weight, is presented in Table 2.

Conjunctiva. Conjunctival injection was generally observed immediately after DSP-Visulex application in all groups. The resolution period of conjunctival injection correlates with DSP concentration. As the DSP concentration increased, it took longer time to resolve to the baseline. The resolution period of conjunctival injection was generally within 1–2 days for 4% and 8% DSP and up to 7 days for 15% and 25% DSP in some cases. The average conjunctival scores for every 4 weeks indicate that the degree of conjunctival injection increased with the DSP concentration and repeated applications (Table 2). The animals treated with 4% and 8% DSP had typical conjunctival injection scores immediately after treatment of 1 or <1 through the whole study. In a rare occasion, a score of 2 was found in the 8% DSP group. The animals treated with 15% DSP had typical conjunctival injection scores immediately after treatment of <1 for the first 4 weeks, and then 2 at week 8 until the end of study. The animals treated with 25% DSP had typical conjunctival injection scores immediately after treatment of <1 for the first 4 weeks, and then 2 or 3 at week 8 until the end of study. Chemosis on the conjunctiva was also observed immediately after DSP-Visulex application. Although chemosis tended to increase in severity with the DSP concentration and with repeated application, the occurrence of chemosis appeared to be sporadic. Conjunctival discharge was noted occasionally, but appeared to be irrespective of DSP concentration and not related to infection.

TABLE 2. CLINICAL OBSERVATIONS AND HISTOPATHOLOGY RESULTS

Dose	Average conjunctival injection score (range)			Body weight	Ocular histopathology
	Weeks 1–4	Weeks 5–8	Weeks 9–12		
4% DSP, 20 min	0.11 (0 to <1)	0.25 (0 to 1)	0.31 (0 to 1)	NCS	NSF
8% DSP, 20 min	0.11 (0 to <1)	0.19 (0 to 1)	0.40 (0 to 2)	NCS	NSF
15% DSP, 20 min	0.27 (0 to 1)	0.72 (0 to 2)	0.93 (0 to 2)	8% loss	NSF
25% DSP, 20 min	0.36 (0 to 1)	1.08 (0 to 3)	1.39 (0 to 3)	13% loss*	NSF

The scores were from treated eye.

* $P < 0.05$.

NCS, not clinically significant change; NSF, no significant findings.

Cornea. Cornea appeared normal after each DSP-Visulex application in all rabbits except in 1 case with a rabbit in the 15% DSP group from week 4 to 8. Corneal haze on the treated eye was immediately observed in this rabbit after the DSP-Visulex application at week 4. The lesion covered about 40% of the corneal surface. The haze was identified as a result of an off-center applicator placement. This caused the drug reservoir to be in direct contact with the cornea during the DSP-Visulex application. The corneal haze grew fainter over time and it was not visible by week 8.

Body weight. There were no significant weight changes in the 4% or 8% DSP-treated rabbits. However, the animals in the 15% and 25% DSP groups showed trends of decreasing body weight. Although only the 25% DSP group showed statistically significant reduction in body weight, the consistent decline in body weights of the animals in these 2 groups indicate that long-term exposure at these levels of DSP-Visulex dosing (ie, 15% and 25% DSP for 20 min) may have significant systemic side effects on rabbit.

Histopathology. All eyes were considered to be morphologically normal, except 1 treated eye in the 8% DSP group showed mild chronic inflammation at the limbus of the cornea. Besides this 1 eye, there were no significant findings with any ocular tissue examined: no edema or congestion of conjunctiva, ciliary body, or cornea was observed in any group, no neovascularization on the cornea was found, no inflammation in conjunctiva, cornea, anterior chamber, trabecular meshwork, iris, ciliary body, vitreous, choroid, and retina tissues, and no test article changes were identified.

