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RESEARCH PAPER

Pharmacokinetics and Efficacy of Bioerodible Dexamethasone Implant in Concanavalin A-induced Uveitic Cataract Rabbit Model

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ABSTRACT

Purpose To advance therapy for the treatment of concurrent uveitis and post-cataract surgical inflammation; we evaluated pharmacokinetics and pharmacodynamics of Bioerodible Dexamethasone Implant (BDI) containing 0.3 mg of dexamethasone (DXM) in Concanavalin A (Con A) induced uveitis followed by phacoemulsification in New Zealand White (NZW) rabbits.

Methods The BDI was implanted in the inferior fornix of the capsular bag after intravitreal injection of Con A and ensuing phacoemulsification in NZW rabbits; standard-of-care topical 0.1% dexamethasone drops served as control. DXM was quantified by liquid chromatography-tandem mass spectrometry and pharmacokinetics of DXM in disease *vs.* healthy eyes was compared. All eyes were assessed clinically using slit lamp biomicroscopy and Draize scoring scale. Retinal thickness and histological analyses were performed to evaluate retinal edema, inflammation and implant biocompatibility respectively.

Results In Con A-induced inflammatory uveitic cataract model the BDI controlled anterior and posterior segment inflammation as well as retinal thickening more effectively than topical drops. The exposure (AUC_{0-t}) of DXM with BDI is superior in all ocular tissues, while topical drops did not achieve therapeutic posterior segment levels and did not control inflammation nor prevent retinal edema and architectural disruption.

Conclusions Our results demonstrate the superiority of the BDI in suppressing Con A-induced inflammation and retinal edema in NZW rabbits and highlight the need for sustained bidirectional delivery of potent anti-inflammatory agents for 5 to 6 weeks to optimize clinical outcomes.

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KEY WORDS bioerodible dexamethasone implant · cystoid macular edema · pharmacokinetics · topical drops · uveitis

ABBREVIATIONS

BDI	Bioerodible dexamethasone implant
BQL	Below quantification limit
CME	Cystoid macular edema
Con A	Concanavalin A
DXM	Dexamethasone
EtO	Ethylene oxide gas
GC-MS	Gas chromatography mass spectrometry
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LLOQ	Lower limit of quantification
MWCO	Molecular weight cut off
NSAIDs	Non-steroidal anti-inflammatory drugs
NZW	New Zealand white rabbits
PCO	Posterior capsule opacification
PLGA	Poly(d,l-lactide-co-glycolide)
PVA	Polyvinyl alcohol
SD-OCT	Spectral domain optical coherence tomography

INTRODUCTION

Cataract extraction is the most frequently performed surgery in the USA: about 3.5 million people have their cataracts removed and replaced with an intraocular lens annually (1,2). In the US, the number of patients with cataracts is expected to be around 30 million by 2020 (2,3). Surgical trauma may induce cystoid macular edema (CME) (4–6), which thickens the central retina and threatens visual acuity. Moreover, cataract surgery is often performed in older

patients who have comorbidities, e.g., diabetes or uveitis (7), which increase the risk of CME or inflammation. Ocular inflammation after cataract surgery can prolong recovery time and increase the likelihood of CME, synechiae formation, and posterior capsule opacification (PCO) (8,9).

To prevent short and long-term postoperative complications, corticosteroids and non-steroidal anti-inflammatory drugs (NSAID's) are administered topically after cataract surgery. However, published compliance rates of topical eye drop therapy are only 10–40% (10,11). Poor patient compliance vis-à-vis topical drug administration stems from difficulties in self-administration secondary to poor aiming, tremulous hands, or forgetfulness, all of which are common in the elderly. The lack of compliance, along with complexity of topical eye drop regimens, can delay recovery from surgery to several weeks or months if CME or uveitis occurs. Systemic or local steroidal injections can be used, but often result in side effects (10,11).

In order to mitigate poor compliance and to enhance therapeutic effectiveness, sustained release of steroidal and antiviral drugs loaded in bioerodible or non-bioerodible implants have been developed for the implantation in the vitreous cavity for the treatment of various ocular diseases in the posterior segment. These systems achieve prolonged therapeutic drug concentrations for 6–12 months (e.g., Retisert®, Ozurdex® and Vitrasert®), but require an invasive intravitreal procedure entailing risks of endophthalmitis, retinal detachment, and retinal hemorrhage (12). Furthermore, all commercially available ocular implants deliver drugs to the posterior segment and do not deliver drugs to the anterior segment of the eye.

We recently reported a novel bioerodible dexamethasone implant (BDI) for the treatment of post-operative inflammation following routine lens extraction in rabbits (12). The BDI was placed in the inferior fornix of the lens capsular bag during routine phacoemulsification, and released dexamethasone (DXM) in a tapering fashion. A biodegradable drug delivery system placed within the lens capsule can increase a drug's exposure in the local tissues for the desired duration and improve clinical outcomes. In addition, it circumvents the limitations and side effects of systemic, oral, and intravitreal therapy and fully degrades over time.

Here, we sought to determine pharmacokinetics and pharmacodynamics of the BDI in a model of uveitic cataract extraction (Fig. 1). Further, we compared the pharmacokinetics of the BDI in uveitic and healthy eyes (12). We used the lectin Concanavalin A (Con A) to induce uveitis in New Zealand White (NZW) rabbits. As a non-specific inflammatory agent, Con A consistently incites uveitis within 2 days after intravitreal injection as shown in immunological studies (13–15). This study thus explores clinically relevant efficacy measures in a high-inflammation challenge setting (immune activation by Con-A plus the trauma of cataract surgery).

MATERIALS AND METHODS

Materials

PLGA or poly(d,l-lactide-co-glycolide; 50:50, Mw-7,000–17,000, acid terminated), Hydroxypropyl methylcellulose (HPMC, 2,600–5,600 cP), dexamethasone, poly vinyl alcohol (PVA, 90.0 kDa), dichloromethane, acetonitrile, methanol, ammonium acetate, and acetic acid were purchased from Sigma-Aldrich, USA. Custom 2.0 mm die sets were purchased from International Crystal Labs, USA. Thermo high-purity C18 HPLC column was obtained from Thermo Scientific, USA. The bench top pellet press was from Carver Instruments, USA. Dexamethasone sodium phosphate 0.1% ophthalmic solution and ciprofloxacin hydrochloride 0.3% ophthalmic solution were from Falcon pharmaceuticals, USA. Povidone iodine was from Purdue Pharma LP, USA, and proparacaine hydrochloride and tropicamide were from Bausch & Lomb, USA. Ketamine was from VEDCO, USA, xylazine from Lloyd Laboratories, USA and euthanasia solution was from VETONE, USA. Concanavalin A was used as obtained from Sigma-Aldrich, USA and phenylephrine hydrochloride from Falcon Pharmaceuticals, USA.

Preparation of PLGA Microspheres

DXM loaded PLGA microspheres were prepared using standard oil-in-water (o/w) emulsion-solvent extraction method as described previously (12). Briefly, 160.0 mg PLGA was dissolved in mixture of methylene chloride and acetonitrile (4:1 ratio), then, 40.0 mg DXM and 10 mg of HPMC was dispersed in the PLGA solution by vortexing for 5.0 min. This organic phase was emulsified in 20.0 ml of a 2.0% (w/v) PVA solution and homogenized at 16,000 rpm for 2.0 min. The resultant emulsion was poured into 200.0 ml of a 2.0% (w/v) PVA solution and stirred at 12,000 rpm in an ice bath for 6.0 min. The contents were stirred for 8.0 h at room temperature on a magnetic stirrer in a fume hood to evaporate the dichloromethane and acetonitrile, allowing the formation of a turbid particulate suspension. Microparticles were then separated by centrifugation at 15,000 rpm for 10.0 min. The pellets are washed two times with deionized water, re-suspended in deionized water, and freeze-dried to obtain lyophilized particles.

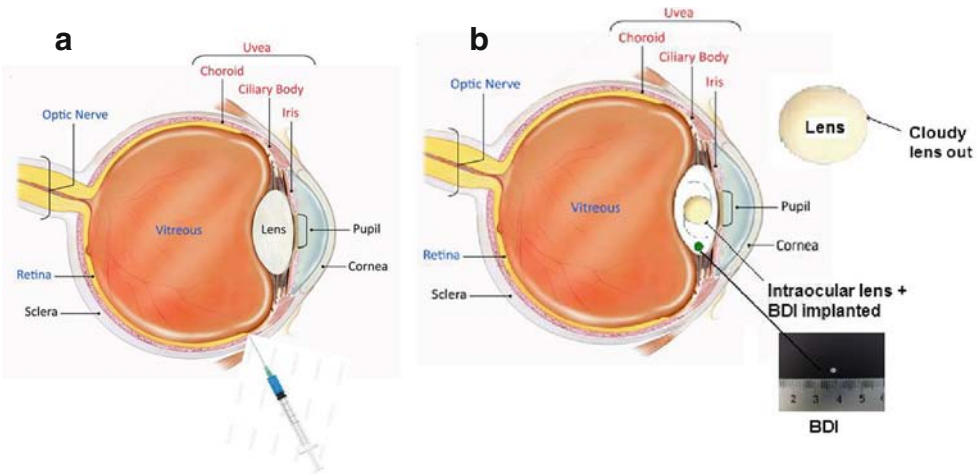

Fig. 1 Intravitreal injection and placement of BDI. (**a**) Showing intravitreal injection of Con A (**b**) phacoemulsification (ultrasonic aspiration of eyes cloudy lens) followed by intraocular lens and BDI implantation. BDI has ~2.0 mm diameter and 0.7 mm thickness with 20% w/w DXM load corresponds to 0.3 mg.

Particle Size Analysis and Drug Loading

Mean particle size of microparticles was analyzed by Zetasizer Nano Z (Malvern Instruments Inc, USA). Approximately 2.0 mg of microspheres were dispersed in 5.0 ml of 0.2% (w/v) PVA solution, diluted 5 times with deionized water and used for particle size analysis. For drug loading, 10.0 mg of microparticle powder was weighed and dissolved in 10.0 ml of acetonitrile. This solution was filtered (Millex® HV, PVDF 0.45 μm syringe filter, Millipore, USA) and the DXM concentration was determined by the LC-MS/MS method. Drug loading was determined as percent drug loading = (weight of drug loaded/weight of microspheres) × 100. All measurements were done in triplicate and the results are reported as mean ± SD.

Preparation of Implants

The BDI implants were prepared using bench top pellet press in a 2.0 mm die set . In brief, 1.6 mg of microparticle powder was weighed and transferred into the opening of the micro collar. The solid anvil with the polished face was placed on the collar and pressure (2.0 t) was applied on the die set for 20.0 s. After releasing pressure, the implant (~2.0 mm diameter and 0.6 mm thickness) was collected. Implants were sterilized by ethylene oxide gas before implantation in rabbits.

Animals, Sample Collection and Storage

Each animal is housed in an individual numbered cage at 19.0–23.0°C with a 12.0 h light–dark cycle and allowed free access to food and water. All animal procedures were performed according to animal care protocols approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with the requirements of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International and all animal handling was performed according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals were sacrificed at each specified time point by injecting 2.0 ml of euthanasia solution intravenously through the marginal ear vein. Individual tissue samples (aqueous humor, vitreous humor, cornea, iris/ciliary body and retina/choroid) were separated and stored. A portion of each tissue sample was immediately transferred to biopsy cassettes submersed in neutral buffered formalin 10.0% for sectioning/staining for histological examination; remaining samples were stored in a freezer (−70.0 ± 2.0°C) until bio-analysis. Approximately 1.0 ml of whole blood was collected (before injecting euthanasia solution), placed into labeled microcentrifuge tubes, and allowed to clot and centrifuged at 6,000 rpm for 6.0 min; serum was collected and stored in a freezer until bio-analysis.

Induction of Uveitis by Intravitreal Injection of Concanavalin A and Study Design

Twenty seven female NZW rabbits (Western Oregon Rabbit Co, USA) weighing 2.5 to 3 kg were anesthetized with a 0.8–1.2 ml intramuscular injection of a 4:1 mixture of 100 mg/ml xylazine and 50 mg/ml ketamine. The fur surrounding the eye was prepared with povidone iodine. Proparacaine hydrochloride was applied topically 1 to 5 min before the intravitreal injections. A lid speculum was inserted and a 30-gauge needle was introduced into the vitreous cavity, approximately 2 to 3 mm posterior to the superotemporal limbus, and 0.1 ml (1 mg/ml) of the Con A solution was injected slowly. Both eyes of all rabbits (standard control group, the BDI group and topical drops group) received 100 μg Con A per eye except normal controls. Rabbits in the normal control ($n = 3$) and

standard control group ($n=9$) did not undergo phacoemulsification, as the surgery requires antibiotic and anti-inflammatory drugs to treat the post-surgical inflammation which would affect the progress of the inflammation. Rabbits in the BDI group ($n=9$) and topical drops group ($n=9$) underwent phacoemulsification on day 3 after Con A injection. Two rabbits each from standard control group, the BDI group and topical drops group were sacrificed on weeks 1, 2 and 4. On week 6 three rabbits from all groups were sacrificed. For all examinations $n=6$ eyes were used except for pharmacokinetic and histological analysis ($n=4$, weeks 1, 2 and 4), (Fig. 2). Topical drops administration schedule: week 1 to 3=q.i.d.; week 4=t.i.d.; week 5=b.i.d.; and week 6=q.d. All the BDI implants were sterilized by ethylene oxide (EtO) gas before implantation in rabbits.