Toxicokinetic study

After single applications of DSP-Visulex, DSP was rapidly absorbed into systemic circulation. Both DSP and DEX were found in plasma for all 4 treatment regimens (ie, 5 or 20 min applications of 4% or 15% of DSP-Visulex). The plasma concentrations of DSP and DEX after single applications of DSP-Visulex are shown in Fig. 3A. $T_{\max}$ of DSP was reached at the first blood drawn (5 min after DSP-Visulex application), whereas $T_{\max}$ of DEX was reached later at 30 min. The maximum plasma concentration ($C_{\max}$) of both DSP and DEX increased with increasing DSP concentration and with longer application time. It appears that the concentration affected the systemic exposure more than the application time; the 4% DSP-Visulex applied for 20 min yielded a lower plasma concentration than the 15% DSP-Visulex applied for 5 min. Within 24 h, the drug plasma concentrations of all groups were approaching or under the lowest detection limit of 1 ng/mL. For the purpose of assessing the systemic exposure of DSP and DEX, the DSP and DEX plasma concentrations were combined and calculated as DSP equivalent. The DSP equivalent is defined as the sum of DSP and DEX in gram equivalents, with 392.5 g of DEX equivalent to 516.4 g of DSP. The pharmacokinetic profiles of DSP equivalent from all 4 treatment regimens are shown in Fig. 3B and the key toxicokinetic parameters are presented in Table 3. The half-life of the drug in the rabbit is ~2–3 h and no statistical difference among all groups is indicated. $C_{\max}$ and AUC increased with increased concentration of DSP and increased application time.

FIG. 3. (A) Mean plasma concentration of DSP (solid line) and DEX (dot line) following single administration of DSP-Visulex at predose, 5, 30, 60, 120, 240, and 360 min, and 24, 48, 72, and 168 h (mean $\pm$ SD, $n=3$ rabbits). (B) Mean plasma concentration of DSP equivalent following single administration of DSP-Visulex. The data were calculated from (A) based on the sum of DSP and DEX in gram equivalent. No SD is given for the DSP-equivalent data. To reveal all pharmacokinetic data, all the graphs were not plotted in a linear time scale on the x-axis. DEX, dexamethasone.

To put these results of systemic DSP exposure in rabbit into human perspective, speculative estimations of $C_{\max}$ of DSP in human are presented in Table 3. $C_{\max}$ values in human were estimated based on $C_{\max}$ data from intravenous (IV) injections in both rabbit²⁷ and human²⁸. An IV injection of 1 mg DSP yields a $C_{\max}$ of 786 ng/mL in rabbit and 10.5 ng/mL in human. Accordingly, these results suggest that the $C_{\max}$ of DSP for rabbit is ~75 times higher than that for human. The estimated $C_{\max}$ in human of the lowest dose (4% DSP, 5 min) and the highest dose of DSP-Visulex (15% DSP, 20 min) are 2 and 25 ng/mL, respectively.

Effect of concentration versus treatment duration on drug distribution

Both the DSP-Visulex concentration and treatment duration have significant effects on drug distribution both locally and systemically. When comparing both factors with respect to the whole eye, it appears that the relative increase in DSP-Visulex concentration affected the ocular tissue

TABLE 3. DEXAMETHASONE SODIUM PHOSPHATE-EQUIVALENT CONCENTRATIONS IN PLASMA

Dose	C_{max} (ng/mL)	$t_{1/2}$ (h)	AUC (ng · h/mL)	Estimated C_{max} in human ^{27,28} (ng/mL)
4% DSP, 5 min	148 ± 71	3.1 ± 2.2	418 ± 93	2 ± 1
4% DSP, 20 min	795 ± 344	2.3 ± 0.6	996 ± 144	11 ± 5
15% DSP, 5 min	1,188 ± 306	1.7 ± 0.9	1,595 ± 418	16 ± 4
15% DSP, 20 min	1,844 ± 664	2.7 ± 0.3	3,779 ± 472	25 ± 9

AUC, the area under the plasma drug concentration versus time curve.

concentrations more than the treatment duration. For instance, in Fig. 2, at the 5-min application time, when the DSP concentration is increased from 4% to 15%, which is about a factor of 4, the total amount of drug in the eye increased about 5-fold from 56 to 288 μ g, but when the application time is increased from 5 to 20 min, which is also a factor of 4, the total amount of the drug in the eye increased by only 2-fold from 56 to 104 μ g. This relationship appears to hold for sclera, conjunctiva, cornea, and anterior chamber, but more subtle for vitreous, retina-choroid, and lens.