Slit Lamp Biomicroscopy and Draize Scoring

All eyes were examined with slit lamp biomicroscopy on days 1, 2 and weekly for 6 weeks and pictures were taken of all the groups. Biomicroscopy findings were graded on a scale from 0 to 4 with 0=none, 1=trace, 2=mild, 3=moderate, and 4=severe. Draize evaluations were done by one observer throughout the study duration for consistency in scoring. Eyes were dilated with tropicamide and phenylephrine hydrochloride before observation.

Retinal Thickness Measurement

Retinal thickness measurements were carried out using SD-OCT (Spectral Domain Optical Coherence Tomography; Heidelberg Engineering GmbH, Heidelberg, Germany). Rabbits were anesthetized and dilated as above. At least 4 measurements were taken from each eye and readings were reported as mean ± SD.

Histological Analysis

Tissue samples were embedded in paraffin and cut on a rotating microtome. Sections were mounted on glass microscope slides for hematoxylin and eosin (H & E) staining (16).

LC-MS/MS Analysis

Quantification of DXM in all the samples was performed using a fully validated and previously published method (12). Briefly, aqueous, vitreous humor, and serum samples were analyzed followed by liquid-liquid extraction using tert-Butyl methyl ether. Extraction of DXM from iris/ciliary body and retina/choroid needed one additional step. To each tissue sample 100 µl of BSS was added and the sample homogenized in an ice bath with sonic dismembrator (Fisher Scientific, USA) at low speed for 1.0 min followed by liquid-liquid extraction using tert-Butyl methyl ether. The lower limit of quantification (LLOQ) was 2.0 ng/ml.

Pharmacokinetic Analysis

DXM pharmacokinetic data was analyzed using non-compartmental analysis model in Phoenix WinNonlin Professional software version 6.3 (Pharsight Corp., USA). The following parameters were determined by visual inspection of the data: highest observed drug concentration in ocular fluids/tissues (C_{max}), time to reach highest observed drug concentration (T_{max}), drug concentration observed at last sampling point (C_{last}), and the time point at which C_{last} was

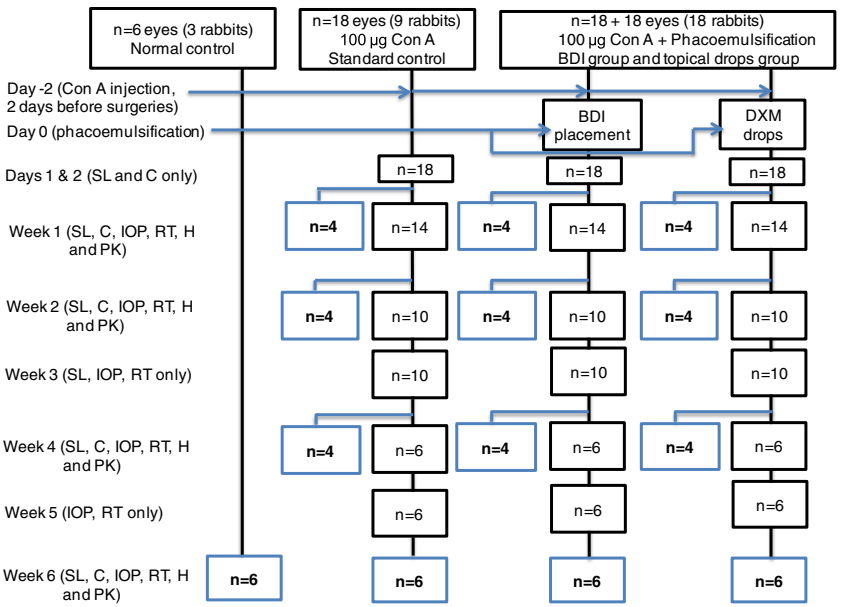

Fig. 2 Study design showing number of animals and examinations performed. Days 1 and 2, preliminary examinations were performed to assess any surgery related adverse events. At weeks 1, 2 and 4 from standard control group, BDI group and topical drops group 2 rabbits (4 eyes) were sacrificed followed by examinations. At week 6, 3 rabbits (6 eyes) were sacrificed from all the groups including normal control group indicated by blue boxes. For SL, C, IOP and RT $n=6$ eyes were used at all time points. *SL* slit lamp examination, *C* clinical examinations, *IOP* intraocular pressure, *RT* retinal thickness, *H* histological analysis, and *PK* Pharmacokinetics.

measured (T_{last}). Area under the ocular fluid/tissue drug concentration-time curve between time 0 and time t (AUC_{0-t}) was calculated using the linear-log trapezoidal method.

RESULTS

Measurement of Particle Size and Drug Loading

Microspheres prepared with standard o/w method resulted in uniform microparticles with mean diameters ranging from 1 to 9 μm as analyzed with Zetasizer Nano Z. The target load of DXM in the microparticles was ~20.0% with a percent recovery of 99.9% (w/w). There was no detectable concentration of methylene chloride or acetonitrile present in microparticles as analyzed by Gas chromatography–mass spectrometry (Limit of Detection: ~1.0 pg in 10.0 mg of sample).

BDI Effectively Suppresses Intraocular Inflammation

Normal control group pictures are presented in Fig. 3a-d. Slit-lamp biomicroscopy and Draize scoring revealed progressively severe inflammation in the standard control group (Fig. 3e-h). The BDI group exhibited less anterior and posterior chamber inflammation one week after implantation compared to topical DXM drops group (Fig. 3i-l); by week 2, all the BDI eyes were clear of inflammation. The BDI was visible at the edge of the intraocular lens but did not affect the visual axis. The anterior chamber in the eyes which received the BDI was clear by week 1. However, in eyes treated with topical DXM drops, flare, fibrin, cells and synechiae were seen up to week 6 (Fig. 3m-p), and none of these eyes had inflammatory resolution before week 4. Furthermore, after 15 days of treatment PCO was minimal in the BDI group; in contrast, there was 1.5+ PCO in eyes which received topical DXM drops. Draize scoring results and slit-lamp biomicroscopy photographs are presented in Table I and Fig. 3 respectively.

Clinical Observations

Clinical examinations were done in all rabbits on days 1, 2 and then weekly until 6 weeks. Normal control group pictures can be seen in Fig. 4a-d. Rabbits in the standard control group (Fig. 4e-h), which received only Con A but no steroids displayed redness, flare, cells, and synechiae in both the anterior and posterior segments up to week 6; however, their normal growth pattern was not affected. All animals in the BDI group (Fig. 4i-l) appeared physically healthy and exhibited no signs of toxicity during the study, gaining weight

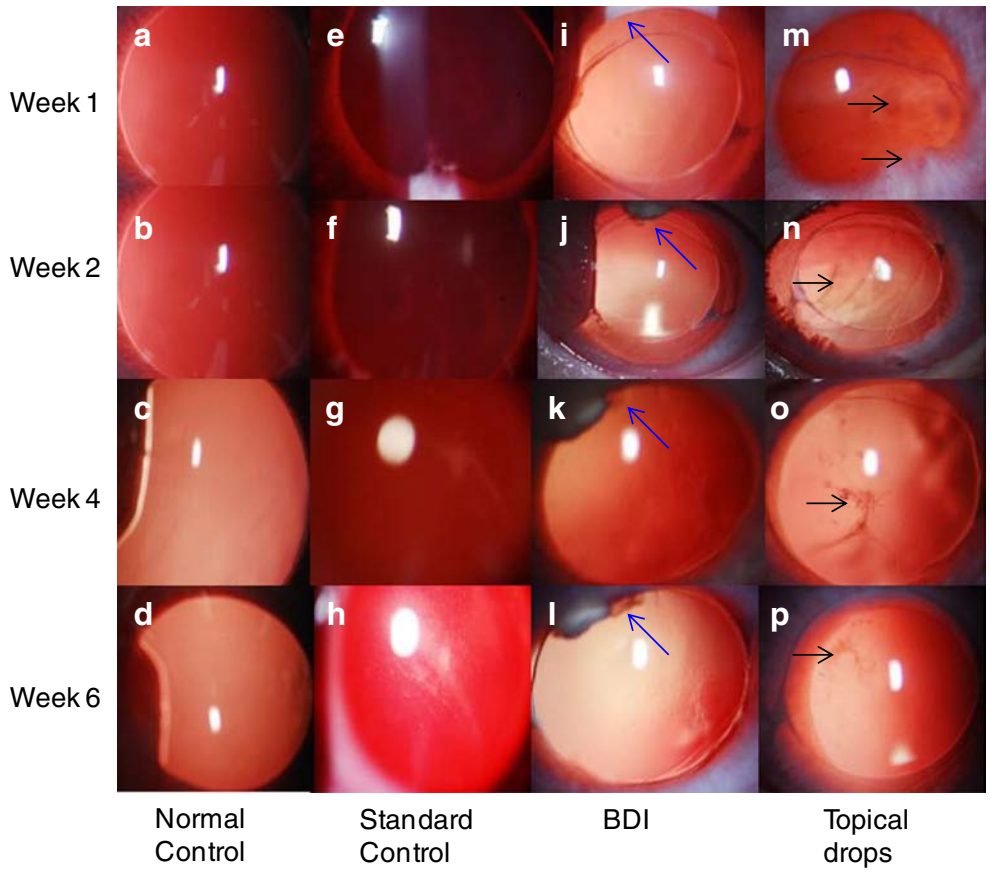

Fig. 3 BDI suppresses intraocular inflammation and does not affect intraocular lens. Slit lamp photographs demonstrate clear lenses in normal control group (**a**–**d**). Standard control group eyes which underwent Con A injection without phacoemulsification and steroid therapy had cloudy lenses with cells, flare and fibrin (**e**–**h**). The BDI group show clear lenses throughout the study duration, the blue arrows point the BDI position, located inferior to the intraocular lens (**i**–**l**)#. Topical drops group show flare, cells, fibrin until week 4 (**m**–**p**). ↗: BDI implant. →: flare, fibrin and synechiae. # BDI implant was located inferior to the IOL, the positional difference seen in the pictures was due to the alignment of the slit lamp channel.

Table I Slit Lamp Grading After Intravitreal Con A Injection Followed By Phacoemulsification: Anterior, Posterior Chamber Flare, Fibrin and Cells ($n = 6$ eyes)

Week	Flare			Fibrin			Cells		
	Std. Control	Topical Drops	BDI	Std. Control	Topical Drops	BDI	Std. Control	Topical Drops	BDI
Anterior Chamber[a]									
1	1.5 ± 0.5	1.0 ± 0	0 ± 0	1.0 ± 0.5	2.3 ± 1.0	1.3 ± 0.5	1.0 ± 0	1.0 ± 0.8	0 ± 0
2	1.0 ± 0.6	1.0 ± 0	0.5 ± 0	0.8 ± 0.4	1.0 ± 0	0 ± 0	2.0 ± 0.8	2.3 ± 0.5	0.5, $n = 2$
3	1.0 ± 0.5	1.0 ± 0	0 ± 0	1.0 ± 0.5	0 ± 0	0 ± 0	1.0 ± 0.5	1.0 ± 0	0 ± 0
4	1.0 ± 0.8	1.0 ± 0	0 ± 0	1.5 ± 0.2	0 ± 0	0 ± 0	0.5 ± 0.5	1.0 ± 0	0 ± 0
6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Posterior Chamber[a]									
1	1.5 ± 0.6	1.8 ± 0	0 ± 0	1.6 ± 0.3	2.8 ± 0.5	1.3 ± 0.6	2.0 ± 0.8	1.5 ± 0.3	0 ± 0
2	3.5 ± 0.9	3.5 ± 0.6	1.4 ± 1.1	3.0 ± 0.7	3.8 ± 0.5	3, $n = 1$	3.5 ± 1.25	3.8 ± 0.5	1.3 ± 1.2
3	1.8 ± 0.4	1.0 ± 0	0.5, $n = 2$	2.5 ± 0.6	0 ± 0	0 ± 0	2.2 ± 1.2	1.0 ± 0	1.0, $n = 1$
4	1.8 ± 0.5	1.0 ± 0	0 ± 0	1.2 ± 0.5	0 ± 0	0 ± 0	2.0 ± 0.9	0 ± 0	0 ± 0
6	2.0 ± 0.8	1.0 ± 0	0 ± 0	1.5 ± 0.5	1.0 ± 0	0 ± 0	2.0 ± 0.8	0 ± 0	0 ± 0