Similarly, the concentration seems to have more effect on the systemic exposure than application duration. For example, when the DSP concentration was increased from 4% to 15%, which is about a factor of 4, the C_{max} increased from 148 to 1,188 μ g (about 8-fold), but when the application time increased from 5 to 20 min, which is also a factor of 4, the total amount of the drug in the eye increased from 148 to 795 μ g (only 5-fold). This was also the case with AUC. The increase in concentration from 4% to 15% increased the AUC by a factor of 4 and it was statistically significant ($P < 0.01$), whereas the increase in the application time from 5 to 20 min increased the AUC only by a factor of 2 and it was not statistically significant ($P > 0.05$).

Discussion

The ocular drug distribution study illustrates the potential for the noninvasive delivery of DSP into the eye tissues using the Visulex system. Although the dynamics of aqueous flow and clearance in the eye are complex, the ocular drug distribution results are in line with an anticipated concentration gradient pattern arising from the outer eye tissues like sclera and conjunctiva, which were adjacent to the DSP drug reservoir and received the most drug, to the innermost tissues like the vitreous humor and lens that received much lesser amounts. In addition, it should be noted that this study was limited only to 1 time point, which was immediately after the DSP-Visulex application. The diffusion time (20 min or less) may not be long enough for drug to distribute in significant amounts into the deeper tissues. More study time points should yield further understanding of the pharmacokinetic profiles of DSP administered by the DSP-Visulex, including drug distribution, onset, duration of action, and half-life of drug in the eye tissues.

Even though the application times of Visulex in this study were significantly different from our previous study, the drug tissue distribution data indicate that the current version of DSP-Visulex is far more effective as an ocular drug delivery system than the previous generation of Visulex reported by Miller et al.²⁰ This is because a much larger surface area, greater drug volume, and better fitting and sealing are utilized

in the current Visulex applicator. For dosing, the DSP concentration in the Visulex applicator appeared to be the more dominant factor compared to the application time. Considering that the application time and administration frequency are likely to be issues for patient compliance, the DSP concentration can be an important factor from the clinical standpoint for dose adjustment.

It is of interest to compare, on a semiquantitative basis, these results to the amounts of DSP in the eye tissues immediately after ocular iontophoresis (ie, cathodal iontophoresis at 2–6 mA for 5 min) of 4% dexamethasone phosphate in the rabbit eye as reported by Güngör et al.²⁹ The 8% DSP-Visulex appears to deliver DSP to retina-choroid tissues roughly to the same extent as the iontophoresis results (2, 4, and 6 mA for 5 min), and the 15% and 25% DSP-Visulex appear to be somewhat better than the iontophoresis results. In the same study,²⁹ the passive delivery using 4% dexamethasone phosphate (0 mA for 5 min) showed similar amounts of DSP in the retina-choroid, vitreous, and anterior chamber compared to the 5 min application of 4% DSP-Visulex.