[a] Anterior and posterior chamber synechiae were commonly seen in standard control group and topical drops group. Only one eye out of 18 had posterior synechiae in BDI group

normally (3.0 *vs.* 3.3 kg on week 0 and week 4 respectively). After 2 weeks, the BDI implant became smaller and progressively decreased in size; no dislocations were observed. By week 6, with enhanced degradation and erosion all implants had only a minimal mass. Rabbits treated with topical drops (Fig. 4m-p) demonstrated redness, flare and inflammation during the first week, which persisted up to week 4, and recovered very slowly compared to rabbits that had received

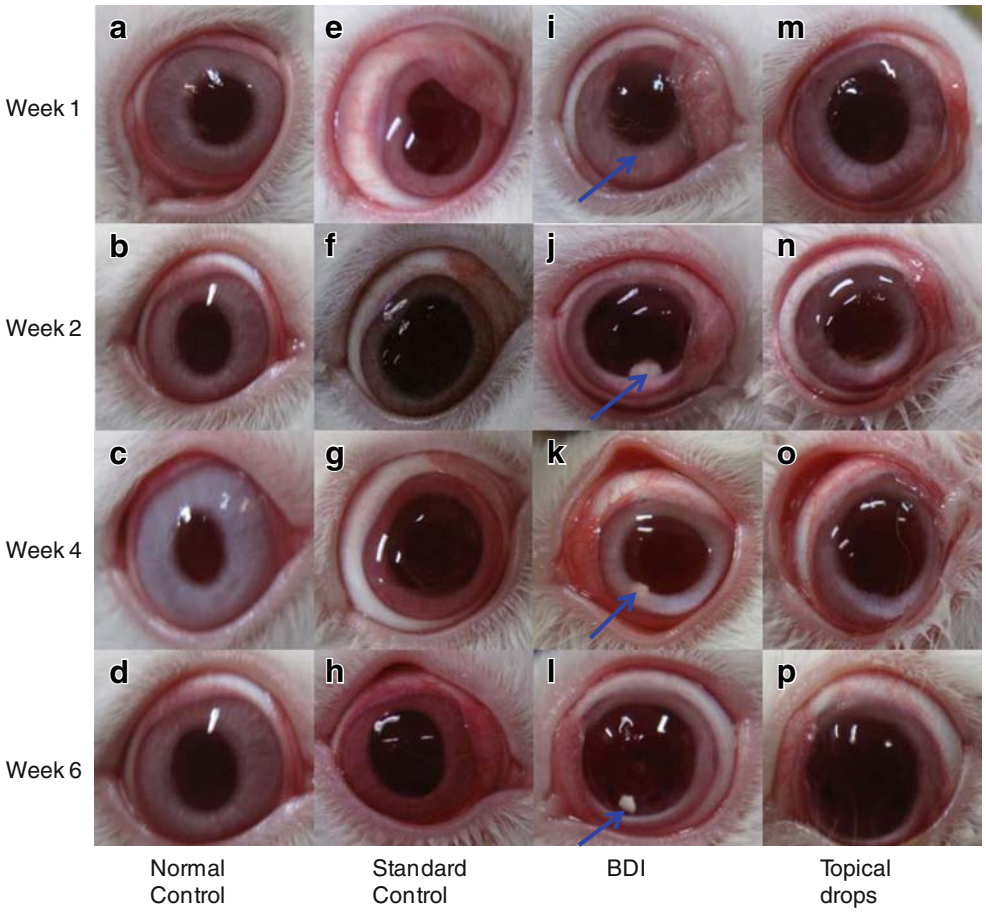

Fig. 4 BDI successfully suppresses ocular inflammation. Clinical photographs of NZW rabbits show normal controls (**a**–**d**), standard control received only Con A intravitreally show redness and inflammation throughout the study (**e**–**h**). The BDI group (**i**–**l**) demonstrated minimal or no inflammation post cataract surgery while rabbits which received topical drops (**m**–**p**) displayed redness and inflammation with slow recovery on week 1, 2, 4, and 6 respectively. ➘: BDI implant.

the BDI. Rabbits in the topical drops group also lost some of their initial body weight by week 4 (2.8 *vs.* 2.5 kg on week 0 and week 4 respectively). The loss in body weight in the topical drops group may be due to systemic exposure of DXM (100.0 *vs.* <5.0 ng/ml, $P<0.001$) (17–19). The total initial weight of the BDI was 1.5±0.1 mg with 20% w/w of DXM, 5% w/w HPMC (hydroxypropyl methylcellulose) and 75% w/w polymer [poly(lactic-co-glycolic) acid, 50:50, Mw 7,000–17,000].

Pharmacokinetics of BDI in Inflamed Eyes

Following BDI placement, sustained release of DXM was evidenced by maintenance of DXM at therapeutic concentrations up to 42 days post implantation. However, with topical drops tissue DXM concentrations declined after week 4 and were below quantifiable limits (BQL) in iris/ciliary body and retina/choroid on day 42. Time to reach highest observed concentration (T_{max}) in the iris/ciliary body and retina/choroid after BDI implantation was faster (7–14 days) compared to topical drops (14–23 days). The opposite was true in the aqueous and vitreous humor in which topical drops attained T_{max} faster. However, this may be moot, considering the exposure (AUC_{0-t}) of DXM after BDI implantation *vs.* topical drops administration (2,329±219 *vs.* 68±5 in vitreous humor). Systemic exposure was minimal with BDI, as serum concentrations of DXM remained below 5.0 ng/ml throughout the study. In line with published results (20–22), topical DXM drops induced much higher systemic concentrations (>100.0 ng/ml on day 7). The pharmacokinetic (PK) profile of the BDI implant *vs.* topical drops in aqueous humor, vitreous humor, iris/ciliary body and retina/choroid are presented in Fig. 5 along with PK parameters in Tables II and III.

BDI Prevents Retinal Thickening Induced by Con A + Phacoemulsification

Retinal thickness measurements were done using SD-OCT. Retinal thickness was defined as the distance between the inner retinal boundary (vitreous–retina interface) and the outer retinal boundary (retina–retinal pigment epithelium interface) (23). Baseline mean retinal thickness was 130±5 μm in all study rabbits as measured by SD-OCT. In the standard control group, retinal edema increased progressively and architectural disruption was seen in $n=4$ eyes by week 4 (Fig. 7). Retinal thickness in the BDI group was controlled effectively and was close to normal at all time points. However, in the topical drop group, retinal thickness increased significantly ($P<0.05$) by week 1 and persisted up to week 6 in comparison to both normal control and BDI groups. Results are presented in Fig. 6 along with OCT scans in Fig. 7.

BDI did not Induce Visible Histological Toxicity

Cornea

There were no significant differences in corneal structure between BDI and topical drops groups. The standard control

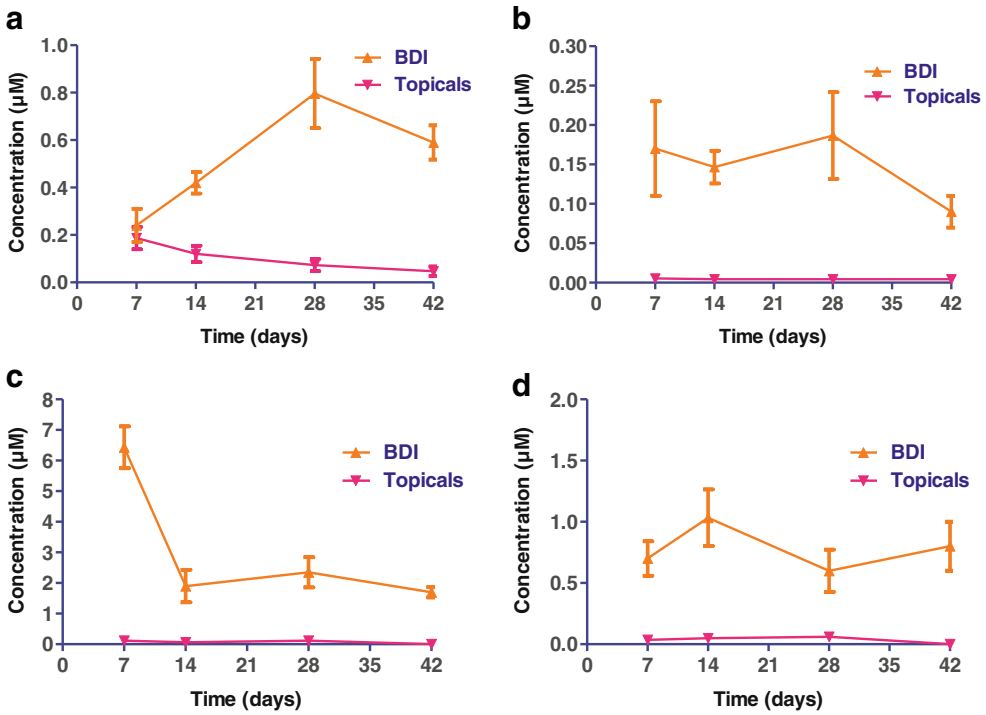

Fig. 5 DXM delivery from BDI exceeds that of topical drops. Pharmacokinetic profile of BDI *vs.* topical drops in NZW rabbits. (**a**) aqueous humor, (**b**) vitreous humor, (**c**) iris/ciliary body and (**d**) retina/choroid. The loading dose of DXM in the BDI was only 0.3 mg, where as with topical drops ~3.8 mg was administered (cumulative). With the BDI, DXM was found in therapeutic concentrations in all ocular tissues throughout the study period while topical drops did not achieve therapeutic concentrations in posterior segment, further, DXM concentrations in iris/ciliary body and retina/choroid were BQL on day 42.

TABLE II Pharmacokinetics of BDI *vs.* Topical Drops in Aqueous Humor and Vitreous Humor of NZW Rabbits

Parameter	BDI		Topical Drops	
	Aqueous humor	Vitreous humor	Aqueous humor	Vitreous humor
C_{max} (μM)	0.8 ± 0.14	0.22 ± 0.03	0.19 ± 0.05	0.01 ± 0.0
T_{max} (day)	33 ± 8	21 ± 12	7 ± 0	7 ± 0
AUC_{0-t} (day*μM)	21.3 ± 2.6	5.9 ± 0.5	3.8 ± 0.8	0.17 ± 0.01
C_{last} (μM)	0.6 ± 0.1	0.09 ± 0.02	0.05 ± 0.02	0.004 ± 0.001
T_{last} (day)	42 ± 0	42 ± 0	42 ± 0	42 ± 0

group (eyes which received only Con A) had severe corneal edema and infiltration of lymphocytes by week 4 (Fig. 8a).

Iris and Ciliary Body

The Standard control group had mild to moderate inflammation of the iris and ciliary body, characterized by infiltration of lymphocytes around the vessels of the ciliary body. These lymphocytes were accompanied by a large number of macrophages. Rabbits treated with topical drops after Con A + phacoemulsification showed increasing severity of ciliary body inflammation which was diffusely infiltrated. In areas where cells migrate from ciliary processes into the vitreous, fibrin exudates and signs of tissue destruction were observed. In the BDI group, the iris and ciliary body were swollen with infiltration of lymphocytes by week 2, but the severity of inflammation was well controlled by week 4, and at week 6, tissues appeared relatively normal (Fig. 8b).

Retina, Choroid and Sclera

The standard control group had moderate to severe inflammation of the retina and choroid. Four eyes that exhibited severe disruption of the retina (as seen on SD-OCT) were excluded from analysis. The BDI group exhibited mild to moderate inflammation during week 1 and 2 but the inflammation was controlled by week 4. In contrast, severe inflammation of the choroid was observed from week 1 through week 6 in rabbits treated with topical drops after Con A injection + phacoemulsification. The formation of lymphoid follicles could be seen in all the eyes (18/18) throughout the study period. Uncontrolled inflammation led to the loss of retinal architecture and massive subretinal exudation led to retinal detachment in some eyes (n=5) treated with topical drops (Fig. 8c). Taken together, these data highlight the efficacy of BDI in overcoming inflammation in eyes receiving Con A and phacoemulsification.

DISCUSSION

Our findings demonstrate that implantation of the BDI immediately after phacoemulsification prevents retinal edema and uveitic inflammation. Unlike topical drops, BDI was effective in limiting inflammation in both the anterior and posterior segments of the Con A-injected eyes following uveitic cataract surgery. We also observed that topical drops were effective in controlling only anterior segment inflammation. Since uncontrolled inflammation in the posterior segment heralds CME, which can lead to permanent vision loss, we believe that BDI-based drug delivery could be effective in therapeutic management of severe posterior segment disorders. While rabbits do not have maculae, our findings that the BDI suppresses retinal thickening in rabbits suggests a high likelihood of efficacy in suppressing CME in humans.