Single application and multiple applications of DSP-Visulex (ie, 8% and 15%) have previously shown to be effective in treating anterior, intermediate, and posterior uveitis in an experimental uveitis rabbit over the course of a 29-day study.²¹ The uveitis model used in that DSP-Visulex study was similar to (if not the same as) the uveitis rabbit model used in the preclinical studies of intravitreal DEX implant.^{30,31} Considering a qualitative (DEX vs. DSP) and indirect comparison (nonhuman primate vs. rabbit) to intravitreal DEX implant (Ozurdex) from a pharmacokinetic study in nonhuman primate,³² the C_{max} of DEX in the retina from Ozurdex was 1.1 μ g/g on Day 60 (ie, T_{max}), whereas the concentration of DSP in the retina-choroid from DSP-Visulex was around 18 μ g/g (or higher) immediately after a single administration ($T = 0$ h). Similarly, the C_{max} of DEX in the vitreous from the DEX implant was 0.2 μ g/mL on Day 60, whereas the concentration of DSP in the vitreous for all DSP-Visulex regimens (except 4% DSP, 5 min) was higher than 1 μ g/g (~ 1 μ g/mL) immediately after administration. This may suggest a more rapid onset of the pharmacological action with DSP-Visulex compared to Ozurdex. However, since Ozurdex, which is a controlled-release product, provides a much longer exposure of DEX in eyes compared to DSP-Visulex, the risks and benefits of the 2 products in the eye diseases will need to be further evaluated, particularly in well control efficacy models.

Comparing the DSP-Visulex in rabbit to periocular injections and oral administration in human,³³ the DSP concentration in rabbit retina-choroid after a single administration of DSP-Visulex ranged from 18 to 351 μ g/g, whereas the estimated maximum DEX concentration in the subretinal fluid in

patients after a typical dose of a peribulbar injection (5 mg), a subconjunctival injection (2.5 mg), and an oral dose of DEX (7.5 mg) was 82, 359, and 12 ng/mL, respectively.³³ When qualitatively comparing DSP-Visulex application in rabbit to the topical DSP eye drop in human,³⁴ the concentration of DSP in the vitreous of rabbit from DSP-Visulex is much higher: the $C_{\max}$ in human vitreous from the DSP eye drop (ie, 1 drop of 0.1% DSP every 1.5 h for a total of 10 or 11 drops) was 1.1 ng/mL, where most DSP-Visulex regimens yield 1 $\mu\text{g/g}$ ($\sim 1 \mu\text{g/mL}$) or more in the vitreous of rabbit. While such indirect comparisons of the DSP-Visulex data in rabbit with the pharmacokinetic studies in human may be to no avail, these at least illustrate the potential significance of the DSP-Visulex approach. A more complete ocular pharmacokinetic study of DSP-Visulex particularly in human is needed to further understand the drug distribution, onset, duration of action, and half-life of the drug in the eye tissue.

The ocular toxicity study suggests that multiple treatments of DSP-Visulex are well tolerated. The only frequent ocular adverse event was conjunctival injection, which appeared to resolve within a week. This sign of irritation correlates with increasing DSP concentration. Some accumulations of conjunctival injection were observed only in the high concentration formulations (ie, 15% and 25% DSP) and after around 2 months into the weekly treatment regimen. The tonicity of the DSP formulation may have played a role in the conjunctiva irritation. The persistence and severity of the conjunctival irritation were found to be much lower in the 4% and 8% DSP formulations (ie, isotonic formulation) compared to the 15% and 25% (ie, hypertonic formulation). It may be noted that conjunctival injection, which is also known as conjunctival hyperemia or conjunctival erythema, is a common side effect found among FDA-approved corticosteroid ophthalmic solutions, including prednisolone, DEX, and difluprednate. A temporary corneal haze was found in 1 rabbit, indicating that the corneal epithelial disruption can occur when the placement of DSP-Visulex is placed off center. This is an adverse effect that can be avoided by checking whether the DSP reservoir has any direct contact with the cornea while in position during treatment.

The systemic drug exposure is believed to be a cause of the weight loss observed in the high-dose groups (15% and 25% DSP) after weekly dosing of DSP-Visulex over 3 months. Since all animals exhibited systemic exposure of both DSP and DEX after single administration of the DSP-Visulex, this is not an unexpected outcome after topical DSP treatment for 3 months.^{35,36} The systemic drug exposure in human is, however, expected to be much lower due to a much larger body size. A $C_{\max}$ of DSP-Visulex in human is speculated to be of the order of 25 ng/mL for the high dose (15% DSP for 15 min) and 2 ng/mL for the low dose (4% DSP for 5 min). When compared to the plasma $C_{\max}$ from a single IVT injections (not detectable), a single topical eye drop (0.7 ng/mL), an oral tablet (62 ng/mL), a single peribulbar injection (60 ng/mL), and a single subconjunctival injection (32 ng/mL),³² it suggests that DSP-Visulex may have a higher systemic exposure than eye drops and IVT injections, but less than oral and periocular injections. In addition, an ocular iontophoretic delivery of dexamethasone phosphate (4% w/v) in uveitis patients showed the plasma $C_{\max}$ of DEX in the range of 2–10 ng/mL.³⁷ Since the ocular iontophoresis delivered approximately the same order of magnitude of dexamethasone phosphate to the rabbit oc-