CME is the most common cause of diminished visual recovery after cataract extraction and occurs in about 8.2%

TABLE III Pharmacokinetics of BDI *vs.* Topical Drops in Iris/Ciliary Body and Retina/Choroid of NZW Rabbits

Parameter	BDI		Topical Drops	
	Iris/Ciliary body	Retina/Choroid	Iris/Ciliary body	Retina/Choroid
C_{max} (μM)	6.4 ± 0.7	1.1 ± 0.2	0.13 ± 0.06	0.06 ± 0.01
T_{max} (day)	7 ± 0.0	14 ± 0	14 ± 12	23 ± 8
AUC_{0-t} (day*μM)	99 ± 19	28 ± 6	2.3 ± 0.7	1.2 ± 0.2
C_{last} (μM)	1.7 ± 0.2	0.8 ± 0.2	0.11 ± 0.06	0.06 ± 0.01
T_{last} (day)	42 ± 0.0	42 ± 0.0	28 ± 0.0	28 ± 0

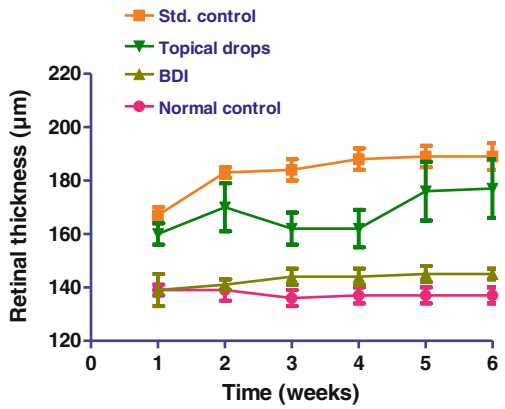

Fig. 6 BDI prevents retinal swelling. Retinal thickness measurements recorded by SD-OCT, expressed as mean ± SD ($n=4$). Topical drops group showed a marked increase in retinal thickness up to 6 weeks, whereas BDI effectively controlled retinal thickness compared to topical drops (145 ± 2 *vs.* 177 ± 11 μm) by the end of 6 weeks. Retinal thickness in topical drops group increased soon after decreasing the drops frequency from t.i.d. to b.i.d and q.d. Normal control group exhibited retinal thickness of 138 ± 1 μm while the standard control group (received Con A, no steroids) demonstrated progressive increase in retinal thickness and reached 189 ± 5 μm by the end of 6 weeks.

of routine cataract surgeries (5,24–28). CME develops as fluid accumulates and pools in the macula, thus decreasing visual acuity (29,30). An increase in macular thickness (of approximately 10–15 μm) visible on OCT occurs in up to 30% of patients undergoing phacoemulsification (31). CME can occur in 10–40% of high-risk cataract extractions (32), which constitute up to 30% of cases depending upon the risk factors and the level of difficulty and complications encountered intra-operatively (32,33). As CME affects the posterior segment, DXM administered topically (which does not reach the retina) is inadequate for treatment and prevention (22).

Not surprisingly, Con A induced inflammation was exacerbated by phacoemulsification (Fig. 8), indicating that cataract extraction in the presence of pre-existing uveitis requires sustained release of a steroidal anti-inflammatory agent for an effective clinical outcome. Retinal thickening was likely worse in Con A injected eyes treated with topical steroid eye drops after phacoemulsification relative to standard control eyes (Con A injection only) as there was no surgery (phacoemulsification) in the standard control group. Further, phacoemulsification is known to exacerbate CME in patients with pre-existing uveitis (34).

We observed that DXM pharmacokinetics in healthy (phacoemulsification only) (12) and inflamed eyes (Con A intravitreal injection followed by phacoemulsification, current study) are significantly different, suggesting that the underlying inflammatory status of the eye influences implant degradation and pharmacokinetics. As reported (35–37), pre-existing inflammation can influence the ocular fluid dynamics, blood-aqueous barrier integrity, retinal permeability, and in turn the absorption, distribution, metabolism and elimination (ADME) of DXM. The concentration-time profiles for DXM were different between healthy rabbit eyes and inflamed eyes for the aqueous humor, vitreous humor, iris/ciliary body and retina/choroid. The cumulative exposure (AUC_{0-t}) of DXM was decreased in inflamed eyes by ~2 times in aqueous humor, 8 times in vitreous humor and iris/ciliary body, and more than 16 times in retina/choroid. In our previous study, in healthy eyes, remnants of the BDI were not seen at the end of 6 weeks and DXM levels were close to BQL both in aqueous and vitreous humor (12). However, in our current study, in inflamed eyes, ~1/10th of the initial mass was recovered, and DXM concentrations were in therapeutic

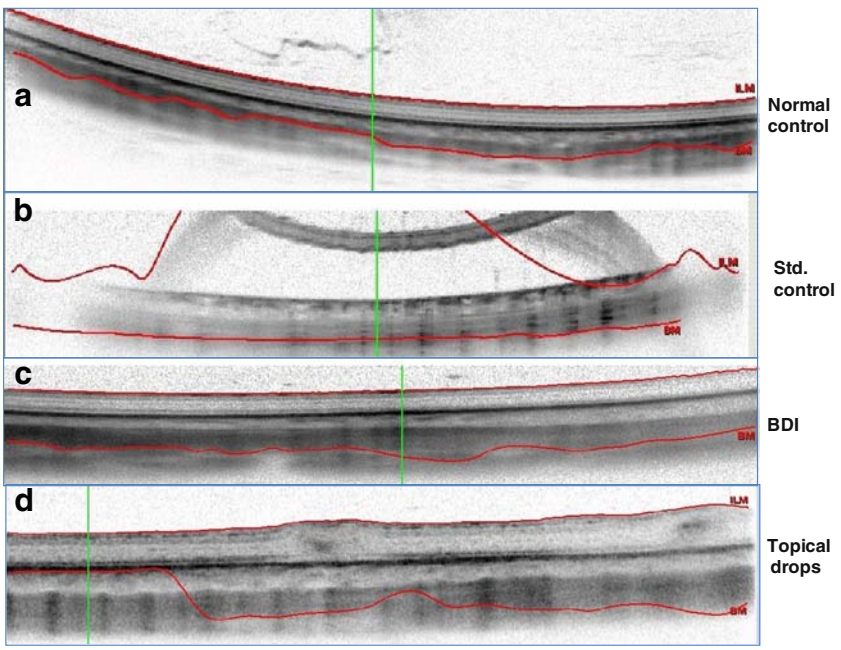

Fig. 7 BDI prevents Con A induced retinal edema, SD-OCT scans show normal retinal thickness (**a**), edema and architectural destruction in eyes injected intravitreally with Con A (**b**). Eyes which received BDI implant demonstrated retinal thickness similar to normal control group (**c**), eyes treated with topical drops had significant edema up to 6 weeks post phacoemulsification/cataract surgery (**d**).

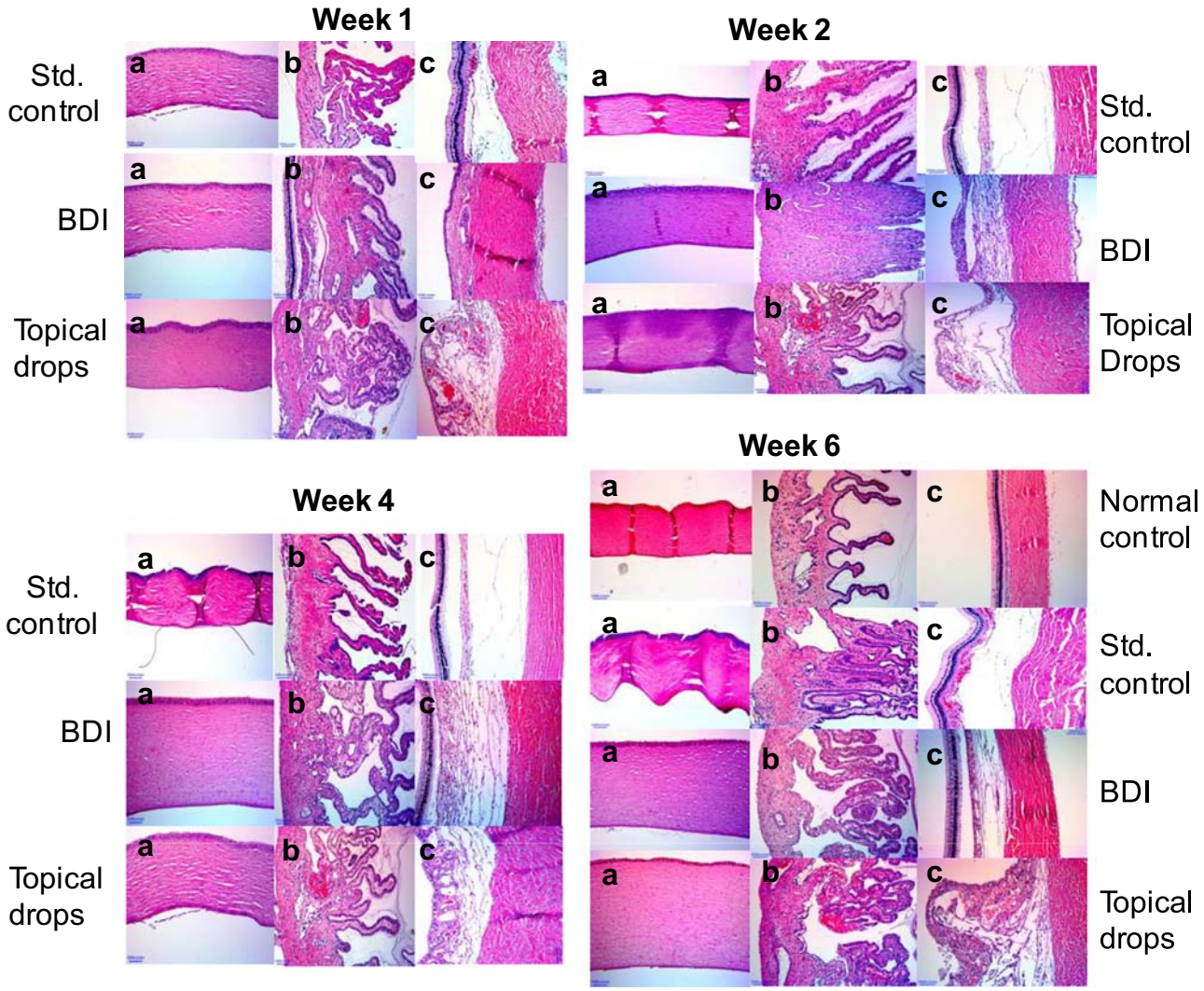

Fig. 8 BDI is biocompatible. Histopathology of the cornea, iris/ciliary body and retina/choroid/sclera in Con A induced experimental uveitis. (**a**) Cornea, (**b**) Iris and ciliary body, (**c**) Retina, choroid and sclera. *Scale bar* − 100 μm.

range at the end of 6 weeks in both the aqueous and vitreous humor. These results suggest that degradation of the polymer and drug release kinetics is different in ocular disease states. Furthermore, it is evident that dosage must be adjusted to ensure that the observed concentrations are within therapeutic window so that drug related adverse effects be avoided.

In the topical drops group, vitreous humor concentrations were negligible, and the levels of DXM decreased tremendously in inflamed eyes in iris/ciliary body (>18 times) and retina/choroid (~40 times). This may indicate the challenges that underlying inflammation pose in the ADME of topically administered drugs. These findings suggest the need for conducting pharmacokinetic and pharmacodynamic studies in appropriate disease/animal models during the development of ocular medications/implants so that, dosage can be adjusted depending on the ocular disease states to achieve therapeutic drug concentrations for an effective clinical outcome.

Currently there is one FDA approved DXM delivery implant indicated for diabetic macular edema: Ozurdex (Allergan Inc.). This intravitreal steroid device has a therapeutic duration of 6–9 months (37,38). A significant limitation of this product is that it does not achieve therapeutic drug concentrations in the anterior segment, limiting its utility only to the posterior segment. *Further, long term (6–9 months) delivery of drugs is not necessary in routine or cataract surgery with pre-existing uveitis*. Persistence of DXM for such a long period has a high risk of elevating intraocular pressure and subsequent adverse effects (39,40).

Clinical and slit lamp examinations revealed that the BDI controlled inflammation successfully. Our results are in line with the published results where topical drops are effective in treating the anterior chamber inflammation but had no effect on posterior segment inflammation (15,20–22). Although anatomic and physiologic differences between rabbit and human eyes must be considered, it appears as though biodegradable

delivery systems from the capsular bag are advantageous for delivering drugs to the eye. The implant stays in place, does not interfere with the line of sight, and erodes over time, thereby eliminating the risk of implant dislocation or need for a second surgery to remove an empty reservoir. Further, combining the implantation procedure with cataract surgery eliminates the safety issues encountered with intravitreal injections or anterior chamber insertions.

CONCLUSION

In conclusion, this study has demonstrated superiority of the BDI over topical 0.1% DXM drops in controlling uveitis induced by intravitreal injection of Con A. We will explore testing of the BDI in other species after successful method transfer for bulk production and then advance to its clinical development.

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