ular tissues as DSP-Visulex (as discussed previously), it is reasonable to speculate that the systemic drug exposure in human of DSP-Visulex would be in the same order of magnitude as the ocular iontophoresis. However, a more thorough systemic toxicity evaluation, including hematology, clinical chemistry, and organ examination, in comparison with other dosage forms typically is recommended for a more quantitative systemic toxicity study.

A limitation of this toxicity study is that no IOP measurements are reported. The increase in IOP from a long-term exposure of DSP is a valid point of concern, although the histopathological examination found no steroid induced glaucoma after 12 weekly applications of DSP-Visulex. It should be noted that IOP measurements have been conducted in subsequent toxicity studies of DSP-Visulex. The IOP increase in those studies was found to be minimal and transient with no evidence of steroid-induced glaucoma (M.J. Larson, DVM, and K. Papangkorn, PhD, unpublished data, 2014). Another limitation of this toxicity study is that DSP-Visulex was evaluated with a single application duration (ie, 20 min). As the application duration might have an impact on toxicity of DSP-Visulex, the application duration of DSP-Visulex should be tested to evaluate its effect on tolerability and toxicity in future studies. In addition, sample size is also a limitation for establishing statistical significance and should be considered in future studies. The present was exploratory and the sample size was not powered for statistical analysis.

Overall, DSP-Visulex has a potential to be considered a new ophthalmic dosage form because it can effectively and safely administer DSP into the eye tissues. DSP-Visulex also shows that it can address certain problems of the other existing corticosteroid dosage forms for eye diseases, including frequent dosing of eye drops, the systemic side effects of oral therapy, and the serious risks associated with intravitreal and periocular injections. DSP-Visulex can possibly benefit both anterior and posterior eye diseases²¹ such as uveitis, diabetic macular edema, diabetic retinopathy, and age-related macular degeneration. In addition, many other drug molecules can be incorporated into the Visulex platform for ophthalmic applications. Currently, DSP-Visulex is being evaluated for its safety and efficacy in a randomized, parallel group, double-masked, active-controlled Phase 1/2 clinical trial for the treatment of noninfectious anterior uveitis (ClinicalTrials.gov Identifier: NCT02309385).

Conclusions

High concentrations of DSP can be administered safely and effectively into the rabbit eye through the Visulex system. Treatment duration and DSP concentrations employed are important factors in achieving targeted delivery of drug into ocular tissues. The DSP concentrations and the numbers of repeated administration correlate with signs of ocular irritation and potential tolerability concerns. Topical administration of 4%, 8%, 15%, and 25% DSP-Visulex for 5, 10, or 20 min shows potential in achieving therapeutically relevant concentrations of DSP, which may be of benefit for various inflammatory diseases of both front and back of the eye. Repeat application of 4% and 8% DSP-Visulex treatments is well tolerated for weekly administration over 3 months, whereas 15% and 25% DSP-Visulex weekly treatment exposure are potentially limited to shorter periods of time, between 4 and 8 weeks. The data suggest that DSP-

Visulex has clinical potential for the noninvasive treatment of ocular diseases including uveitis, macular edema, and postoperative inflammation.

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Author Disclosure Statement

K.P. and J.W.H. are employees of Aciont, Inc.; B.B. is a consultant at Aciont, Inc.; and W.I.H. is a founder and CTO of Aciont, Inc.

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